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Effect of slurry lagoon redesign on reduction of ammonia emission during livestock manure storage

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Abstract. In accordance with the international and EU aims to reduce emissions of ammonia and other air pollution substances farm manure management, including its storage, especially for the animal breeding sector, is one of the most essential stages. One of the preventing steps is covering of the slurry lagoons. The most effective – hard covering can be provided only after the lagoon is constructed. The aim of the research: to develop methodology for calculations of emission reducing lagoon design volume and surface area. In the result of the theoretical research the possibilities of reducing the reflection surfaces at the same capacity of the lagoon have been discovered, as well as the changes of the reflection surface area, if instead of one lagoon several lagoons with less volume are installed and sequential filling of these lagoons is ensured. The article presents the calculation algorithms obtained during the research and recommendations for construction of low emission lagoons.

Key words: manure, lagoon capacity, modelling.

INTRODUCTION

For storage of slurry, cylindrical and lagoon type storages are used in Latvia. Still, in the recent years lagoons have become more popular as they can be more easily installed and they are also cheaper. Nevertheless, lagoon type storages have an essential drawback. They have a large upper surface area of slurry or reflection surface from which unfavorable gas emissions are discharged. Therefore, in the Regulations No. 829 of the Cabinet of Ministers of the Republic of Latvia it has been stated that the closed type storages for slurry have to be installed or the stored slurry should be covered with a permanent natural or artificially made floating covering layer that reduces evaporation.

These measures ensure only partial reduction of the undesirable gas emissions. It is proved, for instance, by the Convention of the Economic Commission for Europe on Transboundary Air Pollution in Large Distances, which states that naturally formed covering layer of the lagoon gives only 40% reduction of ammonia emissions, but covering with the floating layer ensures emission reduction of 60%. Therefore, it is advisable to evaluate also other methods of ammonia emission reduction regarding the reduction of the slurry surface.

Many studies have been conducted lagoon geometry. The criterion for the most part, is the safe and efficient handling of manure and wastewater and requires using proper construction criteria and sizing the structure large enough to meet at least the 180 day storage requirement for a specific size of the operation (Jons & Sutton, 2007, 6). Design parameters must be optimized to ensure greatest degradation of pollutants within the smallest volume possible (Hamilton et al., 2006). Circular or square lagoons facilitate mixing and are usually more economical to construct. Rectangular lagoons may be used, but length-to-width ratios of 3:1 or less are recommended (Pfost & Fulhage, 2000).

As it is shown by our previous research, optimization of the lagoon geometrical parameters is an important factor reducing ammonia emissions. For this purpose we have developed a methodology for calculation of lagoon capacity as well as stated the influence of the lagoon depth, wall sloping angle and side edge length proportion on the area of the reflection surface (Murikov & Priekulis, 2006; Priekulis & Murikov, 2008). In these investigations it was stated that the reflection surface reduces if the lagoon is built possibly deep and with a larger sloping angle as well as with the side edges of equal length. Still, the numerical values of these parameters depend on the particular situation. For instance, the depth of the lagoon is limited by the maximal level of the ground water, as according to the requirements of the Regulation No. 829 of the Cabinet of Ministers of the Republic of Latvia the ground water level should be at least 0.5 m lower than the bottom part of the storage. In turn, the side sloping of the lagoon is limited by the method of its construction as usually the side part of the lagoon is made of ground bank without additional fastening elements.

Still, there is another solution of this problem. Namely: there is a possibility to install several lagoons instead of one large lagoon, for instance, four proportionally smaller ones, the total volume of which is the same as of the large lagoon, but during exploitation to fill these small lagoons sequentially. In such case it is possible to prognosticate that installing the small lagoons the total emissions of ammonia will reduce as the area of the slurry reflection surface, from which the emissions come at the beginning of the slurry filling, is many times smaller than using one large lagoon.

Therefore, the aim of this research was to test the above mentioned hypothesis for this purpose specifying the calculation methods of the lagoon reflection surface as well as its capacity and to state the changes of the reflection surface area filling slurry in one large lagoon as well as in several smaller lagoons with the volume equal of the large lagoon.

MATERIALS AND METHODS

To compare the reflection surface area of slurry, two types of lagoons were examined: with equally long side edges or the square type lagoon and a lagoon with different length of the side edges or the rectangular lagoon.

After that the summary area of the lagoon reflection surface was evaluated in two different cases:

- installing one large capacity lagoon;
- installing four correspondingly smaller lagoons with equal depth, equal volume and equally large side edge angles, but with the total volume equal with the volume of the large lagoon.

In the research it was assumed that slurry filling intensity would be regular and equal. It was also considered that the maximal level of slurry in the storage should be at least 0.2 m lower than the upper edge of the lagoon (Ed. Priekulis, 2012).

Square type lagoon

The first type is a lagoon with a square bottom with the volume V. The task is to fill the given amount of slurry in four equal square type lagoons of a smaller size with equal maximal depth h_0 and the wall angle α (Fig. 1). Lagoons of such kind can be considered as a truncated pyramid and their volume can be calculated accordingly (Priekulis & Murikov, 2008):

$$V = \frac{h_0}{3} \cdot (S_p + S_v + \sqrt{S_p \cdot S_v}) \tag{1}$$

where V – volume of the lagoon, m^3; S_v – surface area of slurry at the highest (maximal) slurry level, m^2; S_p – bottom area of the slurry lagoon, m^2; h_0 – maximal height of the slurry layer in the lagoon, m.

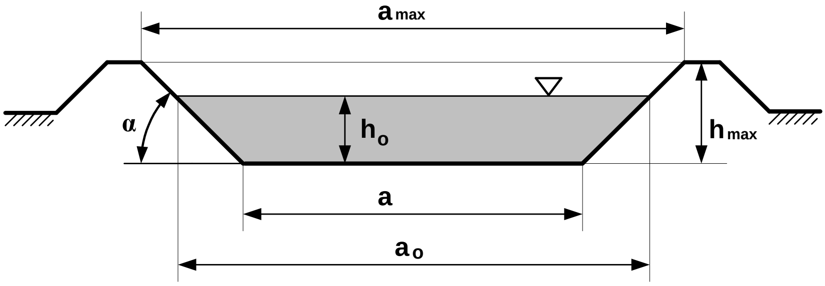

Figure 1. Scheme of lagoon type slurry storage cross section: a – length of the bottom edge; α_{max} – length of the upper edge; h_0 – variable height of the slurry layer in the lagoon $0 \leq h_0 \leq h_{max}$; α_0 – variable length of one edge of the square surface $0 \leq a_0 \leq a_{max}$.

The volume of the large storage is equal to the volume of four equal smaller storages V_m with equal height and wall angle, i.e. $V = 4V_m$:

$$\frac{h_0}{3} \cdot (x^2 + y^2 + \sqrt{x^2 \cdot y^2}) = \frac{4 \cdot h_0}{3} \cdot (a^2 + b^2 + \sqrt{a^2 \cdot b^2}) \tag{2}$$

where x – length of the bottom edge of the large lagoon, m; y – length of the reflection surface edge of the large lagoon at the highest (maximal) level of slurry, m; α – length of the bottom edge of the small lagoon, m; b – length of the reflection surface edge of the small lagoon at the highest (maximal) level of slurry, m.

Filling a part of slurry, the length of the lagoon surface edge will increase by Δ and Eq. (2) takes the following form:

$$x^2+(x+\Delta)^2+(x+\Delta)\cdot x=4\cdot(a^2+(a+\Delta)^2+(a+\Delta)\cdot a) \qquad (3)$$

where Δ – value by which the length of the surface edges has increased compared to the bottom, m;
The simplification of Eq. (3) is below:

$$3x^2+3x\cdot\Delta+\Delta^2=4\cdot(3a^2+3a\Delta+\Delta^2) \qquad (4)$$

The volume of one small lagoon is 0.25 of the volume of the large lagoon, i.e. $V_m = 2.25V$, or:

$$3a^2+3a\cdot\Delta+\frac{3}{4}\Delta^2=\frac{3}{4}x^2+\frac{3}{4}x\cdot\Delta \qquad (5)$$

As $\Delta = \frac{2h_0}{\tan\alpha}$, it is possible to calculate the corresponding bottom edge length of the small lagoons α, if the bottom edge length of the large lagoon *x* is given. For this purpose the quadratic Eq. (5) in relation to the bottom edge α of the small lagoon should be solved:

$$3a^2+3\frac{2h_0}{\tan\alpha}a+\frac{3}{4}\left(\frac{2h_0}{\tan\alpha}\right)^2-\frac{3}{4}x^2-\frac{3}{4}x\cdot\frac{2h_0}{\tan\alpha}=0 \qquad \text{or}$$

$$a^2+\frac{2h_0}{\tan\alpha}a+\left(\frac{h_0}{\tan\alpha}\right)^2-\frac{1}{4}x^2-\frac{1}{2}x\cdot\frac{h_0}{\tan\alpha}=0 \qquad (6)$$

To find the reflection surface of the lagoon the equation system should be solved

$$\begin{cases} V_i=\dfrac{h_i}{3}\cdot(x^2+z^2+xz) \\[2ex] \dfrac{2h_i}{z-x}=\tan\alpha \end{cases} \qquad (7)$$

where V_i and h_i – accordingly the volume of slurry filled in the lagoon and the height; *z* – length of the storage surface edge at the filling height h_i.
Changing the system, obtained equation:

$$(z-x)(z^2+xz+x^2)=\frac{6\cdot V}{\tan\alpha}, \quad \text{or} \quad z^3-x^3=\frac{6\cdot V}{\tan\alpha} \qquad (8)$$

It is possible to determine the length of the square reflection surface edge *z* and after that its area S_i, at a definite volume V_i and the length of the bottom edge *x*:

$$S_i=\sqrt[3]{\left(x^3+\frac{6\cdot V_i}{\tan\alpha}\right)^2} \qquad (9)$$

Rectangular lagoon

The second type is a rectangular lagoon with the volume *V*. Our task is to fill the given amount of slurry in four smaller rectangular lagoons with equal maximal depth h_0 and the wall angle α (Fig. 2).

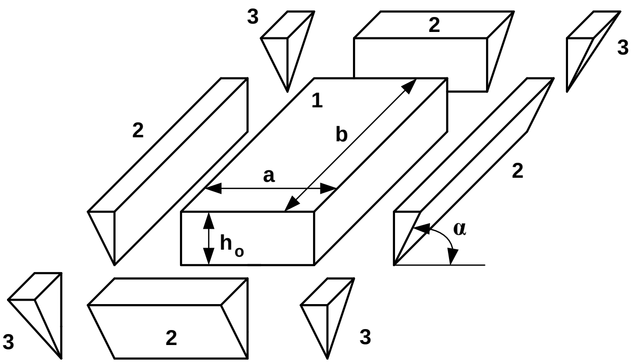

Figure 2. Scheme of calculation of lagoon volume in a general case dividing the volume in elementary components: 1 – parallellepiped; 2 – prism; 3 – pyramid.

The volume of such storages can be calculated:

$$V = a \cdot b \cdot h_0 + \frac{h_0^2}{\tan\alpha}(a+b) + \frac{4}{3}\left(\frac{h_0}{\tan\alpha}\right)^2 \cdot h_0 \ , \quad \text{or}$$

$$V = \frac{h_0}{3 \cdot \tan^2\alpha}(3a \cdot b \cdot \tan^2\alpha + 3 \cdot h_0 \cdot (a+b) \cdot \tan\alpha + 4h_0^{\ 2}) \tag{10}$$

where *a*, *b* – lengths of the lagoon bottom edge.

It is assumed that the relation of the large lagoon bottom edges is known, i.e. $\frac{x_1}{x_2} = k$, that is equal with the relation of the small lagoon bottom edges, i.e. $\frac{a_1}{a_2} = k$, where x_1, x_2 are the lengths of the large lagoon bottom edges; a_1, a_2 are the lengths of the small lagoon bottom edges.

Using (10) and the relation of the given bottom edge lengths an equation can be developed that relates the volumes of the large and small lagoons:

$$\frac{h_0}{3 \cdot \tan^2\alpha}(3k \cdot x_2^2 \cdot \tan^2\alpha + 3 \cdot h_0 \cdot (k+1) \cdot x_2 \cdot \tan\alpha + 4h_0^{\ 2}) =$$
$$= \frac{4h_0}{3 \cdot \tan^2\alpha}(3k \cdot a_2^2 \cdot \tan^2\alpha + 3 \cdot h_0 \cdot (k+1) \cdot a_2 \cdot \tan\alpha + 4h_0^{\ 2}) \tag{11}$$

From Eq. (11) it is possible to find the bottom edge of the small lagoon α_2 that corresponds to the large lagoon, from which the other bottom edge α_1 can be found using the given relation *k*:

$$\begin{aligned} &12 \cdot k \cdot \tan^2\alpha \cdot a_2^2 + 12 \cdot h_0 \cdot \tan\alpha \cdot (k+1) \cdot a_2 + \\ &+ 3[4 \cdot h_0^{\ 2} - k \cdot x_2^2 \cdot \tan^2\alpha - h_0 \cdot (k+1) \cdot x_2 \cdot \tan\alpha] = 0 \end{aligned} \tag{12}$$

Filling a part of slurry V_i the length of the surface area edge varies compared to the bottom by $2\Delta_i$ and the height of filling in the lagoon by h_i, i.e.

$$V_i = h_i \cdot x_1 \cdot x_2 + \frac{4}{3} h_i \Delta_i^2 + h_i (x_1 + x_2)\Delta_i \tag{13}$$

As $\frac{h_i}{\Delta_i} = \tan\alpha$ and $h_i = \Delta_i \cdot \tan\alpha$ we can insert this expression in Eq. (13). After algebraic calculations the equation of the third degree can be obtained, that characterises the value of the surface area edge variations Δ_i depending on the lengths of the rectangular lagoon bottom edges x_1 and x_2, the wall angle α and the volume V_i:

$$\frac{4}{3}\tan\alpha \cdot \Delta_i^3 + \tan\alpha \cdot (x_1 + x_2) \cdot \Delta_i^2 + \tan\alpha \cdot x_1 \cdot x_2 \cdot \Delta_i - V_i = 0 \tag{14}$$

Solving Eq. (11) it is possible to obtain the surface area of the lagoon S_i:

$$S_i = (x_1 + 2\Delta_i)(x_2 + 2\Delta_i) \tag{15}$$

RESULTS AND DISCUSSION

Applying the offered methods numerical experiments were carried out. A square type lagoon with the bottom parameters 15 x 15 m and the height 2 m and four small size lagoons with equal capacity to store slurry, equal depth and side angles $\alpha_1 = \frac{\pi}{4}$ and $\alpha_2 = \frac{\pi}{3}$ were chosen. Using the expression (6) the small storage bottom edge lengths $\alpha_1 = 6.44$ m and $\alpha_2 = 6.90$ m corresponding to the side angles were calculated.

The variations of the lagoon reflection surface during filling stated by calculations are summarised in Fig. 3.

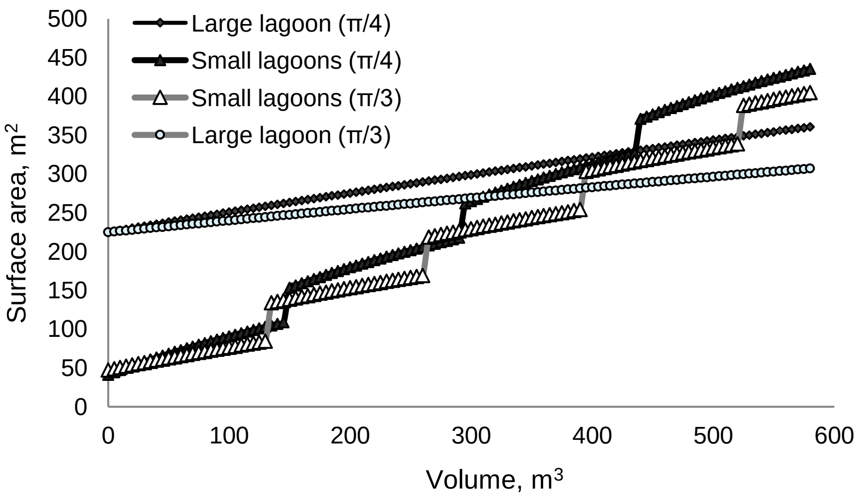

Figure 3. Comparison of the large square type lagoon and small square type lagoon summary reflection surface areas at different side edge sloping angles and the depth of storage h_0 = 2 m with varying amount of filled farm manure.

As it can be seen from the figure, the summary reflection surface of full small lagoons is larger than for the large lagoon. Still, it should be considered that at the beginning of filling the small lagoons, their reflection surface area increases considerably slower and more gradually than for the large lagoon. It is caused by the fact that at the beginning of slurry filling the bottom area of the lagoon is filled first and for the small lagoons it is considerably smaller than that of the large lagoon.

To obtain more precise research results we assume that in all cases lagoons have been filled continuously and with stable intensity. Therefore, the lagoon filling degree can be replaced by the filling time. In turn, the possible value of emissions is characterised by the area integral depending on the filling time of the lagoon. It means that emissions:

$$E = \int S \cdot dt$$

where E – amount of emissions; S – area of slurry reflection surface filled in the lagoon; t – lagoon filling time.

For a large lagoon in a full filling cycle it can be evaluated:

$$E_l = \int_0^1 f(t)dt \tag{16}$$

where E_l – emissions from the surface area of the large lagoon; $f(t)$ – variations of the reflection surface during filling.

For filling the small lagoons, the filling of every lagoon should be evaluated that comprises 25% of the total volume, i.e.

$$E_{sm} = \int_0^{\frac{1}{4}} f_1(t)dt + \int_{\frac{1}{4}}^{\frac{1}{2}} f_2(t)dt + \int_{\frac{1}{2}}^{\frac{3}{4}} f_3(t)dt + \int_{\frac{3}{4}}^{1} f_4(t)dt \tag{17}$$

where E_{sm} – summary amount of emissions from the total surface area of the small lagoons; $f_i(t)$ – variations of lagoon reflection surfaces during filling. The functions $f(t)$ and $f_i(t)$ are found using the expression (6) for square type lagoons and (12), (14) for rectangular lagoons.

By integration it was stated that at the lagoon side sloping angle $\alpha = \frac{\pi}{4}$ usage of four small lagoons reduces emissions by 19% compared to one large lagoon, but at the angle $\alpha = \frac{\pi}{3}$ the reduction is 26%.

Similarly one large rectangular lagoon was compared to four correspondingly smaller lagoons of the same capacity. The bottom edge lengths of the large lagoon are 15 x 20 m, its maximal filling height – 2 m and the side angles $\alpha = \frac{\pi}{4}$, but the volume – 751 m^3. Besides, the large lagoon corresponds to four small lagoons with the bottom sizes 6.58 x 8.78 m (Fig. 4).

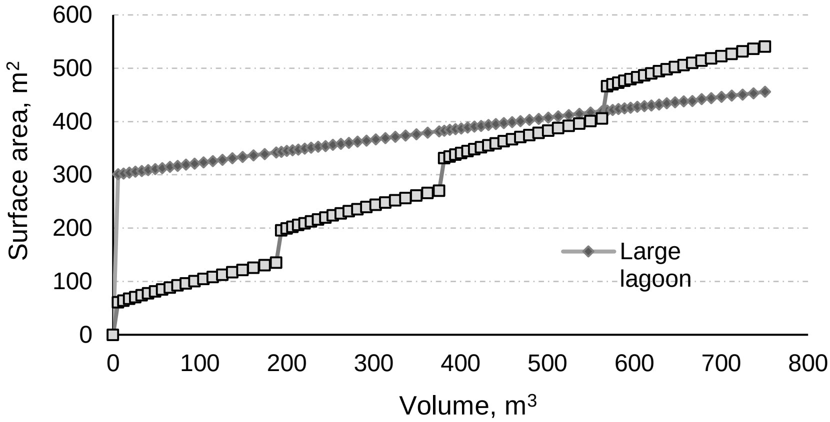

Figure 4. Variations of the reflection surface of the large rectangular lagoon and small rectangular lagoons depending on the filling of these storages if their depth h_0 = 2 m.

Using formulas (16) and (17), can be expected that using small lagoons and filling them sequentially it is possible to reduce ammonia emissions by 27% with accepted sizes in calculations.

If instead of one large volume lagoon four smaller lagoons with the same volume, equal depth and side sloping angles are installed and the small lagoons are filled sequentially, the slurry reflection surface areas from which unfavorable gasses are emitted are changing differently in both cases. Using a large volume lagoon this reflection surface quickly increases at the beginning until the whole lagoon bottom area if completely filled. After that the increasing intensity gets essentially lower as it is caused only by the influence of sloping of the lagoon sides. If, in turn, several smaller lagoons are used and sequentially filled, the increase of the reflection surface at the beginning is small, but its increasing intensity is faster. Therefore, at filling the third lagoon the area of the total reflection surface for the smaller lagoons is larger than for the large lagoon.

CONCLUSIONS

1.Methodology for calculation of volumes and reflection surface areas of different type slurry lagoons depending on their geometrical parameters has been developed.

2.It was found that within the input data used in the research replacing one large lagoon by four correspondingly smaller lagoons and their sequential filling can gives the possible ammonia emission reduction by 19–27%.

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Production and analysis of non-traditional beer supplemented with sea buckthorn

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Abstract. In recent years, there has been a growing demand for non-traditional beer (craft) with different flavours hence the main aim of this research is to produce beer with sea buckthorns (SBT). Brewing software BeerSmith was used to design the Kölch beer. After one month of primary fermentation, SBT were sanitised and crashed before adding into the green beer. Carbonation was done using keg with 1.8 bar of carbon dioxide. Physicochemical properties, microbial load and sensory evaluation of Kölch fruit beer (KFB) were determined. All the physiochemical parameters measured were significantly ($P < 0.05$) influenced by the fermentation time. The pH dropped from 5.8 ± 0.1 to 3.9 ± 0.1 toward the end of the fermentation. There was no microbial growth when KFB was inoculated in the media. °Brix likewise decreases from 13.3 ± 0.43 to 3.86 ± 0.25. There was a change in the colour of the wort throughout the fermentation from 11.2 ± 0.44 to 32.5 ± 0.56 EBC. A decrease from 1.48 ± 0.02 to 0.86 ± 0.02 mg maltose per 100 mL in the reducing sugar was observed during the entire period of fermentation. A total of 32 volatile compounds were identified. All assessed sensory variables of KFB were significantly different ($P < 0.05$) and preferred by the panellists, however, foaminess and clarity of KFB should have to be improved. KFB showed higher DPPH radical scavenging activity as compared to other types of beer examined due to biologically active substances contributed by SBT.

Key words: sea buckthorns (SBT), Kölch fruit beer (KFB), sensory analysis, physicochemical and organoleptic properties.

INTRODUCTION

Beer is the world´s most established and broadly consumed liquid refreshment and the third most prevalent drink after water and tea (Nelson, 2005). It is an alcoholic beverage obtained by fermentation of wort, which is a mixture of malt, hops and water (Catarino, 2010). According to Leitao et al. (2011), beer is rich in nutrients i.e. carbohydrates, amino acids, minerals, vitamins and cancer preventing phenolic compounds. Hence, moderate drinking would have a positive impact on human health.

Fruit beers have been known around for centuries. Sugar from the fruits have been utilised as a principal constituent in fermentation processes and it was routine for ancient people to mix beer with fruits using the sugar from fruits to help in the maturation process.

According to Mosher (2004), fruit beer is moderately present day creation of the 20th century. Egyptians alluded to the utilisation of dates and pomegranates, however, just a few passing references can be found for this type of beer in interceding years until the 1930's. These beers contain no real natural products, rather a mixture of light beer with a simulated natural product to give a flavour of fruits. Home brewers can do a similar thing by obtaining fruits from shops and adding a suitable amount to their beers. Some brewery industries likewise utilise fruit extract. This functions admirably for natural products like raspberry and apricots that keep up their flavour after ageing. Examples of some commercial fruits beer are New Glarus Belgian Red, Raspberry Tart, Bell's Cherry Stout, Dogfish Head Aprihop, Melbourne Apricot Beer and Strawberry Beer (Strong et al., 2008). Ducruet et al. (2017) added goji berries to amber ale type beer. They brewed at different stage of the production process with the aim of developing beverage with high antioxidant and sensory characteristics. Sour cherry has been also added to mid-young lambic beer (Gert et al., 2014). However, other authors have utilized various fruits in designing their beers (Osta, 2017).

Sea buckthorn (SBT) is a deciduous shrub, generally conveyed everywhere throughout the world, including Russia Federation. It contains various types of nutrients and bioactive substances, such as, vitamins, carotenoids, flavonoids, polyunsaturated unsaturated fats, free amino acid and basic compounds. These compounds change generously among geographical locations, however, their presence is essential for human health. Scientific reviews in the 20th century affirmed therapeutic and wholesome advantages of SBT. Studies are right now experiencing to utilise this natural product in cancer treatment, cardiovascular illness, skin issue, and gastrointestinal ulcer and as liver defensive operator (Mingyu, 1994; Li & Schroeder, 1996; Xing et al., 2002; Zeb, 2004; Ruan, 2007). The fundamental SBT processing products are juice, wine, jam, preserves, compote, and tea (from leaves) (Li & McLoughlin, 2007).

In recent years, there has been a growing demand for beer with fruit flavours. The main purpose of this study is to produce fruit beer using SBT since in present there is no single scientific publication on beer with SBT.

MATERIALS AND METHODS

Some of the materials and equipment use in brewing the KFB are presented in Table 1. A brewing software BeerSmith (version 2.3) (USA) was used in designing the Kölch beer.

Table 1. Raw materials and Equipment

Malt	Hops	Yeast	Equipment	Disinfectants	Fruit
Château Pale Ale (4.9 kg)	Hallertauer Hersbrucker (100 g)	Kölsch yeast (Wyeast Labs #2565, UAS)	Mash turn	Ethanol (90%)	Sea buckthorn
Château Pilsen 2RS (2.5 kg)	Huel Melon (40 g)		Mash paddle	Mira acid NV liquid (Mirachem Industries)	
Château Wheat Blanc (0.4 kg)			Siphon		
			Hydrometer Containers		

Mashing protocol and wort production

Single infusion method was used in this study. Before starting the brewing, all the equipment was washed and sterilised using Mira acid NV liquid. 7.8 kg of malted grains were measured and milled with roller miller. The milled malt was mixed with 30 L distilled water in mash tun (hydromodule 1:3.8) and heat up to 47 °C for 20 minutes. The temperature was increased to 65 °C after 5 min with a step time of 40 min. Again after the step time, the temperature was once again increased to 78 °C after 10 minutes. Filtration was carried out and the filtrate (wort) then boiled at 100 °C for 90 minutes. 22 L of distilled water was used for sparging process. Mash Paddle was used in stirring the mixture throughout the whole process. Hallertauer Hersbrucker hops were added 60 min before the end of the boiling time. When the boiling time was over the Huel Melon hops was then added. Clean sterilised copper wort chiller was then lower into the boiled wort for cooling. With the aid of sterilised syphon, the cold wort was transferred into fermentation bucket equipped with airlock bubbler.

Pitching and fermentation

Wyeast smack-pact™ sealed with internal nutrient was purchased and activated by shaking package vigorously to release nutrient 3 h before the pitching time. 95% alcohol was use to sanitise the package before opening and pitched the wort in the fermentation bucket. Fermentation was carried out at a temperature of 18 °C for 4 weeks.

Addition of SBT

SBT (Fig. 1) was purchased from the local market in Yekaterinburg, washed with distilled water peracetic acid and sodium hydroxide and was rinsed with distilled water. It was then mashed in a sterilised plastic bowl and added into the new fermentation bucket with the 4 weeks old Kölch green beer (KGB) for further two weeks fermentation at 18 °C.

Conditioning

Stainless steel keg was used for the carbonation. The keg was initially washed and sterilised with antiseptics (peracetic acid and sodium hydroxide) and dry for several hours before usage. The 6 weeks old KFB was transferred into the keg with the aid of sterilised syphon and pumped 1.8 bar of carbon dioxide (CO_2) from CO_2 tank fitted with an automatic gas pressure regulator that can be set at a predetermined pressure. It was then stored at 4 °C for 1 week.

Physicochemical parameters

°Brix was measured using a refractometer RSG-100ATC. pH was determined using digital pH meter (Hanna, model HI 98127). Titratable acidity (TA) was determined by titrating the sample with 0.1 N NaOH solution with 1% phenolphthalein until slightly pink colouring remains for 30 seconds. Lane-Eynon method was used to quantify the reducing sugars (ASBC, 2007). Colour was determined by measuring the absorbance at 430 nm using spectrophotometer (ParkinElmer, Lambda 35) against distilled water in cuvettes of 10 mm length.

Enumeration of Microorganisms

Using the laminar flow cabinet, a serial dilution of KFB have been performed mixing 1 mL of KFB with 9 mL of sterile peptone physiological saline solution (5 g peptone, 8.5 g NaCl, and 1,000 mL distilled water, pH = 7.0) and homogenised with the help of pipette. Decimal dilutions were plated. Aerobic mesophiles were enumerated by pour plate on nutrient agar (15 g peptone, 3 g yeast extract, 6 g NaCl, 1 g dextrose and 12 g agar, pH = 7.5) incubated at 30 °Cfor 3 days. Yeasts and molds were numerated by spread plate on yeast mold agar (3 g yeast extract, 3 g malt extract, 5 g peptone, 10 g dextrose, and 1,000 mL distilled water, pH = 4.0) and potato dextrose agar (200 g of sliced unpeeled potatoes boiled in 1 L distilled water, filter through cheesecloth, 20 g dextrose, 20 g agar, pH = 5.6) and incubated at 25 °Cfor 5 days.

Volatile compounds analyses by Gas chromatography–mass spectrometry (GC-MS)

Major volatile compounds were identified, by analysing the beer directly with no pre-treatment. GCMS-QP2010 Ultra, Shimadzu, gas chromatograph outfitted with a Mass spectrometer, and a capillary column DB-5 ms (30 m x 0.25 mm, 0.25 μm) covered with 5% diphenyl-95% dimethyl polysiloxane was utilised. Helium was used as the carrier gas at a constant flow rate of 1.5 mL min^{-1}. Without pre-treatment 1 μL of the KFB was injected in the splitless mode (vent time, 60 s) and the Compounds were identified by comparing of mass spectra obtained with Mass Spectral Library of the National Institute of Standards and Technology (NIST).

DPPH radical scavenging activity

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radicals scavenging activity was estimated by their Electron paramagnetic resonance (EPR) (Bruker Elexys E-500, X-band) spectra. The determination of antioxidant activity of beer samples was carried out by the difference in the amount of paramagnetic particles of a stable free radical, measured before and after complete proceeding the chemical reaction between a free radical and an analyte, which is accompanied by a decrease in the intensity of the EPR spectrum of the free radical after adding the analyte by the formula:

$$AOA = \frac{(n_{s1} - n_{s2}) \cdot C_{DPPH}}{n_{s1}}, \tag{1}$$

where AOA – the antioxidant activity (meq); C_{DPPH} – the DPPH concentration in an initial solution (M); n_{s1} – the initial amount of paramagnetic centers of DPPH, units; n_{s2} – the amount of paramagnetic centers of DPPH after interaction with the analyte (beers), units (Ivanova et al., 2017).

DPPH (1 mmol) was dissolved in ethanol and without pre-treatment 10 μl of the samples were pipetted into Eppendorf tube containing 1 mL of DPPH. EPR spectra of every 30 seconds for 15 minutes were recorded during the reaction with an antioxidant using EPR spectrometer Bruker Elexys E-500, X-band.

Organoleptic properties assessment

A total of 15 panellists consisting of unequal numbers of males and females of different ages were randomly selected at the beer festival organised by the Ural craft beer association, Yekaterinburg, Russia. The flavour (aroma), clarity, colour, foaming and saturation with carbon dioxide, mouthfeel, alcohol strength and the overall acceptability of the fruit beer were evaluated according to the Hedonic Rating Scale with the following keys; Liked extremely = 9, like very much = 8, like moderately = 7, like slightly = 6, neither like nor dislike = 5, dislike slightly = 4, dislike moderately = 3, dislike very much = 2, dislike extremely = 1. Regular consumers of beer were selected for recruitment as panellists for sensory evaluation.

Statistical Analysis

Data generated were subjected to analysis of variance (ANOVA) using Origin statistical software (version 8.1) at 5% significance. All measurements were made at least in triplicate. Results were reported as means ± standard deviations. For sensory analysis a one-way ANOVA and a post-hoc Tukey-Scheffe test were conducted to test the means results obtain at 5% significance.

RESULTS AND DISCUSSION

Reducing sugars content

The reducing sugars content in the wort is vital characteristic in the process of fermentation since yeast transform sugars to liquor, carbon dioxide, and different aggravates that impact the essence of fermented foods and drinks. The pattern in exhaustion of reducing sugars appears in the Fig. 1. There was a general diminishing in reducing sugars content from 1.48 ± 0.01 to 0.88 ± 0.01 mg maltose per 100 mL at the start and at the 4th week of the fermentation, respectively. SBT was added in green beer at the 4th week that the decreasing sugar expanded again in view of the sugars in the fruit itself from 0.94 ± 0.02 till 0.85 ± 0.03 mg maltose per 100 mL at the 5th week and 6th week before the carbonation started. The wellspring of the sugars can likewise influence on fermentation through contrasts in supplements and flavour antecedents. While the most widely recognised hotspot for beer is malted grain, brewers around the globe utilise numerous diverse starches.

The sort of sugars present in wort relies on upon the mashing temperature administrations. Monosaccharides are easier fermentable than polysaccharides and brewers all over the globe utilizes this finding in brewing especially while selecting priming sugars for carbonation. Also, this kind of sugars in wort influences on the flavor of the final beer.

For instance, ageing of wort, which is high in glucose, produces lagers with higher content of esters than typical groupings of esters (especially ethyl acetic acid derivation and isoamyl acetic acid derivation). On the other hand, wort, which is high in maltose, brings about lower centralizations of these esters. This decreasing trend was also reported by other investigators (Demuyakor & Ohta, 1993; Villicaña & Saldivar, 2004) in sorghum beer and barley wort.

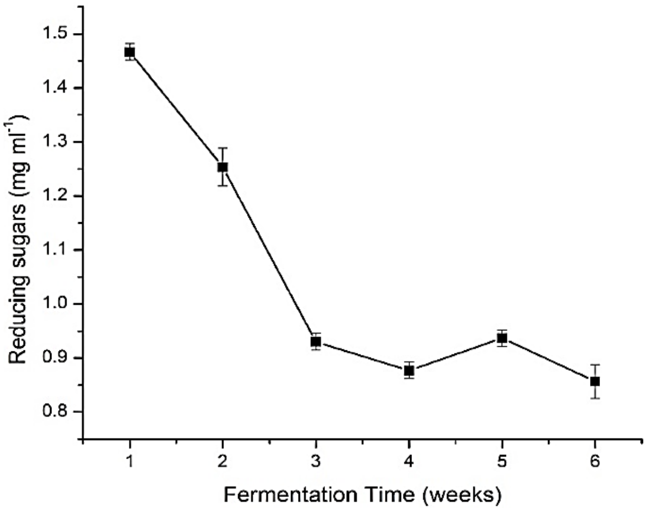

Figure 1. Change in reducing sugars over time.

pH and Titratable acidity

The pH of the wort begins to diminish as the fermentation time advances which differs statistically ($P < 0.05$). A decrease in pH from 5.8 ± 0.1 to 4.6 ± 01 at the 4th week was observed in Fig. 2. Additionally, there was no further diminishing in pH the after the 4th week when SBT was added in the green beer. pH falls amid ageing accordingly due to utilisation of buffering materials (free amino nitrogen) by yeast and the release of natural acids (Bamforth, 2001). We realized that malt worts are the phenomenal wellspring of nitrogen, minerals, and vitamins. Wort supplies the greater part of the supplements yeast requirement for an appropriate fermentation. As the yeast take up minerals and vitamins from the wort, they begin to produce enzymes important for development and this might likewise be a factor for dropping pH (Zainasheff & White, 2010). The further drop in pH after the 5th week may be assigned to the presence of various phytochemicals (bioactive substances) in SBT which might have excreted and mixed with the green beer.

All parts of the SBT plant are thought to be a decent wellspring of substantial number of bioactive substances like vitamins (A, C, E, K, riboflavin, folic acids), carotenoids (α, β, δ-carotene, lycopene), phytosterols (ergosterol, stigmasterol, lansterol, amyrins), natural acids (malic and oxalic acids), polyunsaturated unsaturated fats and some fundamental amino acids (Beveridge et al., 1999; Yang & Kallio, 2001; Pintea et al., 2005). The hops (Hallertau-Hersbrucker and Huel Melon) used in this study may likewise be one of the elements that contribute in the mode of a change in pH since they contain alpha and beta acids in various amounts. As pH values of the beer diminishes over the normal range from 4.5 to 3.9 there is expanded protection against microbial deterioration, colloidal strength, froth stability (for reasons which were not completely comprehended), diminished flavour solidness, sense of taste, smoothness and drinkability (Melm et al., 1995).

TA is one of the essential parameters for beverages analysis, aside from carbonic acids content. There was a radical increment in the TA from an underlying estimation of 0.65 ± 0.04% to 2.18 ± 0.03% of the last month of the fermentation. The dependences of these parameters on fermentation time were shown in Fig. 2. From the general dependable guideline, the lower a pH value, the higher TA. However, not specifically corresponded to this trend, which was mentioned in this study and can be affirmed from Fig. 2. Generally speaking, TA well correlates with the 'acid taste' of the beer. The acidic substances of beer are of incredible hugeness for the protection and tactile attributes (Rajković et al., 2007). TA most likely increases during fermentation processes due to that the substrates in wort are spent and metabolites (lactic, malic, citrus, acidic acids) start to aggregate subsequently affecting TA.

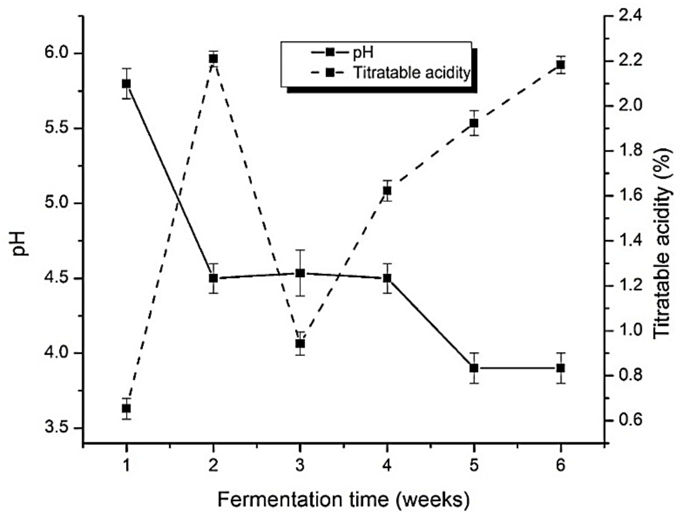

Figure 2. Change in pH and titratable acidity over the fermentation period.

Volatile compounds

Yeast can create 500 distinctive flavour and aroma substances (Zainasheff & White, 2010). Yeast metabolites, which impact beer flavour, consist of natural acids, medium chain-length (8–10 carbon particles) aliphatic alcohols, sweet-smelling alcohols, esters, carbonyls and different sulfur-containing compounds (Boulton & Quain, 2001).

A total of 32 volatile compounds were identified by gas chromatography in the beer during maturation and presented in Table 2. There were 10 acids, 6 alcohols and 16 esters. After adding SBT berries to the beer on the stage of its maturation, more volatile compounds were identified due to the fact that SBT plant itself contained a considerable number of compounds with therapeutic effect. These compounds gave an impact in flavour, pH, TA and mouthfeel. For instance, the acidic acids, such as propanoic and isobutyric acids as well as others contributed fundamentally in pH of both the green and final beer.

Mostly, volatile acids can contribute with vinegary, mushy, and fatty smells and, in addition, provides with sharpness, stringency and rancidity (Pinho et al., 2006). In the present study 10 of such acids were identified.

Esters significantly impact the character of beer. They contribute in the fruity fragrances and flavours of beer. According to Meilgaard (1975), even the 'cleanest-tasting' lagers contain esters in quantities up to 50. With no esters a beer would appear to be very insipid. The esters of ethyl acetic, ethyl caproate, and isoamyl acetic acids in beer appear due to a specific yeast strain activity and ageing conditions (Zainasheff & White, 2010).

Table 2. Volatile compounds found Kölch fruit beer

2-methyl-3-Buten-2-ol	Hexanoic acid
3-methylButanal	Ethanol
3-methyl-1-Butanol	Fusel oil
Propanoic acid	Ethyl esters
Isobutyric acid	Octanoic acid
Butyl isobutyrate	Ethyl butanoate
Butanoic acid	Ethyl caprylate
1-methylbutyl ester	2-Methylbutyl isobutyrate
3,7-dimethyl-1,6-Octadien-3-ol	Isovaleric acid
Acetaldehyde	Ethyl isovalerate
Ethyl aldehyde	Isoamyl acetate
Ethyl Acetate	Bicyclo[4.2.0]octa-1,3,5-triene
Acetic acid	Pentanoic acid
Ethyl ethanoate	Isopentyl pentanoate
Isoamyl alcohol	3-Methylbutanoic acid
2-methylButyraldehyde	Caprylic acid, Ethyl octanoate

Volatile natural compounds are typical constituents of many matured nourishments and drinks and are classified as esters, alcohols, aldehydes, acids, terpenes, ketones, sulphur mixes, amines, phenols etc. (Cortacero-Ramírez et al., 2003; Lui et al., 2005). The greater part of these organic constituents do not influence on beer, whereas there are various pathways to generate numerous different compounds which spill out of the cell and affect beer flavour. Volatile compounds are seen as fundamental beer constituents in brewery business as they affect the nature of beer and upgrade purchaser acknowledgement (Erny et al., 2009; Lui et al., 2005). Similar volatile compounds were identified by Shale et al. (2013). Some of these compounds had positive impact on fragrance (i.e. esters), whereas others affect negatively (i.e. fusel oil).

°Brix

The °Brix gives the information of sugars and substances content in the wort. A general decrease in their quantities was observed throughout ageing due to their utilisation by fermenting yeast (Fig. 3) which differs statistically ($P < 0.05$). A decrease from 13.3 ± 0.43 to 3.8 ± 0.25 °Brix can be caused by consumption of sugars that was mentioned in early work by Avicor et al. (2015).

There was no supplementary decrease in °Brix observed after addition of SBT presumably because no discharge of sugars from the organic product (SBT) in the green beer. Sugar utilisation started quickly after pitching and the most elevated rate of sugar consumption may happen after the cell biomass has achieved maximal density. From Fig. 3 °Brix decreased drastically from 13.3 ± 0.43 to 3.9 ± 0.25 during 3 weeks of the ageing time. The catabolism of sugars gives yeast energy and carbon skeletons for anabolic pathways. This basic activity results in an extensive decline of the solutes in the wort (Briggs et al., 2004). Embden-Myerhof-Parnas cycle is the real sugar catabolic pathway in yeast, functions under both oxygen and anaerobic conditions and is the cause by which around 70% of exogenous sugars are absorbed (Briggs et al., 2004).

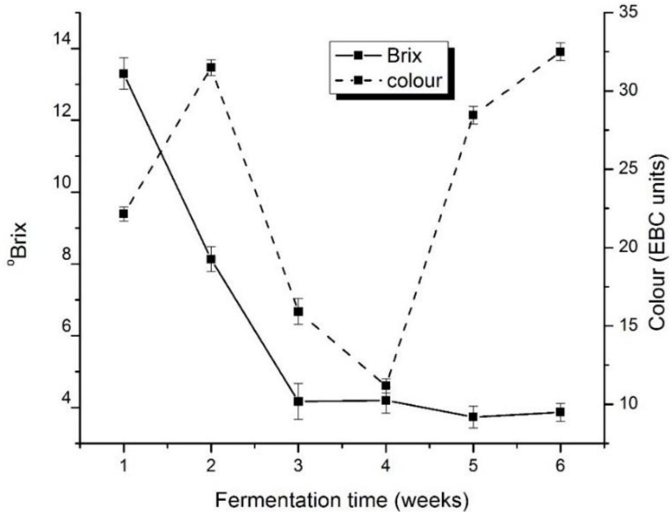

Figure 3. °Brix and colour of wort vs. fermentation time.

Colour

We observed an increase in colour measured in EBC units after the 1st week of maturation which are statistically different ($P < 0.05$). This fact can be explained by extraordinary fermentation and massive foam production accompanying with evolving yeast metabolites. However, in the next two weeks there was a decrease in colour due the consumption of the specific solutes in the wort resulting in the less intensive fermentation accompanying with lower foam production. The addition of SBT was found to increase the colour of the Kölch green beer. It should be noticed that SBT contains fermentable sugars and other substances that subsequently increase in gravity of the green beer at the 4th week. However, colour continues to develop until the 6th week (Fig. 3).

In food and beverage products, the consumers regularly survey the nature of products by their colour and appearance. The colour and appearance of products are essential characteristics of their quality (Lawless & Heymann, 1998; Cao, 2013). They affect the impression of different qualities, i.e. smell and flavour (DuBose et al., 1980).

Most consumers expect to taste clear beer; they may presume low quality and reject beer that is not clear (Cao, 2013). Producing clear beer is a fundamental prerequisite of all brewers. The outcomes were in concurrence with the early report by Cao (2013).

Microbial analysis of fruit beer

No microbial growth was observed on the plated Petri dishes inoculated with KFB on different media.

Strict hygienic conditions were observed in the whole brewing processes by utilizing a sanitised equipment. Processes such as mashing, wort boiling, filtration, aseptic bottling, conditioning and cold storage in the brewing decrease the potential for microbial contamination.

Different parameters are vital for the microbial stability of beer and include pH, concentration of hops bitterness, content of ethanol, CO_2, SO_2, natural acids, acetaldehyde and different metabolites as well as supplements and storage temperature (Jespersen & Jakobsen, 1996). In our study pH and ethanol content of final beer were found to be 4 and 5.2%, respectively. Natural acids such as octanoic, caprylic acid and hexanoic acids as well as hops (Hallertauer Hersbrucker, Huel Melon) of KFB may be the factors which inhibit microbial growth in the plated dishes.

The primary contaminants originate from the raw materials and brewery equipment, whereas the secondary contaminants are acquainted with the beer before packaging and kegging (Moretti, 2013). In the research (Sarlin et al., 2005) there has been reported that usually grains can be contaminated with *Fusarium graminearum* and *F. moniliforme* resulting in gushing (spontaneous ejection of beer from its container) of beer. The barley used in this study was acquired from credible producers in Germany who are guarantees of its microbial safety.

The use of clean water in brewery cannot be underestimated. The fundamental concern is the presence of spoilage organism in water used for brewing, for instance, dilution of beer after high gravity fermenting or from vessels rinsed with contaminated water (Hill, 2009). Filtered water was used in the entire brewing process and it assists to avoid introducing contaminated water into wort/beer. However, boiling also helps to eliminate contamination.

Hops are known for their antibiotic properties. As known, the majority of gram positive microorganisms are inhibited by hops, despite the fact that gram negative organisms are unaffected (Hill, 2009). The usage of two sorts of hops in brewing KFB provided higher antibiotic properties to inhibit microbial contamination.

Sensory analysis

The final KFB brewed within 6 weeks was evaluated for consumer acceptability using 15 panellists. The average values of the analysis were used to plot a radar chart presented in Fig. 4, which consists of the sequence of equi-angular spokes, representing 7 variables. All the variables assessed were significantly different ($P < 0.05$).

Esters and alcohols identified in the present study were found to contribute to aroma (8.60 ± 0.82) with a higher average score 9. It is obvious for an aroma variable. The hops in the present study were used for a dual purpose as flavour and bitterness contributors. Nevertheless, SBT in its turn contain some natural aroma substances as well. The responses from the panellists with respect to mouthfeel were also impressive and can be confirmed by score 8 for this variable in the chart. The mouthfeel (8.40 ± 0.63) is important to the aroma when it comes to beer products as they correlate. The filtration processes during the wort production affect the clarity of KFB, because poor filtration results in non transparency of beer. KFB obtained after the 4th week was transparent enough, but it was altered after the mashed SBT was added providing with score 7 for this variable in the chart.

The average score for foaminess (6.86 ± 2.20) was good, although not excellent like for other variables, which indicate that 1.8 bar CO_2 utilised for the carbonation was not sufficient enough to replace the lost CO_2 in the KFB. CO_2 content needs to be increased up to 2 bar to achieve excellent foaminess.

The amount of alcohol in KFB is assessed by alcoholic strength. The score 8 of alcohol strength (8.40 ± 0.73) on the chart indicates the acceptance of the alcohol percentage (5.2%) by the panellists. 13 out of 15 testers assessed alcohol strength of the beer brewed as extremely similar to calculated one by BeerSmith (version 6.0).

The colour (7.92 ± 0.79) of the final beer is very important for both a brewer and consumers. Pleasant colour will attract more consumers and the brewer will earn more profit in return. SBT contributed to colour of KFB and panellist generally liked it. The score 8 on the radar chart supports this point (Fig. 4). 4 panellists marked score 9 (liked extremely) while 6 assessors gave score 8 (like very much), 7 – (like moderately) for this variable.

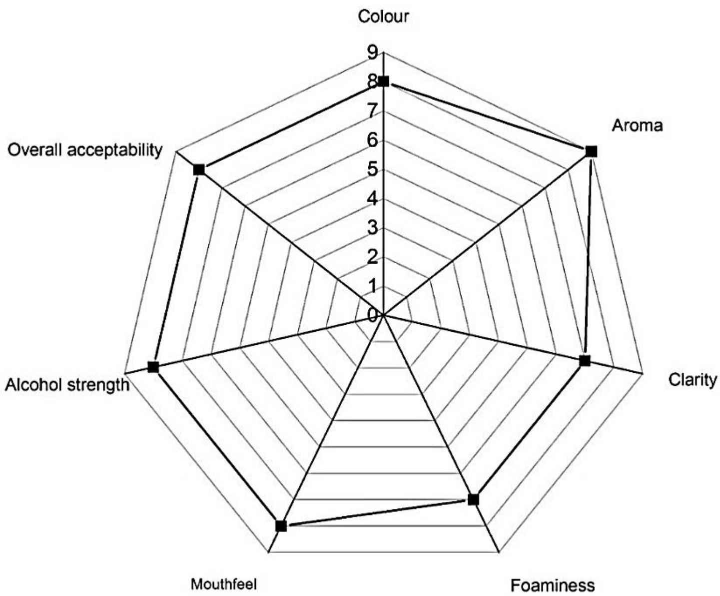

Figure 4. Average hedonic scores of sensory analysis of KFB brewed within 6 weeks.

The overall acceptability (8.33 ± 0.72) are utilised for various goals in a product research, such as product, product optimisation, new product development, appraisal of market potential. Overall acceptability rating of KFB was between 9 (like extremely) and 8 (like very much), with the exception of one score 7 (like moderately). The acceptability was impressive judging from the panellists' scores given on the heptagon radar chart (Fig. 4). There was no statistically significant difference ($P > 0.05$) between variable means except for foaming and aroma ($P < 0.05$).

Antioxidant activity

Antioxidant activity of any substance is defined as its ability to capture free radicals. It is a vital guard of living systems, contrary to oxidative stress (Valko et al., 2006; Oliveira et al., 2009). Diseases like cancer, diabetes, cardiovascular disease, Alzheimer's disease, and other neurodegenerative disorders are caused by oxidative stress (Halliwell, 1994; Giacco & Brownlee, 2010). Numerous antioxidant compounds have anti-inflammatory, antiatherosclerotic, antiproliferative, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities to a more noteworthy or lesser degree (Liu et al., 2002; Ratnam et al., 2006). Fruit juices, beverages and hot drinks contain high amounts of antioxidants, like polyphenols, vitamin C, vitamin E, Maillard reaction products, β-carotene, and lycopene (Ramadan-Hassanien, 2008).

Beer is considered as one of the main sources of antioxidants to consumers. The amount and composition of the antioxidants vary not only on the qualities of the materials, also on the brewing processes. For example, grains and malt are the principal source of antioxidant compounds in beer and hops contribution is lower (Jurková et al., 2012).

Antioxidant activity of KFB in the comparison with two other industrial alcoholic beverages (Nectar from Bosnia and Baltica from St Petersburg, Russia) was determined using EPR spectroscopy of free radicals.

The DPPH radical scavenging activity of the alcoholic beverages is shown in Fig. 5. KFB sample showed higher DPPH radical scavenging activity of 1.53×10^{-4} mol × equ ($R^2 = 0.93$) than Nectar beer 1.16×10^{-4} mol x equ ($R^2 = 0.69$) and the least Baltica beer 9.85×10^{-5} mol x equ ($R^2 = 0.96$). The higher antioxidant activity of KFB is thought to be caused by incorporating SBT, which possesses its own antioxidant activity due to presence of essential substances mentioned above.

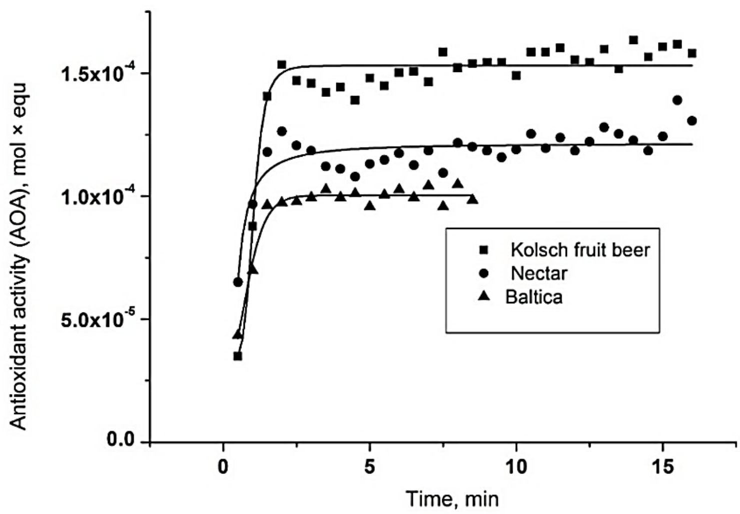

Figure 5. DPPH radical scavenging activity of different beers vs. time.

CONCLUSION

The present study provided information on the use of SBT in producing fruit beer. Fermentation time had an influence on the pH, reducing sugars, °Brix, colour and TA. There was a decrease in reducing sugar content and °Brix as the fermentation proceeded until when the SBT has added that it increase and decreased again. A total of 32 volatile compounds were identified, 10 acids, 6 alcohols and 16 esters. KFB showed higher DPPH antiradical activities than two other alcoholic beverages, which were used for comparison. Panellists generally had a higher preference for the KFB because of the taste and flavour contributed by SBT.

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Research on solid biofuels from cotton waste biomass – alternative for Tajikistan's energy sector development

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Abstract. An increasing awareness of the negative environmental cost associated with the combustion of fossil fuels and concerns over the geopolitical instability of the main oil producing regions is driving the development of renewable energy sources and biofuels. Use of solid biofuels made of different types of biomass became perspective alternative to conventional fuels in many countries. Such positive indicators as low cost of the final product that meets the quality of standards, not capital intensive production, possibility of producing briquettes/pellets from almost any agricultural waste or combination of raw materials are undoubted advantages of biomass based fuels. The main challenges for Tajikistan's energy sector, which is depended on energy imports, are: to increase energy supply through better exploitation of hydropower and other renewable energy sources such as wind, solar and primary biofuels. Within the agricultural sector of Tajikistan, which is highly agrarian country, cotton accounts for 60% of agricultural output. According to the Ministry of Agriculture of Tajikistan 199,400 hectares of lands have been allocated to cotton cultivation in the year of 2014. Plenty of unused cotton residual biomass could be effectively utilized for winter heating in rural areas. The main focus of the research was to investigate and assess physical, chemical and mechanical properties of pellets and briquettes produced form cotton waste biomass.

Key words: cotton residues, biofuel, standards, pellets, briquettes, quality assessment.

INTRODUCTION

Cotton (*Gossypium*) is one of the world major cultivated non-food crops (Kasimov, 2013) and the top consumed natural fibre (Esteve-Turrillas & de la Guardia, 2017). Thus, the global cotton production has continuously increased up to current estimates of about 25,000,000 t (Hamawand et al., 2016; Egbuta et al., 2017).

For Tajikistan cotton is an important dominant of an agricultural sector and the primary crop for its economy; with one-third of the total arable area, 75–90% of agricultural exports and significant portion of country's GDP (World Bank, 2012; Kasimov, 2013). According to TAJSTAT (2016) about 370,000 t of cotton was produced in 2015. Agricultural sector employs two-thirds of the Tajik population and cotton industry is the largest employer, which supports 75% of rural population (Boboyorov, 2012; MOA, 2012; Kasimov, 2013). Trends in the value of cotton output therefore have a major impact on overall sectors' growth and people well-being. Agriculture, in particular cotton production has made a powerful contribution to country's post-war economic recovery. However, Tajikistan still remains deeply poor (World Bank, 2012) and almost three-quarters of the extreme poor live in cotton growing areas (Boboyorov, 2012). According to Kasimov (2013) 'cotton has the potential to be an avenue for rural poverty reduction', but the current production, processing and marketing techniques being applied in Tajikistan do not develop the potential gains to be beneficial for local farmers. By MOA (2012) an important task of a present state policy programme for 2012–2020 is dedicated to improving cotton sector management. Resource-poor farming households all over the Tajikistan have a great level of self-sufficiency in food, fodder and fuel (Ruppen et al., 2016). Current energy situation, i.e. irregular supply of electricity and the lack of coal, which is also often expensive and of poor quality, forces reliance on scarce locally available resources (Mislimshoeva et al., 2014). Moreover, Tajikistan is characterized by harsh winters (World Bank, 2012; Mislimshoeva et al., 2014) and long heating period, which lasts from November to March or April (Ruppen et al., 2016). In many villages animal dung and firewood from fruit trees and cultivated vines are the main sources of energy for cooking and heating (Mislimshoeva et al., 2014; Ruppen et al., 2016).

Worldwide utilization of crop residues other than cotton for energy purposes has been an interesting subject for years (Mythili & Venkatachalam, 2013; Hamawand et al., 2016; Egbuta et al., 2017). Recently, the energy potential of cotton waste started to draw scientists' attention, too (Hamawand et al., 2016). Cotton cultivation results in tonnes of waste (Hamawand et al., 2016; Egbuta et al., 2017) and faces the producing countries to serious environmental issues (Eissa et al., 2013; Ranjithkumar et al., 2017). Cotton waste production was estimated to be 2.9–3.8 times larger than cotton production (Coates, 2000; Mythili & Venkatachalam, 2013). There are three types of wastes generated during growing and processing: post-harvest field trash (PHT), cotton gin trash (CGT) and seed meal after oil extraction (Egbuta et al., 2017); where PHT represents the biggest source of waste biomass (Hamawand et al., 2016). According to calculations about 5.2–5.6 t ha^{-1} of cotton waste is left in the field after harvesting and many growers usually burn it (Hamawand et al., 2016) or slash and leave on the field (Egbuta et al., 2017). However, PHT has little value as a soil amendment and tillage operations have high energy requirements and often degrade soil structure (Coates, 2000; Hamawand et al., 2016). Moreover, Li & Zhang (2016) observed allelopathic effects of naturally decomposed cotton stalks that caused autotoxicity, and much lower and unstable crop production in China. In contrast, Hamawand et al. (2016) published that PHT is important for minimising losses in soil carbon; it provides surface protection and has positive impacts on soil quality. Therefore, answering the question of how much PHT should be retained in the field and how much should be utilised for other purposes Sahoo et al. (2016) proved that 80% of PHT can be removed from the majority of the cotton land keeping the sustainability indicators within the limit. Additionally, by Hamawand et al. (2016) use of PHT as livestock feed is not suitable due to Endosulfan contamination and very poor feeding value.

For these reasons, PHT is considered as a negative value biomass (Coates, 2000), but it seems to be a good source of bioenergy (Hamawand et al., 2016). PHT can be processed to all kinds of biofuels: liquid, gaseous and solid, nevertheless, there are still little studies about it. According to Keshav et al. (2016) PHT is a promising feedstock for ethanol production due to high holocellulose content, but it is practically applicable only if technical issues associated with this process (especially pre-treatment) are solved (Hamawand et al., 2016; Ranjithkumar et al., 2017). Quality bio-oil can be produced from PHT by pyrolysis (Ji-lu et al., 2008; Hamawand et al., 2016); however, a number of barriers need to be overcome such as reducing the amount of char and energy required (Hamawand et al., 2016). By Iscia & Demirer (2007) PHT is a good source of biogas, per contra by Hamawand et al. (2016) the biogas production and conversion is low, and it seems to be non-feasible due to the little revenue generated. Several researches were focused on solid biofuels production: Chen et al. (2017) has studied chemical characteristics of cotton stalk briquettes; mechanical properties of briquettes produced by screw press were analysed by Eissa et al. (2013); research of Coates (2000) showed that cotton residues can be incorporated with pecan shells to manufacture commercially acceptable briquettes; Mythili & Venkatachalam (2013) concluded that PHT briquettes are well suited for the energy generation due to high gas production in gasifier; and Hamawand et al. (2016), Sahoo et al. (2016), Stavjarská (2016) stated that PHT can be feasible used to produce fuel pellets.

Thus, PHT presents available source of energy to cotton growers (Hamawand et al., 2016), but assumed high cost associated with harvesting the trash for other uses is considered as a major economic hurdle (Egbuta et al., 2017). However, Coates (2000) and Hamawand et al. (2016) have stated that the energy required to collect and process PHT into briquettes or pellets is a small percentage of the energy content of the residue itself. And, the complexity, capital and operating costs of such application are lower compering to other options (Hamawand et al., 2016). Therefore, today solid biofuels' production is the most viable solution of recycling PHT into useful products (Eissa et al., 2013). In addition, utilization of PHT as a bioenergy feedstock can offer new incentives to cotton growers (Sahoo et al., 2016); briquettes/pellets can be commercialized (Avelar et al., 2016). Still there is a lack of research in the area of cotton PHT utilization as solid biofuel and more studies are needed (Hamawand et al., 2016). The aim of this research is to determine the properties of both pellets and briquettes produced from cotton field residues originated from Tajikistan and to evaluate their quality through solid biofuels' standards.

MATERIALS AND METHODS

The cotton waste biomass used in the present research was brought from Tajikistan. The waste biomass (post-harvest trash) included different parts of the plant: predominantly stalks, some flowers/pods, roots, leaves and negligible amount of fibers.

Production of pellets and briquettes

Before densification the material was processed by two-steps crushing. For primary cutting up to 5 cm the shredder Murena (Bystroň) was used and the hammer mill 9FQ – 40C with screen holes' diameter of 6 mm was used for secondary crushing.

Production of pellets was carried out on pelletizing line Kovo Novak 200 with a size of matrix holes 6 mm and briquettes were produced by hydraulic briquette press Brikstar 50 with working pressure 18 MPa and diameter of pressing cylinder 65 mm.

Determination of pellets and briquettes properties was done by the methodology of International and European standards for solid biofuels. For further testing representative sample of waste cotton biomass was prepared according to EN 14780. Homogenized analytical sample was made by laboratory grinding knife mill Grindomix GM 100.

Moisture Content test (w) – determination of moisture content was carried out in accordance with EN ISO 18134-3 (2015) using laboratory dryer Memmert 100–800 and calculated by following formula (1):

$$\mathrm{w} = \frac{\mathrm{m_2} - \mathrm{m_3}}{\mathrm{m_2} - \mathrm{m_1}} \cdot 100, \% \tag{1}$$

where m_1 – mass of empty crucible, g; m_2 – mass of crucible with sample before drying, g; m_3 – mass of crucible with sample after drying, g.

Ash Content test (AC) – measurement of ash content was performed in muffle furnace LAC by burning the sample in regulated temperatures with respect to EN ISO 18122 (2015). Formula for determination of ash content is (2):

$$\mathrm{AC} = \frac{(\mathrm{m_3} - \mathrm{m_1})}{(\mathrm{m_2} - \mathrm{m_1})} \cdot 100 \cdot \frac{100}{100 - \mathrm{M_{ad}}}, \% \tag{2}$$

where $\mathrm{m_1}$ – mass of empty crucible, g; $\mathrm{m_2}$ – mass of crucible with sample, g; $\mathrm{m_3}$ – mass of crucible with ash, g; $\mathrm{M_{ad}}$ – water content in a sample expressed as a mass fraction, %.

Gross Calorific Value test (GCV) – determination of gross calorific value was carried out according to the standard EN 14918 (2009) using semi-automatic bomb calorimeter LECO AC-600 under compressed oxygen at temperature 22 °C. GCV was calculated by calorimeter taking into account heat capacity of calorimeter, weight of the material sample and different corrections.

Net Calorific Value (NCV) was calculated from GCV by the following Eq. (3):

$$\mathrm{NCV} = \mathrm{GCV} - 24.42 \cdot (\mathrm{w} + 8.94 \cdot \mathrm{H_a}), \mathrm{J\ g^{-1}} \tag{3}$$

where GCV – Gross calorific value, J g^{-1}; 24.42 – coefficient of 1% water in the sample at 25 °C (J g^{-1}); w – water content in the sample, %; 8.94 – coefficient for the conversion of hydrogen to water, $\mathrm{H_a}$ – hydrogen content in the sample, %.

Volatile Matter Content Test (VM) – volatile matter content was determined according to EN ISO 18123 (2015) by burning of material analytical sample for seven minutes at 900 °C in oxygen free environment in Muffle furnace ELSKLO MP5. Formula for calculation of volatile matter content is (4):

$$\mathrm{VM} = \left[\frac{100(\mathrm{m_2} - \mathrm{m_3})}{\mathrm{m_2} - \mathrm{m_1}} - \mathrm{M_{ad}}\right] \cdot \left(\frac{100}{100 - \mathrm{M_{ad}}}\right), \% \tag{4}$$

where $\mathrm{m_1}$ – mass of empty crucible and lid, g; $\mathrm{m_2}$ – mass of crucible with sample and lid before heating, g; $\mathrm{m_3}$ – mass of crucible with sample and lid after heating, g; $\mathrm{M_{ad}}$ – moisture percentage by mass in the general analysis sample, %.

Durability Test (DU) – determination of mechanical durability of pellets was conducted by EN ISO 17831-1 (2015) using pellet tester and mechanical durability of briquettes was done by EN ISO 17831-2 (2015) in rotation drum. Mechanical durability of pellets and briquettes was further calculates as (5):

$$\mathrm{DU} = \frac{\mathrm{m_A}}{\mathrm{m_E}} \cdot 100, \% \tag{5}$$

where $\mathrm{m_A}$ – sample weight after crumbling, g; $\mathrm{m_e}$ – sample weight before crumbling, g.

Carbon, Nitrogen, Hydrogen and Sulfur Content Test (C, H, N, S) – determination of C, H, N was carried out with the respect to International standard EN ISO 16948 (2015) and S content was determined according to EN ISO 16994 (2015). Elementary analyzer LECO CHN628 + S was used for these measurements.

Heavy Metals Content Test (Cr, Ni, Cu, Zn, As, Cd, Hg, Pb) – determination of heavy metals content was done according to standard EN ISO 16968 (2015). After required preparation of the sample solution the element contents were measured by inductively coupled plasma mass spectrometry using ICP-MS, Agilent 7700x.

RESULTS AND DISCUSSION

Quality of solid biofuels depends on a number of parameters (physical-mechanical and chemical properties). In the Table 1, all parameters assessed in this research are presented and compared to the standards of graded wooden and non-wooden biofuels (class A1).

From the Table 1 it is visible that pellets from cotton residues do not meet A1 class requirements of graded wood pellets, specifically due to high ash content and slightly lower $NCV_{a.r.}$ According to the same standard ash content for A2 class pellets is 1.2% and B class is 2.0%; $NCV_{w.b.}$ for A2 class is ≥ 16.5 and B class is ≥ 16.5. From above mentioned value it is seen that cotton pellets do not meet standard requirements of any class of graded wooden pellets. Also content of sulfur in cotton based pellets is higher than requirements of all the classes (class A2 ≤ 0.05 and class B ≤ 0.05) of graded wooden pellets. Nitrogen content in cotton pellets corresponds only to class B (EN ISO 17225-2, 2014). Beside ash content, NCV, S and N contents by all other parameters produced pellets achieved wood biomass quality. Comparing to non-wooden pellets standards, pellets from cotton biomass shows much better properties and fully fulfils A1 class requirements.

The same as pellets briquettes made of cotton fully correspond to the best A1 quality of non-wooden briquettes. In comparison with wood briquettes, cotton briquettes do not fulfill requirements of A1 class due to higher ash content and higher content of S and N. In contrast with cotton pellets, cotton based briquettes achieved the A1 class by NCV (see Table 1).

According to Coates (2000) and Hamawand et al. (2016) $GCV_{a.r.}$ of cotton stalks ranges from 17.1 to 18.1 MJ kg^{-1}, which is in correspondence with the present research results. $GCV_{d.b.}$ of cotton textile industry residues (CGT) published by Avelar et al. (2016) is 17.9 MJ kg^{-1} and $NCV_{d.b.}$ is 16.7 MJ kg^{-1}, i.e. the values are lower than the measured values. This can be explained by different composition of CGT. According to Egbuta et al. (2017) CGT contains of leaves, fibre, flowers, immature seeds, sticks and soil, and more attention was previously given to CGT utilization because it is centrally stockpiled at gins and collected with existing infrastructure. Comparing to another typical raw material used for solid biofuels production: $GCV_{d.b.}$ of cotton biomass is higher than the average calorific value of a mixture of wheat and rape straw 15.3 MJ kg^{-1} (Niedziółka et al., 2015), it is almost equal to the value of Miscanthus 19 MJ kg^{-1}, but lower than $GCV_{d.b.}$ of wood logging residues 19.7 MJ kg^{-1} (broad-leaf wood) and 20.5 MJ kg^{-1} (coniferous wood) (EN ISO 17225-1, 2014). In comparison, fossil non-renewable brown coal has $GCV_{d.b}$ 22.3 MJ kg^{-1} (Tsuchiya & Yoshida, 2017).

Table 1. Properties of pellets and briquettes based on cotton waste biomass

Parameters	Pellets	Briquettes	Standards for graded wooden*		Standards for graded non-wooden*	
			Pellets	Briquettes	Pellets	Briquettes
Moisture content ($w_{a.r.}$), %	6.7		≤ 10	≤ 12	≤ 12	≤ 12
Ash content ($AC_{d.b.}$), %	3.22		≤ 0.7	≤ 1.0	≤ 6	≤ 6
Gross calorific value ($GCV_{a.r.}$), MJ kg^{-1}	17.66		–	–	–	–
Gross calorific value ($GCV_{d.b.}$), MJ kg^{-1}	18.93		–	–	–	–
Net Calorific value ($NCV_{a.r.}$), MJ kg^{-1}	16.34		≥ 16.5	≥ 15.5	≥ 14.5	≥ 14.5
Net Calorific value ($NCV_{d.b.}$), MJ kg^{-1}	17.69		–	–	–	–
Volatile matter ($VM_{d.b.}$), %	88.4		–	–	–	–
Length (L), mm	30–40	55–65	3.15 < L ≤ 40 3.15 < L ≤ 40	–	3.15 < L ≤4 0 3.15 < L ≤ 50	–
Diameter (D), mm	6–8	65	6 ± 1 8 ± 1	–	6 < D < 10 12 < D < 25	–
Mechanical durability (DU), %	7.82	97.63	≥ 97.5	–	≥ 97.5	–
$C_{d.b.}$, %	8.56		–	–	–	–
$H_{d.b.}$, %	.69		–	–	–	–
$N_{d.b.}$, %	.90		≤ 0.3	≤ 0.3	≤ 1.5	≤ 1.5
$S_{d.b.}$, %	.13		≤ 0.04	≤0.04	≤ 0.20	≤ 0.20
$O_{d.b.}$, %	1.50		–	–	–	–
Chromium (Cr), mg kg^{-1}	.090		≤ 10	≤ 10	≤ 50	≤ 50
Nickel (Ni), mg kg^{-1}	.170		≤ 10	≤ 10	≤ 10	≤ 10
Copper (Cu), mg kg^{-1}	.080		≤ 10	≤ 10	≤ 20	≤ 20
Zinc (Zn), mg kg^{-1}	.890		≤ 100	≤ 100	≤ 100	≤ 100
Arsenic (As), mg kg^{-1}	.080		≤ 1	≤ 1	≤ 1	≤ 1
Cadmium (Cd), mg kg^{-1}	.009		≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5
Mercury (Hg), mg kg^{-1}	.002		≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1
Lead (Pb), mg kg^{-1}	.170		≤ 10	≤ 10	≤ 10	≤ 10

* – the values were obtained from international standards of graded wooden and non-wooden pellets and briquettes class A1 EN ISO 17225-2:2014; EN ISO 17225-3-2014; EN ISO 17225-6-2014 and EN ISO 17225-7:2014; all values vary according to the raw material and used compacting technology; a.r. – as received; d.b. – dry basis.

Table 1 also shows that ash content of cotton based pellets and briquettes is at least three times higher than ash content of graded wooden biofuels, but twice lower than required one for non-wooden pellets and briquettes. Avelar et al. (2016) have measured dry basis ash content of biofuels made of cotton residues 8.93%, which is much higher (three times) than ash content measured in this research, but textile industry residues were used in that case. For example, in comparison to ash content of rice straw 9.44% (Yang et al., 2016) or Pendopo brown coal 10.94% (Kim et al., 2000) content of ash in cotton wastes is significantly lower. According to Kim et al. (2000) high ash content causes high dust emissions and negatively affects combustion efficiency. Three other researches have presented very different values of cotton stalk waste's ash content (as received): 2.54% (Chen et al., 2017), 14.80% (Mythili & Venkatachalam, 2013) up to 17.3% (Hamawand et al., 2016). This difference can be probably explained by different origin of biomass (different soil conditions), different varieties or amounts of used defoliants.

High nitrogen and sulfur content in the fuel can negatively affect formation of harmful emissions, mainly nitrogen oxides (NO_x, principally NO) and sulfur oxide (SO_2) (Tumuluru et al., 2012). However, it was found several studies (Sun et al., 2008a; Sun et al., 2008b) dedicated to the combustion of poor cotton stalks, which have been considered the pollutant emissions of NO and SO_2 to be quite good (NO emission ranged from 110–153 ppm, SO_2 emission from 32–55 ppm, at 6% oxygen concentration; NO_2 emission were negligible – less than 1 ppm). Additionally, Sun et al. (2008b) observed a close linkage between the oxygen and NO emissions, so the emissions may be reduced by appropriate measures, e.g. air staging. Coates (2000) and Hamawand et al. (2016) have also published that cotton stalks are characterized by the highest burning efficiency and longest burn time in comparison with other residues such as corn stover and soybean.

Analysis of mechanical durability has showed that produced solid fuels are of high mechanical quality (see Table 1). Rajkumar & Venkatachalam (2013) have determined even better value of mechanical durability of briquettes made of cotton residues – 99.56%; in contrast Eissa et al. (2013) have measured slightly lower value – 97.06%. Durability of cotton pellets published by Stavjarská (2016) – 97.9% is almost equal to the present finding.

CONCLUSIONS

The research results showed that pellets and briquettes from cotton waste biomass (PHT) fully correspond to quality requirements for pellets and briquettes such non-wooden biofuels stated by standards. The standard requirements for graded wooden pellets and briquettes were not fulfilled due to higher ash content, higher nitrogen and sulphur content and slightly lower $NCV_{a.r.}$ (the last only for pellets). The contents of all other elements and heavy metals are within the limits. Moreover, pellets and briquettes produced from cotton waste biomass are characterized by high mechanical durability equal to A1 class wood pellets/briquettes. To summarize the results, PHT-based solid biofuels (both pellets and briquettes) showed very good quality.

According to the literature, cotton PHT is considerably lacking suitable utilization, furthermore accumulating large amounts of PHT has negative environmental impacts and generates social costs by insufficient or expensive disposal and difficulties in cultivation due to possible unfavourable effects on soil, etc. Taking into account positive fuels properties, sufficient energy content and abundance of waste biomass, especially in the country like Tajikistan where cotton is planted in a large scale, PHT utilization in form of solid biofuels can solve not only waste management and associated problems, but also significantly contribute to energy situation and sustainable development, primarily in rural areas. To conclude, cotton post-harvest residues (as also possibly in combination with gin trash) should be considered as an energy source/viable option for Tajik energy sector.

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Study of clonal variation of 'Bidaneh Ghermez' grapevine cultivar in Iran

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Abstract. Grapevine (*Vitis vinifera* L.) is a well-known plant including different cultivars and clones. In spite of the extensive works at the cultivar level, identification and determination of clonal genetic variation has remained as a challenge. To assess the genetic variation between clones of grapevine cv. 'Bidaneh Ghermez', 20 selected clones were analyzed for cluster weight (CW), cluster length (CL), cluster width (CWI), berry weight (BW), berry length (BL), berry width (BWI) and total soluble solids (TSS) in randomized complete block design with three replications. Analysis of variance revealed considerable genetic variation for all measured traits (except cluster width) among clones. Cluster analysis, discriminant function analysis and principal component analysis (PCA) showed same results and all clones assigned in 2 groups. First group was including 9 clones and second group was including 11 clones. Overall, our results indicated C7, C10, C12 and C14 clones were best clones and have potential to introduce promising clones for stablishing new vineyard with high yield.

Key words: Clone, genetic variation, *Vitis vinifera* L., Bidaneh Ghermez.

ABBREVIATIONS: CW: Cluster weight; CL: Cluster length; CWI: Cluster width; BW: Berry weight; BL: Berry length; BWI: Berry width; TSS: Total soluble solids; PCA: Principal component analysis.

INTRODUCTION

Grapevine is one of the most economically important horticultural crops in the world and mainly used for wine production, fresh fruit, raisins and grape juice. It has been domesticated about 6,000 to 8,000 years ago in the Near East (Iriti and Vitalini, 2012). International trade in wine, its vegetative propagation and distribution in the different climatic conditions has produced great diversity of varieties (Seyedimoradi et al., 2012; Rusjan, 2013). More than 9,600 different grape cultivars are documented in the world (Rao et al., 2014). Moreover, there are a large number of clones that have different morphological characteristics which cause broad adaptability to different environments and planting techniques.

Iran is one of the top countries in grape production. According to the FAO statistic, Iranian vineyards with 215,000 ha cultivated area supplied more than 2 million tons of world grape market in 2012. Financial contribution of grape production in the economy of Iran has been more than 13 million dollars. Besides the financial performance, due to variable climate, Iranian germplasm has high diversity including cultivars, wild populations and clones (Tafazoli et al., 1993). Vegetative propagation system together with the sole use of a few cultivars has led to decrease in grapevine diversity. In addition pests and diseases attacks have contributed to this genetic erosion (This et al., 2006).

Nowadays with the growing population, the establishment of new vineyards or improvement of old vineyards is unavoidable and requires identifying of high yield and good quality cultivars and clones. In spite of the extensive works at the cultivar level, identification and study of clones has remained as a challenge (Moncada and Hinrichsen, 2007; Baneh et al., 2009; Loureiro et al., 2011). However, due to importance of clones in modern vineyards, identification of their variability is crucial for increasing grape production (Gotor et al., 2008).

Shinde et al. (2013) studied clonal diversity in 'Centennial Seedless' cultivar by 1,093 AFLP markers. Three polymorphic markers were reported that be useful for establishing genetic identity, variety registration and protection of breeder's right. Combination of SSR and AFLP markers were used to investigate the genetic difference between clones of 'Keshmeshi' cultivar. All clones were assigned to 2 groups based on the AFLP data. The first group included white berry skin clones and the second one with red berry skin clones. They concluded that AFLP could only distinguish the red berry clones of 'Keshmeshi' from other white berry clones (Baneh et al., 2009). Genetic variation was found in grapevine clones by many other reports (Moncada and Hinrichsen, 2007; Loureiro et al., 2011; Miotto et al., 2014). Genetic diversity is critical to success in breeding programs (Aremu, 2011). Genetic variations enable plant breeder to create new gene combinations and select best individuals for different breeding objectives (Glaszmann et al., 2010). Due to long history of grapevine cultivation, *Vitis vinifera* L. indicate considerable diversity in morphology, disease resistance, abiotic stress tolerance and etc. Since grape species are maintained by vegetative propagation, so most of them are heterozygous plants. Regarding grape literature review, clone implies asexual propagation without meiotic recombination resulting identical offspring. This definition means clones will not be able to adapt to environmental changes. However, mutations (in genes or genome level) are often source of clonal variation. Mutations occur spontaneously in nature and many desirable clones have arisen by this mechanism (Anhalt et al., 2011). Recent studies demonstrated that white grape has derived from red grape by mutations that affected the anthocyanin synthesis (Walker et al., 2007). Moreover, accumulation of epigenetic mutations and presence of pathogens could be causes of somatic polymorphism in grape clones (Imazio et al., 2002; Espinoza et al., 2007).

Clonality is a dynamic concept and new genetic variation is added by numerous mechanisms to provide an open system for adaptation and facing with environmental changes (Forneck, 2005). The ampelography is a science that concerned with identification and classification of grape genotypes using morphological traits of leaves, shoots, clusters and berries. Berry and cluster characters are important quality parameters that are considered for grape export and are major contributing parameters (Somkuwar et al., 2006). In table grape, the overall flavor is critical index for consumer preference. Among the flavor compositions, total soluble solids (TSS) are associated with market quality including fruit taste (Shiraishi et al., 2010). Khadivi-Khub et al. (2014) studied sixteen fruit parameters in 23 grape cultivars and found high variability in the evaluated cultivars. Significant differences were detected among the cultivars in fruit yield, cluster size, berry size, TSS and titratable acidity (TA). This evaluation methods based on morphological, agronomical and physiological traits can help breeder to focus on promising clones or genotypes (Cruz et al., 2004). Also genetic diversity assessment is essential to germplasm characterization and genetic resources conservation which in turn are important to improve or substitute present cultivar and genotypes (Khadivi-Khub et al., 2014). The objectives of present research are to investigate the clonal variation of 'Bidaneh Ghermez' grapevine cultivar and to identify best clones that could be used in the new vineyard establishment.

MATERIALS AND METHODS

Twenty clones of 'Bidaneh Ghermez' cultivar were selected for this study from vineyards of Qazvin, Iran. ‘Bidaneh Germez’ is a seedless, red-skinned and medium maturity cultivar (Nejatian, 2013). Some other important features of 'Bidaneh Ghermez' are shown in Table 1. It is one of the most popularly consumed table grapes in Iran, but it is not well known in other countries. Qazvin is one of the main areas of Iranian vineyards and located in about 153 km west of Tehran (Fig. 1). Vineyards were frequently screened for applied management and sanitary status (control of fungal disease and virus symptoms free plants) during spring and summer 2014. Clones were taken from well management and health vineyards and localized using GPS (Fig. 2). Cluster related traits including cluster weight (gr), cluster length (cm) and cluster width (cm) and berry related traits including berry weight (gr), berry length (mm), berry width (mm) and total soluble solids (%) were measured. All cluster related traits were recorded as average measurement of five clusters. The widest and longest parts of cluster were calculated as cluster width and cluster length respectively. Measuring the berry length and width (based on the average of 10 berries) was done by caliper and total soluble solids (TSS) were calculated by refractometer. Due to our study was in situ, so data were collected from three different directions (west, east and center) of each clone and directions were taken as replications in the randomized complete block design.

Descriptive statistics (average, variance and standard deviation) and normality test (Kolmogorov-Smirnov and Shapiro–Wilk tests) were calculated by SPSS software. Genetic variation between selected clones was determined by the analysis of variance. Data were subjected to multivariate analysis using PAST software (Hammer et al., 2011). Cluster analysis using UPGMA algorithm and Euclidian distances method were carried out and all clones were grouped. Discriminant function and principal component analysis were used to infer clones relationships and to determine promising clones.

Table 1. Some important features of 'Bidaneh Ghermez' grape cultivar

time of bud burst	very late
status of tip	half open
attitude (before tying)	horizontal
density of erect hairs on tip	none or very low
color of dorsal side of internodes	green
color of ventral side of internodes	red

Table 1 (continued)

shape of blade	pentagonal
size of blade	medium
number of lobes	three
petiole sinus limited by vein	not limited
length of teeth	medium
sexual organ	fully developed stamens and fully developed gynoecium
cluster size (peduncle excluded)	medium
cluster density	medium
veraison	medium
berry size	short
berry shape	broad ellipsoid
color of berry skin	red
thickness of skin	very low
firmness of flesh	soft
particular flavor	none
formation of seeds	none
Nejatian & Doulati Baneh, (2016)	

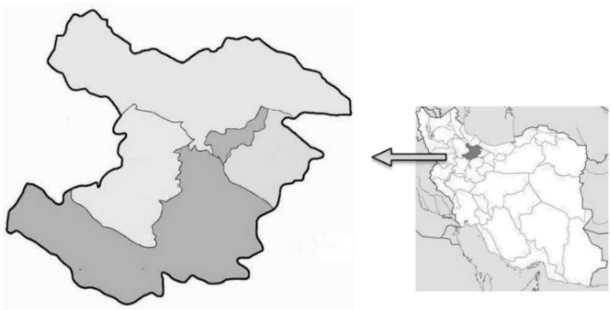

Figure 1. Map of the location of study in Qazvin (right), Iran (left).

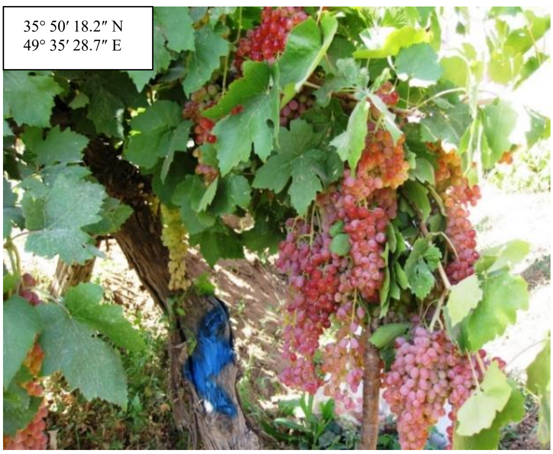

Figure 2. Selected clone and localized by GPS.

RESULTS AND DISCUSSION

Morphological descriptive statistic of measured traits showed cluster weight was more variable (standard deviation was 31.14) characteristic while berry weight had lowest standard deviation (0.33) among recorded traits (Table 2). Due to majority of statistic functions are based on normal distribution, we investigated the normality of experimental data using Kolmogorov-Smirnov and Shapiro–Wilk tests. Non-significance of these tests (especially Kolmogorov-Smirnov test that is designed for large sample size) showed that our experimental data is drawn from a normal distribution and could proceed further to next analyses (Table 3).

Table 2. Descriptive statistics (clones mean, total mean, standard deviation and variance) for recorded traits

Clones	CW (gr)	CL (cm)	CWI (cm)	BW (gr)	BL (mm)	BWI (mm)	TSS (%)
1	68.13	24.33	13.33	1.91	18.17	14.23	25.00
2	55.53	23.00	14.33	1.99	17.50	14.50	25.63
3	104.00	25.00	16.67	1.86	17.47	13.67	23.63
4	74.07	24.33	12.00	1.87	17.50	13.63	23.80
5	56.20	22.67	11.33	2.33	18.67	14.50	21.47
6	63.93	27.00	14.67	1.82	17.20	13.47	24.50
7	105.87	22.00	15.00	2.56	19.57	14.65	25.00
8	60.67	23.67	13.67	1.60	16.47	13.17	25.73
9	127.40	29.00	16.33	1.82	17.07	13.57	28.07
10	118.07	23.00	14.67	2.01	16.33	13.53	25.07
11	74.87	30.33	13.33	1.26	14.53	13.72	21.07
12	93.60	31.33	15.67	1.82	16.43	13.30	19.73
13	107.27	22.33	15.67	1.78	16.23	13.07	21.67
14	111.40	27.00	13.67	2.12	17.07	13.93	25.50
15	116.27	21.50	13.67	1.78	16.83	13.20	23.20
16	45.33	21.00	12.67	1.91	17.60	13.43	24.07
17	78.67	25.50	14.33	1.73	17.50	12.73	23.63
18	75.50	29.00	16.00	2.20	18.27	13.80	18.73
19	99.93	26.67	16.00	1.81	15.60	13.00	21.67
20	88.20	29.67	14.67	1.29	14.97	13.65	21.17
Total Mean	86.3	25.4	14.3	1.87	17	13.6	23.4
Standard deviation	31.14	4.44	2.6	0.33	1.2	0.65	2.52
Variance	970.46	19.7	7.2	0.11	1.6	0.43	6.3

Table 3. Kolmogorov-Smirnov and Shapiro–Wilk tests for normality of data

	Kolmogorov-Smirnov			Shapiro–Wilk		
	Statistics	d.f*	Sig.	Statistics	d.f	Sig.
CW	0.115	58	0.055	0.938	58	0.005
CL	0.090	59	0.200	0.975	59	0.257
CWI	0.095	60	0.200	0.965	60	0.080
BW	0.091	60	0.200	0.982	60	0.505
BL	0.106	60	0.090	0.974	53	0.318
BWI	0.097	53	0.200	0.975	53	0.318
TSS	0.076	60	0.200	0.981	60	0.490

* – Difference in the degree of freedom (d.f) is due to estimation of missing data.

Analysis of variance showed that all traits (except cluster width) were significant indicating considerable genetic variation for all measured traits (except cluster width) among selected clones (Table 4).

Table 4. Analysis of variance for measured traits in randomized complete block design

Sources of variation	Mean Square						
	CW	CL	CWI	BW	BL	BWI	TSS
Replication	95.48	25.04	1.82	0.03	0.135	0.076	0.277
Clones	1,739.64**	30.08*	6.29ns	0.27**	4.34**	0.841**	16.08**
Error	612.76	14.16	8.06	0.04	0.364	0.243	1.849
R^2	0.601	0.542	0.287	0.778	0.857	0.645	0.813

* and ** significant at 5% and 1% probability level respectively; ns – non-significant.

Our research showed difference in studied clones taken from different vineyards. Diversity between different vineyards can be explained by microclimate of each vineyard and on the other hand as a result of different rootstocks, canopy management or fertilization (Spayd et al., 1994; Main et al., 2002; Loureiro et al., 2011). In our study rootstocks and management (including canopy, irrigation and fertilization management) was same in all vineyards. On the other hand, clones taken from one same vineyard were different. We could conclude that existence of genetic factors is main source of observed variation.

Hierarchical cluster analysis clarified the relationships among clones. Cluster analysis indicated 2 groups of similar clones. First group was including nine clones and second group was including eleven clones (Fig. 3). Cluster analysis have been used in many other researches to detect similarity between grape clones (Rakonjac et al., 2010), cultivars (Martínez et al., 2003) and wild populations (Franco-Mora et al., 2008; Ekhvaia & Akhalkatsi, 2010).

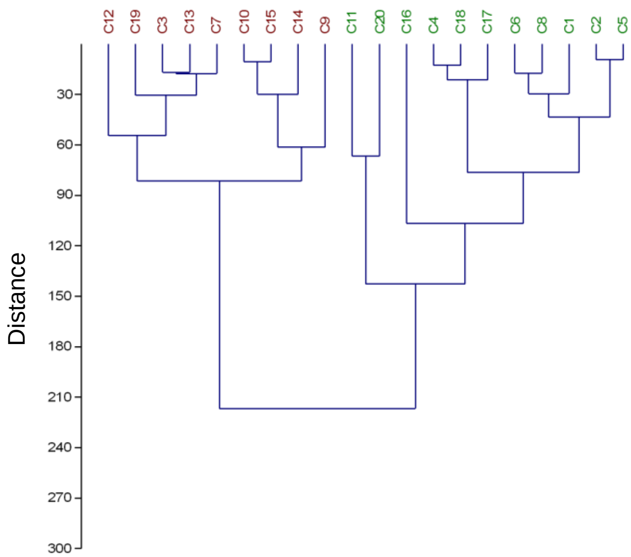

Figure 3. Dendrogram of the grapevine clones using UPGMA algorithm and Euclidian distances method. First group includes 9 clones (red) and second group includes 11 clones (green).

Assuming that created groups by cluster analysis is true; discriminant function analysis based on linear combinations of the predictor variables for 2 groups was fitted to develop a predictive model of group membership. Zero assumed to be cut off point and classification results showed that fitted function assign all clones to correct groups when 2 groups are considered (Fig. 4).

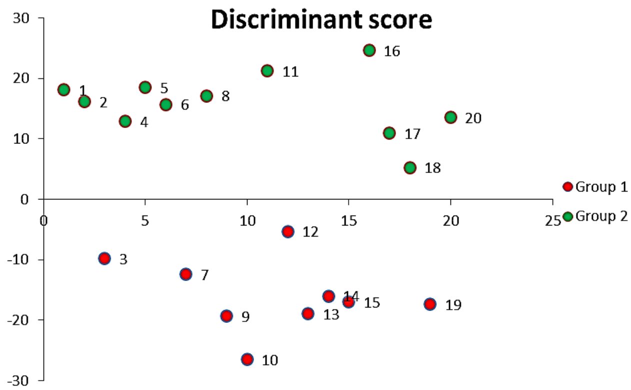

Figure 4. Discriminant score plot shows two groups (is shown with red and green color) in cutoff point (zero).

Principal component analysis (PCA) was performed to estimate morphological differentiation between clones revealed the first 2 components explain 99% of variance. Considering the loading factors indicated first component correspond to cluster related traits (cluster weight, cluster length and width) and second component was correlated with berry related characters (berry weight, berry length, berry width and TSS). PCA analysis separated all clones in 4 regions (Fig. 5). Clones in the first region of coordinate axis (C7, C10, C12 and C14) had highest value of cluster related traits and berry related traits while the rest of the clones had just high value for one out of two components (high value for cluster related characters or for berry related characters).

The PCA, as a powerful tool in statistical investigations, is widely used in the analysis of multivariate data in the agricultural sciences to evaluate genetic diversity and population's classification. For instance, Ekhvaiva & Akhalkatsi (2010) have studied seven wild grape populations from three geographic regions and showed not only PCA and multivariate discriminant analysis are powerful techniques but also could classify the populations correctly, when three geographic regions were considered. In the current study, PCA has separated studied clones in the best possible way. Similar to cluster analysis and discriminant analysis results, PCA showed that C7, C10, C12 and C14 clones are the best ones. Moreover, clones in the first region of coordinate axes, didn't have same value. The C10 clone had high value of the second component and low value of the first component, whereas C7 clone had high value of the first component and low value of the second component. Accordingly it can be concluded that C10 is a distinguished clone when berry related traits are more important than cluster related traits. Conversely, C7 is the preferential clone when cluster related traits are more important than berry related traits. In addition, in cases that berry and cluster related traits have similar importance, C14 can be considered as an alternative clone for selection in breeding programs.

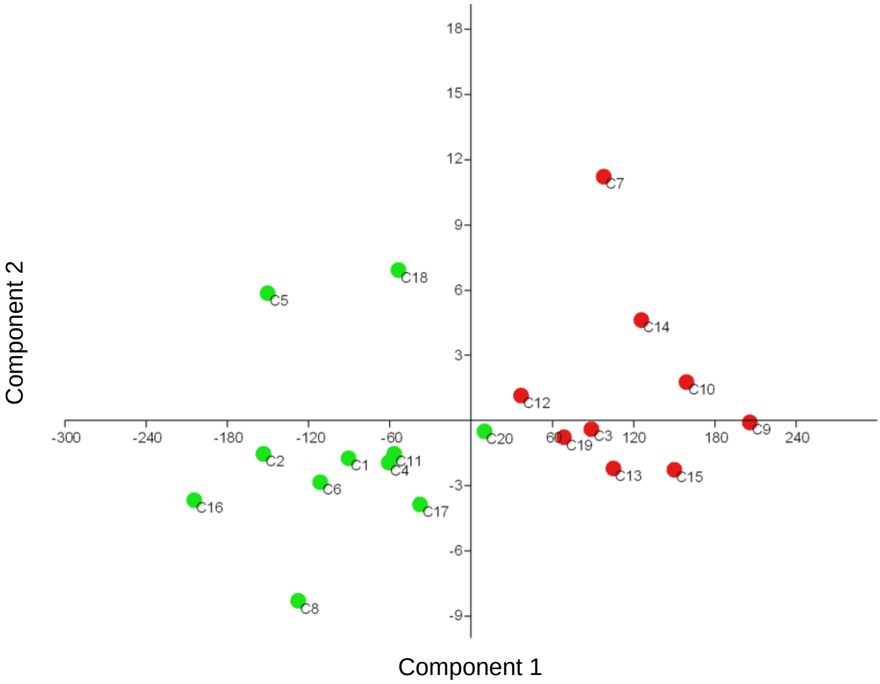

Figure 5. Principal component analysis (PCA) and two separated groups.

CONCLUSIONS

Grape *(Vitis vinifera* L.) is a well-known fruit, botanically a berry, cultivated with different cultivars and clones across the world. The 'Bidaneh Germez' is a local cultivar, consumed as table grape with several diverged clones in Iran. Identifying the grape cultivars and determining their potential is important in improving vineyards and grape quality. The current study indicated a considerable genetic variation among clones that could be used in future breeding programs and clonal selection. According to the results, cluster analysis, discriminant function analysis and principal component analysis showed same results and all clones were assigned in two groups. The low level of divergence might be due to this fact that the studied traits were correlated so further work should be performed to detect more differentiation between the clones. This investigation showed that evaluation of genetic diversity in Iranian grape germplasm, using morphological traits, is highly efficient for future applied and basic researches. According to the results, C7, C10, C12 and C14 clones were found to be distinguished clones and have shown potential to be introduced as promising clones in future breeding programs.

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Quality evaluation of local apple varieties: physicochemical and antioxidant proprieties at harvest and after cold storage

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Abstract. A wide apple germplasm is present in Italy in which numerous local genotypes of specific cultivation environment have to be still evaluated for fruit quality attributes. This is the case of a long-established fruit area located in central Italy (Tuscany) where several ancient apple varieties survive with the perspective to be re-introduced for their excellent quality. The objective of this work was to determine over a 2-year period the physicochemical traits, total antioxidant capacity (TAC), and polyphenols (TP) content of three old local apple varieties: 'Paganina', 'Paradisa' and 'Rosa'. These characteristics were also assessed after 90 and 150 days at 4 °C cold storage, in normal atmosphere refrigerated cellars. For comparison purpose, the commercial apple cultivar 'Fuji' was considered. In general, the old varieties showed valuable quality properties, particularly due to a greater antioxidant power of fruits, although a variability between the two crop seasons, characterized by different weather conditions, was observed. After a dry summer, 'Rosa' showed very high TAC and TP values (2-fold higher than other varieties) that, after long-periods of cold storage, markedly decreased. These losses were not so noticeable in the others, suggesting a tendency to maintain a major stability during storage. These findings could meet the preference of demanding consumers for healthier foods who appreciate fresh fruits with protective properties by free radicals scavenging activity. Moreover, farmers who are focused on nearby and niche markets could have interesting in these local varieties to promote their valorization.

Key words: *Malus domestica* Borkh, antioxidant properties, pomological traits, post-harvest, storability.

INTRODUCTION

In a context characterized by a growing consumer's interest in locally produced foods, the safeguard of ancient fruit varieties appears relevant. These genotypes, characterized by high hardiness and suitable for niche markets, are considered by consumers more genuine and healthier (Darby et al., 2008; Denver & Jensen, 2014). The attractiveness is also due to increasing evidence about their high nutritional value. Recent researches showed that ancient varieties have higher nutraceutical traits than modern ones, particularly apple, one of the most commonly consumed fruits throughout the world (Donno et al., 2012; Jakobek et al., 2013). It has been proved that apple fruits are rich source of antioxidants, mainly phenolic compounds, able to neutralize free radicals and protecting towards degenerative disorders in humans (Vrhovsek et al., 2004; Scafuri et al., 2016). In Italy, a wide apple germplasm is present but, while for the most popular varieties the antioxidant power is known, for varieties from specific cultivation areas the knowledge is often lacking or limited to few genotypes. This is the case of Lucca province in central Italy, a long-established fruit area in which ancient apple varieties survive with the perspective to be re-introduced for their excellent quality and sensory characteristics (Bartolini et al., 2015a).

Apples are generally stored over long periods using a wide range of technologies to extend the commercial life of fruits and keep them in the market longer (Farooq et al., 2012). For local farmers a regular cellar refrigerated storage is better than the sophisticated and expensive controlled-atmosphere technique. However, low temperatures can adversely affect the apple quality inducing some post-harvest changes on physicochemical attributes and antioxidant compounds content (Jan et al., 2012; Maragò et al., 2015).

Therefore, the main objective of this work was to determine the physicochemical traits, total antioxidant capacity, and polyphenols content in three old local apple varieties 'Paganina', 'Paradisa' and 'Rosa'. In addition, for the first time, the evaluation of cold storage effect on fruit quality characteristics was assessed.

MATERIALS AND METHODS

Plant material and cultivation site. The research was conducted over two consecutive harvesting seasons (2014–2015) on full bearing apple trees of three old local varieties: 'Paganina', 'Paradisa' and 'Rosa'. Trees, cultivated in a traditional area at high agricultural vocation (Tuscany, Lucca province, lat. 44.02 N, long. 10.27 E) were grown in three farms with similar pedo-climatic characteristics, using low input farming practices and a regular drip irrigation system. From fruit-set to harvest time the main climatic parameters were provided by the Hydrological Service of Tuscany (SIR). For comparison purpose, the popular apple cultivar 'Fuji' grown under the same cultivation conditions, was considered.

At physiological maturity, stage samples of fruits were analyzed to determine the main physicochemical parameters, total antioxidant capacity and total phenol content. In the second crop season, these analyses were also carried out on additional samples which were stored at 4 ± 0.5 °C, 90% relative humidity (RH) in a normal atmosphere for 90 and 150 days (d). The storage modality was selected as a representative conservation method used for apples by small farms.

Physicochemical analysis. Measurements of fruit weight, peel and flesh color, fruit firmness, pH, total soluble solids (TSS) and titratable acidity (TA) were obtained individually on 30 fruit/each variety. The size of fruits by weight was defined according to the following classification (Vitagliano et al., 2000): small (≤ 100 g), medium (100–190 g), large (> 191 g). Peel and flesh color (PC, FC) was evaluated using color charts according to UPOV Code (International Union for the Protection of New Varieties of Plants, Geneva) for apple. The relative area of red cover color of peel (CC) was visually estimated. Firmness was evaluated with a manual penetrometer on two peeled opposite areas at the equatorial region of apples, using an 11-mm-wide plunger. TSS was measured using a refractometer (°Brix) and TA was determined on fruit juice by titration a known volume of juice with 0.1 N NaOH to pH 8.1 endpoint. TA was expressed as milliequivalents of malic acid per 100 grams of fresh weight (meq malic ac. 100 g FW^{-1}). Fruit weight loss was calculated as [(Initial weight-final weight)/ Initial weight]*100.

Total antioxidant capacity (TAC) and total phenol (TP) analysis. Analyses were made on the same fruits (N = 30) previously subjected to the physicochemical determinations. Samples of 3 g (in triplicate) of fruit slices, constituted by flesh with peel, were immediately frozen and stored at –20 °C until extraction. Samples were homogenized using an ultra-Turrax blender at 4 °C to avoid oxidation. The extraction was performed in 80% ethanol for 1 h in a shaker in the dark and subsequently centrifuged at 4 °C for 10 min at 2,600 g. The supernatant was used for TAC (Total antioxidant capacity) and TP (total phenol) analysis.

TAC was evaluated using the improved Trolox equivalent antioxidant capacity (TEAC) method (Arts et al., 2004). The TEAC value was calculated in relation to the reactivity of Trolox, a water-soluble vitamin E analogue that was used as an antioxidant standard. In the assay, 40 μl of the diluted samples, controls or blanks added to 1,9690 μl ABTS$^{\bullet+}$ solution, resulted in a 20–80% inhibition of the absorbance. The decrease in absorbance at 734 nm was recorded 6 min after an initial mixing, and plotted against a dose–response curve calculated for Trolox (0–30 μM). Antioxidant activity was expressed as micromoles of Trolox equivalents pergram of fresh fruit weight (μmolTE gFW^{-1}). Trolox was purchased from Sigma Chemical Co. (St. Louis, MO).

TP content was determined according to the improved Folin-Ciocalteu (F-C) method (Waterhouse 2001). The assay provides a rapid indication of the antioxidant status of the studied material and is valuable for different food samples. The standard compound for the calibration curve was gallic acid (GA, Sigma Chemical Co, – St. Louis, MO). Total phenol content was calculated as milligrams of GA equivalent (GAE) per gram of fresh fruit weight (mg GAE gFW^{-1}). The absorbance of the blue colored solutions was read at 765 nm after incubation for 2 h at room temperature.

Statistical analysis. Instrumental data are reported as mean ± standard error of the mean (SEM). Analysis of variance (ANOVA), Student's *t*-test procedure and correlation analysis were performed using the package GraphPad Prism 5 (GraphPad Software, Inc.).

RESULTS AND DISCUSSION

Weather conditions. Climatic data, from May to October over two consecutive crop seasons (2014 and 2015), differed in terms of temperatures and distribution of rainfall (Table 1).

The summer 2015 was characterized by warmer temperatures by mean values of about 2 °C than 2014 which, instead, was noticeable for the very rainy condition during the early summer season Indeed, up to August more than 400 mm of rainfall were recorded, of which 80% in July. This occurrence was infrequent for the considered geographical area where, average precipitations of the last 10 years, for the same period, ranged from 8.6 to 60.2 mm (SIR Toscana). By contrast, as usually happens, in 2015 conspicuous rainfall (more than 300 mm) occurred in October, close to harvest time. The total rainfall recorded during the fruit growth-ripening period (May–October) was 646 and 513 mm in the first and second year, respectively. An average of about 500 mm may be considered conforming to the multiannual data about Lucca province (SIR Toscana).

Table 1. Monthly minimum and maximum temperatures (°C) and cumulative rainfall (mm) from May to October, over a 2-year period (2014–15)

Month	2014				2015			
	Min.	Max.	Average	Rainfall	Min.	Max.	Average	Rainfall
May	11.8	23.7	17.8	39.8	13	24.5	18.8	34.4
June	16.0	29.4	22.7	48.0	16.8	30.2	23.5	29.8
July	17.4	28.7	23.2	314.6	20.7	33.9	27.3	7.4
August	17.3	29.1	23.2	4.0	18.4	31.9	25.2	80.6
Av. temp.	*15.6*	*27.7*	*21.7*		*17.2*	*30.1*	*23.7*	
Total rainf.				*406.4*				*151.2*
September	14.9	26.2	20.6	142.8	15.4	27.1	21.3	36.2
October (1–15)	14.9	24.6	19.7	63.0	14.4	21.4	17.9	230.0
October (15–31)	10.2	21.1	15.7	34.2	8.7	18.7	13.7	95.2
Av .temp. (Sept.-Oct.)	*13.3*	*24.0*	*18.7*		*12.8*	*24.9*	*17.6*	
Total rainf.(Sept.-Oct.)				*240.0*				*361.4*
Av. temp.(May-Oct.)	*14.9*	*26.6*	*20.8*		*15.9*	*27.9*	*21.9*	
Total rainf.(May-Oct.)				*646.4*				*513.6*

Physicochemical and antioxidant parameters of fruits at harvest. The harvest time and the main pomological traits of fruits are reported in Table 2. The physiological maturity of apples occurred in the second and third ten days of September for 'Fuji' and 'Paradisa', and in the second and third ten days of October for 'Paganina' and 'Rosa', respectively. Fruits presented the following morphological characteristics: 'Paganina'-medium size, red-green peel (40–50% red cover colour), cream flesh and flat shape; 'Paradisa' – medium size, grey-yellow peel (5–10% red cover colour), greenish flesh and truncated cone shape; 'Rosa' – medium-large size, green-yellow peel (70–80% red cover colour), cream flesh and truncated cone shape; 'Fuji' (reference variety) – medium-large size, green peel (30–40% cover colour), cream flesh and spherical shape.

Table 2. 'Paganina', 'Paradisa', 'Rosa' and 'Fuji' apple varieties: harvest time, peel and flesh colour, red cover colour (CC, %), longitudinal shape and size of fruits

	Paganina	Paradisa	Rosa	Fuji
Harvest time	10–20 October	20–30 September	20–30 October	10–20 September
Peel colour	red-green, 40–50CC	grey-yell., 5–10CC	green-yell.,70–80CC	green, 30–40CC
Flesh colour	cream	greenish	cream	cream
Shape	flat	truncated cone	truncated cone	spherical
Size	medium	medium	medium-large	medium-large

Apple fruits showed different physicochemical traits according to the variety and the harvest year (Table 3). As regards the mean values of the two years, the flesh firmness ranged between 4.6 and 8.2 kg 0.5 cm^{-2}, above the minimum threshold of 4.4 kg 0.5 cm^{-2} considered an important qualitative index for apples (Kupferman, 1992). The highest TSS content (about 14 °Brix) was detected in 'Paganina' and 'Rosa' which were significantly different from 'Fuji' (about 11 °Brix). TA values ranged from 4.4 to 7.3 meq malic ac. 100 g FW^{-1} in 'Fuji' and 'Paganina', respectively. As a consequence, variations in TSS/TA ratio from 1.8 to 2.7 were found.

Within each variety, apple pomological attributes in response to different summer weather conditions were observed. In 2015, year characterized by a warm and dry summer season, values of FF, TSS, TA and TSS/TA ratio were generally higher than the previous year. It has been observed that, among the environmental factors determining the apple quality, temperature was the most important correlating with fruit hardness, soluble sugar content, and sugar-acid ratio (Wei et al., 1998). In addition, conditions of moderate water deficit stress caused a TSS and TA increase (Navarro et al., 2010). Among the local varieties, 'Paradisa' had less variability of fruit parameters in response to summer stresses due to high temperatures and drought conditions.

In order to establish the influence of environmental conditions on physicochemical characteristics of fruits, analysis of variance, considering two main effects (years and varieties), was performed (Table 4). Significant interactions 'year x variety' for FF, TA and TSS/TA ratio were observed, confirming the role of weather conditions which can impact with the physiological responses of genotypes during the grown-ripening season (Bartolini et al., 2015b).

Concerning the antioxidant properties of apples, expressed by total antioxidant capacity (TAC) and total phenol (TP) as average of the two crop seasons (Table 3), local varieties proved values higher than the commercial variety 'Fuji' showing about 36 μmol TE g FW^{-1} for TAC and 1 mg GAE g FW^{-1} for TP. Indeed, in the other three varieties the concentrations were from about 44 to 63 μmol TE g FW^{-1} for TAC, and from 2 to 5 mg GAE g FW^{-1} for TP, confirming that the ancient cultivars may have higher levels of bioactive compounds than the modern ones (Jakobek & Barron, 2016). Changes in TAC and TP values in relation to the crop season were observed. In general, the lowest concentrations were found in 2015 by a mean decrease ranging from 30 to 70% respectively, in comparison to the previous year. On the contrary, 'Rosa' showed an increase of 2-fold higher, reaching values of about 90 μmol TE g FW^{-1} for TAC, and around 7 mg GAE g FW^{-1} for TP. The raise in antioxidants recorded for 'Rosa' in 2015 was in agreement with findings of authors who found an antioxidant enhancement in apples and other fruits such as grape, acerola and apricot, under warm and dry summer conditions (Lima et al., 2005; Leccese et al., 2012). It has been suggested that water stress, caused by the deficit irrigation technique, can imply an activation of phenolic biosynthesis in the plants (Roby et al., 2004). The behavior of the other varieties was in accordance to Lata (2007) who has reported higher significant concentrations of total phenolics in wetter and cooler growing seasons. The influence of different climatic conditions on antioxidant power of fruits has been debating, confirming the importance of genotype who can differently react to the environment and, consequently, responses are cultivar-dependent (McGhie et al., 2005).

The 2-way ANOVA results (Table 4) showed that TAC and TP variations among varieties were greater than between harvest years, as found in other fruit species (Howard et al., 2003; Leccese et al., 2012). Nevertheless, the significant interaction 'variety x year' revealed that environmental growing conditions may impact the capacity of genotype to synthesise antioxidants which are influenced by biotic and abiotic factors (Besiada & Tomczak, 2012).

Table 3. 'Paganina', 'Paradisa', 'Rosa' and 'Fuji' apple varieties over a 2-year period (2014–15): main physicochemical and antioxidant parameters: flesh firmness (FF, kg 0.5 cm^{-2}), total soluble sugars (TSS, °Brix), titratable acidity (TA, meq malic ac. 100 g FW^{-1}), sugars/acids ratio (TSS/TA), total antioxidant capacity (TAC, µmol TE g FW^{-1}), total phenols (TP, mg GAE g FW^{-1}). Mean ± SEM. Within each variety, asterisk indicates significant differences between years by Student *t*-test ($P \leq 0.05$). Among varieties, different letters indicate significant differences at $P \leq 0.05$

	Paganina			Paradisa			Rosa			Fuji		
	2014	*2015*	*mean*	*2014*	*2015*	*mean*	*2014*	*2015*	*mean*	*2014*	*2015*	*mean*
FF	3.9 ± 0.3	5.3 ± 0.5*	4.6 ± 0.4c	5.4 ± 0.3	5.9 ± 0.5	5.6 ± 0.3b	6.2 ± 0.1	10.2 ± 0.7*	8.2 ± 0.4a	5.9 ± 0.2	6.8 ± 0.3*	6.3 ± 0.2b
TSS	12.6 ± 0.2	15.1 ± 0.6*	13.8 ± 0.4a	12.2 ± 0.4	13.1 ± 0.2	12.6 ± 0.3ab	13.8 ± 0.3	14.9 ± 0.2*	14.3 ± 0.3a	11.0 ± 0.3	11.6 ± 0.3	11.3 ± 0.3b
TA	6.8 ± 0.2	7.9 ± 0.1*	7.3 ± 0.2a	8.4 ± 0.2	5.9 ± 0.1*	7.1 ± 0.2a	3.7 ± 0.1	8.3 ± 0.2*	6.0 ± 0.1ab	3.6 ± 0.1	5.2 ± 0.1*	4.4 ± 0.1b
TSS/TA	1.8	1.9	1.8b	1.4	2.2*	1.8b	3.7	1.8*	2.7a	3.1	2.2*	2.6a
TAC	64.9 ± 0.8	43.2 ± 0.6*	54.0 ± 0.7a	51.5 ± 8.5	37.6 ± 0.1	44.5 ± 4.5a	35.4 ± 3.3	89.9 ± 1.1*	62.6 ± 4.4a	42.3 ± 1.5	30.4 ± 0.4*	36.3 ± 1.0a
TP	3.4 ± 0.5	0.8 ± 0.1*	2.1 ± 0.3ab	3.9 ± 0.8	0.5 ± 0.1 *	2.2 ± 0.4ab	2.7 ± 0.2	7.4 ± 0.3*	5.0 ± 0.2a	1.9 ± 0.3	0.9 ± 0.5*	1.4 ± 0.4b

Table 4. Two-way ANOVA results. Variables: FF (FF, kg 0.5 cm^{-2}), TSS (°Brix), TA (meq malic ac. 100 g FW^{-1}), TSS/TA, TAC (µmol TE g FW^{-1}) and TP (mg GAE g FW^{-1})

Main effects	FF	TSS	TA	TSS/TA	TAC	TP
	P	*P*	*P*	*P*	*P*	*P*
Year (Y)	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***	0.0109*	0.8940 ns
Variety (V)	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***
Interac. YxV	< 0.0001***	0.0519 ns	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***

Physicochemical and antioxidant parameters of fruits after cold storage. To assess the storability of apples, in the second crop season (2015) physicochemical and antioxidant performances were tested after 90 and 150 d at 4 °C cold storage (Table 5).

Table 5. 'Paganina', 'Paradisa', 'Rosa' and 'Fuji' apple varieties (year 2015). Physicochemical parameters of fruits at harvest time (0) and after 90 and 150 days at +4 °C cold storage: weight loss (%), flesh firmness (FF, kg 0.5 cm^{-2}), total soluble sugars (TSS, °Brix), titratable acidity (TA, meq malic ac. 100 g FW^{-1}), and sugars/acids ratio (TSS/TA). Mean ± SEM. Within each variety, different letters indicate significant differences at $P \leq 0.05$; ns not significant. For the weight loss, asterisks indicate significant differences between storage days by Student *t*-test ($P \leq 0.05$)

Varieties	Storage (days)	Weight loss (%)	FF	TSS	TA	TSS/TA
Paganina	0	-	5.3 ± 0.5 a	15.1 ± 0.6 ns	7.9 ± 0.1 a	1.9 c
	90	4.5	3.6 ± 0.3 b	14.4 ± 0.1	4.8 ± 0.2 b	2.9 b
	150	11.9*	3.4 ± 0.2 b	13.8 ± 0.1	3.9 ± 0.1 c	3.5 a
Paradisa	0	-	5.9 ± 0.5 ns	13.1 ± 0.2 a	5.9 ± 0.1 a	2.2 ns
	90	5.0	5.4 ± 0.3	13.4 ± 0.3 a	6.1 ± 0.1 a	2.0
	150	10.3*	4.5 ± 0.2	11.6 ± 0.4 b	4.9 ± 0.2 b	2.4
Rosa	0	-	10.2 ± 0.7 a	14.9 ± 0.2 ns	8.3 ± 0.2 a	1.8 c
	90	1.9	5.0 ± 0.1 b	16.0 ± 0.3	6.2 ± 0.1 b	2.6 b
	150	3.8*	6.7 ± 0.6 b	14.9 ± 0.1	4.6 ± 0.4 c	3.2 a
Fuji	0	-	6.8 ± 0.3 ns	11.6 ± 0.4 ns	5.2 ± 0.1 a	2.2 c
	90	4.6	6.9 ± 0.5	10.0 ± 0.4	2.9 ± 0.1 b	3.5 b
	150	6.7*	6.6 ± 0.4	10.1 ± 0.1	2.6 ± 0.1 b	3.8 a

Concerning the physicochemical parameters, apple fruit-weight loss ranged from about 2% to 12%, similarly to whom reported for several apple cultivars (Golias et al., 2008; Guerra et al., 2010). This occurrence, as a consequence of the increase in transpiration and respiration rates (Blampired, 1981; Erturk et al., 2003), was particularly strong in 'Paganina' after the longest storage. On the other hand, the lowest weight loss was found in 'Rosa', probably due to its peel structure which appears thick with presence of wax (data not shown).

Flesh firmness, one of the most storage-susceptible parameter, did not change over time in 'Paradisa' and 'Fuji', while 'Paganina' and 'Rosa' had a significant firmness decrease after 90 d, without further declines. This finding supports several investigations in which a gradual FF reduction with an extending storage period was observed (Ghafir et al., 2009; Jan et al., 2012). Apple's softening in post-harvest, due to respiration and evapo-transpiration phenomena (Kov et al., 2005; Fuller, 2008), could negatively influence the fruit quality, predisposing fruits to diseases (Hoehn et al., 2003).

With the exception of 'Paradisa', TSS content did not change in comparison to harvest, in agreement with results obtained by Golias et al. (2008). About the total soluble sugars, literature reports some contradictions (stability, increase or decrease) as a function of the variety rather than storage conditions (Ghafir et al. 2009; Guerra et al., 2010). Thus, uncertainty for TSS as a valid parameter assessing the apple tolerance to cold storage arises (Farooq et al., 2012). During storage, as a result of the respiration process consuming organic acids, a progressive and significant decrease of TA was recorded (Jan et al., 2012). Changes in TA had a significant effect on TSS/TA ratio, widely considered as an important feature for apple flavor (Abbott et al., 2004). The ratio gradually increased, but ‘Paradisa’ maintained a mean value close to 2, denoting a good balance between sugars and acids, main factors for the eating apple quality (Sadar et al., 2016). This variety, object of a sensory evaluation carried out by people with visual disability able to assess the intrinsic quality attributes, was well appreciated for sweetness, juiciness and flavor (Bartolini et al., 2015a).

TAC and TP values, recorded at harvest time and at the end of the two cold storage periods, are showed in Fig. 1.

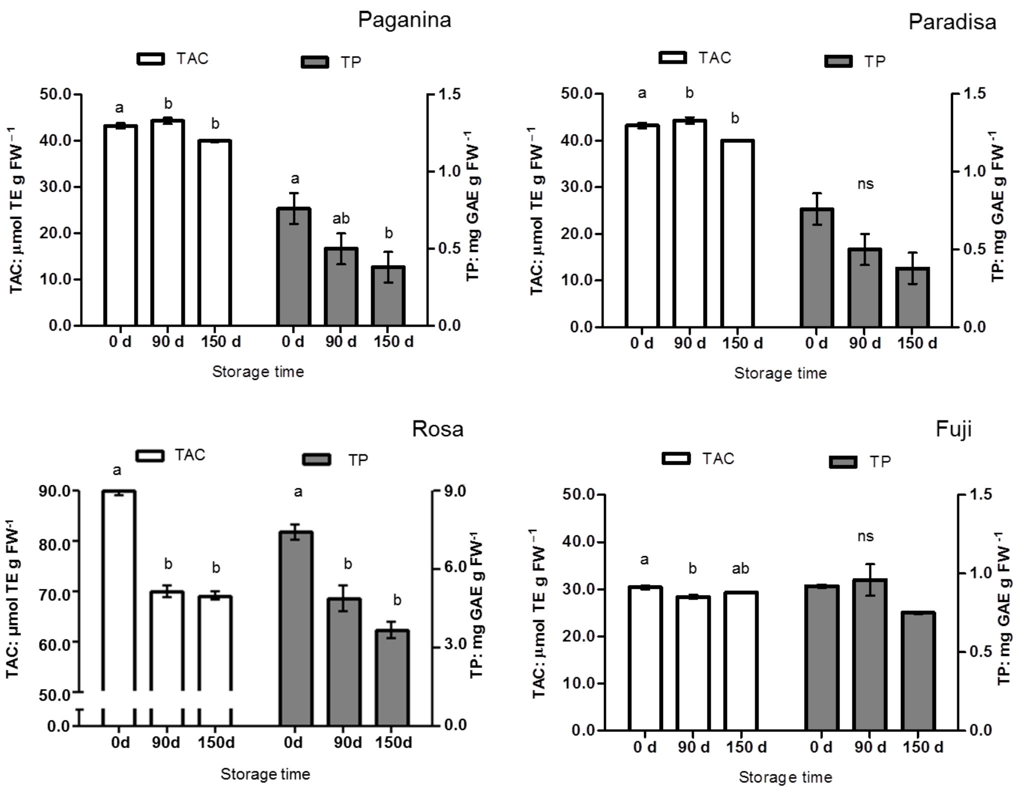

Figure 1. ‘Paganina’, ‘Paradisa’, ‘Rosa’ and ‘Fuji’ apple varieties (year 2015). Total antioxidant capacity (TAC) and total phenols (TP) at harvest time (0) and after 90 and 150 days at +4 °C cold storage. Mean ± SEM. Different letters indicate significant differences at $P \leq 0.05$.

Significant differences between fresh and stored samples were observed and varieties showed a similar trend in TAC and TP content decreases. In comparison to the initial average antioxidant values, ‘Fuji’ had the lowest declines while the local varieties, characterized by the highest contents at the beginning of storage, showed values which dropped over time from 7.5 to 22% for TAC and 26 to 50% for TP. The greatest losses were found in ‘Rosa’ that, however, preserved elevated contents because, in the year of storage trials, it was characterized by the highest antioxidant values at harvest (more than 2-fold higher than the other varieties). This general decline of antioxidant power was in agreement with Tarozzi et al. (2004) who have found that cold storage impoverishes the antioxidant properties of apples, particularly in skin tissue. In contrast, other authors have observed that cold storage did not affect the TP content of certain apple varieties which may show stable or increased antioxidant concentrations (Napolitano et al., 2004; Matthes & Schmitz-Eiberger, 2009). The different evidence existing in literature suggest that the capacity to maintain over time the antioxidant content of fruits would be primarily related to variety, hence the importance to extend the knowledge within the wide apple Italian germplasm.

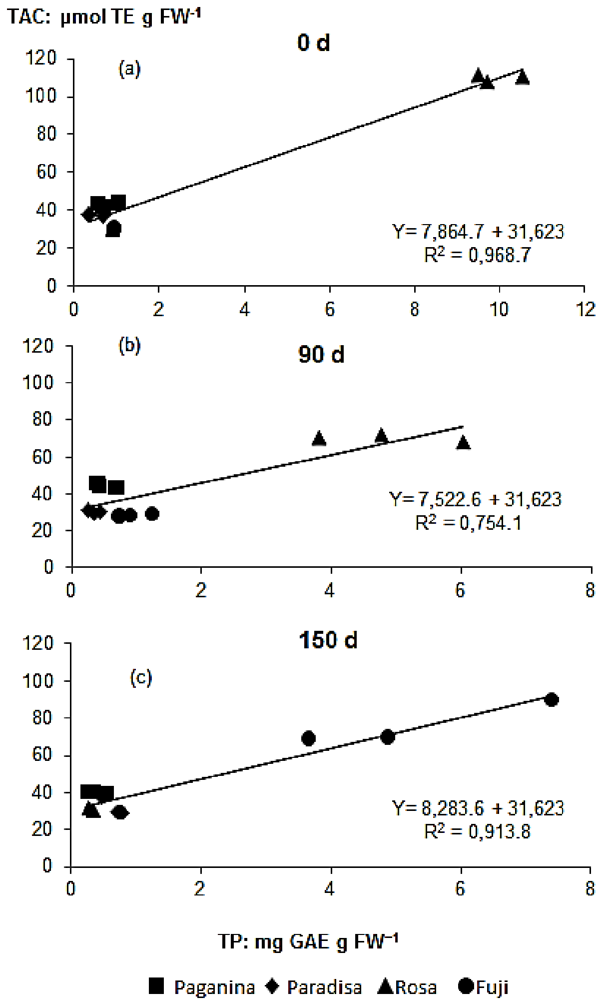

Figure 2 (a, b, c). Linear regression between total antioxidant capacity (TAC) and total phenols (TP) at harvest time (0) and after 90 and 150 days at +4 °C cold storage.

In order to verify a possible relation between TAC and TP of the studied varieties, regression analysis was carried out. From harvest to the end of both cold storage periods, a significant and positive relationship was shown (Fig. 2, a, b, c). As it has been observed for other genotypes, polyphenols are important bioactive compounds contributing to the antioxidant properties of apples (Leccese et al., 2009; Donno et al., 2012). Although the local varieties showed higher TAC values in comparison to 'Fuji', over time the correlation coefficients were influenced depending on variety. Indeed, after the longest storage period, the standard variety 'Fuji' was able to maintain more constant level of polyphenols, important nutraceutical suppliers with health-promoting effects for humans (Scafuri et al., 2016).

CONCLUSIONS

Results of this study provide new knowledge about the physicochemical and antioxidant properties of apple fruits belonging to old varieties grown under a long-established fruit area in central Italy. Compared with the world-wide-cultivated 'Fuji', the three local varieties, 'Paganina' 'Paradisa' and 'Rosa', showed valuable quality properties. In particular, they stood out for a greater antioxidant power of fruits, although a variability, linked to the weather conditions of the two investigated crop seasons, was observed. After a dry summer, 'Rosa' showed very high TAC and TP values that markedly decreased after long-periods of cold storage. These losses were not so noticeable in the other varieties, suggesting a tendency to maintain a major stability during storage in normal atmosphere refrigerated cellars. Further investigations will be likely extended to better ascertain the effect of cold-storage time on antioxidant properties of local apples**.** Anyway, the quality traits established for the old analyzed varieties could meet the preference of demanding consumers for taster and healthier foods, who appreciate fresh fruits for high antioxidant power with protective properties by free radicals scavenging activity. Moreover, the adopted simple storage technique might be useful to farmers who are focused on nearby and niche markets.

In conclusion, consumers and farmers should have ready access to information on the valuable quality characteristics of the old but relevant apple varieties. This is a further and valid attribute to take into account for a re-introduction of local varieties to promote their valorization and also contributing to the preservation of biodiversity. Moreover, breeders could be interested in these genotypes to create apple parental lines with high quality profile.

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Instrumental and sensory evaluation of seven apple (*Malus domestica* Borkh.) cultivars under organic cultivation in Sicily

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Abstract. In this trial we examined the quality of 7 clones belonging to more diffused apple polyclonal varietal groups, using chemical/physical and sensory analyses during two consecutive years. *Galaxy*, and their ameliorative clones *Gala Annaglò*® and *Dalitoga* (Gala clones) that ripen in summer, *Erovan* Early Red One*® and *Scarlet Spur*-Evasni*® (Red Delicious clone), *Corail Pinova* and its ameliorative clone *RoHo 3615 * Evelina*® that ripen in autumn were studied. *Gala Annaglò*® is interesting for the fruit size and peel color, *Dalitoga* for the early ripening and *Galaxy* for the crunchiness and consistency. All the Gala clones reached very high total solid soluble content confirmed by the panel judgment of the sweetness and acidity descriptors. The Red Delicious clones confirm the larger size and the high colorimetric standard of all covered red fruits; the new clone *Scarlet Spur*-Evasni*® reached an interesting fruit size and peel colour intensity and uniformity, and the best total solid soluble content to total acidity ratio confirmed by the sensory descriptors of acidity and sweetness. Moreover, it reached very high values of crunchiness, consistency and interesting values of apple flavour, honey flavour and fruit flavour. The ameliorative clone *RoHo 3615*Evelina*® was characterized by well uniform and intense coloured fruits and a more balanced total solid soluble content to total acidity ratio, and interesting values of crunchiness, consistency, apple flavour, honey flavour and fruit flavour. This study confirms the relationship between instrumental and sensory analysis.

Key words: *fruit quality, soluble solid content, titratable acidity, ground colour, panel test.*

INTRODUCTION

In Sicily apple cultivation is very ancient Nicosia (Nicosia, 1735) and it is witnessed by local genotypes selected for growing and cultivated mostly in fairly remote mountainous and hilly areas (750–850 m a.s.l.). In the last decade, a progressive renewal of these traditional orchards was based on the introduction of affirmed commercial cultivars (Lo Bianco & Farina, 2012). The choices of the apple varieties are determined by the request of a market based on quality, consistency and continuity in availability of the product. As a result, apple cultivation in Italy is oriented towards cultural standardization through 4–5 polyclonal varietal groups (Golden Delicious, Red Delicious, Fuji and Gala) characterized by specific and recognized quality characteristics (Farina & Di Marco, 2009) following European trends. These commercially affirmed cultivars, although they have reached high levels of quality, are characterized by a progressive varietal turnover with the introduction of new ameliorative clones (Sansavini et al., 2005, Kellerhals et al., 2008). In fact, over the past decade, there have been some major changes in the marketplace that are likely to have resulted in an increasing expectation of the eating quality of the apples. So even when the quality of apples remains the same, outside influences are driving up consumer expectations of apple taste (Harker et al., 2008). In particular, recent studies indicate that there is interest for both local and domestic apple production among the consumers (Denver & Jensen, 2014).

Quality of fruit is made of its external and internal factors such as size, shape, colour, taste, aroma, crunchiness and firmness (Abbott et al., 2004, Talluto et al., 2008).

Generally, researchers have tried to use measurements of flesh firmness, soluble solids content and titratable acidity to define quality (Hoehn et al., 2003).

Unfortunately, eating quality is difficult to measure objectively. Analytical measurements, soluble solids content, titratable acidity must be correlated with sensory perceptions of sweetness and sourness (Harker et al., 2002b). Flesh firmness relates to the mechanical properties (texture) of the apple flesh, mouthfeel, and juiciness and is important for consumers (Daillant-Spinnler et al., 1996) which associate them with positive characteristics such as freshness (Fillion & Kilcast, 2002). Several researchers have found a good relationship between sensory scores (obtained using a trained panel) and instrumental measurements of apple texture (Abbott et al., 1994; Karlsen et al., 1999; Harker et al., 2002a), total soluble solids content (TSSC), titratable acidity (TA) and consumer acceptability of fruit (Vangdal, 1985; Fellers, 1991; Mitchell et al., 1991). Other studies indicated that quantitative measurements of acids, TSSC and TSSC/TA ratio did not appear good predictors of taste and flavour in apples whereas TA was always amongst the best predictors of sensory attributes of acid taste, overall flavour, and apple flavour (Harker et al., 2002b).

The recent developments in the field of sensory evaluation and instrumental analysis further accentuate the interface between humans (sensory science) and machines (instrumental analysis) (Ross, 2009). More recently, several studies have focused on the quality of many fruits using instrumental and sensory analyses (Allegra et al., 2015; Montevecchi et al., 2013; Sortino et al., 2015; Gentile et al., 2016), and specifically on apples (Karlsen et al., 1999; Donati et al., 2006; Skendrović Babojelić et al., 2007). Taste panel data have been used to assess preferences among standard and newer apple cultivars (Stebbins et al., 1992; Røen et al., 1996) and as a selection tool in apple breeding (Hampson et al., 2000). Other studies have used sensory analysis to evaluate differences and preferences among clones of Gala, Red Delicious and Jonagold (Kappel et al., 1992; Greene & Autio, 1993). Several researches have demonstrated a relationship between the sensory perceptions of apple texture (firmness, crispness, crunchiness) and instrumentally measured parameters in order to predict fruit quality (Abbott et al., 1994; Mehinagic et al., 2004).

However, the relationship between instrumental data and consumer acceptability is cultivar specific, and there is insufficient information to make recommendations.

The aim of this work was to define the quality characteristics of seven apple cultivars and their ameliorative clones under organic cultivation in mountainous areas of Sicily investigating the relationship between instrumental (firmness, soluble solids content and titratable acidity) and sensory (taste, odour and flavour) measurements.

MATERIALS AND METHODS

The trial was conducted in an experimental orchard in Caltavuturo (Palermo, 37° 49' N and 850 m a.s.l.) in central Sicily, Italy. Seven apple varieties were considered in this work: *Galaxy, Gala Annaglò*® and *Dalitoga* (Gala clones) that ripen in summer, *Erovan* Early Red One*®, *Scarlet Spur*-Evasni*® (Red Delicious clone), *Corail Pinova* and its clone *RoHo 3615 * Evelina*® that ripen in autumn.

Three 10-year-old trees per each cultivar were selected. Trees were grafted on M9 rootstock, trained to a central leader and planted in North-South direction with an inter-trees spacing of 1.5 m and 5 m between rows.

The soil was classified as sandy clay loam with pH 7.3 and 1.8% active carbonates. The irrigation system was drip irrigation and trees were submitted to organic farming cultural care (Reg. 834/2007 ex-EEC EEC 2092/91 no).

Fruits were collected at commercial ripening stage (Table 1) using starch pattern index (rated between 1 = no staining and 5 = complete staining) as the maturity index (Peirs et al., 2002).

Table 1. Harvest dates and starch indexes (1–5 scale) of the 7 apple cultivars in the trial

Cultivar	Harvest date		S. Index	
	1st year	2nd year	1st year	2nd year
Galaxy	18-Aug	14-Aug	2.80	3.05
Gala Annaglò®	23-Aug	18-Aug	2.78	2.93
Dalitoga	16-Aug	12-Aug	2.45	2.71
Erovan Early Red One*®	07-Sep	01-Sep	2.10	2.20
Scarlet Spur-Evasni*®	11-Sep	03-Sep	1.95	2.11
Corail Pinova	06-Sep	01-Sep	3.62	3.51
*RoHo 3615*Evelina*®	06-Sep	02-Sep	3.47	3.71

At commercial ripening a sample of 30 fruits x tree x cultivar were collected and submitted to analytical and sensory evaluations. Biometrical and physical-chemical characteristics were also observed: flesh firmness (FF) expressed in kg cm^{-2}, total soluble solid content (TSSC) expressed in % (Brix°), titratable acidity (TA) expressed in g L^{-1} of malic acid and TSSC/ TA ratio. Fruit weight (FW) was determined by digital scale, longitudinal diameter (LD) and transversal diameter (TD) by digital caliper TR53307 (Turoni, Forlì. Italy), FF by digital penetrometer TR5325 (Turoni, Forlì. Italy), TSSC by digital refractometer Atago Palette PR-32 (Atago Co., Ltd. Tokyo. Japan), TA and pH using a CrisonS compact titrator (Crison Instruments. SA. Barcelona. Spain).

Ground colour and cover colour as well as starch index were determined via digital image analysis of each fruit by Fruit Analysis System (F.A.S.) (Francaviglia et al., 2013). Digital images were used to determine percentage and intensity of peel red color. In particular, we used an algorithm that converts images from RGB (additive color model in which red, green and blue light are added together in various ways to reproduce a broad array of colors) to CIE L*a*b* (mathematic description of all perceivable colors in the three dimensions ***L*** for lightness and ***a*** and ***b*** for the color opponents green–red and blue–yellow) format. It extracts the fruit from the image (removing the image background), separates the total fruit area into two sub-regions: cover colour (closer to red) and ground colour (closer to green) according to an adjustable green–red threshold, and quantizes colour characteristics of each region as the weighed distance of each pixel in the image from pure green (ground colour) or pure red (cover colour). The output is an index (CI) for the cover colour ranging from 0 (no red) to 1 (red). Percentage of cover colour was calculated dividing the number of pixels of the red region by the number of pixels of the entire fruit area. Cover colour fruit area was expressed as a percent of total surface (CP).

The sensory profile (UNI 10957, 2013) was defined on a subsample of 10 fruits per tree per cultivar by a panel of 10 judges (five female and five male) who evaluated the intensity of each attribute by assigning a score between 1 (absence of the sensation) and 9 (extremely intense). All panelists were trained at Catania University and have a wide expertise in sensory evaluation of foods and in particular in fruits (Farina et al., 2010; Mazzaglia et al., 2010; Farina et al., 2011; Liguori et al., 2014; Farina et al., 2016). In a preliminary meeting, 19 attributes were generated, on the basis of frequency of citation (> 60%), as listed below: six for odour (Apple – AO; Vegetal – VO; Honey – HO; Fruit – FO; Almond – ALO; Off – OFO) three for tastes (Acid – A; Sweet – S; Bitter – B), four for rheological (Juiciness – J; Consistency – C; Crunchiness – CR; Sponginess – SP), six for flavour (Apple – AF; Vegetal – VF; Honey – HF; Fruit – FRF; Almond – ALF; Offtaste – OFF). The evaluations were carried out from 10.00 to 12.00 a.m. in individual booths with controlled illumination and temperature. The study was carried out during three different sessions. In each session, the panelists tested all cultivars under study; the sample order for each panelist was randomized and water was provided for rinsing between apple samples. The judges evaluated the intensity of each attribute by assigning a score between 1 (absence of the sensation) and 9 (extremely intense). A computerized data collection program was used (FIZZ. Software Solutions for Sensory Analysis and Consumer Tests. Biosystemes. Couternon. France).

Data analysis was carried out using the program SYSTAT v.11 (SYSTAT Software Inc.). Statistical significance was defined at $P < 0.05$. Differences between apple samples according to physicochemical characteristics were investigated with a one-way ANOVA using cultivars as main factors. The sensory data for each attribute were submitted to analysis of variance (one-way ANOVA), with samples as effects. The significance of these effects was assessed by F-tests.

RESULTS AND DISCUSSIONS

Gala fruits were ready to be harvested in the second half of August, in particular, *Dalitoga* had the earliest maturation followed by *Galaxy* and *Gala Annaglò*®; Red Delicious clones and Pinova clones reached commercial harvest during the first half of September (Table 1). Although the three Gala clones ripen in the summer, earlier than the other, Red Delicious and Pinova clones were harvested in early autumn, so the ripening times were quite similar for all clones. Dalitoga was harvested before Galaxy enlarging the harvest window. Starch index indicated that all fruits had reached their commercial ripening (Angelini et al., 2008).

The analysis of pomological traits showed wide variability among the different clones. The analysis of variance of the internal (Table 2) and external (Table 3) quality parameters highlights significant differences between different polyclonal groups and between clones. All examined clones reached the legal standards for the edible quality of apples that have been in place in many apple-growing regions of the world (Harker et al., 2008) through a numerical lower limit for commercial size, diameter or fruit weight (Reg. CE n.85/2004). In particular, fresh weight ranging from 162.12 g of *Dalitoga* and 235.69 g of *Scarlet Spur*-Evasni*®. Among Gala clones, *Gala Annaglò*® have the best fresh weight. Red Delicious clones produce the biggest fruits, in particular *Scarlet Spur*-Evasni*, with a very interesting size. The two Pinova clones had the same fresh weight. An elevated commercial size is more appreciated by the consumer and it sprouts prices that are more profitable for the producers. In this case, all the fruit were classified in the 'extra' size.

Table 2. Pomological traits of 7 apple cultivars in the trial. Mean of two years ±SD (n = 90)

Varietal group	Cultivar	FW (g)	LD (mm)	TD (mm)	LD/ TD	FF (kg cm^{-2})	GCI	CP (%)
	Galaxy	158.00 ± 4.21d	63.22 ± 2.21 d	60.02 ± 2.25 b	1.05 ± 0.04 c	6.90 ± 0.89 ns	0.876 ± 0.004 b	92.4 ± 2.15 b
	Gala Annaglò®	168.48 ± 4.21 c	67.36 ± 3.25 c	63.32 ± 2.42 b	1.06 ± 0.05 c	6.78 ± 0.75 ns	0.901 ± 0.005 ab	97.3 ± 2.12 a
	Dalitoga	162.12 ± 5.42 cd	58.40 ± 2.68 d	73.88 ± 2.65 a	0.79 ± 0.04 d	6.81 ± 0.70 ns	0.871 ± 0.004 b	90.1 ± 2.05 b
	Erovan Early Red One*®	187.56 ± 6.25 b	77.47 ± 3.24 ab	63.56 ± 2.67 b	1.21 ± 0.03 a	6.44 ± 0.58 ns	0.902 ± 0.005 ab	92.5 ± 2.65 b
	Scarlet Spur -Evasni*®	235.29 ± 8.22 a	82.94 ± 2.41 a	72.39 ± 2.89 a	1.14 ± 0.06 ab	6.24 ± 0.98 ns	0.931 ± 0.004 a	98.2 ± 2.03 a
	Corail Pinova	165.00 ± 5.21 c	70.22 ± 2.65 b	63.58 ± 2.68 b	1.10 ± 0.05 b	6.20 ± 0.87 ns	0.872 ± 0.005 b	92.3 ± 2.23 b
	RoHo 3615 Evelina*®	166.25 ± 5.68 c	71.59 ± 3.21 b	64.12 ± 2.41 b	1.11 ± 0.04 b	6.51 ± 0.78 ns	0.903 ± 0.004 ab	97.1 ± 2.41 a

Fruit Weight – FW; Longitudinal Diameter – LD; Transversal Diameter – TD; LD/TD Ratio; Flesh Firmness – FF; Colour Index – CI; Percentage of cover colour – CP. The values marked with different letters in the same column indicate significative significant differences ($P \leq 0.05$).

Table 3. Physicochemical parameters of 7 apple cultivars in the trial. Mean of two years ±SD (= 90)

Varietal group	Cultivar	TSSC (°brix)	AT (g L^{-1})	TSSC/AT	PH
	Galaxy	16.50 ± 0.42 a	4.10 ± 0.20 d	4.02 ± 0.09 a	3.05 ± 0.05 ns
	Gala Annaglò®	15.50 ± 0.45 b	4.83 ± 0.18 b	2.29 ± 0.07 c	3.04 ± 0.04 ns
	Dalitoga	16.80 ± 0.65 a	4.23 ± 0.21 c	3.97 ± 0.08 a	2.95 ± 0.05 ns
	Erovan Early Red One*®	16.15 ± 0.51 a	7.31 ± 0.19 a	2.51 ± 0.09 c	2.93 ± 0.04 ns
	Scarlet Spur-Evasni*®	13.80 ± 0.42 c	4.46 ± 0.17 c	3.09 ± 0.07 b	3.02 ± 0.04 ns
	Corail Pinova	16.50 ± 0.39 a	6.80 ± 0.23 a	2.43 ± 0.08 c	3.02 ± 0.05 ns
	*RoHo 3615*Evelina*®	14.05 ± 0.38 c	7.02 ± 0.20 a	2.00 ± 0.08 d	3.05 ± 0.06 ns

Total soluble solids content – TSSC; Titratable Acidity – TA; TSSC/TA ratio and pH. The values marked with different letters in the same column indicate significantly differences ($P \leq 0.05$).

LD/TD ratio indicated uniform oval rounded shape for *Galaxy* and *Gala Annaglo*® and oblong shape for Pinova clones and Red Delicious clones*:* Gala clones had the typical rounded shape whereas Red Delicious the typical heart-shaped fruit. Firmness and SSC can explain an important portion of consumer preferences for apples (Harker et al., 2008). FF values were no less than 6.00 kg cm^{-2} and were similar for all the observed cultivars ranging from 6.20 of *Corail Pinova* to 6.90 of *Galaxy.* In every case, FF is compatible with the commercial standard and favourable to postharvest operations and storage. Moreover, our data are similar to the research of Harker et al. (2008) that showed a substantial increase in consumer acceptance as firmness increased from 3.7 to 6.3 kg cm^{-2}.

As expected, Red Delicious clones presented the highest CI value whereas CP was different inside of each clonal group (Table 2). Considering Gala clones, *Gala Annaglo*®, *Galaxy, Dalitoga* were characterized by the typical red coloration over the entire surface with often indistinct red over-striping. Moreover, the ameliorative clone *Gala Annaglo*® presented the highest CI and CP in respect to the other two clones. These results are in accordance with those previously reported by Iglesias et al. for Galaxy (2008). Moreover, the intensity of red colouration was strongly related to the total surface area covered as reported by White & Johnstone (1991).

Among Red Delicious clones*, Scarlet Spur*-Evasni* showed the appearance with the highest CI and CP values. *RoHo 3615*Evelina*® fruit was characterized by very attractive uniform intense red coloured peel. This last was selected as an ameliorative clone of Pinova and confirms this aim with higher values of colour index and cover colour percentage in respect to *Corail Pinova*. The ameliorative clones of each polyclonal group that present more intense fruit coloration and uniformity could have a higher economical value. In fact, today retail groups (GD) and supermarket chains (DO) tend to show some preference for more uniform and more intense coloured fruits. Colour must be considered as a primary trait to select for, since consumers are strongly attracted to well coloured fruits, especially for Gala clones (Iglesias et al., 2008).

TSSC values were, for all cultivars, compatible with commercial standards of fresh fruit, but the highest contents were recorded in *Galaxy*, *Erovan* Early Red One*® and *Corail Pinova,* the oldest clones of each group. In every case, all observed clones reached very high values ranging from 13.80 to 16.50 in respect to the standard varietal values (Angelini et al., 2008). Fruit acceptability was positively affected by high TSSC (Harker et al., 2008) The lowest TA content was observed in Gala clones followed by Red Delicious, whereas Pinova clones, as expected, showed the highest values.

In our experiment, TSSC/TA ranging from 2.00 in *RoHo 3615*Evelina*® to 4.02 in *Galaxy*. As expected the Gala clones produced the sweetest fruit and Pinova the more acidic fruit. pH does not differ significantly between the varieties.

By the results of the sensory attributes (Table 4) the observed cultivars differed significantly for the descriptors VO, FO, A, S, B, C, CR, SP, J, AF, VF, HF, FRF, and OFF. Sensory profiles are described in Fig. 1.

Table 4. Sensory profiles of the 7 observed apple cultivars as evaluated by a trained panel. Mean of two years ±SD (n = 90)

Descriptors	*Galaxy*	*Gala Annaglò®*	*Dalitoga*	*Erovan* Early Red One®*	*Scarlet Spur* - Evasni®*	*Corail Pinova*	*RoHo 3615* Evelina®*
Apple odour	$6.6^{n.s.}$	6.0	6.5	5.8	7.0	5.6	6.0
Vegetal odour	5.5^{a}	4.5^{b}	5.5^{a}	5.1^{ab}	4.3^{b}	5.1^{ab}	5.5^{a}
Honey odour	$4.3^{n.s.}$	4.1	4.1	4.0	3.6	3.8	4.5
Fruit odour	4.8^{ab}	3.5^{c}	4.4^{ab}	5.1^{a}	4.5^{b}	5.0^{a}	4.7^{ab}
Almond odour	$3.1^{n.s.}$	3.3	3.0	3.3	3.0	3.6	3.5
Off odour	$2.5^{n.s.}$	2.4	2.0	2.3	2.1	1.8	2.1
Acid	3.4^{b}	3.3^{b}	6.1^{a}	5.8^{a}	3.3^{b}	5.1^{a}	5.8^{a}
Sweet	7.3^{a}	5.8^{ab}	5.2^{bc}	5.5^{b}	6.3^{a}	4.6^{c}	5.2^{bc}
Bitter	2.2^{b}	2.4^{b}	3.0^{ab}	2.7^{b}	2.4^{b}	3.3^{a}	2.8^{ab}
Consistency	5.4^{b}	4.6^{c}	5.2^{b}	4.3^{c}	7.0^{a}	5.1^{bc}	6.4^{ab}
Crunchiness	6.1^{ab}	4.3^{c}	5.5^{bc}	4.7^{bc}	6.3^{ab}	5.4^{bc}	7.5^{a}
Sponginess	5.4^{b}	7.1^{a}	5.8^{ab}	6.4^{a}	5.0^{b}	6.6^{a}	5.5^{b}
Juiciness	6.2^{b}	4.7^{c}	5.2^{bc}	6.8^{ab}	7.1^{a}	6.2^{b}	7.4^{a}
Apple flavour	6.2^{ab}	5.4^{b}	5.3^{b}	5.4^{b}	7.2^{a}	6.0^{ab}	6.3^{ab}
Vegetal flavour	5.1^{a}	4.0^{b}	4.5^{ab}	4.4^{ab}	4.5^{ab}	4.6^{ab}	4.2^{b}
Honey flavour	3.3^{b}	4.5^{a}	3.2^{b}	3.6^{b}	3.8^{ab}	3.6^{b}	4.7^{a}
Fruit flavour	6.1^{a}	3.6^{c}	4.3^{bc}	4.3^{bc}	4.5^{bc}	5.7^{ab}	5.0^{b}
Almond flavour	$2.8^{n.s.}$	2.8	2.6	2.3	2.6	3.6	3.7
Off flavour	2.8^{ab}	3.2^{a}	2.1^{b}	3.5^{a}	2.2^{b}	1.7^{c}	1.7^{c}

The values marked with different letters in the same column indicate significant differences (P ≤ 0.05).

In particular, for odour descriptors, vegetal odour ranged from 4.3 for *Scarlet Spur* -Evasni®* to 5.5 of *Galaxy, Dalitoga,* and *RoHo 3615* Evelina®*. The greatest intensity of fruit odour was observed in *Erovan* Early Red One®* (5.1) and *Corail Pinova* (5.0) whereas *Gala Annaglò®* (3.5) showed lower intensity. As expected, *RoHo 3615* Evelina®* (5.8) and *Corail Pinova* (5.1) had the highest intensity of acid descriptor: this fact confirms the high value of TA and the low TSSC/TA ratio. These fruit was perceived as less sweet due to the fact that this apple is the ones that presents higher acidity. On the contrary, *Dalitoga* (6.1) showed a high value of acid descriptor whereas chemical-physical data indicated not acid fruit. In this study, °Brix/titratable acidity ratio was the best predictor of consumer response. The sweetest fruits were *Galaxy* (7.3), *Gala Annaglò®* (5.8) and *Scarlet Spur* -Evasni®* (6.3), and this confirmed the TSSC/AT ratio. As observed by Harker et al. (2002) there is a good relationships between °Brix/titratable acidity ratio and consumer acceptability of fruit.

Bitterness ranged from 3.3 for *Corail Pinova* to 2.2 for *Galaxy*. The judges expressed a low appreciation in regard to consistency for *Gala Annaglò®* (4.6) and *Erovan* Early Red One®* (4.3) fruits; on the contrary the highest values were observed in *Scarlet Spur* -Evasni®* (7.0) and *RoHo 3615* Evelina®* (6.4). The sensory evaluation of fruit consistency was not directly correlated with because analytical evaluation did not consider many parameters such as texture as observed by Harker (2002a). Similar behaviour was observed for crunchiness: *RoHo 3615* Evelina®* (7.5) and *Scarlet Spur* -Evasni®* (6.3) showed the highest values with the exception of Galaxy (6.1) while *Gala Annaglò®* reached the lowest intensity. For the sponginess, *Gala Annaglò®*, *Corail*

Pinova and *Erovan* Early Red One*® followed by the other three cultivars. On the other hand, *Scarlet Spur* -Evasni*® (7.1) and *RoHo 3615* Evelina*® (7.4) has the greater intensity of juiciness, whereas *Gala Annaglò*® has the lesser intensity. For flavour descriptors, apple flavour was greater in *Scarlet Spur* -Evasni*® (7.2) followed by *RoHo 3615* Evelina*® (6.3), *Galaxy* (6.2) and *Corail Pinova* (6.0); *Galaxy* (5.1) had the highest value while *Gala Annaglò*® (4.0) and *RoHo 3615* Evelina*® reached the lowest values (4.2); Honey flavour ranged from 4.7 of *RoHo 3615* Evelina*® and 3.2 of *Dalitoga*; *Galaxy* (6.1) and *Corail Pinova* (5.7) showed the highest fruity flavour values; on the contrary, *Gala Annaglò*® reached the lowest value (3.6). Finally, relating to the off flavour, all the values were very low ranging from 3.2 to 1.7.

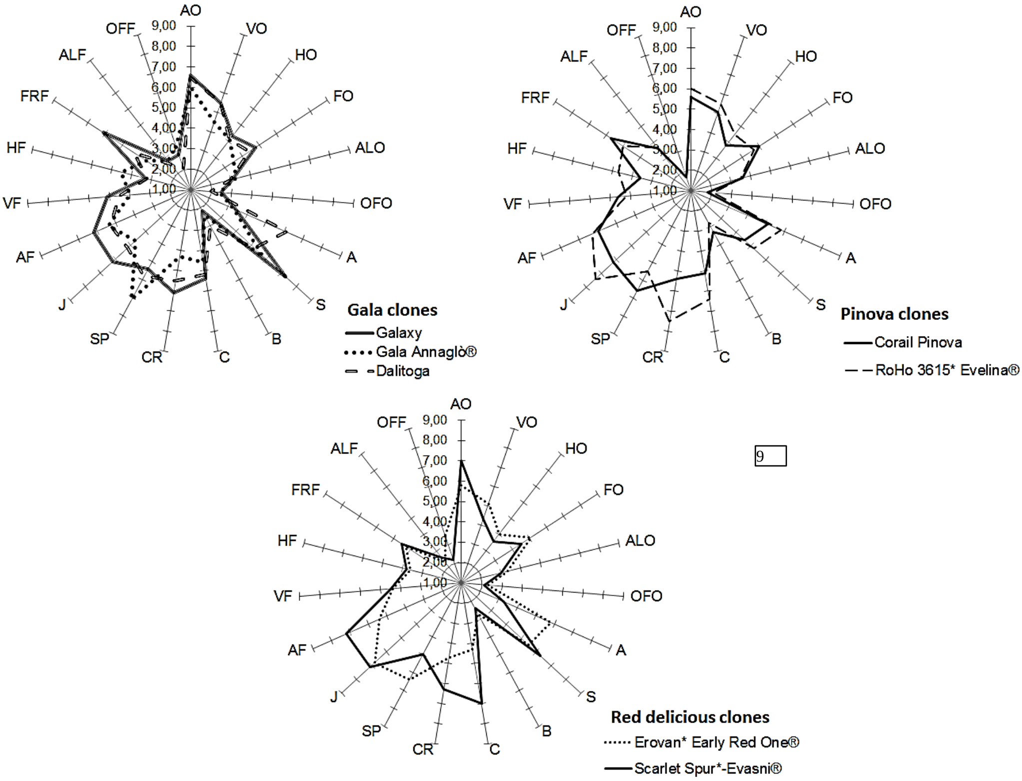

Figure 1. Spider plot of the observed apple fruits. AO – Apple odour; VO – Vegetal odour; HO – Honey odour; FO – Fruit odour; ALO – Almond odour; OFO – Off odour; A – Acid; S – Sweet; B – Bitter; C – Consistency; CR – Crunchiness; SP – Sponginess; J – Juiciness; AF – Apple flavour; VF – Vegetal flavour; HF – Honey flavour; FRF –Fruity flavour; ALF – Almond flavour; OFF – Off flavour.

The comparison between sensory and chemical attributes showed some interesting relations (Table 5). The instrumental quality measurements for apple cultivars were well correlated with the several sensory attributes. Flesh firmness was positive correlated with off odour (0.6638) and negative correlated with honey odour (-0.6458). Total solid soluble content was positive correlated with sweet (0.8973), apple flavour (0.6637) whereas negative correlated with consistency (-0.8092*) and honey flavour (-0.6349).

TSSC/TA was inverse correlated with vegetal flavour (**-0.7643)**. Inverse correlations were determined between tritatable acidity and apple odour (**0.6502)**, and positive correlation between tritatable acidity and almond odour (**0.7697)**.

Table 5. Coefficients of correlation between flesh firmness (FF), total soluble solids (TSSC), titratable acidity (TA) and sensory attributes in apple fruits

Sensory attributes	FF	TSCC	TA	TSSC/TA
Apple odour	-0.3943	-0.2556	-0.6502*	0.4826
Vegetal odour	0.4049	0.4203	0.1829	0.2833
Honey odour	-0.6458*	0.0535	0.1116	-0.0413
Fruit odour	-0.4456	0.2281	0.5210	0.0873
Almond odour	-0.4833	0.1717	0.7697	-0.7105
Off odour	0.6638*	-0.0589	-0.3723	0.2481
Acid	-0.1291	0.1623	0.5910	-0.2000
Sweet	0.4654	0.8973*	-0.6371	0.5902
Bitter	-0.4410	0.2460	0.5331	-0.2988
Consistency	-0.3372	-0.8092*	-0.2105	0.0637
Crunchiness	-0.1533	-0.5891	0.0595	0.0289
Sponginess	0.0536	0.5014	0.3325	-0.4859
Juiciness	-0.5989	-0.5163	0.4984	-0.2430
Apple flavour	-0.4940	0.6637	-0.1468	0.0463
Vegetal flavour	0.1439	0.4553	-0.3380	-0.7643
Honey flavour	-0.1177	-0.6349*	0.3499	-0.8025
Fruit flavour	-0.0852	0.3014	0.0670	0.3111
Almond flavour	-0.2840	-0.1715	0.4237	-0.4795
Off flavour	0.3778	0.3229	-0.0815	0.0568

The values marked with * indicate significant differences ($P \leq 0.05$).

CONCLUSIONS

The ameliorative clone *Gala Annaglò*® is interesting for the fruit size and peel color, *Dalitoga* for the early ripening and *Galaxy* for the crunchiness and consistency. All the Gala clones reached very high total solid soluble content confirmed by the panel judgment of the sweetness and acidity descriptors. Referring to the odour and flavour *Galaxy* clone produced a more aromatic fruit with the highest values of fruit odour, apple flavour and fruit flavour. The Red Delicious clone confirmed the larger size and the high colorimetric standard of all red covered fruits; the new clone *Scarlet Spur*-Evasni*® reached an interesting fruit size and peel colour intensity and uniformity, and the best total solid soluble content to total acidity ratio confirmed by the sensory descriptors of acidity and sweetness. Moreover, it reached very high values of crunchiness, consistency and interesting values of apple flavour, honey flavour and fruit flavour. The ameliorative clone *RoHo 3615*Evelina*® was characterized by uniform and intense coloured fruit and a more balanced total solid soluble content to total acidity ratio and good values of crunchiness, consistency, apple flavour, honey flavour and fruit flavour. The combined approach for perceptible quality profiling of apples based on sensory and instrumental techniques permitted to study a correlation between sensory and instrumental data.

Finally, although, all the varieties were cultivated in organic farming, they had reached required commercial parameters. The higher colour index, found in ameliorative clones, may result in a much greater acceptance of the fruits from the consumer generally attracted by more intense shades of red. Sensory results obtained by new clones did not exhibit any losses in the characteristics of the affirmed variety. This presents a possibility to the Sicilian apples, in particular in the organic industry, as these represent quality fruits obtained with sustainable methods.

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Qualitative classification of mulchers

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Abstract. Mulchers are helpful in forest establishment and tending. Numerous mulchers are available, so buyers can become disoriented when choosing one. This paper was aimed at creating a classification of mulchers based on the evaluation of the most important parameters: weight, required engine performance, and mulching diameter. Through ANOVA, and regression and correlation analyses of our database, we created six machine classes, devised their upper limits, and assigned mulchers to the classes. Class K1 (weight up to 1,300 kilograms; performance up to 75 kilowatts; mulching diameter up to 22 centimetres) was the most popular one with 88 mulchers. It was followed by class K2 (1,800 kilograms; 100 kilowatts; 27 centimetres) with 61 mulchers, class K4 (3,200 kilograms; 175 kilowatts; 41 centimetres) with 44 mulchers, class K3 (2,300 kilograms; 125 kilowatts; 31 centimetres) with 34 mulchers, class K5 (4,100 kilograms; 225 kilowatts; 51 centimetres) with 18 mulchers, and class K6 (no upper limit) with nine mulchers.

Key words: mulchers, mulching diameter, engine performance, weight, classification.

INTRODUCTION

Machinery producers have broad product ranges to satisfy the specific needs of their customers. They use the latest scientific knowledge in design, technology, materials and other fields of research to keep up with the competition. However, they cannot meet the needs of every single potential client, as this could theoretically cause the production of an almost infinite number of machine types, thus significantly increasing production costs. Sloboda et al. (2008) and Majdan et al. (2012) described the process of selecting machines suitable for various applications. They state that one of the most important factors in product innovation and production economics improvement is the optimisation of the product range, reflected in a finite number of machine classes. This enables producers to apply unification in design, contributes to cost effectiveness, and increases customer satisfaction. Producers need to know, which machines (or machine classes) are the most marketable to be able to focus their resources on effectively.

Bukoveczky et al. (2007) and Štollman & Slugeň (2009) focused on the classification of forestry machines. They devised their classification systems for different kinds of machines however, and their classifications are not fully applicable to forestry mulchers. They approached the classification of forestry machines and mechanisms only through evaluating two variables (performance and weight). They did not consider the joint effects of technical (e.g. weight of the machine), and operational (e.g. required engine performance of the machine, diameter of the processed growth, etc.) parameters. Furthermore, they designed their classification system for machinery used in forest harvesting. In our article, we have tried to consider both the technical and operational parameters simultaneously, because we believe that dividing machines in this manner provides valuable information for the consumers and the producers.

Rao (1992) described the possibilities of using mathematical data processing in creating models, such as classifications. Their work gives one valuable information on what statistical analyses to use. Forestry mulchers and their most important characteristics came into the focus of Eisenbarth (2000), who determined the most influential parameters for the quality of their work. He evaluated two mulchers during the maintenance and construction of forest roads. Based on measurements from 145 newly constructed roads he determined that the performance, costs and technique influence the quality of the work of the mulcher the most. Zemánek et al. (2004) evaluated the innovation of machines based on their technical and economical parameters.

Čedík et al. (2016) and Kumhála et al. (2016) define of mulching is among energy intensive crop harvesting operations. It is therefore interesting to deal with mulching energy demands in more details. The energy intensity of mulching or shredding of plant material is dependent on the type of processed crop, parameters of the cut (mass performance, cutting speed etc.) and shape and condition of the cutting tool (Syrový et al., 2008; Hosseini & Shamsi, 2012; Kronbergs et al., 2013; Pecenka & Hoffmann, 2015).

Mulchers are multipurpose machines. The wide range of their shapes and sizes documents this fact. Despite the variety of the machines, no classification of mulchers was found when the literature survey for this paper was carried out. This article is aimed at creating a classification of mulchers with mechanical drives according to their main technical and operational parameters – required performance of the base machine, the diameter of the processed growths, and the weight of the mulcher.

MATERIALS AND METHODS

The proposed classification of mulchers with mechanical drives is based on a method described by Bukoveczky et al. (2007). The method was modified to meet the needs of this study. To elaborate the classification of mulchers, a comprehensive database of mulchers with mechanical drives was created, statistical analyses were carried out, and the criteria for dividing mulchers into classes were devised.

The comprehensive database (statistical sample) of mulchers with mechanical drives produced globally was created according to the method described by Hnilica et al. (2012). Each database entry contained information on the brand, mulcher designation, weight, required performance of the base machine (furthermore: required performance), power torque output of the mulcher, and the maximal diameter of processed growth (furthermore: mulching diameter).

The analysis of variance (ANOVA) was used to prove whether the required performance had a significant influence on the weight and mulching diameter. The relationship between the required performance and weight, and between the required performance and mulching diameter was studied further through a regression and correlation analysis. Individual mulchers were divided into groups and categories through a frequency analysis, i.e. the detection and characterization of the distribution of the crushers' abundance in terms of performance, weight and average growth.

An orthogonal projection of the individual database entries (mulchers) to a regression line was created. This lead to the creation of 10 orthogonal groups (I to X). The mulchers were divided into orthogonal groups according to the following criteria: (i) weight of the mulcher; (ii) mulching diameter; (iii) required performance, as provided by the producers of the individual mulchers.

The producers did not have a consistent method of stating the required performance of the mulchers they produce. Mulcher producers stated the required performance in four different ways. If producers provided the upper and lower performance limits, their mean was calculated and then the mulcher was assigned to the corresponding orthogonal group. If the producers provided the required performance as one value, this value was used to assign the mulcher to the corresponding group. If the producers provided the minimal required performance, the number was multiplied by a coefficient of 1.2 and then the mulcher was assigned to the corresponding group. If the producers provided the maximal required performance, the number was multiplied by a coefficient of 0.8 and then used to assign the machine to the corresponding group.

The frequency p1 to p10 was determined in every orthogonal group and a frequency diagram was constructed. The length of the class intervals for the required performance (Scheer & Sedmák, 2010) was set, and the orthogonal groups were divided into classes K1 to Kn. Each class consisted of three groups maximally, and contained as many mulchers as possible.

RESULTS AND DISCUSSION

A database containing 254 mulchers from 17 different producers was created. Table 1 shows a sample of the database. The complete database is available from the authors upon request.

The data from the database was divided according to the relationship between the weight and the required performance and the relationship between the mulching diameter and the required performance. ANOVA proved a statistically significant effect upon the required performance on the weight (F: 1,211.67; p: < 0,001). The regression and correlation analysis showed a strong correlation between the required performance and the weight (R: 0.91; R2: 0.83; standard deviation – SD: 0.44; p: < 0.001). A ten kilowatt increase in the required performance resulted in a 190 kg (95% confidence interval – CI: 180–200 kg) increase in weight. The variability of the required performance explained

83% of the variability in weight. Fig. 1, a shows the graphic depiction of the relationship. It also shows that most mulchers in the database were in the 50 to 150 kW required performance range and weighed 500 to 2,200 kg.

Table 1. A Sample from the Comprehensive Database of Mulchers with Mechanical Drives

Brand	Mulcher designation	Weight (kg)	Required performance (kW)	PTO (rpm)	Grinding diameter (cm)
FAE	UML/SS 150	970	45–82	540/1,000	20
FERRI	MC 180	545	41–52	540	6
AHWI	M700	4,350	185–300	1,000	30
SERRAT	FX3 T-1200	580	32–44	540	8
MERI	MJS-2.0 DT	1,610	82–135	1,000	25
FAE	UMH 175	2,910	97–112	1,000	40
SERRAT	FX5 T-2000	2,470	110–150	1,000	20
VENTURA	TFVJ 130	625	*min* 30	540	18
TEAGLE	EKR/S250	645	45–60	540/1,000	3
OSMA	TLPF 220	1,290	89–104	1,000	25
FAE	SSH 225	4,581	164–261	1,000	69
FECON	BH200	2,449	104–336	1,000	50
SEPPI M_	FORST M	4,900	125	1,000	30
RYETEC	TRB 180	1,220	60–82	1,000	15
BUGNOT	BFO 1800	2,560	*max* 186	1,000	50

PTO – power torque output; kg – kilograms; kW – kilowatt; rpm – revolutions per minute; cm – centimeter.

ANOVA proved that the required performance significantly affected the mulching diameter too (F: 384.62; p: < 0.001). The correlation between the mulching diameter and the performance proved to be strong as well (R: 0.78; R2: 0.60; SD: 8.05; p: < 0.001). A ten kilowatt increase in the required performance resulted in a 1.95 cm (95% CI 1.75–2.14 cm) increase in the mulching diameter. The variability in the required performance explained 60% of the variability in the mulching diameter. Fig. 1, b shows the graphical depiction of the relationship and that less than 50% of the mulchers can process growths thicker than 35 cm.

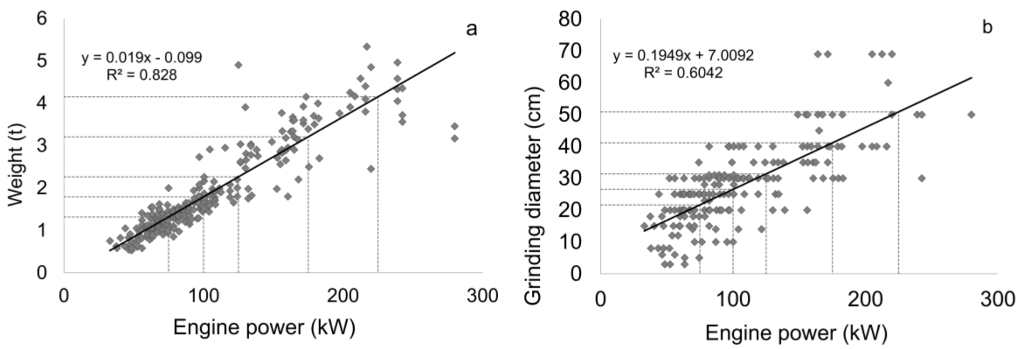

Figure 1. Relationship between the weight of the mulchers and their required performance (a) and the mulching diameter of the mulchers and their required performance (b); dashed lines denote the individual classes.

The mulchers were assigned into orthogonal groups based on their parameters and a frequency diagram of the mulchers in individual groups was constructed (Fig. 2, a). The orthogonal groups were divided into six classes K1 to K6. Class K1 contained groups I and II, K2 contained group III, K3 contained group IV, K4 contained groups V and VI, K5 contained groups VII and VIII, and K6 contained groups IX and X.

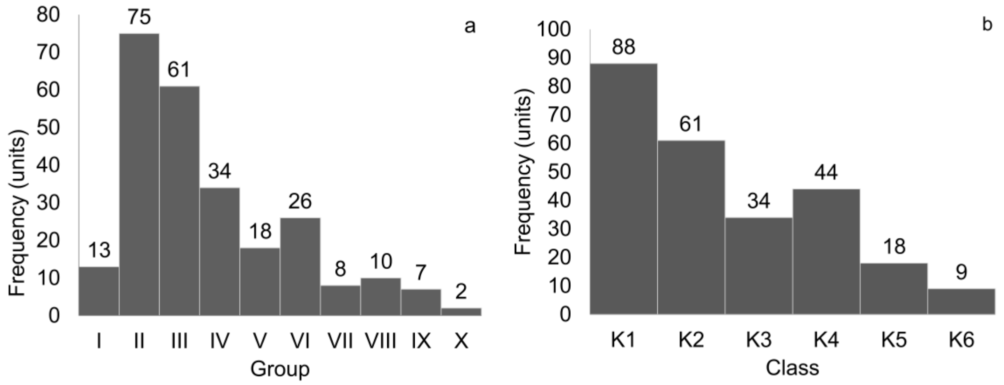

Figure 2. Frequency diagrams of the mulchers assigned to the individual orthogonal groups (a) and classes (b).

The class upper limits for weight, required performance, and mulching diameter were determined from the corresponding regression lines (Fig. 1). Table 2 shows the characteristics of each class. Fig. 2, b shows the population of each class. Classes K1 to K4 are the most relevant for producers. Mulchers in the K1 class are the most frequently used in practice. Classes K5 and K6 contain high performance mulchers with lower production quantities. Mulchers included in these classes are produced almost exclusively on demand and are optimised for the specific needs of the individual customers.

Table 2. Specification of lower and upper class limits for required engine performance, weight, and mulching diameter of mulchers with mechanical drives

Parameter	K_1*	K_2*	K_3*	K_4*	K_5*	K_6*
Engine performance (kW[a])	0–75	76–100	101–25	126–175	176–225	over 225
Weight (kg[b])	0–1,300	1,400–1,800	1,900–2,300	2,400–3,200	3,300–4,100	over 4,100
Mulching diameter (cm[c])	0–22	23–27	28–31	32–41	42–51	over 51

*K_i – class designation; [a]kW – kilowatt; [b]kg – kilogram; [c]cm – centimeter.

Classifications of machinery based on more than two parameters are common in practice. Eisenbarth (2000) stated that performance, costs, and technique influence the quality of terrain preparation the most. This information validates the parameters we chose for the proposed classification – mulcher performance (defined by the mulching diameter) and weight (the quality and quantity of material used throughout the machine production affects its price). Zemánek et al. (2004) evaluated the innovation of machines based on their technical and economical parameters. They also considered the technological process when selecting appropriate machines. Incorporating economic parameters of mulchers into the classification is problematic, because the price is not a fixed value and depends on the time of the purchase and the accessories of the mulchers. Operational economic parameters of mulchers, such as variable costs, also change with the conditions in which the mulchers operate (e.g. terrain, base machine, processed material, natural conditions in which the mulchers are used, etc.).

We shall further verify the proposed classification of mulchers by including new types of mulchers and expanding the database. We shall remain focused on the area of machines used in forest establishment and tending.

CONCLUSIONS

This classification reflects the needs of forestry in practice. It can serve as a lead for machine producers when they optimise their product range. Meeting the market demand with minimum product types is especially important for the economics of machine production and the unification of machine parts. After assigning mulchers into classes, one can see the popularity of each class. This is beneficial to the producers of mulchers, as they will have valuable information about the state of the market and their competitors. This categorization reflects customer specifications (forestry) after crushers these dimensional types and can be for machine producers support in determining the of optimal construction crushers series. Meet market demand with the minimum number of types is especially important for the producer in economic terms and in view of unification the construction. They can reach an informed decision on what types of mulchers to produce. The proposed classification can also improve the decision-making process of consumers. They will simultaneously acquire information on which mulcher they can mount on the base machines they already own and whether it is going to be sufficiently powerful for their intended purposes.

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Carbon content of below–ground biomass of young Scots pines in Latvia

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Abstract. Forest ecosystems play crucial role in global carbon cycling, therefore, increasing afforestation of agricultural land in Europe has been recognized as important contribution of carbon sequestration. In carbon reporting systems, root carbon content (CC) default value has been set to 50%. The study aimed to estimate CC in below-ground biomass and in relation to tree age in young Scots pine stands on forest and former agricultural land. The below–ground CC of young (8 to 40 years) managed Scots pine (*Pinus sylvestris* L.) stands growing on nutrient poor mineral soils in Latvia was carried out. In total 62 sample trees (43 in forest land, 19 in former agricultural land) were randomly selected for destructive sampling to estimate the CC within below-ground biomass. Below–ground biomass weighted mean CC was 49.7 ± 0.4%, being slightly lower than the default CC value used to calculate carbon budgets. Root fractions stump, small roots (diameter 2–20 mm), coarse roots (diameter > 20 mm)) differed ($p < 0.001$) in their CC. Stumps (50.6 ± 0.6%) had highest ($p < 0.001$) CC in the below–ground biomass, followed by coarse (49.5 ± 0.4%) and small (49.1 ± 0.4%) roots, which did not differ from each other in their CC. Results demonstrated age–dependent increase of CC ($p < 0.001$) from 48.2 ± 0.3% to 51.7 ± 0.5%, indicating overestimation of the default value during the first two decades, but underestimation for older trees (24 to 40 years).

Key words: root biomass, coarse roots, stump, abandoned agricultural land.

INTRODUCTION

Forest ecosystems contain the majority of the carbon (C) pool on the Earth and play crucial role in global C cycling both as C sink and source (Dixon et al., 1994; Uri et al., 2012). Increasing afforestation of former agricultural land in Europe also has been seen as important contribution for C sequestration in future (Paul et al., 2002; Vesterdal et al., 2002). Both above- and below-ground biomass is an essential C pool of forest ecosystems (Helmisaari et al., 2002; Karsenty et al., 2003; Tang et al., 2015), however, the uncertainties in below-ground biomass C estimation remains (Varik et al., 2013; Addo–Danso et al., 2016). The importance of below-ground C pool inclusion in total C estimation is recognised thought the Kyoto protocol and Paris Agreement under the United Nations Framework Convention on Climate Change (UNFCC) (Petersson et al., 2012; Merilä et al., 2014). In accordance to both international agreements, member states are required to monitor, report, and reduce greenhouse gas

(GHG) emissions (Frederici et al., 2008) to achieve ambitious climate goals worldwide (Rogelj et al., 2016).

In national and international GHG reporting systems for C stock estimates, the mean carbon content (CC) of tree roots is set 50% of the dry biomass (Lamlom & Savidge, 2003; Martin & Thomas, 2011). This default value is widely used for CC calculations in different forest zones (Saatchi et al., 2011) and in various forest management systems as managed forests, plantations and agro–forestry. However, this approach can lead to an accounting error of approximately 5% (Martin & Thomas, 2011). Partly due to methodological challenges (Finér et al., 2011), the accurate C estimation in forest below–ground biomass and its potential to mitigate global climate change in Europe are not always clearly defined (Addo-Danso et al., 2016; Sochacki et al., 2017). Efforts reducing uncertainty in the below-ground biomass studies on GHG attraction and C turnover cycle in the forest ecosystem recently have grown (Liski et al., 2003; Thomas & Martin, 2012; Sochacki et al., 2017).

Forest sector has high importance in Baltic States. Nearly half of the territory of Baltic States are covered by forests (Eurostat, 2016). According to the Latvian State Forest Service, forests cover 52% of the total land area. The forest area has almost doubled from 1935 till 2015, mostly due overgrowth of agricultural land (Nikodemus et al., 2005). Scots pine (*Pinus sylvestris* L.) is the most common and economically important in Latvia, occupying 34% of the total forest area, of which nearly half are located on dry mineral soils (Baumanis et al., 2014). Despite the intensive forest management practices in region, studies of C accumulation in tree root biomass and their role in C budgets of forests in this region have been scarce.

Carbon sequestration and amount of root biomass in forest are dependent on tree species, site types, soil properties, environmental factors and forest management practices (Haynes & Gower, 1995; Gill & Jackson, 2000; Pregitzer et al., 2000; Brassard et al., 2011). Moreover, previous studies suggest that CC in below–ground biomass has been strongly influenced by the stand age (Peichl et al., 2006; Jain et al., 2010; Uri et al., 2012; Bijak et al., 2013). With the increase of forest harvesting in Europe for the substitution of fossil fuels in energy production (Levers et al., 2014; Merilä et al., 2014), due to slow decomposition and life–cycle features, roots and stump may play essential role for long term C input in soil (Bardgett et al., 2014; Kaarakka et al., 2016). Therefore, due to changes in forest age–structure, it is crucial to understand the contribution of young tree root biomass growth to C fluxes in forest and woodland (Pajtík et al., 2008; Finér et al., 2011; Fonseca et al., 2011).

The aim of this study was to estimate CC in below–ground biomass and in relation to tree age in young Scots pine stands on forest and former agricultural land (FAL).

MATERIALS AND METHODS

Site characteristics

The study was carried out in young Scots pine stands in Latvia (Table 1). Latvia is located in the north-western edge of the East European Plain within hemiboreal forest zone with mixed broad-leaved and coniferous forests (Ahti et al., 1968; Hytteborn, 2005). The climate of Latvia is moderate. According to data from the Latvia Environment, Geology and Meteorology Agency, mean annual temperature is around 6 °C. January is the coldest month with mean temperature –5.3 °C, but warmest month is July when mean temperature is 17.2 °C. The mean annual precipitation is 645 mm, though half of annual precipitation is recorded during summer period May-September.

Table 1. Characteristics of Scots pine experimental stands

Land type	Stand location	N	Stand age	Tree height (m)	dbh (cm)	Tree below-ground dry-mass (kg)	Basal area (m^2 ha^{-1})	Stand density (trees ha^{-1})
	56°50 N 24°38 E	5	8	2.7	2.4	0.7	2.3	3,600
	56°50 N 24°38 E	7	8	2.4	2.2	1.0	4.6	8,800
	56°34 N 25°01 E	3	12	4.0	6.6	7.5	7.3	2,145
	56°24 N 25°01 E	6	13	4.6	5.2	4.1	4.8	2,215
	56°51 N 24°35 E	6	14	4.8	5.3	4.8	7.2	3,200
	56°41 N 24°27 E	10	24	10.0	11.0	17.4	13.2	1,385
	56°43 N 24°34 E	3	40	17.7	18.8	38.9	18.0	583
	56°43 N 24°34 E	3	40	17.1	18.6	41.6	16.2	570
	56°34 N 24°08 E	5	12	7.7	11.2	14.7	21.1	2,145
	56°32 N 24°04 E	5	14	8.2	10.3	21.8	24.3	2,925
	56°41 N 26°01 E	3	38	17.3	14.4	19.6	28.9	1,680
	56°41 N 26°09 E	6	38	17.0	14.4	19.3	18.6	1,260

FAL – former agricultural land; N – number of sampled trees; dbh – tree diameter at breast height.

All sampled stands were on dry sandy nutrient poor mineral soils, representing typical Scots pine forest growth conditions in Latvia. The study material was collected in 8 Scots pine stands on forest land and 4 stands on FAL at the age of 8 to 40 years. Except one naturally regenerated 8 years old Scots pine stand, all studied stands was planted after soil preparation with 2 years–old bare–rooted or containerised seedlings.

One circular sample plot was established in each stand: in stands younger than 15 years the area of sample plot was 100 m^2 (R = 5.64 m), in older – 500 m^2 (R = 12.62 m). In each plot tree diameter at breast height (dbh) (± 0.1 cm), tree height (h) (± 0.1 m) and age was measured.

Sampling

In total 62 sample trees (43 in forest land, 19 in FAL) were randomly selected for destructive sampling of the root biomass to estimate the CC allocation in root fractions (Table 1). The sample trees, 3–10 trees per plot, were selected based on quality criteria (health, vitality, single tree top) from different size classes. Samples were collected during the vegetation period from June to August. The dbh and h of sample trees ranged from 1.2 to 26.5 cm and 1.9 to 20.8 m, respectively on forest land. In FAL at age of 12 to 38 years the dbh and h corresponding figures were 3.7 to 26.0 cm and 5.9 to 20.8 m (Fig. 1).

Figure 1. Tree diameter at breast height and height of the sample trees.

For CC analysis, the sample tree root biomass was divided into fractions: small roots (diameter 2–20 mm), coarse roots (diameter > 20 mm) (Fujii & Kasuya, 2008; Finér et al., 2011) and stump. Stump biomass included both the above–ground (5–8 cm above the root collar) and below–ground monolithic part.

The stumps and roots were excavated and washed using high pressure water jet. The entire root system was weighted (± 0.02 kg), divided into fractions and each root fraction was weighted (± 0.02 kg). CC analysis were performed for small roots, coarse roots and stump samples. For 16 sample trees (12 trees at age of 8 years and 4 trees at the age of 14 years) coarse roots were not detected, suggesting that coarse roots are developed later. All root components were dried to constant mass at 105 °C temperature and weighted. The mean total below-ground biomass of the sampled trees varied between 0.1 and 155.3 kg with an average of 17.1 kg on forest land. The mean total below-ground biomass of the sampled trees on FAL was 27.3 kg and ranged from 1.3 to 104.2 kg. For CC analysis of individual tree, small roots and coarse roots were divided on 3 diameter classes. In each diameter class, from randomly selected roots on average 2 cm long cuts of boths ends and centre of the root were collected in one homoginised sample (150–200 g) for each root fraction. Whole stump (if it was less than 200 g) or two 2 cm samples from the radial cut zone were obtained. Air–dried samples were milled and 0.25–0.50 g samples for carbon determination were taken.

Data analysis

Carbon content was analysed with an LECO CR–12 Carbon analyser set at 900 °C and the CC was assessed directly by measurement of the CO_2 using infrared radiation (LECO Corporation, 1987). The instrument was calibrated using calibration substance – carbon powder containing 64.8% C and an empty test without a sample was performed. The mean CC of sample tree below–ground biomass was calculated as weighted average of total CC.

All statistical analyses were performed using R v.3.3.1 statistical environment (R Core Team, 2016). Analysis of covariance ANCOVA was used for estimating the effect of the land type and different root fractions on the root CC. Stand age was added as a numeric covariate in analyses (Peichl & Arain, 2006; Seedre et al., 2015). The Turkey Honest Significant difference (HSD) test was employed to perform multiple post hoc comparisons between CC for different root fractions.

RESULTS AND DISCUSSION

Below–ground biomass CC was compared in each root fraction among and within forest land and FAL (Fig. 2). Mean CC of below–ground parts of young Scots pine trees was 49.7 ± 0.4%. In forest land and FAL the mean below–ground CC was 49.4 ± 0.4% and 50.4 ± 0.7%, respectively. The observed mean root CC of young Scots pine showed slight differences (0.3%) from generally accepted CC of 50%, as shown for other conifers (Ritson & Sochacki 2003, Thomas & Martin, 2012). Such differences have been related to ecological factors (e.g. stand density, tree dimensions, forest type) effecting the assimilation of C as well as sampling methodology of studies (Vucetich et al., 2000; Lamlom & Savidge, 2003; Jain et al., 2010, Thomas & Martin, 2012).

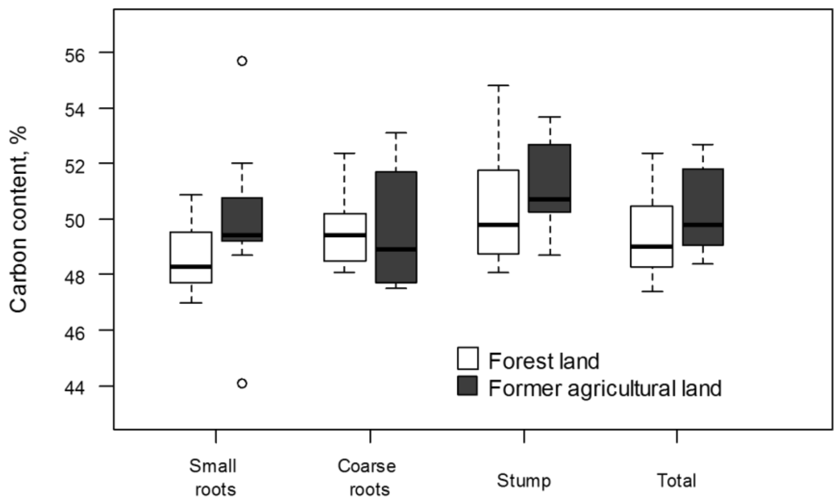

Figure 2. Carbon content in different root fractions in afforested former agricultural land and forest land. Total is carbon content of weighted means based on the weights of different components (stumps, small roots, coarse roots). Line shows the median of dataset, box represents 1st and 3rd quartile, whiskers mark range (not exceeding 150% of interquartile distance) and circles indicate outliers of the datasets.

The observed difference in the mean CC between land types was non–significant ($p > 0.05$) (Table 2), however, higher variations of CC at the individual tree level were observed in FAL (Fig. 2) likely due to physical and biological features, such as content of lignin, different age and composition of root fraction (Lamlom & Savidge, 2003; Peichl & Arain, 2006; Bennett et al., 2014; Seedre et al., 2015).

Table 2. The effect of root component and growing conditions on CC in roots according to the results of ANCOVA; the age of the stand have been added as covariate in analysis

Model Anova (Type II test)				
Variable	Sum of Square	Degree of freedom	F value	*p*–value
Root fraction	93.91	2	45.389	< 0.001
Land type	1.85	1	1.789	0.183
Age	269.55	1	260.549	< 0.001
		$R^2 = 0.69$ (Adjusted $R^2 = 0.68$)		
Multiple Comparisons of Means of Tukey HSD Test				
Root fraction	Difference	Standard Error	*t*–value	*p*–value
Coarse roots – Stump	–1.51	0.20	–7.566	< 0.001
Small roots – Stump	–1.57	0.18	–8.608	< 0.001
Small roots – Coarse roots	–0.06	0.20	–0.302	0.951

Root fraction differed in their CC (Table 2). The CC were estimated to be 49.5 ± 0.4% and 49.1 ± 0.4% in the coarse roots and small roots, but there were not statistically significant ($p > 0.05$) difference observed between both root fractions. The CC of both root fractions is also comparable to earlier study (cf. Janssens et al., 1999; Bert & Danjon, 2006). Stumps had significantly ($p < 0.001$) hig her CC (50.6 ± 0.6%), that might be explained by the differences in length of the life cycle of different root fractions (Palviainen et al., 2010; Uri et al., 2012), hence lignification of tissues (Bert & Danjon, 2006).

At the age from 8 to 40 years, mean below–ground biomass CC increased significantly ($p < 0.001$) from 48.2 ± 0.3% to 51.7 ± 0.5%, approving our hypothesis that CC is age dependent, as it has been previously observed for other *Pinus* species by Ritson & Sochacki (2003), Peichl &Arain (2006,) Jain et al. (2010). Steeper increase of mean below–ground CC was observed for younger trees, reaching 50.6 ± 0.3% at the age of 24 years (Fig. 3).

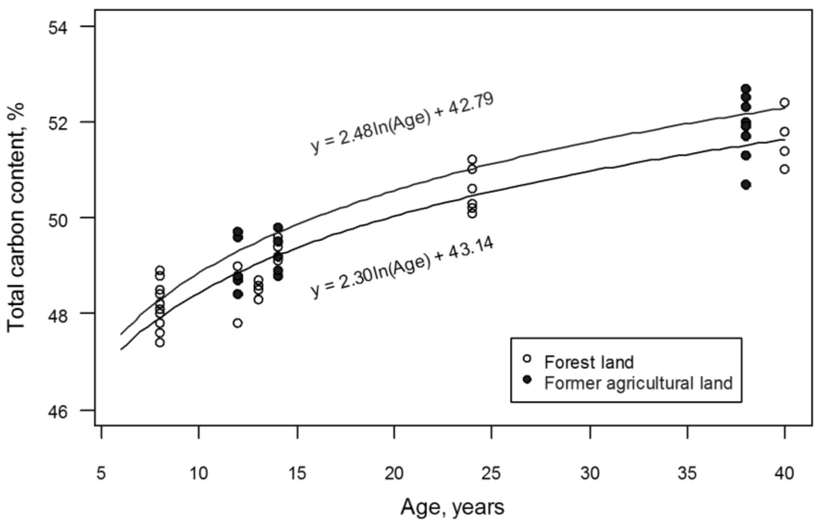

Figure 3. Effect of stand age on the total root CC on forest land and former agricultural land.

The age–dependent increase of root CC followed logarithmic curve (Fig. 3), indicating overestimation of the default value during the first two decades of tree life, but underestimated CC at older age 24 years). Considering that, the below–ground biomass CC could be determined as a function of stand age to improve C estimation within reporting systems for climate change mitigation (Bert & Danjon, 2003; Lamlom & Savidge, 2003). Further studies shall address older trees and other tree species in order to improve overall accuracy of below–ground C assessment.

CONCLUSIONS

Below–ground CC was age dependent, but was not affected by land type. More accurate below-ground biomass CC values for young trees had been established.

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Determination of mechanical properties of poppy waste pellets

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Abstract. The work deals with evaluation of mechanical properties three types of pellet samples produced from poppy waste. The pellets were submitted to compressive loading. The compressive loading curves of dependencies of force on strain and force on time were realised. Certain mechanical parameters were determined, namely the diameter of the sample, length of the sample, force at 10% of strain, force in the first maximum of the force – strain curve, strain in the first maximum of the force – strain curve, modulus of elasticity, force in the inflex point of the force – time and force – strain curves and strain and stress in the inflex point of the force – time and force – strain curves. The work lists correlations of mechanical parameters of individual pellet types. The pellet type 1 made only of ground poppy head mass has shown the best results, the pellet type 3 consisting of ground poppy heads after harvest and waste from sieving of poppy seeds in mass proportion 1 : 1 has shown the worst results.

Key words: compression loading, modulus of elasticity, force, strain.

INTRODUCTION

Pellet biomass is currently utilized as a fuel in energetics, bedding and feed for livestock, as well as a fertilizer in primary agricultural production. The most common material for pellet production is wood, however, the usage of biomass from agricultural production and waste biomass, potential of which is considerable, is becoming more and more prominent. Due to this, the aim of the paper is to determine the mechanical properties of poppy waste pellets.

Duncan (2010), Obenberger & Thek (2010), Macák et al. (2015), Spirchez et al. (2016) and others deal with pellet quality in their research papers and works.

Pellet mechanical properties evaluation by compressive loading test began to receive increased attention in the last decade. Our research and experiments are based on the results of studies carried out by Oloso & Clarke (1993), Vursavuş & Özgüven (2004), Kubík et al. (2016). Pellet quality is usually evaluated by means of their density and durability. Low pellet durability is undesirable, as it can result in malfunctions of feeding system, dust emissions, and increased risk of explosion and fire during handling and storage (Zafari & Kianmehr, 2012).

Mechanical durability of pellets is influenced by many factors, including force and temperature of compression, particle size and chemical properties of input biomass (Kaliyan & Morey, 2009). Križan (2014) states that fraction content significantly influences the compressed material quality when evaluating the pellet mechanical properties crucial in terms of handling and storage.

MATERIALS AND METHODS

The evaluated pellets are made of ground poppy heads and waste from sieving of poppy seeds (that is, small impurities and oilseed of poppy (*Papaver somniferum*) with smaller diameters, not captured in sieving). The poppy heads were ground using hammer mill. Pellet type 1 was made solely of ground poppy heads; pellet type 2 was made of material consisting of ground poppy heads and waste from sieving of poppy seeds in mass ratio 2 : 1; pellet type 3 was made of material consisting of ground poppy heads and waste from sieving of poppy seeds in mass ratio 1 : 1.

The pellets were made by the granulating machine MGL 200 (output 50–150 kg per hour for alternative pellets, power 8.85 kW, weight 310 kg, maximum height 2,230 mm). In the production of the pellets no binding agent was used, except for water. The moisture of pellets 1 was 8.89%, the moisture of pellets 2 was 8.75% and the moisture of pellets 3 was 9.77%. The density of pellets 1 was 796.3 kg m^{-3}, the density of pellets 2 was 776.33 kg m^{-3} and the density of pellets 3 was 655.98 kg m^{-3}. The difference in density was caused by the differences in sample composition.

Material fraction size (Feret diameter) of pellets is listed in Table 1. Materials of pellet type 1 and 3 are shown Figs 1 and 2.

Table 1. Material fraction size of pellets, percentage

Fraction, mm	0.0–1.5	1.5–3.0	3.0–4.5
Pellet type 1	70.83	18.75	10.42
Pellet type 2	75.00	16.67	8.33
Pellet type 3	86.42	11.31	2.27

Figure 1. Material of pellet type 1.

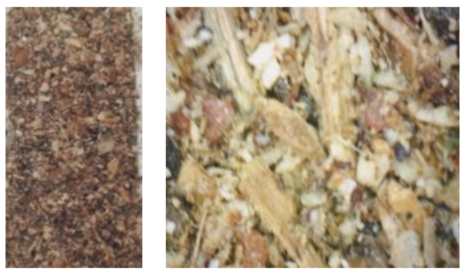

Figure 2. Material of pellet type 3.

The pellets were subjected to the compressive loading. The strength of the pellets was evaluated via the quasi static compression test. Ten compression tests were carried out for each pellet type. The result of the single test is the compression curve, that is, the dependence between compression force F and compressive strain ε of the pellet. The basis for the determination of compressive strain is the initial length of the pellet L_0. The pellets were compressed in the axial direction. The upper steel plate of the Andilog Stentor 1000 test stand, compressed the cylinder at a speed of 10 mm min^{-1}, until failure was observed. The compression curves were determined as the dependences $F(\varepsilon)$ and the dependences $F(t)$. Time of compression was calculated from the speed of the test stand (10 mm min^{-1}), the elongation ΔL was also measured by the stand.

The cubic regression equations of the dependence $F(\varepsilon)$ were evaluated on the intervals (0, F_m):

$$F = a\varepsilon^3 + b\varepsilon^2 + c\varepsilon + d \tag{1}$$

where F – compression force, N; ε – compressive strain, mm mm^{-1}; a, b, c, d –regression coefficients, N.

The cubic regression equations were evaluated also on the intervals (0, F_m) and the inflection points were determined for the dependencies $F(t)$:

$$F = et^3 + ft^2 + gt + h \tag{2}$$

where F – compression force, N; t – time of compression, s; e – regression coefficient, N s^{-3}; f – regression coefficient, N s^{-2}; g – regression coefficient, N s^{-1}; h – regression coefficient, N.

Several mechanical parameters of the pellets were enumerated on the basis of the dependence of force on time and strain and on the basis of the inflection points of the compression curves. The compressive strain in the first maximum of the compression curves was enumerated from the equation:

$$\varepsilon_m = \frac{v\, t_m}{L_0} \tag{3}$$

where ε_m – compressive strain in the first maximum of the compression curve $F(\varepsilon)$, mm mm^{-1}; v – speed of deformation, mm min^{-1}; t_m – time in the first maximum, min; l_0 – original length of the sample, mm.

The compressive stress in the first maximum of the compression curves was enumerated from the equation:

$$\sigma_m = \frac{4\, F_m}{\pi\, d^2}(1 - \varepsilon_m) \tag{4}$$

where σ_m – compressive stress in the first maximum of the compression curve $\sigma(\varepsilon)$, MPa; ε_m – compressive strain in the first maximum of the compression curve $F(\varepsilon)$, mm mm^{-1}; F_m – force in the first maximum of the compression curve $F(\varepsilon)$, N; d – original diameter of the sample, mm.

The compressive stresses σ_{mp} were also evaluated from the first maximum of the compression loading curves of the dependence $F(\varepsilon)$.

Time t_m was determined in the first maximum of the compression loading curves of the dependence $F(t)$. The compressive strains ε_{mp} were also evaluated from the first maximum of the compression loading curves of the dependence $F(\varepsilon)$. The inflection point (F_{inf}, ε_{inf}) of the compression curve was determined. The times t_{inf} and forces F_{inf} were determined in the inflection points on the curves $F(t)$. Compressive strains ε_{inf} and compressive stresses σ_{inf} in the inflection points of the compression curves and compressive strains ε_m and stresses σ_m in the first maximum of the compression curves were also enumerated. The initial firmness of the pellets was determined as the force F_{10} at the 10% of the compressive strain on the compression curve. The second parameter of the firmness was the force F_{inf} in the inflection point of the compression curve at the compressive strain ε_{inf} . The third parameter of the firmness of the pellets was the force F_m at the first maximum at the ε_m compressive strain on the compression curve.

The compressive strain in the inflection point of the compressive curves was enumerated from the equation:

$$\varepsilon_{inf} = \frac{v\, t_{inf}}{L_0} \tag{5}$$

where ε_{inf} – compressive strain in the inflection point of the compression curve $F(\varepsilon)$, mm mm^{-1}; v – deformation speed, mm min^{-1}; t_{inf} – time in the inflection point, min; L_0 – original length of the sample, mm.

The compressive stress in the inflection point of the compression curves was enumerated from the equation:

$$\sigma_{inf} = \frac{4\, F_{inf}}{\pi\, d^2}\left(1 - \varepsilon_{inf}\right) \tag{6}$$

where σ_{inf} – compressive stress in the inflection point of the compression curve $\sigma(\varepsilon)$, MPa; ε_{inf} – compressive strain in the inflection point of the compression curve $F(\varepsilon)$, mm mm^{-1}; F_m – force in the inflection point of the compression curve $F(\varepsilon)$, N; d – original diameter of the sample, mm.

Young's modulus of elasticity was calculated from the equation:

$$E = \frac{4\, \Delta F\, L_0}{v\, \Delta t\, \pi\, d^2} \tag{7}$$

where E – Young's modulus of elasticity, MPa; $\Delta F/\Delta t$ – the slope of the linear part of dependences the compression curve $F(t)$, N s^{-1}; v – speed of deformation, mm s^{-1}, L_0 – original height of the sample, mm; d – original diameter of the sample, mm.

RESULTS AND DISCUSSION

For each pellet type, ten pellet samples were subjected to measuring.

The Fig. 3 depicts the compression diagrams of pellet type 1; these are dependencies $F(\varepsilon)$. The slope is almost all the diagrams is the same. In the diagrams for samples 4, 6 and 10, it is clearly visible that in the beginning of the test, significant sample compression took place even at small values of compression force, reaching the deformation rate of 6%. In sample 6, after the reaching of the first maximum – after which the sample was not yet destroyed – an increase of density, paired with the increase of compression force, took place, with the sample being destroyed only at reaching the second maximum of force.

The Fig. 4 shows dependencies $F(t)$ of pellet type 1. Time necessary for reaching of the first maximum was in the range of 7 to 10 s; the lowest time was observed for sample 3 and the highest time was observed for sample 10.

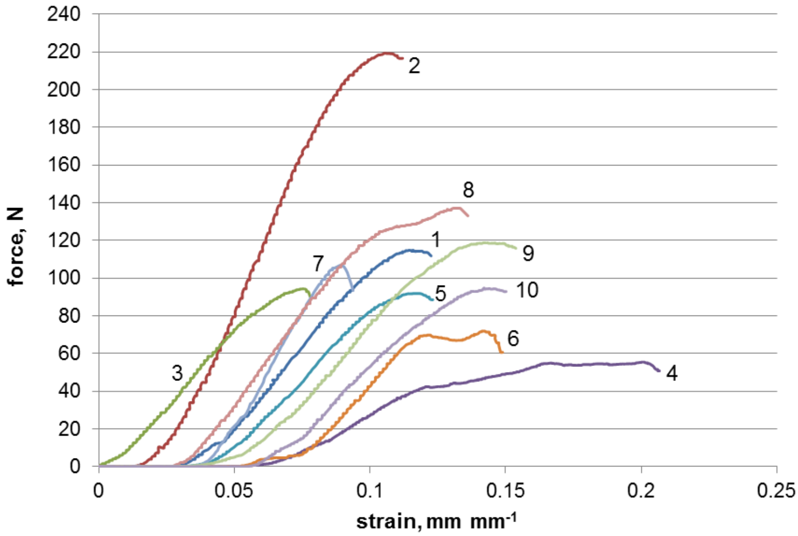

Figure 3. Compression diagrams of pellet type 1 – dependence $F(\varepsilon)$.

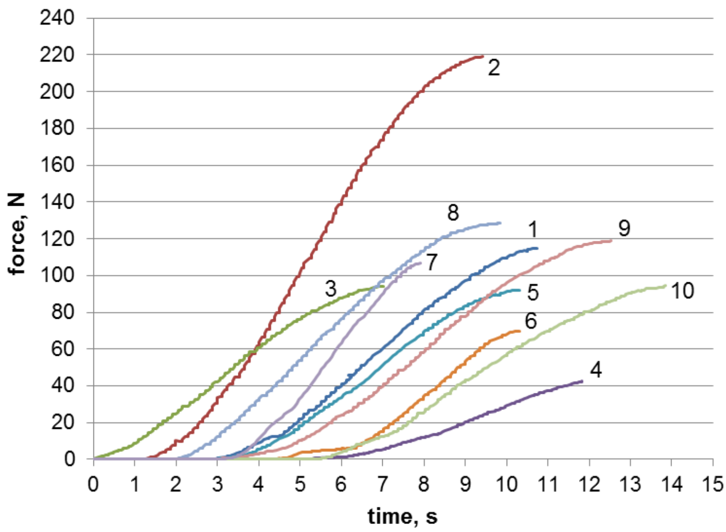

Figure 4. Compression diagrams of pellet type 1 – dependence $F(t)$.

Fig. 5 shows the compression diagrams observed at compressive loading test of pellet type 2, these are dependencies $F(\varepsilon)$.

All the compression diagrams have approximately the same slope. On the slopes of samples 1, 5 and 8, it can be observed that, after reaching the first maximum of the compression force, increase in pellet density takes place, resulting in the increase of compression force to the second maximum. On the slopes of samples 7 and 8, non-linearity of dependence $F(\varepsilon)$ was observed. The compression diagrams $F(t)$ depicted in Fig. 6 shows that time necessary for reaching of the first maximum of the loading force F_m was in the range of 6.8 to 16.3 s; the lowest time was measured for sample 5 and the highest time was measured for sample 9.

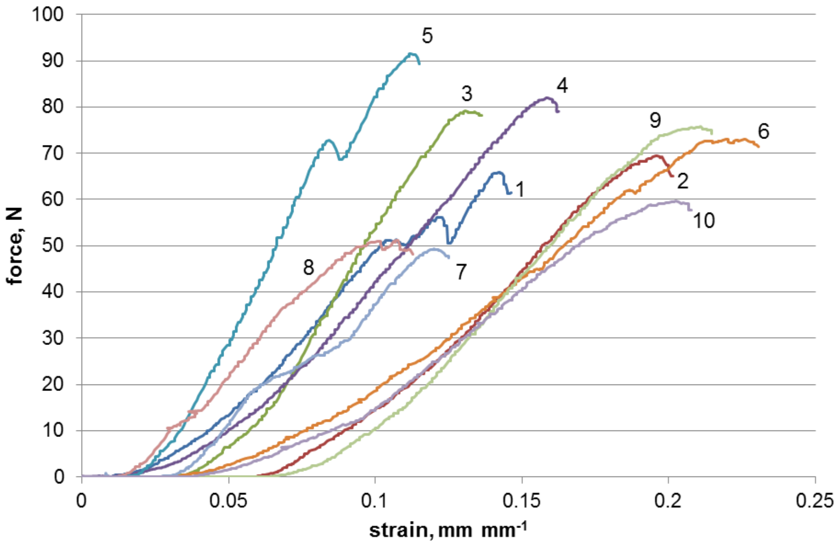

Figure 5. Compression diagrams of pellet type 2 – dependence $F(\varepsilon)$.

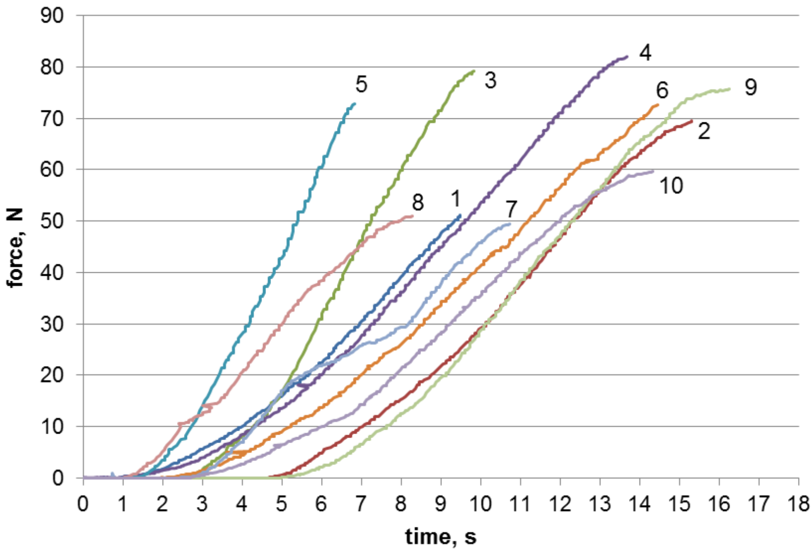

Figure 6. Compression diagrams of pellet type 2 – dependence $F(t)$.

Fig. 7 depicts the compression diagrams of dependence $F(\varepsilon)$ obtained from compression loading test of pellet type 3.

Behaviour of individual pellets during the compression test differs. The values of compression force in the first maximum and strain of pellets have a high variance.

Development of the force on pellet 5 is nonlinear, pellets 3 and 7 have a more prominent yield point, typical for brittle materials. Diagrams for pellets 8 and 10 show properties typical for materials without prominent yield point. This signifies that, with increase in the amount of poppy waste from sieving added to the base ground poppy head mass, the mechanical properties of pellets, produced by same means as pellet type 1 and 2, become significantly imbalanced.

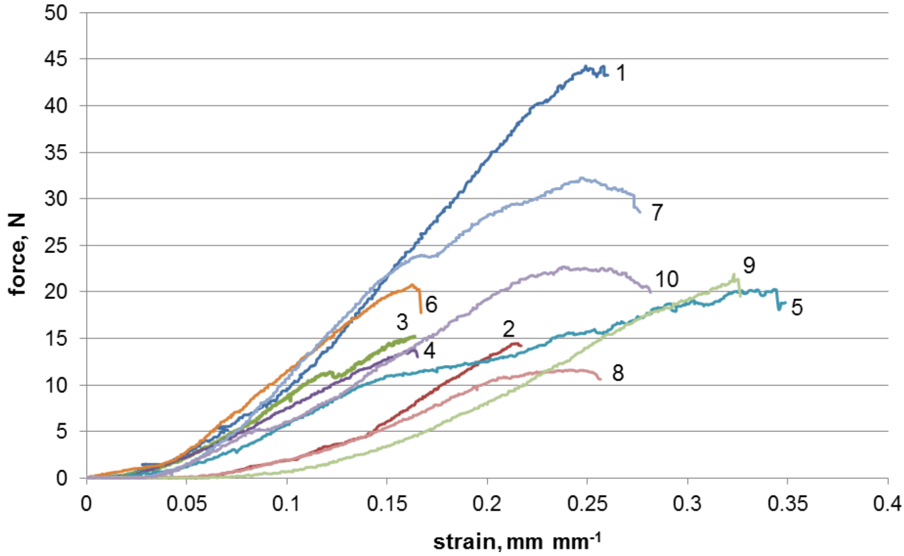

Figure 7. Compression diagrams of pellet type 3 – dependence $F(\varepsilon)$.

The Fig. 8 shows the compression diagrams $F(t)$ of pellet type 3.

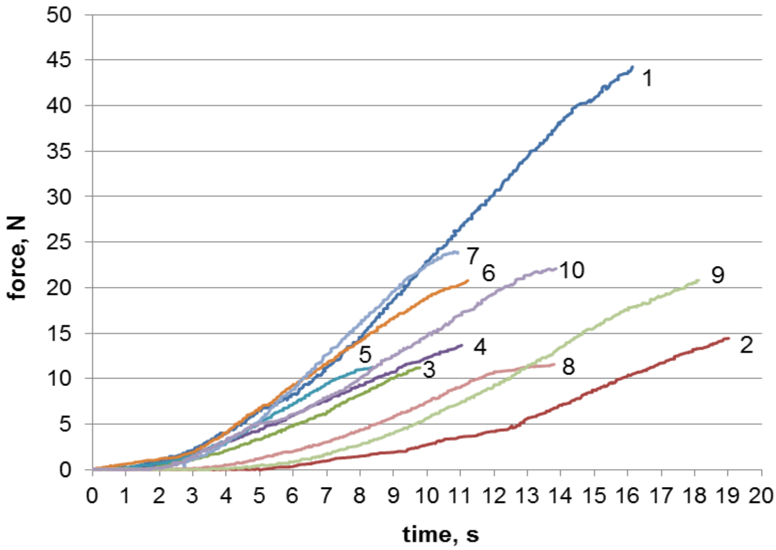

Figure 8. Compression diagrams of pellet type 3 – dependence $F(t)$.

The geometrical parameters and mechanical properties of the pellets are shown in Table 2.

Table 2. Geometrical parameters and mechanical properties of observed pellets

	Pellets	1	2	3
	$\bar{x}$	6.29	6.33	6.50
	S	0.13	0.18	0.07
	s (%)	2.14	2.92	1.06
	$\bar{x}$	15.19	13.36	11.11
	S	0.64	1.22	1.81
	s (%)	4.21	9.12	16.26
	$\bar{x}$	107.96	66.31	19.40
	S	44.08	11.78	9.50
	s (%)	40.83	17.76	48.97
	$\bar{x}$	0.12	0.15	0.20
	S	0.02	0.05	0.06
	s (%)	15.78	32.10	27.81
	$\bar{x}$	3.52	2.11	0.62
	S	1.51	0.40	0.30
	s (%)	42.98	18.81	48.26
	$\bar{x}$	0.11	0.15	0.20
	S	0.02	0.05	0.06
	s (%)	17.50	32.10	27.81
	$\bar{x}$	3.13	1.79	0.46
	S	1.36	0.35	0.22
	s (%)	43.50	19.71	46.88
	$\bar{x}$	56.72	24.48	4.80
	S	20.58	8.89	1.67
	s (%)	36.28	36.32	34.77
	$\bar{x}$	79.67	37.36	6.37
	S	55.96	21.70	3.62
	s (%)	70.24	58.09	56.91
	$\bar{x}$	64.04	45.18	13.22
	S	21.32	11.25	6.33
	s (%)	33.29	24.90	47.89
	$\bar{x}$	0.09	0.12	0.15
	S	0.04	0.04	0.06
	s (%)	44.44	34.15	39.01
	$\bar{x}$	1.91	1.27	0.33
	S	0.67	0.32	0.15
	s (%)	34.90	24.89	46.17

The diameter of all pellets was of similar values – this is also proven by statistical evaluation.

Small deviations from the mean value for strain compression ε_{mp} observed form diagrams (Fig. 1) and strain compression ε_m calculated according to relation (3) were observed. Similar deviations from the mean were detected for the values of mechanical compression stress σ_{mp} observed by means of diagrams and values of σ_m calculated by means of relation (4) and also at values of compressive strain and mechanical stress in the inflexion point. The greatest deviations from the mean were observed in the initial durability of pellets F_{10}.

The values of mechanical properties of the pellet type 2 show only small deviations from the mean values. However, the variability of measured and calculated values is higher; the greatest variability can be seen in the initial durability of pellets F_{10}. Its values are in the range of 10.52 N to 82.22 N.

All the quantity values observed in pellet type 3 showed very small or small deviations from the mean values. However, the variability of measured and calculated values is higher for all quantities, except the diameter and length of the pellets. The initial durability of the pellets F_{10} varies the most; its values are within the range of 0.68 N to 11.53 N.

The mean value of the modulus of elasticity of the pellet type 1 was 56.72 MPa. The mean value of the modulus of elasticity of the pellet type 2 was 24.48 MPa, what is 2.3 times lower than the value of modulus of elasticity of the pellet type 1, made solely of ground poppy heads. The mean value of the modulus of elasticity of the pellet type 3 was 4.8 MPa, what is 11.8 times lower than the value of modulus of elasticity of the pellet type 1.

It can be seen that the values of compressive strain ε_{mp} and ε_m in pellet type 3 are nearly twice as high as pellet type 1 and 1.3 times higher than those of pellet type 2.

It can be therefore concluded that with increasing addition of the waste from the sieving of poppy seeds to the pellet content, pellets become softer, what is reflected in their mechanical properties. The values of modulus of elasticity, initial durability and force in the first maximum are the lowest for pellet type 3.

Experiments show that differences can be observed even in the pellets of the same type, meaning that pelletization of the given material under operational conditions does not result in the pellets with the same mechanical properties,. In addition to this, with increasing amount of poppy waste from sieving, the differences in pellet properties also grow, this material type therefore negatively affects the particle integration in pelletization.

The Table 3 provides the correlation coefficients for mechanical properties of the pellet type 1, absolute value of which is higher than 0.5. Coefficients with absolute value higher than 0.8, signifying a high degree of linear dependence between quantities, are highlighted. The greatest degree of dependence – the coefficient value almost equals 1 – is between quantities $\sigma_m - \sigma_{mp}$, $F_m - \sigma_{mp}$ and $F_m - \sigma_m$.

Table 3. Value of correlation coefficients for the quantities evaluated in the compression test of pellet type 1

	d	L_0	F_{10}	F_m	ε_{mp}	σ_{mp}	ε_m	σ_m	E	F_{inf}	ε_{inf}	σ_{inf}
d	1											
L_0		1										
F_{10}			1									
F_m	-0.55		**0.90**	1								
ε_{mp}					1							
σ_{mp}	-0.61		**0.91**	**1.00**		1						
ε_m					**0.98**		1					
σ_m	-0.62		**0.89**	**0.99**		**1.00**		1				
E	-0.66		**0.86**	**0.96**		**0.97**		**0.97**	1			
F_{inf}			0.73	0.77		0.73		0.70	0.63	1		
ε_{inf}											1	
σ_{inf}			**0.80**	**0.84**		**0.82**		**0.80**	0.73	**0.98**		1

Only correlation coefficients with the absolute value higher than 0.5 are provided.

The correlation coefficients for mechanical properties of the pellet type 2 are shown in Table 4. Correlation coefficient of $\varepsilon_m - \varepsilon_{mp}$ reaches the value of 1; the values of compressive strain obtained from the dependence $F(\varepsilon)$ are corresponding with values of compression strain calculated by means of relation (3). For the pellet type 2, there occurs a significant degree of linear dependence between diameter and F_{10}, ε_{mp}, ε_m and E.

Table 4. Values of correlation coefficients for the quantities evaluated in the compression test of pellet type 2

	d	L_0	F_{10}	F_m	ε_{mp}	σ_{mp}	ε_m	σ_m	E	F_{inf}	ε_{inf}	σ_{inf}
d	1											
L_0		1										
F_{10}	**-0.87**		1									
F_m				1								
ε_{mp}	**0.81**	-0.66	**-0.91**		1							
σ_{mp}				**0.94**		1						
ε_m	**0.81**	-0.66	**-0.91**		**1.00**		1					
σ_m	-0.54		0.52	**0.82**		**0.96**		1				
E	**-0.87**		**0.88**		-0.79		-0.79	0.71	1			
F_{inf}				0.70		0.64		0.54		1		
ε_{inf}	0.79	-0.51	**-0.85**		**0.91**		**0.91**			0.54	1	
σ_{inf}						0.74		0.75		**0.91**		1

The correlation coefficients of the observed values obtained from the test of the pellet type 3 are given in Table 5. The diameter, initial length and durability of the pellets do not show any degree of dependence with other quantities that would be worth of consideration. $\varepsilon_m - \varepsilon_{mp}$ have correlation coefficient equal to 1. High degree of correlation was discovered for the couples of quantities $\sigma_{mp} - F_m$, $\sigma_m - F_m$, $\sigma_{mp} - \sigma_m$, $\sigma_{inf} - F_{inf}$.

Table 5. Value of correlation coefficients for the quantities evaluated in the compression test of pellet type 3

	d	L_0	F_{10}	F_m	ε_{mp}	σ_{mp}	ε_m	σ_m	E	F_{inf}	ε_{inf}	σ_{inf}
d	1											
L_0		1										
F_{10}			1									
F_m				1								
ε_{mp}			-0.61		1							
σ_{mp}	-0.54			**0.99**		1						
ε_m			-0.61		**1.00**		1					
σ_m			0.51	**0.99**		**0.99**		1				
E	-0.66		0.65	0.77		**0.82**		**0.84**	1			
F_{inf}	-0.52			**0.94**	0.58	**0.93**	0.58	**0.89**	0.62	1		
ε_{inf}			-0.71		0.76		**0.97**				1	
σ_{inf}	-0.55			**0.98**		**0.97**		**0.95**	0.73	**0.99**		1

The issue of influence of fraction size on compressed material was investigated by Kaliyan & Morey (2009). The fact that structure of input material has an impact on the properties of the compressed material is also proven by this evaluation of the mechanical properties of the pellets made of poppy waste. The pellet type 1 made only from ground poppy heads shows the best mechanical properties. This is due to the fact that the crushed poppy mass has a high proportion of fine fraction. This result proves the observations of Križan (2014) that if the material contains a larger amount of fine particles, the compression to pellet form is easier.

According to Gil et al. (2010), the usage of several raw materials can also have adverse effects on the final compression of the product.

Swietochovski et al. (2016) also focused on the determination of the mechanical properties of the pellets and carried out compression tests for the pellets that had identical diameter as those observed here, with feed speed of the loading plate of the testing device also the same as in experiments presented here. In their study on force bondings in pellets made of wood and agricultural biomass, Stelte et al. (2011) performed a compressive loading test that is comparable to compressive loading test carried out by testing device Stentor Andilog 1000 in terms of technical parameters and conditions. The dependence course of compression force on deformation corresponds with the compression diagrams obtained in testing of pellets presented here. The force necessary for mechanical damaging of the pellet integrity was slightly higher than the observed values.

Mechanical properties of the evaluated pellets prove high variability of behaviour of the pellets in the fields of elastic and plastic deformation. This conclusion was also reached by Stelte et al. (2011) and Miao et al. (2015). The compression diagrams are very similar to compression testing of biological materials presented by Shirmohammadi et al. (2011), proving that the observed pellets show behaviour typical for biological materials in general.

CONCLUSION

The paper provides an examination and evaluation of three pellet types, differing in composition of the input material. The pellet mechanical properties were changing with the alternations in the material composition. With increasing proportion of waste from sieving of poppy seeds to the basic matter, the values of the mechanical properties were worsening.

The results show that the examined pellets differ from each other in their mechanical properties; in comparison with pellets made of dendromass, they show lower values of the observed parameters. The pellet type made only of ground poppy head mass has shown the best results, the pellet type consisting of ground poppy heads and waste from sieving of poppy seeds in mass proportion 1 : 1 has shown the worst results. Young's modulus of elasticity is equal to 56.72 MPa for pellet type 1, 24.48 MPa for pellet type 2. In pellet type 3, it amounts only to 4.8 MPa. The force in the first maximum develops in the same manner: it is 107.96 N for pellet type 1, 66.31 N for pellet type 2 and only 19.4 N for pellet type 3.

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Chemical composition of seeds and green beans of common bean varieties, breeded in Omsk State Agrarian University under conditions of southern forest-steppe zone of Western Siberia

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Abstract. The article considers the biochemical composition of green beans and seeds of common beans varieties, breeded in Omsk State Agrarian University named after P.A. Stolypin (OmSAU). The research was conducted in 2014–2016. Varieties of locally breeded beans, in comparison with the standards, have advantages in the content of protein, zinc, iodine, calcium, iron, sugar; green bean technological properties and tenderizing of seeds during cooking, which becomes an indispensable component of the diet.

Key words: Legume breeding, microelements contain, kidney beans, green beans.

INTRODUCTION

Beans originated and domesticated in Latin America in two geographically differentiable geographic origins (Mesoamerica and the Andes) derived from a common 100,000-year-old ancestor. In Mexico and South America, the bean were domesticated independently about 8,000 years ago. There are records of cultivated seeds of Phaseolus vulgaris 3,000 years old (Lara Flores, 2015).

Common bean (*Phaseolus vulgaris L.*) is the grain legume with the highest volume of direct human consumption in the world. Among food leguminous crops, common beans are distinguished by nutritional value and a variety of uses for food purposes. It has excellent taste qualities and medicinal properties. There is all the essential amino acids necessary for the human body in the protein of bean seeds, so beans are often called the 'concentrate of essential amino acids'. The bean protein is highly digestible (Beebe et al., 2014).

For Russia, green beans are a relatively young crop compared to cereals. In Western Siberia, on an industrial scale, it is not cultivated, mainly grown as garden crops. The main reasons for the weak introduction of beans into production are the absence of varieties adapted to a specific soil and climate zone, a small amount of seed production, a high labor intensity of harvesting, and insufficient propaganda of valuable cultural qualities (Kazydub & Marakayeva, 2015).

Due to the features of the climate in Western Siberia, the bean varieties of foreign breeding can not realize the productive potential inherent in them, the production of such varieties is unprofitable (Kazydub et al., 2012).

In modern conditions, breeding work with beans should be aimed at satisfying of processing requests, as well as expanding the range of cultivation of the crop and the scope of its use (Kazydub & Marakayeva, 2015; Javaloyes, 2016).

Common bean is a valuable source of protein, minerals and vitamins. In terms of biofortification, improvement of mineral content is advantageous precisely because the baseline grain iron content is high at 55 ppm (mg kg^{-1}) and variability for the trait is great, ranging up to 110 ppm, allowing initial breeding attempts to be much more successful than in the cereals in overall iron and zinc content increases (Beebe et al., 2000). In addition, unlike many cereals that are polished before eating, resulting in significant loss of nutrients, common beans are consumed whole, thus conserving all their nutritional content. Estimates for the Harvest Plus challenge program on biofortification are that an addition of approximately 40 ppm to baseline iron levels in common bean can meet a large proportion of the recommended daily intake of iron (Blair, 2009).

Bean varieties must have good product quality (green beans and seeds). Seeds should have a good tenderizing (during cooking) and high taste qualities. Frozen vegetable mixtures, in which green beans are added, must fit the technological requirements. These indicators are closely related to the chemical composition of seeds and beans (Kazydub et al., 2016).

The bean varieties, existing in Russia, are not always satisfy the quality requirements and are inferior to the foreign varieties (Kazydub et al., 2012).

Breeders of Department of Agronomy, Breeding and Seed Production of Omsk State Agrarian University created new high-yield varieties of common beans: for green beans use – Pamjati Ryzhkovoj, Zoloto Sibiri, Marusja and Sibirjachka; for grain use – Lukerja, Olivkovaja, Omskaja Jubilejnaja, Sizaja, Sibskovskaja 100, Omichka.

The results of the scientific researches testify to the prospects of cultivation of this legume crop in the conditions of Western Siberia, since valuable protein products in our zone can be obtained fairly early. The use of beans in the diet of the population will increase the assortment of vegetables and leguminous crops of the Siberian region and increase their role in the system of 'health, nutrition, resources'. Need to note that local varieties of leguminous crops are the basis of 'healthy food', due to their high content of vitamins, essential organic acids and other biologically valuable substances.

MATERIALS AND METHODS

The research was conducted in 2014–2016 on the fields of the Educational Experimental Farm of the Omsk State Agrarian University, located in the southern forest-steppe zone of the Omsk Region.

The southern forest-steppe zone is characterized by a warm, moderately humid climate. The sum of average daily temperatures over a period with a temperature above 10° is 100–130 days average. The frost-free period in this region averages 110–120 days, the period with a temperature above 0 °C – 185, above 5 °C – 157, above 10 °C – 123 days. Night frosts in the air in spring time are stop on May 21–22, and appear in the autumn of September 10–22. The abundance of sun and heat largely compensates the short duration of the frost-free period and ensures vegetation of plants. The southern forest-steppe of the Omsk region belongs to the zone of unstable hydration. The average long-term annual precipitation is 300–350 mm, for a period with a stable average daily air temperature above 10° precipitation, 190–220 mm falls out. Provision of plants with moisture in the area is characterized by a hydrothermal coefficient of 1.0–1.2, which indicates a satisfactory average moisture supply in the period of active vegetation. By the time of sowing, moisture reserves in the soil are usually sufficient. The soil of the field is ordinary black chernozem, medium humus.

The years of research varied according to meteorological conditions in 2014 was very dry (hydrothermal coefficient = 0.60), 2015 and 2016 were moisture-insulated (the hydrothermal coefficient was 1.02 and 1.1 respectively).

The object of research were common bean varieties of local breeding: of vegetable (green bean) use – Zoloto Sibiri, Pamjati Ryzhkovoj, Marusja and Sibirjachka; grain use – Lukerja, Olivkovaja, Omskaja Jubilejnaja, Sizaja, Sibskovskaja 100, Omichka. As a standard (control variety) for the evaluation of vegetable (green) beans used zoned variety Zolushka (breeding of the All-Russian Research Institute of Breeding and Seed Production Of Vegetable Crops, Moscow), grain beans - Nerussa (breeding of All-Russian Research Institute of leguminous crops, Orel).

Samples in the nursery were sown manually on single-row plots in fourfold repetition according to the scheme of 60 x 10 cm to a depth of 4–5 cm. The size of the plot is 5.2 m^2. Rows were arranged from north to south. The extreme rows were sown as protective bands.

The chemical analysis of green beans and seeds was carried out in the laboratory of the Omsk Agrochemical Center according to the following normative documents: state standard 13496.2-91 (crude fiber), state standard 26570-95 (calcium), state standard 26657-97 (phosphorus), state standard 13496.4-93 (raw protein), state standard 30692-00 (copper, zinc), state standard 27998-88 (iron), state standard 27997-88 (manganese).

Evaluation of seeds for tenderness was carried out in the Omsk laboratory of the State Inspectorate in accordance with the guidelines ‘Technological evaluation of grains of peas, lentils, beans’ (Komarov, 1992).

The plants were manually harvested during the full ripeness phase of the beans. The number of beans on the plant, the number of seeds in the bean, and the height of the attachment of the lower bean were taken into account. The weight method was used to determine the mass of seeds from one plant and the mass of 1,000 seeds.

Observations, surveys and analyzes in the field were carried out according to the methodological guidelines for studying the collection of grain legumes (All-Russian Institute of Plant Production, 1975) and studying samples of the world bean collection (All-Russian Institute of Plant Production, 1987).

Biochemical analysis of green beans was carried out in the testing laboratory of the Omsk branch of the federal state budget institution ‘Federal Center for Evaluation of Safety and Quality of Grain and Products of Its Processing’. The content of sucrose in green beans is determined by the refractometer ‘Refracto 30P’. The statistical processing of the experimental data (mean, standard deviation, variance analysis, etc.) was carried out according to the method described in the manual of B.A. Dospekhov (1985).

RESULTS AND DISCUSSION

Legumes are an affordable source of food and feed protein, balanced by amino acid composition. Synthesis of proteins from simple nitrogen compounds carried out through the root system of plants from the soil, and the role of assimilating factor are playing nitrogen fixing bacteria used only by legumes (Pivovarov, 2006).

The protein content in seeds (dry weight) of grain beans breeded in Omsk SAU varied from 21.22 to 24.06%. The four varieties of grain beans were significantly higher than the standard: Sizaja (24.06%), Lukerja (23.38%), Omskaja Jubilejnaja (22.60%), Olivkovaja (23.13%), Table 1.

Table 1. Chemical composition of seeds (dry weight) of grains bean varieties breeded in Omsk SAU (2014–2016)

Variety	Content				
	Protein, %	Zinc, mg kg^{-1}	Iodine, mg kg^{-1}	Calcium, mg kg^{-1}	Iron, mg kg^{-1}
Nerussa, control	19.0	20.7	0.1	0.1	21.1
Sibakovskaja 100	21.2	25.5*	0.2*	0.2*	10.0
Sizaja	24.0*	24.3	0.2*	1.4*	54.0*
Lukerja	23.4*	20.9	0.2*	0.3*	80.0*
Omskaja Jubilejnaja	22.6*	27.8*	0.2*	0.2*	12.0
Olivkovaja	23.1*	28.1*	0.2*	0.9*	17.0
Omichka	21.8	26.9*	0.2*	0.6*	10.0
Average	22.2	24.9	0.2	0.5	26.4
LSD_{05}	3.3	3.7	-	0.1	4.0

* significantly at $P > 0.05$.

In the research work of Gorbataya (2010–2013), which was performed on the basis of the Training and Experimental Farm of Omsk State Agrarian University, it was noted that lysine, methionine, tryptophan, cysteine are considered the most valuable for human nutrition. Her studies showed that the seeds of the common bean varieties under conditions of southern forest-steppe of Western Siberia contained on average 2.46 g 100 g^{-1} of lysine, 1.20 g 100 g^{-1} of methionine, 1.11 g 100 g^{-1} of tryptophan, 0.43 g 100 g^{-1} of cysteine.

The mass fraction of amino acids, including essentials, in the seeds of beans characterizes its high biological value. Our researches have shown that in the conditions of southern forest-steppe zone of Omsk region, the seeds of grain beans contain around 27.7 g 100 g^{-1} of amino acids, of which 12.7 g are essential. The highest content of amino acids was observed in varieties: Lukerja (29.89 g 100 g^{-1}, of which 14.0 g are essential) and Olivkovaja (28.96 g 100 g^{-1}, of which 13.40 g 100 g^{-1} are essential).

The zinc content in the seeds of the studied grain bean samples varied from 20.9 to 28.1 mg kg^{-1}. High contain of zinc in the seeds, significantly higher than the standard, have had varieties of Omsk breeding: Sibakovskaja 100 (25.5 mg kg^{-1}), Omskaja Jubilejnaja (27.8 mg kg^{-1}), Olivkovaja (28.1 mg kg^{-1}), Omichka (26.9 mg kg^{-1}).

The average iron content in grain bean varieties ranged from 10.0 to 80.0 mg kg^{-1}. With its maximum content was characterized varieties Lukerya (80.0 mg kg^{-1}) and Sizaja (54.0 mg kg^{-1}).

The iodine content in beans of Omsk varieties was more than 2 times higher than the standard, and ranged from 0.15 to 0.23 mg kg^{-1}. The highest iodine content was found in the varieties Lukerja (0.23 mg kg^{-1}) and Olivkovaja (0.21 mg kg^{-1}).

The average content of calcium in new varieties of grain beans varied from 0.24 to 0.85 mg kg^{-1}. The maximum content had varieties Olivkovaja (0.85 mg kg^{-1}) and Omichka (0.60 mg kg^{-1}).

All varieties created in Omsk SAU for iodine and calcium content significantly surpassed the control variety Nerussa.

For grain beans an important feature is the rate of seed tenderizing during cooking, which depends on water-absorbing capacity and shape of the seeds, the percentage and thickness of the seed coat, the conditions of mineral nutrition, and on the conditions under which seed formation and maturation took place. In order to assess the consumer benefits of beans, was made the evaluation of bean sample's tenderizing by the method developed in the laboratory of technological evaluation of crops of the All-Russia Institute of Plant Growing.

For the classification of seed tenderness is proposed the scale: I group – excellent (up to 90 minutes); Group II – good (91–124 min); Group III – satisfactory (125–161 min); IV group – unsatisfactory (162–299 min).

All new Omsk varieties of beans had excellent tenderness (57 to 67 min.), and were assigned to group I. The shortest cooking time had varieties Sizaja (57 min.) and Lukerja (58 min), Table 2.

Table 2. Tenderness time of bean varieties of OmSAU breeding (2014–2016)

Variety	Seed coloring	Tenderness, min			
		2014	2015	2016	Average
Nerussa, control	White	58	60	59	59
Sibakovskaja 100	White, dark cherry pattern at the rib	60	61	60	60
Sizaja	Blue-grey	57	58	57	57
Lukerja	Black	57	59	58	58
Omskaja Jubilejnaja	Beige with brown pattern	65	67	66	66
Olivkovaja	Olive	60	63	61	61
Omichka	White, blue strokes	58	60	59	59
LSD_{05}	-	8.9	9.2	9.0	9.0

Varieties of grain beans of Omsk SAU breeding are distinguished by a high level of yield. During the years of testing, the yields of varieties ranged from 1.5 to 5.7 t ha^{-1}, Table 3. The maximum yield of varieties was 4.1 t ha^{-1} in 2014, the minimum in 2015 – 2.3 t ha^{-1}. Varieties Sibakovskaja 100, Lukerja, Sizaja, Omskaja Jubilejnaja and Olivkovaja were significantly exceeded the control variety for the yield.

Green beans are valuable food product, in which there are almost all substances necessary for normal human nutrition. Vegetable beans are rich in organic and mineral substances, as well as potassium, iron, calcium, zinc, iodine, etc. (Javaloyes, 2016). When choosing varieties for processing, account should be taken of the roundness and fleshiness of the bean, the absence of the parchment layer and fibers in the pod of the bean.

Most of the production of green beans available on the Russian market is imported from countries with a more favorable climate for their cultivation, for example, from Poland. Therefore, it is important to study the chemical composition and technological qualities of green beans breeded in Omsk SAU for various types of processing (Kazydub et al., 2012).

Table 3. Yield of grain beans varieties breeded in Omsk SAU (2014–2016)

Variety	Seed yield, t ha^{-1}			Average
	2014	2015	2016	
Nerussa, control	2.6	1.5	2.8	2.3
Sibakovskaja 100	4.5	2.8	3.4	3.6
Sizaja	3.5	2.1	3.7	3.1
Lukerja	4.8	3.1	3.8	3.9
Omskaja Jubilejnaja	4.3	2.6	3.1	3.3
Olivkovaja	5.7	2.2	3.2	3.7
Omichka	3.0	1.5	2.6	2.4
LSD_{05}	0.6	0.3	0.5	0.5

* Significant at $P > 0.05$.

In the phase of technical maturity we evaluated the green beans varieties according to their processability. The results of the studies are presented in Table 4.

Table 4. Indicators of processability of green beans varieties breeded in Omsk SAU (average for 2014–2016)

Variety	Cross-sectional shape	Fleshiness, point	The parchment layer, +, - *	Bean thickness, cm
Zolushka, control	round	2	-	0.5
Zoloto Sibiri	round	3	-	0.8
Pamjati Ryzhkovoj	round	3	-	1.0
Marusja	round	3	-	0.8
Sibirjachka	round	3	-	1.0
LSD_{05}	-	-	-	0.2

* + *presence*, - *absence.*

The green beans of Omsk breeding varieties in the stage of technical maturity were differed from the control variety by the higher fleshiness and the cross-sectional thickness of the bean (from 0.8 to 1.0 cm), had long straight thick beans of round shape, without a parchment layer, yellow and green. These parameters characterize the suitability of bean varieties for freezing and canning.

The nutritional value of grain and green beans is high and depends on the content of organic and mineral substances, vitamins, micro- and macro-elements (Gamzikova, 1979). Research had shown that the chemical composition of common beans is unstable and subject to variability depending on the species, variety, and also varies due to growing conditions (Kazydub, 2013).

The protein content, as well as micro- and macro-elements in green beans of Omsk varieties are presented in Table 5.

Table 5. Chemical composition of green beans of Omsk State Agrarian University (2014–2016)

Variety	Mass fraction in absolutely dry matter			
	Protein,%	Zinc, mg kg^{-1}	Iodine, mg kg^{-1}	Iron, mg kg^{-1}
2014				
Zolushka, control	21.13	19.63	0.014	2.0
Zoloto Sibiri	18.31	21.23	0.010	2.0
Pamjati Ryzhkovoj	20.63	28.68	0.014	3.1
Marusja	23.60	23.74	0.014	3.2
Sibirjachka	22.16	26.21	0.016	3.2
Average	21.17	23.90	0.011	2.7
LSD_{05}	3.17	3.58	0.002	0.4
2015				
Zolushka, control	19.13	20.14	0.014	1.9
Zoloto Sibiri	17.75	20.95	0.017	1.6
Pamjati Ryzhkovoj	17.81	27.54	0.018	1.8
Marusja	20.94	22.14	0.017	1.8
Sibirjachka	19.38	24.84	0.018	1.8
Average	19.00	23.12	0.028	1.8
LSD_{05}	2.85	3.47	0.003	0.3
2016				
Zolushka, control	18.17	20.05	0.014	1.1
Zoloto Sibiri	19.79	25.30	0.014	1.1
Pamjati Ryzhkovoj	18.63	21.20	0.012	1.2
Marusja	18.79	22.10	0.013	1.4
Sibirjachka	18.19	27.30	0.014	1.8
Average	18.71	23.19	0.013	1.3
LSD_{05}	2.81	3.48	0.002	0.2

The protein content in green beans of studied bean varieties over the years of research ranged from 17.75 in wet and cool conditions to 23.60% in arid conditions. By highest content of protein was characterized variety Marusja – 23.60% (in 2014), 20.94% (in 2015) and Zoloto Sibiri – 19.79 (in 2016).

Iron plays a big role in immunobiological and oxidation-reduction reactions, with its lack is possible developing of anemia. The biggest amount of iron was found in green beans of varieties Pamjati Ryzhkovoj (1.2–3.1 mg kg^{-1}) and Marusja (1.4–3.2 mg kg^{-1}).

It should be emphasized the importance of zinc presence in the green beans, which is necessary for the normal operation of the pancreas and prostate gland. The high content of zinc was recorded in green beans of the following varieties: Pamjaty Ryzhkovoj – 28.68 mg kg^{-1} (in 2014), 27.54 mg kg^{-1} (in 2015) and Sibirjachka – 27.30 mg kg^{-1} (in 2016).

With a lack of iodine, nervous and irritability is noted in the body, memory and intellect are weakened. Over time, there is an arrhythmia, decreases the level of hemoglobin in the blood. This is especially important for iodine deficiency areas, including the Omsk Region. By the highest content of iodine in green beans were characterized varieties – Sibirjachka (0.014–0.018 mg kg^{-1}), Marusja (0.013–0.017 mg kg^{-1}) and Pamjaty Ryzhkovoj (0.012–0.018 mg kg^{-1}).

Green beans and seeds of common beans contain a significant amount of sugars (up to 6%). The main sugar contained in all kinds of legumes, including beans, is sucrose (from 0.66 to 1.23%). As the beans mature, the content of sucrose decreases. In 2016, we evaluated the green beans for the content of sucrose during the technical maturity during the second and third harvesting (July 18 and August 3, respectively), Table 6.

Table 6. The content of sucrose in green beans of Omsk SAU breeding (2016), %

Variety	Technical maturity		
	II harvest (18.07)	III harvest (03.08)	Average
Zolushka, control	3.5	0.6	2.1
Zoloto Sibiri	3.8*	1.8*	2.8*
Pamjati Ryzhkovoj	4.1*	2.1*	3.1*
Marusja	3.9*	1.1*	2.5*
Sibirjachka	3.9*	1.4*	2.7*
Average	3.8	1.4	2.6
LSD_{05}	*0.2*	*0.3*	*0.3*

* Significant at $P > 0.05$.

The research revealed that the content of sucrose in green beans at the second harvest (July 18) varied from 3.5 to 4.1%. In the subsequent harvest (August 3) the value of this indicator is less than 2.7 times (from 0.6 to 2.1%). Consequently, a high content of sucrose in green beans provides harvesting in the second half of July, with later harvesting times, the content of sucrose is significantly reduced.

During the period of the experiments, the yield of green beans varied on average from 3.9 to 5.6 t ha^{-1}. All studied varieties of Omsk SAU have surpassed the control variety for this parameter. The highest yield was registered for variety Pamjaty Ryzhkovoj – 5.3 t ha^{-1} (in 2014), 5.8 t ha^{-1} (in 2015 and 2016).

The formation of yields in green beans of vegetable beans was greatly influenced by meteorological conditions. Thus, under conditions of insufficient humidification in 2014 and 2015 (hydrothermal coefficient = 0.7), the yield was the lowest, Table 7. With the ensured wetting of 2016 (hydrothermal coefficient = 1.0), the yield of green beans was increased by an average of 7%. In conditions of sufficient heat and moisture availability in the studied varieties, this indicator varied from 3.0 to 5.8 t ha^{-1}.

Table 7. The yield of green beans varieties of Omsk SAU (2014–2016 years), t ha^{-1}

Variety	2014	2015	2016	Average
Zolushka, control	3.3	2.9	3.0	3.0
Zoloto Sibiri	4.0	3.8*	4.1	3.9*
PamjatiRyzhkovoj	5.3*	5.8*	5.8*	5.6*
Marusja	4.3*	4.4*	4.9*	4.5*
Sibirjachka	4.2*	4.4*	4.8*	4.5*
LSD_{05}				0.4

* Significant at $P > 0.05$.

CONCLUSIONS

1.The results of our studies indicate the possibility of growing beans with high potential yields of grain and green beans and excellent product quality in the southern forest-steppe of the Omsk region.

2.Varieties of Omsk beans are characterized by a complex of valuable features:

- high potential yield of: green beans – Pamjaty Ryzhkovoj (5.3–5.8 t ha^{-1}), and grains – Sibakovsaja 100 (3.6 t ha^{-1}), Sizaja (3.7 t ha^{-1}), Lukerja (3.9 t ha^{-1}), Olivkovaja (3.7 t ha^{-1});
- high content of protein: in grain – Sizaja (24.06%), Lukerja (23.38%), Omskaja Jubilejnaja (22.60%), Olivkovaja (23.13%), and green beans – Marusja (23.60–20.91%), the Zoloto Sibiri (19.79%);
- high content of zinc in: grain – Sibakovskaja 100 (25.5 mg kg^{-1}), Omskaja Jubilejnaja (27.8 mg kg^{-1}), Olivkovaja (28.1 mg kg^{-1}), Omichka (26.9 mg kg^{-1}), and in green beans – Pamjaty Ryzhkovoj (28.68–27.54 mg kg^{-1}) and Sibirjachka (27.30 mg kg^{-1});
- high iron content in: grain – Lukerja (80.0 mg kg^{-1}), Sizaja (54.0 mg kg^{-1}), and green beans – Pamjaty Ryzhkovoj (1.8–3.2 mg kg^{-1}) and Marusja (1, 8–3.3 mg kg^{-1});
- high content of iodine in: grain – Lukerja (0.23 mg kg^{-1}) and Olivkovaja (0.21 mg kg^{-1}), and in green beans – Sibirjachka (0.014–0.018 mg kg^{-1}), Marusja (0.014–0.018 mg kg^{-1}) and Pamjaty Ryzhkovoj (0.012–0.018 mg kg^{-1});
- high sugar content in green beans – Pamjaty Ryzhkovoj (2.1–4.1%).

3.Omsk beans varieties are characterized by a high content of amino acids in the grain: Lukerja (29.89 g 100 g^{-1}, of which 14.0 g are essential) and Olivkovaja (28.96 g 100 g^{-1}, of which 13.40 g 100 g^{-1} are essential).

4.Varieties of beans of Omsk SAU breeding have excellent technological indexes and can be recommended for processing: excellent grain tenderness (from 57 to 67 minutes), high fleshiness, greater cross-sectional thickness and length of green beans, without parchment layer, attractive yellow and green color of beans, and high sugar content.

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Effects of irrigation applied at different growth stages on chickpea yield

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Abstract. This study was conducted over the experimental fields of Erciyes University in 2016 to investigate the effects of irrigations applied at different growth stages on chickpea yields. Experiments were conducted in randomized blocks design with 3 replications. There were 7 irrigation treatments as of I_1: rainfed, I_2: pre-bloom single irrigation, I_3: single irrigation at the beginning of blooming, I_4: single irrigation at 50% pod set, I_5: two irrigations at 50% bloom and 50% pod-set, I_6: two irrigations at pre-bloom and 50% pod-set, I_7: full irrigation. The amount of applied irrigation water varied between 85.6–323 mm. Plant water consumptions varied between 262–569 mm. The greatest yield was obtained from I_4 treatment with 273 kg da^{-1} and the lowest yield was obtained from I_1 treatments with 146 kg da^{-1}. It was concluded for chickpea cultivation under deficit water resources conditions that water deficits may be applied at different growth stages except for 50% pod-set period.

Key words: Chickpea, irrigation, yield, ET.

INTRODUCTION

Chickpea kernels contain more than 20% protein, more than 40% carbohydrate, oils, phosphorus and calcium. Therefore, chickpea has always a great significance in human nutrition. Worldwide chickpea production is used as foodstuff in different fashions. It also a legume and thus able to fixate free atmospheric nitrogen into the soil and therefore it is used as a well intercropping plant (Degirmenci et al., 2009).

Chickpea is the most important legume worldwide and 87% of world production comes from Asia, 4.5% from Africa, 4.1% from America and 0.9% from Europe (FAOSTAT, 2014). It is produced over 14 million hectares and annual world production is around 13.7 million tons. In Turkey, chickpea is produced over 388 thousand hectares and annual production is about 450 thousand tons (FAOSTAT, 2015).

Global warming, climate change and rapid increases in world population exert ever-aggravating pressure on water resources (Yılmaz, 2011). Food demand of increasing population can only be met with the optimum utilization of soil and water resources. Sustainable agriculture primarily depends on proper irrigation practices.

Drought is the greatest limiting factor for agricultural production activities (Kalefetoglu & Ekmekci, 2005). Plants are subjected to various levels of droughts from sowing till the harvest (Gunes et al., 2006). Droughts have two major impacts on agriculture. The first one is insufficient emergence and the second one is the decrease in growth and yields because of water deficits (Saxena et al., 1993).

Chickpea plants are quite resistant to droughts, but do not like humid conditions. They can have quite high yield levels with slight irrigations during the dry periods. Besides a proper field preparation, use of high-yield seeds, fertilization and the other cultural practices, irrigation scheduling and amounting are also quite significant issues in chickpea culture (Yolcu, 2008).

In semi-arid climate zones, chickpea culture is commonly carried out under quite dry conditions because of deficit water resources of these regions. One or two irrigations throughout the growing season will greatly improve yield levels in chickpea and consequently will increase the agricultural income of farmers.

The present study was conducted to investigate the effects of supplementary irrigations performed at different growth stages on yield and plant water consumption of chickpea.

MATERIALS AND METHODS

Experiments were carried out over the experimental fields of Erciyes University Agricultural Research and Training Center in 2016. Experimental site has an altitude of 1,094 m and located at 34°56′ north longitude and 36°59′ east longitude. Some weather data for chickpea growth season of 2016 are presented in Table 1. Total rainfall from seeding (13 April) to harvest (11 August) was 179.4 mm for the growth season.

Table 1. Some weather data for the growth season of 2016

Weather data	Months				
	April	May	June	July	August
T_{mean} (°C)	14.02	14.83	20.41	23.33	25.38
T_{max} (°C)	20.4	26.7	34.6	37	34.8
T_{min} (0°C)	4.5	4.4	7.5	10.8	14.5
Wind speed (m s^{-1})	1.57	1.88	1.75	1.81	1.81
Rainfall (mm)	0	151.8	25.6	2	0
RH_{max} (%)	65.2	80.0	78.2	66.1	62.4
RH_{min} (%)	25.5	34.4	30.8	21.1	19.9

T_{mean}, T_{max} and T_{min}: Daily mean, maximum and minimum temperatures of the related month, respectively; RH_{max} and RH_{min}: Monthly averaged maximum and minimum relative humidity.

Soil samples were collected from 1.2 m soil profile at three points. Texture was found as loamy. Soil pH and salinity were suitable for production (Table 2).

Water samples were taken from a deep well within the experimental site. Irrigation water class was C_1S_1 according to Wilcox (1948) (Table 3). Infiltration tests were performed at three different locations and average infiltration rate was found as 23.3 mm per hour.

Table 2. Some soil properties of the experimental site

Soil property	Soil depth			
	0–30 cm	30–60 cm	60–90 cm	90–120 cm
Texture	Loamy	Loamy	Clay-Loamy	Loamy
Salinity (dS m^{-1})	0.22	0.173	0.258	0.191
pH	8.13	8.17	8.14	8.23
Field capacity (%)	23	26	26	25
Wilting point (%)	10.73	11.38	9.3	9.37
Bulk density (g cm^{-3})	1.27	1.24	1.22	1.28
Organic matter (%)	1.25	1.05	0.69	0.73
Lime (%)	2.54	5.83	3.15	6.2
Nitrogen (kg ha^{-1})	2.15	1.05	0.4	0.4
Phosphorus-P_2O_5 (kg ha^{-1})	20.5	11.5	6.0	2.0
Potassiun-K_2SO_4 (kg ha^{-1})	271.6	376.4	310.1	310.1

Table 3. Some properties of irrigation water

pH	EC (μS)	Na^+ (mg L^{-1})	K^+ (mg L^{-1})	Ca^{+2} (mg L^{-1})	Mg^{+2} (mg L^{-1})	HCO_3^- (mg L^{-1})	CO_3^{-2} (mg L^{-1})	SAR	Class
7.6	242	11.6	4.57	26.4	6.63	12.2	< 1.0	2.86	C_1S_1

Electrical conductivity and pH of irrigation water were 242 mS m^{-1} and 7.6, respectively.

A completely randomized blocks design with three replications was used. Row spacing was 35 cm, on-row plant spacing was 5 cm and each plot (5 x 1.75 m) had 6 rows, sowing was performed manually. Observations and harvests were performed from the inner 4 rows. Two side rows were omitted as to consider the side effects. A spacing of 1.2 m was left between the experimental plots and 2.5 m was left between the blocks to prevent interactions among the treatments.

Drip irrigation system with 16 mm laterals at each plant row was used for irrigation. The dripper discharge rate was 2 L h^{-1} at 4 atm pressure and dripper spacing was 0.25 m. Plant efficient root depth was taken as 60 cm (Allen et al., 1998)

Soil moisture content was determent with TDR probes with 60 cm long and deficit moisture was brought to field capacity in each irrigation.

Irrigation treatments: I_1 – Rainfed; I_2 – Pre-bloom single irrigation; I_3 – Single irrigation at the beginning of blooming; I_4 – Single irrigation at 50% pod set; I_5 – Two irrigations at 50% bloom and 50% pod-set; I_6 – Two irrigations at pre-bloom and 50% pod-set; I_7 – Full irrigation.

Chickpea seeds were planted manually on 13 April, 2016 and they were harvested between July 18, 2016 and August 11, 2016. In June 1, 2016 programmed irrigations were initiated.

Amount of irrigation water to be applied was calculated by using the following Eq. 1:

$$d = \frac{\left(P_{vfc} - P_{vp}\right)}{10} \cdot D \cdot P \qquad (1)$$

where d – Amount of water applied, mm; P_{vfc} – volumetric water content at field capacity %; P_{vp} volumetric water content before irrigation %; D – depth of soil to be irrigated, cm, and P – canopy percentage.

Actual plant water consumption (ET) was determined based on James (1993) using water balance Eq. 2:

$$ET = I + R + Cr - Dp - Rf \pm \Delta s \quad (2)$$

ET is plant water consumption (mm); I is irrigation water applied (mm); R is effective rainfall (mm); Cr is capillary rise (mm); Dp is deep percolation (mm); Rf is runoff (mm) and Δs is soil moisture storage difference between the seeding and harvest.

RESULTS AND DISCUSSION

The effects of irrigations performed at different phenological stages on plant water consumption and yield of chickpea plants are provided in Table 4. The amount of irrigation water applied in different treatments varied between 85.6 (I_3) – 323 (I_7) mm. For reliable emergence levels, before the initiation of programmed irrigations, 17 mm water (5 mm on 28 Nisan 2016 and 12 mm on 13 May 2016) was applied to all treatments. Seasonal plant water consumption in I_1, I_2 , I_3, I_4, I_5, I_6 and I_7 treatments were respectively measured as 262, 374, 326, 360, 391, 438 and 569 mm. As compared to dry treatment without any irrigations (I_1), 54.13% decrease was observed in plant water consumption in full irrigation treatment (I_7).

Table 4. Effects of different irrigation water levels on yield and plant water consumption

Treatments	Irrigation (mm)	ET (mm)	Yield (kg da^{-1})
I_1	17	262	146
I_2	142,7	374	157.0
I_3	85,6	326	212.0
I_4	119,7	360	273.1
I_5	164	391	221.7
I_6	199	438	224.7
I_7	323	569	217.7

Different irrigation water treatments had significant effects on yields at $P < 0.05$ significance level. The greatest yield was obtained from I_4 treatments with 273 kg da^{-1} and the lowest yield was obtained from I_1 treatment with 146 kg da^{-1} (Table 5). There were four different statistical groups and the treatments of I_5, I_6 and I_7 were placed in the same group (Table 6). Irrigations significantly increased chickpea yields. Thusly Soltani et al. (2000) carried out a study in Iran and reported chickpea yields as 276.6 kg da^{-1} for full irrigation and 90.9 kg da^{-1} for rainfed treatment. Oweis et al. (2004) carried out a study in West Asia and North Africa and reported chickpea yields as 255 kg da^{-1} for full irrigation and 144 kg da^{-1} for rainfed treatments. Yolcu (2008) investigated the effects of irrigation performed at different growth stages of chickpea under Diyarbakır conditions and reported significant increases in yields with irrigations at pod-set period.

Table 5. Effects of different irrigation water levels on chickpea yield

Sources	SD	Sum of squares	Means square	F
Block	2	197.429	98.715	0.09 ns
Irrigation level	6	33,725.143	5,620.86	5.21*
Error	10	12,944.571	1,078.71	
General	17	46,867.143		

ns – not-significant;
* – significant at 5% level;
** – significant at 1% level.

Table 6. Duncan's test groups for yields (kg da^{-1})

Irrigation treatments	Mean
I_1	146.0 d
I_2	157.0 cd
I_3	212.0 bc
I_4	273.0 a
I_5	221.7 ab
I_6	224.7 ab
I_7	217.7 ab

The relationship between the amount of irrigation water and ET was identified as ET= 0.9896 I – 234.26 and R^2 = 0.9951 (Fig. 1).

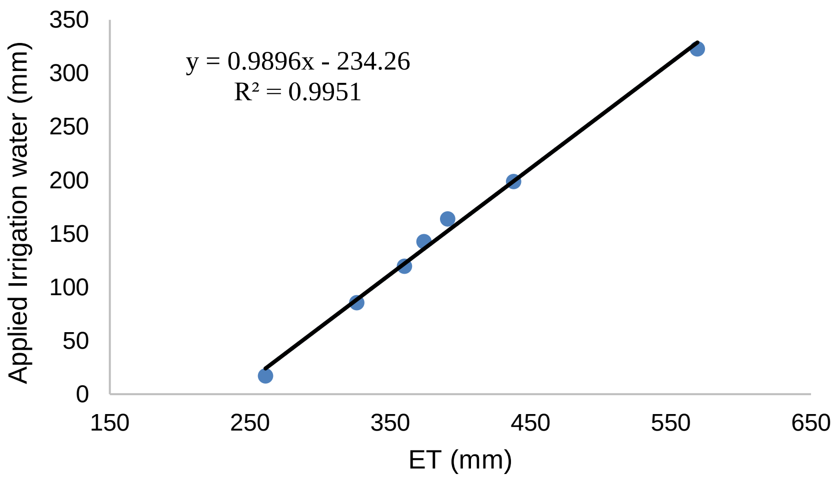

Figure 1. The relationship between amount of irrigation water and ET.

Soil moisture contents at measurement days are presented in Fig. 2 and it was observed that moisture levels were brought to field capacity later on with irrigation.

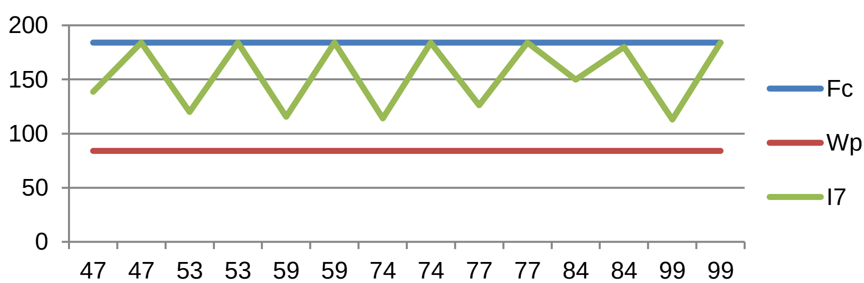

Figure 2. Variation in soil moisture levels.

CONCLUSIONS

In present study, effects of irrigations applied at different growth stages of chickpea on yields were investigated under Kayseri conditions. Results revealed that irrigations at 50% pod-set period improved yield levels of chickpea and single irrigation at 50% pod set had higher yield than full irrigation. Such a case revealed that water deficits can be applied in chickpea culture at proper produces. Therefore, it is recommended that irrigation should be performed at 50% pod-set period of chickpea in places with semi-arid climate conditions and deficit water resources.

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The content of weed seeds in the soil based on the management system

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Abstract. In 2008 an experiment was set up on the field in Eerika experimental station (Estonian University of Life Sciences) as a 5-field crop rotation: barley (*Hordeum vulgare* L.) with undersown red clover, red clover (*Trifolium pratense* L.), winter wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.), potato (*Solanum tuberosum* L.). The objective of the study was to measure the content of weed seeds in the soil and to evaluate the diversity of the species at the beginning of the period of organic production in 2011. In conventional farming systems without fertilizer (Con I) and conventional farming with mineral fertilizer (Con II) herbicides were used for weed control. All the crops in Con II system received P 25 kg ha^{-1} and K 95 kg ha^{-1}, but the application rates of mineral nitrogen fertilizer differed. In organic systems (Org I – organic farming based on winter cover crop and Org II – organic farming based on winter cover crop and manure), the winter cover crops (ryegrass after winter wheat, winter oilseed rape after pea, winter rye after potato) were sown after the harvest and were ploughed into the soil as green manure in spring. Organic farming systems (Org II) had a negative effect on the content of weed seeds in the soil (2.0–22.7% less seeds than in other variants). The seeds of *Chenopodium album* were the most abundant among summer annual weeds and the seeds of *Viola arvensis* among winter weeds in the soil. Organic farming measures increased the domination of *Chenopodium album* – the dominance index *D'* was increased by 0.09–0.14 compared to conventional variants. The content of seeds of winter weed *Viola arvensis* in Org II variant was decreased by 82%. The index of species evenness *J'* and Shannon Wiener diversity index *H'* were lower in organic plots by 0.10–0.18 and 0.60–0.19, respectively. Org II variants showed the best results based on the decrease of soil weed seed bank and distribution of the weed species.

Key words: organic farming, winter cover crops, weed seeds, species diversity.

INTRODUCTION

The weed seed bank consists of many species, whereas some dominant species may comprise 70–90% of the total seed bank causing the most problems (Wilson, 1988). Plant protection practices without the use of herbicides has been pointed out as one of the main problems of successful organic cultivation system (Gianessi & Reigner, 2005). In organic farming weeds are the main crop yield decreasing factors. In ecologically friendly cultivation weeds can be controlled by tilling, crop rotation, harrowing, mulching, growing legumes in rotation and cover crops (Watson et al., 2002; Thorup-Kristensen et al., 2003). Soil movement on no-tilled field may lead to much larger number of emerging weed seeds compared to tilled soils (Chauhan et al., 2006; Tamm et al., 2016). The increase of number of soil weed seeds has also been observed in reduced tillage system (Tørresen et al., 2003; Woźniak & Kwiatkowski, 2012). A vigorous cover crop can change the environment for weed seeds on and in the soil. Previous results indicate, that winter cover crops reduced the dry biomass and density of weeds compared to the conventional control system (Madsen et al., 2015).

The aim of the study is to investigate the influence of different winter cover crops and their combination with composted cattle manure on soil weed seed bank at the start of organic farming. The effect of the yield of winter cover crops and preceding crops on the number of weed seeds in the soil was studied in detail.

MATERIALS AND METHODS

The five-field crop experiment with three different organic systems was started in 2008 and was set up in test site of the Estonian University of Life Sciences in Eerika (58°22′ N, 26°40′ E). A 5-year crop rotation contains: red clover *RC* (*Trifolium pratense* L.), winter wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.), potato (*Solanum tuberosum* L.) and barley (*Hordeum vulgare* L.) undersown with red clover. First 5-year rotation ended in 2012. The experiment was established in four replications, each plot (60 m^2) situated in a systematic block design (80 plots). Organic and conventional plots were separated with an 18 m long section of mixed grasses to avoid contamination with synthetic pesticides, mineral fertilisers and winter cover crops. In systems Con I (conventional farming without fertilizers, as control) and Con II (conventional farming with mineral fertilizers) herbicides (Roundup 4 L ha^{-1} were used in last autumn). In conventional system Con II all the crops received phosphorous (P 25 kg ha^{-1}) and potassium (K 95 kg ha^{-1}). The amount of nitrogen (N) varied depending on the crop: for pea N 20 kg ha^{-1}, for barley undersown with red clover N 120 kg ha^{-1}, for winter wheat and potato N 150 kg ha^{-1}. Plots with red clover did not receive any mineral fertilizers and chemical pest control. Two organic farming systems (Org I and Org II) were investigated. In both systems winter cover crops as green manure were used. Cover crops were sown right after the harvest: ryegrass – *RG* (*Lolium perenne* L., sowing rate 25 kg ha^{-1}) after winter wheat, winter oilseed rape *WOR* (*Brassica napus* L., *var. oleifera, subvar. biennis*, 7.1 kg ha^{-1}) after pea and winter rye *WR* (*Secale cereale* L., 220 kg ha^{-1}) after potato. Before sowing the subsequent crop all cover crops were ploughed into the soil as green manure. The growth stage (according to BBCH) of cover crops before ploughing was determined (ryegrass 21–22, winter oilseed rape 24–26 and winter rye 23–26. In the organic system Org II fully composted cattle manure was added in the autumn 2010 and in spring 2011 at a total rate of 40 t ha^{-1} and ploughed into soil. The cover crops were sown with the Kongskilde sowing machine right after the harvesting of the main crop and in the beginning of May they were ploughed into the soil. Red clover was cut twice: middle of June and during the second half of July and ploughed into the soil in all organic systems. Cereals and peas were harvested at the beginning of August, using a Sampo Rosenlew experimental harvester. Potato tubers were hand-collected between August and September. Yield data were adjusted to dry matter content. Samples for measuring the weed seed bank were collected in September

2011, at the start of first year to full organic production. The weed seed bank samples were taken with soil borers (15 mm diameter) after crop harvest and before autumn ploughing. From each plot 16 soil samples were taken from the depth of 0–25 cm soil layer. Samples of each plot were mixed together in a bucket. The samples were air-dried and 500 g of each sample were sieved and washed through a 0.25 mm sieve. Weed seeds were separated from the soil by potassium carbonate (K_2CO_3) aqueous solution. For preparation of the solution 2.0 kg of potassium carbonate was dissolved in 1.8 L of water. The number of weed seeds in seed bank was calculated to an area of 1 m^2 of plot area using the formula Eq. 1 (Kuht et al., 2016) as shown below:

$$N = \frac{h \cdot \rho \cdot n \cdot 10}{W\mathrm{d}} \tag{1}$$

where N – number of viable seeds, n m^{-2}; h – depth of plough layer, cm; ρ – soil bulk density, g cm^{-3}; n – counted number of seeds in the soil sample; Wd – weight of dry soil sample, g.

The species composition of weed seed communities and the number of seeds of each species were used to assess the biodiversity.

The diversity indexes (Eq. 2) of weed seed species were calculated as Shannon-Wiener diversity index (Shannon, 1948; Shannon & Wiener, 1949) of weed seed species diversity *H'*:

$$H' = -\sum_{i=1}^{s} pi \ln(pi) \tag{2}$$

The community dominance was determined based on the Simpson index, Eq. 3. Simpsons' index of species dominance (Simpson, 1949) was calculated as follows:

$$\lambda = \sum_{i=1}^{s} pi^2 \tag{3}$$

The formula to calculate Pielou evenness index, Eq. 4 (Pielou, 1966; Boyce, 2005) is as follows:

$$J' = \frac{H'}{H'max} \tag{4}$$

The richness index, Eq. 5, was measured based on the Margalef index (Margalef, 1958) as shown below:

$$R = \frac{S - 1}{\ln N} \tag{5}$$

where the *pi*'s are the proportion of all observations in the i^{th} species category; $H'max = \ln(S)$; S – number of species (the total number of weed types in a single treatment); N – total number of individuals of all the species in the block.

The results were analysed by using STATISTICA 7.0: ANOVA, Fisher (LSD) test (Statsoft Inc, 2005). Correlation analysis was used to study the correlation between different number of weed seeds and some indicators of soil physical properties. Linear correlation coefficients between variables were calculated, the significance of coefficients being $P < 0.01$, $P < 0.05$.

RESULTS AND DISCUSSION

As an average, there were significantly less viable annual weed seeds in conventional variants with mineral fertilizers and herbicides (Con II) and organic variants with winter cover crops and cattle manure (Org II) compared to other cultivation methods by 3.1–6.1 and 2.5–5.6 (thousand seeds per 1 m^2) respectively (Table 1). In the soil of Org II variant, there were less seeds (as average) in the plots of potato and barley undersown with clover.

Table 1. Number of annual (summer, winter and total) weed seeds in the soil of different crops in 2011

Crop and preceding crops	Weed seeds	Number of seeds, 1,000 seeds per m^2			
		Con I	Con II	Org I	Org II
Red clover	Summer annual	24.22^a	20.58^a	28.42^a	21.00^a
	incl. *Chenopodium album*	19.04^a	18.20^a	27.16^a	19.88^a
1) barley, us. r. clover	Winter annual	0.98^a	0.70^a	1.12^a	2.10^a
2) potato	incl. *Viola arvensis*	0.14^a	0.56^a	0.14^a	0.28^a
3) pea	Total	25.20^a	21.28^a	29.54^a	23.10^a
Winter wheat	Summer annual	25.06^b	20.30^a	20.16^a	20.44^a
	incl. *Chenopodium album*	19.88^a	17.78^a	19.32^a	19.60^a
1) red clover	Winter annual	1.54^a	2.80^{ab}	1.82^a	3.78^b
2) barley, us. r. clover	incl. *Viola arvensis*	0.84^a	2.38^b	0.70^a	0.70^a
3) potato	Total	26.60^b	23.10^a	21.98^a	24.22^{ab}
Pea	Summer annual	27.72^b	20.02^a	20.30^a	23.24^{ab}
	incl. *Chenopodium album*	25.20^a	17.78^a	19.74^a	22.82^a
1) winter wheat	Winter annual	4.90^b	3.36^{ab}	1.68^a	1.54^a
2) red clover	incl. *Viola arvensis*	4.62^a	2.52^a	0.42^a	0.28^a
3) barley, us. r. clover	Total	32.62^b	23.38^a	21.98^a	24.78^{ab}
Potato	Summer annual	20.80^a	19.24^a	21.71^a	15.73^a
	incl. *Chenopodium album*	18.46^a	18.98^a	20.80^a	14.82^a
1) pea	Winter annual	0.52^a	3.38^b	1.30^a	1.56^{ab}
2) winter wheat	incl. *Viola arvensis*	0.52^a	2.47^b	0.65^a	0.26^a
3) red clover	Total	21.32^a	22.62^a	23.10^a	17.29^a
Barley, us. red clover	Summer annual	27.02^c	14.84^a	21.42^b	13.16^a
	incl. *Chenopodium album*	24.22^b	13.16^a	19.60^b	12.60^a
1) potato	Winter annual	1.40^a	0.98^a	0.98^a	1.12^a
2) pea	incl. *Viola arvensis*	0.70^a	0.56^a	0.14^a	0.00^a
3) winter wheat	Total	28.42^c	15.82^{ab}	22.40^{bc}	14.28^a
Average	Summer annual	24.96^b	19.00^a	22.40^{ab}	18.71^a
	incl. *Chenopodium album*	21.36^b	17.18^a	21.32^b	17.94^{ab}
	Winter annual	1.87^a	2.24^a	1.38^a	2.02^a
	incl. *Viola arvensis*	1.36^{ab}	1.70^b	0.41^a	0.30^a
	Total	26.83^b	21.24^a	23.78^{ab}	20.73^a

Note. Within the same row, values with different letters are significantly different (ANOVA, Fisher (LSD) test); 1) – preceding crops in 2010; 2) – preceding crops in 2009; 3) – preceding crops in 2008.

There were a lot more (11 times more as an average) weed seeds of summer annuals than winter weeds, dominated by the seeds of common lambsquarter (*Chenopodium album* L.). The domination of the seeds of common lambsquarter in various cultivation systems has been observed by many authors (Caroca et al., 2011). It was found that the proportion of the seeds of common lambsquarter among summer annuals was significantly higher in organic plots than in conventional variants. It was 95.2% in Org I, 95.9% in Org II and 85.6% and 90.4% in Con I and Con II systems (4.8–10.3% more in organic systems). Except the Con II variant with potato, the domination of the seeds of common lambsquarter was apparent in all the organic variants.

Among winter annual weeds the most dominant species was field pansy (*Viola arvensis* Murr) and as an average they were present in significantly smaller numbers in organic variants compared to the conventional plots (Table 1). On the contrary to the seeds of common lambsquarter which share among summer annuals was higher in organic variants, the proportion of field pansy seeds in summer annuals in Org I and Org II treatments was 0–50.0% in winter annuals (50.0–100% in conventional plots).

The yield of preceding crop proved to be one of the main factors influencing the number of weed seeds. Clear negative correlation (r = 0.56) between dry matter yield of crops (without the clover) and the number of weed seeds was observed in 2010 (Fig. 1). Moreover, very strong relationship (r = 0.83) between them was apparent in Org II and Con II systems. It was found that with higher dry matter yield of a preceding crop there were less weed seeds in the soil. For most of the crops studied, weed densities were lower when a crop was grown in rotation (Liebman & Dyck, 1993).

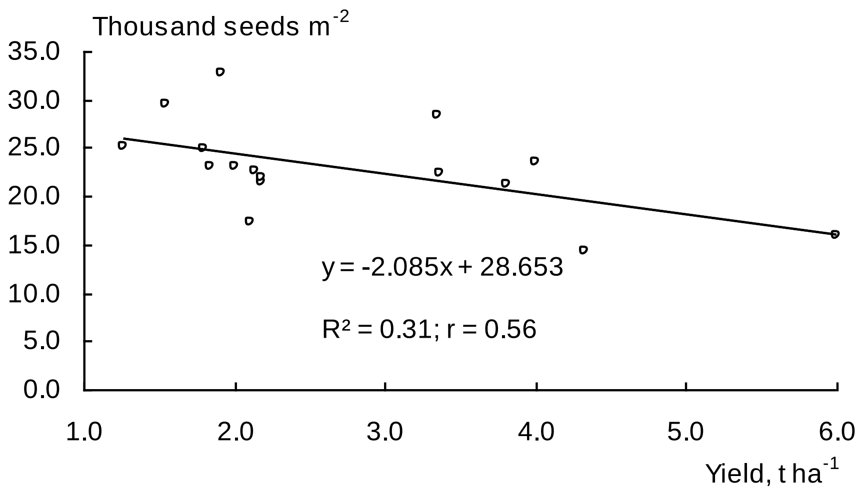

Figure 1. The correlation between total yield of the preceding crop (DM, t ha^{-1}) in 2010 and the number of weed seeds in the soil in 2011 (seeds per m^{-2}).

The correlation between dry matter yield of Org I variants and the number of weed seeds was r = 64, but there was no significant relationship with the yield of Con I system. In 2011, the correlation between yields and the number of weed seeds was r = 0.32. The number of weed seeds was mostly affected by winter rye (sown as green manure for barley preceding crop and ploughed into the soil in spring) and ryegrass (ploughed into the soil before sowing the pea) the reduction in the number of weed seeds by 17.2% and 16.4% respectively (Fig. 2). Similar effect of reducing the density of weed plants was seen in the same plots where winter cover crops were used. Also, Miura & Watanabe (2002) found that living mulch is effective for weed control and Liebman & Dyck (1993) concluded that weed biomass was lower in the living mulch system. The use of cover crops has an effect on the germination and sprouting of weed seeds, by influencing the light conditions, soil temperature and soil moisture (Teasdale, 1996). Weed presence was strongly influenced by green manure crop, rye being the most suppressive one and ryegrass the least. This had a carry-over effect on weed density in the next pea crop (Barberi et al., 2014). Davis et al. (2005) observed that weed seed banks in the organic systems were positively correlated with weed biomass whereas seed banks in the conventional systems were less predictive. The small reduction (7.2%) of weed seeds in organic variants with red clover was probably due to the effect of the cover crops sown after the preceding pea and potato crops. The effect of growing potato and red clover on the weeds and their seeds was similar in conventional and organic systems.

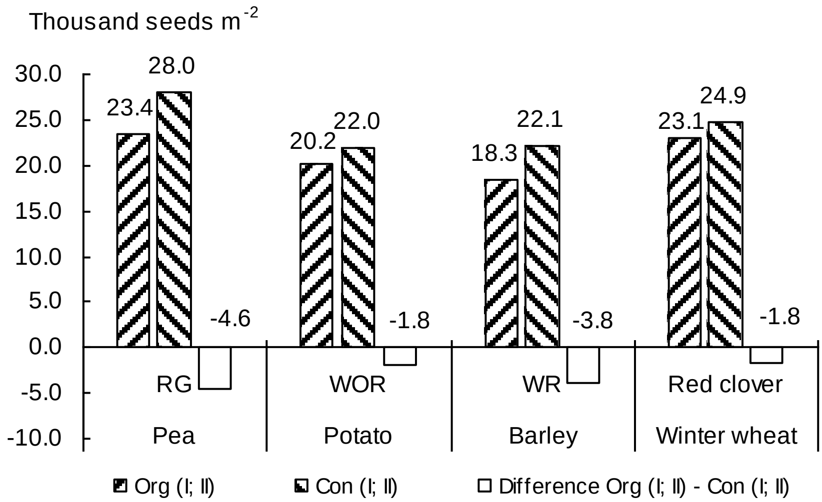

Figure 2. The number of weed seeds in the soil in 2011, depending on the winter cover crops or red clover sown in 2010. *Note*. RG – ryegrass; WOR – winter oilseed rape; WR – winter rye.

In potato plots it was because of the mechanical weeding and in clover plots it was due to the dense canopy that competed with annual weeds during the whole growing period. Also the red clover undersown to barley reduced the density of weeds. Albrecht (2005) also found that the soil weed seed bank was reduced by 39.0% when grasses undersown with clover were used.

Cover crops increase destruction of weed seed in fields. Additionally the increase in the activity of weed seed predator insects was observed. Our results showed that the increase in number of omnivorous predators was observed in areas where winter cover crops had been cultivated previously (Org I and Org II systems, Kuht et al., 2016). The dominant species was *Harpalus rufipes,* that was significantly more present on all organic plots compared to conventional ones. Previous studies have not revealed any significant changes in the number of seed beetles between crops. In our present experiment the *Harpalus rufipes* was significantly more present in pea treatments compared to other crops. As an average of 2 year experiment, diversity index of seed beetles (species per area) was higher by (35%) in organic systems with winter cover crops, compared to the conventional treatments. This is partly due to the better wintering conditions for seed beetles offered by winter cover crops. Blubaugh et al. (2016) also indicated that the cultivation of cover crops increased the activity of weed seed eating insects (omnivorous predators) on the field by enabling their activity on the individual level.

As an average, the Shannon-Wiener diversity indexes (*H'*) were lower in organic variants than in conventional variants (by 0.06–0.19, Table 2), while the largest reduction (by 0.32–0.44) of species diversity was seen in weed seeds in the pea variant.

Simpson dominance indexes (λ) were higher in organic variants than in conventional variants (by 0.09–0.14), especially in the plots with pea. It was obvious that higher dominance index was due to the increase in the share of common lambsquarter seeds in the soil of organic systems.

Table 2. Species diversity index, dominance index and richness index of the weed seed bank in different crops in 2011

Crops	Shannon-Wiener diversity index, *H'*				Simpson's dominance index, λ				Margalef richness index, *R*			
	Con I	Con II	Org I	Org II	ConI	Con II	Org I	Org II	ConI	Con II	Org I	Org II
Barley	0.65	0.68	0.48	0.66	0.60	0.61	0.78	0.66	0.39	0.31	0.60	0.42
R. clover	0.67	0.49	0.30	0.60	0.61	0.74	0.85	0.74	0.39	0.30	0.39	0.70
W. wheat	0.49	0.56	0.56	0.47	0.73	0.70	0.77	0.78	0.20	0.30	0.40	0.40
Potato	0.48	0.62	0.46	0.67	0.76	0.72	0.82	0.74	0.30	0.60	0.60	0.82
Pea	0.73	0.79	0.41	0.35	0.62	0.60	0.81	0.85	0.38	0.50	0.60	0.40
Average	0.61	0.63	0.44	0.55	0.66	0.67	0.80	0.76	0.33	0.40	0.52	0.55

The values of Margalef species richness index (*R*) were higher in organic variants (by 0.12–0.22) compared to conventional variants. Higher species richness indexes in organic variants were observed in plots with red clover and potato. The reason for this here lies also in the agrotechnological characteristics where the effect on the weeds is expressed by intensive mechanical weeding and manure applied with potato and dense canopy and manure applied to the preceding barley crop with red clover.

As an average the evenness index values of Org I and Org II weed seeds were lower than respective values for conventional systems: by 0.18 and 0.12, compared to Con I and by 0.16 and 0.10, compared to Con II variants. The changes in the evenness indexes appeared more clearly in the experiment than species richness and dominance indexes and these are presented in Fig. 3.

The lowest values for evenness index were observed in Org II and the highest values in control variant Con I (except for winter wheat plot), whereas the biggest difference (by 0.24) between conventional and organic systems was seen in plots with pea. The results of correlation analysis indicated that as an average there was a strong relationship (r = 0.87) between dominance and evenness indexes, while very strong relationships were seen with pea (r = 0.98), barley (r = 0.93) and clover (r = 0.96).

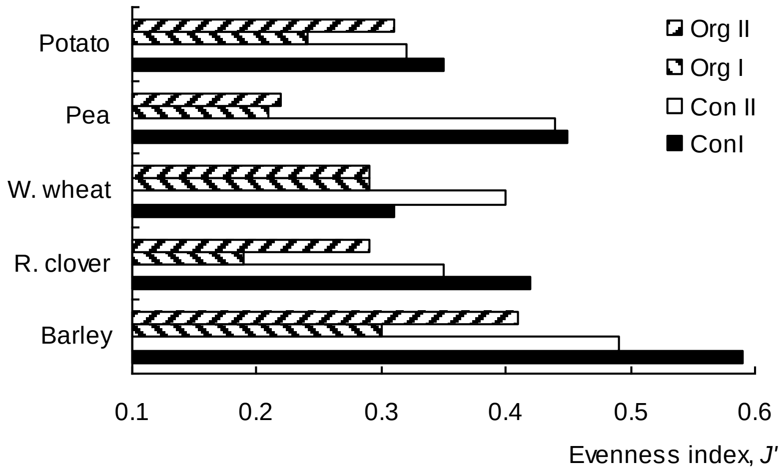

Figure 3. The values of evenness index (*J'*) of weed seeds in the soil of various crops depending on the cultivation system in 2011.

CONCLUSIONS

The yield of preceding crops and cultivation of post-harvest winter cover crops proved to be strong factors for reducing the number of weed seeds in the soil. There were significantly less viable annual weed seeds in conventional variants with mineral fertilizers and herbicides (Con II) and organic variants with winter cover crops and cattle manure (Org II) compared to other cultivation methods. In the soil of Org II variant as an average less weed seeds were found in the soils of potato and barley plots undersown with clover. Common lambsquarter in summer annuals and field pansy in winter annuals were the dominant species. Clear relationship ($r = 0.56$; $P < 0.05$) was found between the dry matter yield of crops in 2010 and the number of weed seeds in the soil in 2011, whereas very strong correlation was seen between Org II and Con II variants. The number of weed seeds was mostly affected by winter rye (sown as green manure for barley preceding crop and ploughed into the soil in spring) and ryegrass (ploughed into the soil before sowing the pea) the reduction in the number of weed seeds by 17.2% and 16.4% respectively. As an average, the Shannon-Wiener species richness indexes were lower in organic variants than in conventional variants (by 0.06–0.19). The lowest values for evenness index were observed in Org II and the highest values in control variant Con I (except for winter wheat plot), whereas the biggest difference (by 0.24) between conventional and organic systems was seen in plots with pea. The values of dominance and species richness index increased in organic variants compared to the conventional variants.

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Assessment of different growing conditions for enhanced postharvest quality and shelf-life of leaf lettuce (*Lactuca sativa* L.)

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Abstract Lettuce is the most important leafy vegetable. It is exclusively used as freshly raw form but sometime also as cooked. However, its quality depends on several pre- and postharvest factors. The effects of growing conditions and cultivars on the postharvest quality of leaf lettuce were investigated. In this experiment the interaction of variable factor; like growing conditions and fixed factor; cultivars are also observed. The leaf lettuce cultivars ‘Cheongchima’, ‘Cheongchuckmyeon’, ‘Geockchima’ and ‘Geockchuckmyeon’ were grown under favourable (natural condition) and unfavorable growing condition (with excess soil water and 50% shading) to evaluate their shelf-life and postharvest qualities. Plant height and the number of leaves were significantly varied in different growing conditions and by cultivars. In addition, fresh weight was affected by only growing condition, whereas leaf thickness was influenced by cultivars, growing conditions and the cultivars influenced the degree of fresh weight loss and respiration rate during postharvest storage. However, the postharvest storage qualities differed with cultivars and in combination with growing conditions. Leaf thickness and quality of leaf lettuce before and after harvest were also varied by cultivars and growing conditions. It could be concluded that postharvest leaf lettuce quality remains acceptable when growing under excess soil water with 50% shading.

Key words: Growing condition, shelf-life, postharvest quality, leaf lettuce.

INTRODUCTION

Leaf lettuce (*Lactuca sativa* L.) is an annual plant of the family *Compositae*. It is grown as leafy vegetables. Lettuce is normally used for salads, although it is also used with other foods, like wraps the meats, fish and so on. It is also grown for eating either as raw or cooked. Fresh lettuce leaves contain good amounts of folates and vitamin C. Among them folates is part of co-factors in the enzyme metabolism required for DNA synthesis and thereof, play a vital role in prevention of the neural defects in fetus during pregnancy (Agüero et al., 2008; Lettuce nutrition facts, 2016). Leaf lettuce can be grown year-round and is one of the most widely used fresh vegetables in Korea. Many studies of leaf lettuce have been focused on improving productivity, while postharvest qualities have not been coupled to production factors so far (Jang et al., 2007; RDA, 2007; Ryu et al., 2011).

The quality of fresh harvest leaf lettuce depends on number of factors such as genotypes, cultivation methods, soil conditions, growing season, and diseases (Park et al., 1999). In addition couple of postharvest factors like storage temperature, relative humidity, packaging materials, and packaging method are also determined the quality of leaf lettuce (Lee et al., 2008a). Currently, leaf lettuce is grown in open fields, greenhouses, and hydroponic culture (Lee et al., 2005) abundantly. But leaf lettuce has poor storage properties after harvest due to high respiration rates (Lee et al., 2007). Hence, most of the researches are concentrated on storage management for increasing its shelf-life. However, the effect of growing conditions on postharvest qualities of leaf lettuce has not been extensively investigated.

Leaf lettuce is cultivated year-round in Korea under plastic-film greenhouses (polyvinyl house). However, these plastic greenhouses are not well protected against cold and heavy rain compares to well-designed greenhouse. Therefore, this crop is easily influenced by the changes of outsides environment, that could directly influence the inside conditions of greenhouse. Thus, these changes can directly affect the growth performance and postharvest qualities. Along with uncertain rainy weather also reduces photosynthetic light intensity and sometime causes water logging in soil. The water logging conditions negatively affects the oxygenation status of the root environment, leading to wilting or shriveling (Lee, 1997). Therefore, this study focused on the effects of amount of excess watering and poor illumination conditions on postharvest qualities relevant to freshness and storability of leaf lettuce.

MATERIALS AND METHODS

Plant materials and experimental conditions

This experiment was conducted to assess the effects of growing environments and cultivars on the production and postharvest storage qualities of leaf lettuce. Four popular available leaf lettuce cultivars namely ‘Cheongchima’, ‘Cheongchuckmyeon’, ‘Geockchima’, and ‘Geockchuckmyoen’ were collected from Nongwoobio Seed Company, South Korea. Among them ‘Cheongchima’, ‘Cheongchuckmyeon’, are greenish type and ‘Geockchima’ and ‘Geockchuckmyoen’ are red colour type cultivars widely used by Korean people as wrapper of meat with different types of sauces. They were grown under conventional cultivation protocol and unfavorable environmental conditions. The unfavorable conditions were achieved by 28.4% excess soil water and 50% shading of the plants. The experiment was carried out in plastic greenhouses (polyvinyl house) at the National Institute of Horticultural & Herbal Science, Suwon, Korea. Firstly, the polyvinyl house was separated into two parts, one part was covered with black net intercepting 50% sunlight and other part had normal sunlight without interception. The experiment was conducted in the soil with 3.5% organic matter, 2.1% N, 445.7 mg kg^{-1} P2O5, 1.6 cmol kg^{-1} K, 7.7 cmol kg^{-1} Ca, and 2.2 cmol kg^{-1} Mg with the pH 6.5, EC 0.7 mS cm^{-1}. One month aged leaf lettuce seedlings (previously raised in nursery using multi-tray with 162 wholes) were transferred to experimental polyvinyl house. The seedlings were transplanted with 20 cm × 20 cm spacing (plant to plant × row to row) in well raised soil beds inside the polyvinyl house. In case of excess soil water, furrows were made in the bed with 20 cm apart to each other and 4 furrows/bed were made. The raised part of furrows was covered with black polythene and continuously irrigated into furrows using irrigation pump regulated by sensor with 28.4% excess soil water. Whereas, only subsistence watering was done in case of normal growing condition plants. Data recorded in leaf lettuce prior to harvest were plant height, number of leaves, shoot fresh weight, leaf thickness, crude fiber content, and leaf anatomy. Harvested plants were wrapped in plastic film package and stored in a retail showcase refrigerator at 5 °C. In addition, the postharvest parameters were measured on fresh weight loss, respiration rate, leaf color change, and external appearance.

Experimental design and application of treatments

This experiment was conducted following Split-Plot design with two factors and three replicates. The factors were genotypes (four cultivars), soil moisture and light interception (normal moisture with normal light condition and excess soil water with 50% shading). The excess soil moisture treatment was prepared by following the procedure of Lee (1997). In this method, soil water movement was prevented by piling up the soil wall (~30 cm thickness) and overlaying it with polyethylene film. Soil moisture was adjusted and maintained to 28.4% using a soil moisture control device (WT-1000 TDR, AgroNet, Seoul, Korea). The average soil moisture content under the control condition was 21.1%, whereas in excessive watering condition it was 28.4%. For the shade treatment, a high-density black polyethylene net was used to reduce 50% light. Because outside light intensity was on average 1.6 MJ/day during the cultural period.

Other growth factors were constant as standard cultivation procedures followed as Rural Development Administration (RDA, 2007), Korea. Seeds were sown on August 17th, seedlings were transplanted on September 9th, and crop was harvested on October 10th. Seedlings were planted in commercial nursery soil (Bio Bed Soil No. 1, Seminis Korea Inc., Seoul, Korea). Fertilizer was applied to achieve 200, 59 and 128 kg ha^{-1} nitrogen, phosphate and potassium, respectively. Leaf lettuce was planted with an inter-plant distance of 20 cm. The growth parameters of lettuce was recorded following standard criteria of Rural Development Administration (RDA, 2003), Korea. Crude fiber content was determined using a Fibertec System (M1020, Fosstecator, Höganäs, Sweden). Cross-sections of specimens were observed under light microscopy following the procedures of Luft (1961) and Chang (1973). Specimens were taken from below the leaf apex at position of 90% distance of leaf length from the base. 1,500-nm thick sections were stained with periodic acid Schiff and examined under a Carl Zeiss Axioscope binocular light microscope (Carl Zeis, Zena, Germany).

Shelf-life and postharvest qualities

Storage shelf-life was recorded by comparing the harvested plants of unsuitable environment and control (suitable) conditions. Three to ten upper leaves were harvested and used for determining shelf-life and postharvest qualities. Each lettuce sample (150 g) was packaged in a polypropylene film bag (32 × 22 cm, 0.05 mm) and stored at 5 °C in a refrigerator. Storage was terminated when samples had visibly deteriorated (yellowing/spoil). The fresh weights were measured before and after storage of the specimens to calculate weight losses. Four different packaged samples were weighed at one day intervals throughout the duration of storage. Respiration rates were measured by inserting 150 g leaf lettuce samples into gas-tight containers for 1 h at 5 °C under airflow of 1 mL min^{-1} and four replications were used for each sample. CO_2 production was recorded to estimate respiration rate. Measurements were done using an automated sampling flow-through system (Lee et al. 2007) equipped with gas chromatograph (HP6890, Hewlett-Packard, Palo Alto, CA, USA) fitted with stainless steel Porapak Q column (80–100 mesh, 1 m × 3.2 mm) and a thermal conductivity detector [150 °C, He (50 mL min^{-1})].

Surface color was measured with a chromameter (CR-300, Konica-Minolta, Tokyo, Japan). Color was recorded using CIE-Lab uniform color spacing procedures (Hunter Lab, 2001). Ten positions of each leaf of the four samples were measured and average values were used for recoding color changes. The total difference (ΔE) was calculated using the following equation:

$$\Delta E = \sqrt{\{(L^*_0 - L^*)^2 + (a^*_0 - a^*)^2 + (b^*_0 - b^*)^2\}} \qquad (1)$$

where L^*_0, a^*_0, and b^*_0 represent readings at initial time (zero), and L^*, a^*, and b^* represent individual readings under a given storage condition. Total color changes were grouped as follows: 0–1.5 = detectable, 1.6–6.0 = appreciable, > 6.1 = noticeable.

A survey on external appearance changes was conducted referring the methods of Jeong et al. (1990), Lee et al. (2007), and Yang et al. (1991). Sensory properties, including external appearance and color change, were assessed by a three member's panel. Samples were scored on 0–6 scale with a five-step rating (6 = excellent, 4.5 = good, 3.0 = moderate, 1.5 = poor, and 0 = inferior).

Statistical analysis

Analyses were performed using the SAS software ver. 9.1 (SAS Institute, Cary, NC, USA). Data collected before plant harvest was subjected to split-plot analyses with three replicates. Postharvest data were subjected to analyse with four replicates. Analyses of variances (ANOVAs) were based on the Generalized Linear Model (GLM) initiated by the PROC GLM statement in SAS. LSD tests at 5% significance level were used to identify significant difference among pair of means, where ANOVA revealed a significant difference among means. The OriginLab software ver. 8.0, (OriginLab Co., Northampton, MA, USA) was used to generate graphical analyses and plots.

RESULTS AND DISCUSSION

Effects of growing conditions on growth parameters

Growth of leaf lettuce was varied with growing conditions and type of cultivars (Table 1). The growth of the four cultivars was affected by various growing conditions. Under the excessive soil moisture with shading treatments, plant height was increased but number of leaves, shoot fresh weight, and crude fiber content were decreased. Although, increased growth was observed in excess soil moisture with shading conditions, however the quality of the lettuces was decreased under that poor and unfavorable environment (Table 1).

Excess soil moisture with shading, and cultivars had significant effects on plant heights and numbers of leaves. Fresh weight was varied significantly with growing conditions but not by cultivars; leaf thickness varied significantly by cultivars but not with growing conditions. A significant interaction effect of growing conditions and cultivars on fiber content was observed (Table 1). 'Geockchima' plants were the tallest in excessive water with shading conditions, whereas the shortest plants was observed in

‘Cheongchuckmyeon’ (oak leaf type with green color) grown under control conditions. Plant height changed according to cultivars type, when the growing conditions were changed. However, plants grown under excess watering with shading conditions were taller than each of the cultivars grown under control conditions.

Table 1. Growth parameters of four lettuce cultivars subjected to excessive soil water and shading treatments and control conditions grown in a plastic greenhouse

Cultivar	Culture condition	Plant height (cm)	No. of leaves	Shoot fresh weight (g/plant)	Leaf thickness (mm)	Crude fiber (%)
Geockchima	Control	41.3	23.1	467.3	0.2	1.87
	Excessive soil water with shading	45.2	14.3	169.7	0.2	1.11
increase/decrease (%)		9.4	-38.1	-63.6	0.0	-40.6
Geockchuckmyeon	Control	35.0	22.3	467.3	0.4	0.90
	Excessive soil water with shading	37.2	11.0	132.0	0.5	0.82
increase/decrease (%)		6.2	-50.6	-71.7	25.0	-8.8
Cheongchima	Control	35.4	27.8	495.3	0.3	0.68
	Excessive soil water with shading	37.5	20.7	184.0	0.3	0.63
increase/decrease (%)		5.9	-25.5	-62.8	0.0	-7.3
Cheongchuckmyeon	Control	31.7	24.2	472.3	0.5	0.86
	Excessive soil water with shading	34.8	13.8	139.3	0.6	0.63
increase/decrease (%)		9.7	-42.9	-70.5	20.0	-26.7
Cultivar (A)		**	**	NS	**	**
Growing condition (B)		**	**	**	NS	**
A × B		NS	NS	NS	NS	**

NS, not significant at $P < 0.05$; *, ** Significant at $P < 0.05$ and 0.01, respectively.

The number of leaves also differed based on cultivars and growing conditions. In where, cultivar ‘Cheongchima’ had the highest number of leaves, followed by ‘Cheongchuckmyeon’ (oak-leaf type mixed with red color), ‘Geockchima’, and ‘Geockchuckmyeon’ in the plants grown under control conditions. The higher number of leaves was also produced in all cultivars grown under control conditions compare to excess water with shading conditions. Shoot fresh weight was differed little among cultivars when grown under control conditions; whereas, it was adversely affected by excess watering with shading (Table 1). Meanwhile, leaf thickness was varied among cultivars. ‘Cheongchuckmyeon’ had the thickest leaf, followed by ‘Geockchuckmyeon’, ‘Cheongchima’, and ‘Geockchima’. However, there was a non-significant effect of growing conditions on this trait. Although tendency was produced bit thicker leaves in excess watering with shading in cultivars ‘Geockchuckmyeon’ and ‘Cheongchuckmyeon’.

Shoot fresh weight was affected significantly by the growing conditions. It was severely affected than other parameters due to excess soil water with shading. Shoot fresh weight was led to about 63–72% decrease in excess soil water with shading compared to control for all cultivars (Table 1). Therefore, it might be concluded that shoot fresh weight was affected by growing conditions but not by the cultivars. The ‘Cheongchuckmyeon’ and ‘Geockchuckmyeon’ cultivars showed a greater decrease in shoot fresh weight than that of ‘Cheongchima’ and ‘Geockchima’. Morphologically similar cultivars showed similar trend of decreasing of fresh weight. The fresh weight of 'Cheongchuckmyeon' and 'Geockchuckmyeon' was decreased by 71% and 72%, respectively under excess water with shading treatment as compared to the control. Whereas, fresh weights of ‘Cheongchima’ and ‘Geockchima’ was decreased by 63% and 64%, respectively (Table 1).

Crude fiber content was significantly affected by all three factors: cultivar, growing conditions, and their interactions. Fiber is composed of long-chain polymers, including lignin and cellulose (Kwon, 1990; Choi et al., 2014; Kye, 2014). Fiber supports the plant structures in aerial environment. The plants grown in control condition contained more crude fibers than that of excess water with shading condition. Among the cultivars, the ‘Geockchima’ cultivar contained the highest crude fiber (Table 1). Lee et al. (2005) showed, in leaf lettuce cell osmotic solution, moisture, and cell structural fibers are affected by external environment. This experiment also showed crude fiber contains also changed due to cultural methods.

Growth was influenced by cultivars rather than growing conditions. Morphologically similar cultivars showed similar growth pattern. Thus, it was assumed that leaf lettuce cultivars of similar appearance have similar responses to the external environment. However, a leaf internal structure was changed due to presence of excessive soil water with shading (Fig. 1). Excessive soil water with shading increased plant height, but reduced both the number of leaves and plant biomass. Furthermore, in case of excess soil water and low light, intercellular spaces and bundle sheaths were widely spaced compared with those in the control conditions’ growing plants. In addition, the density of the leaf structures differed among the cultivars due to excess soil water with shading. Lettuce grown under excess soil moisture with shading showed reduced growth but increased size of individual cells. Loosen cell structure would be expected because of increase thickness of leaves as a result of presence excessive water inside cells. Lettuce grows well even under low light, but Nam (1996) reported that shading reduced the leaf thickness of lettuce. By contrast, we found that leaf thickness differed on growing conditions and also by cultivars. ‘Geockchima’ leaves showed little change in leaf thickness, either grown under excess water with shading or control condition. Simillarly, ‘Geockchuckmyeon’ leaves tended to be thicken in excess soil water with 50% shadingand also control conditions. Thus, it is clear that leaf thickness depends on cultivars rather than growing conditions.

Lettuce growth is not affected by shading down to 50% ambient illumination (Wolff & Coltman, 1989), but shading in combination with reduced nitrogen fertilization reduces growth rates in comparison with controls (Lee et al., 1998). Additionally, this experiment showed that shading can reduce growth in combination with other unfavorable conditions, like excessive soil water, in all cultivars. Lee et al. (1998 and 2005) also reported that growing conditions influence lettuce leaf morphology and finally effects on growth. Lee et al. (2008b) reported that the leaf thickness and volume of Chinese cabbage was affected by environmental conditions and postharvest salting on the characteristics of salted Chinese cabbage.

A

B

C

D

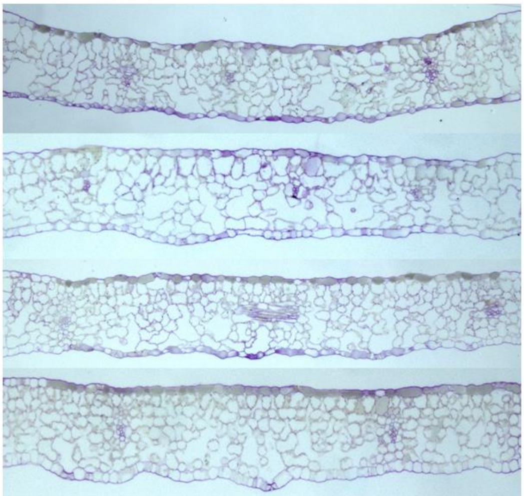

Figure 1. Cross-section micrographs of the lettuce cultivars ‘Geockchima’ and ‘Geockchuckmyeon’ under various culture conditions. (A) ‘Geockchima’ control, and (B) ‘Geockchima’ subjected to excess soil water with shading; (C) ‘Geockchuckmyeon’ control, and (D) ‘Geockchuckmyeon’ subjected to excess soil water with shading grown in a greenhouse.

Three leaf lettuce cultivars ‘Geockchuckmyeon’, ‘Cheongchuckmyeon’, and ‘Cheongchima’ responded differently to unfavorable environments. For example, leaf thickness of three of the four cultivars was changed differently with growing environments, whereas that was not significantly affected by excess soil water with shading in ‘Geockchima’ cultivar. Therefore, it could be marked that some cultivars are not susceptible to growing conditions. Our findings confirmed that excess soil water with shading affected growth, cell morphology and crude fiber content of leaf lettuce.

Effects of growing conditions on the postharvest qualities

Fresh weight loss ratio during postharvest storage at 5 °C was influenced by growing conditions and cultivars (Fig. 2). Weight loss ratio was increased in three cultivars except ‘Geokchima’ in case of plants grown under excess soil water with shading condition, which is confirmed by the findings of Lee et al. (2005). According to Yang et al. (1991), fresh weight loss is more affected by postharvest factors, such as packaging and storage conditions, than by growing conditions. However, growing conditions could become crucial if storage conditions are fixed.

Furthermore, the postharvest respiration rate was also influenced by growing conditions. In general, the shelf-life of harvested crops is reduced by high rates of respiration (Kader, 2002). Postharvest respiration rates were higher in three cultivars except ‘Geokchima’ grown under excess soil water with 50% shading than that of control (Fig. 3). The respiration rate of cultivar ‘Cheongchuckmyeon’ was double at 10 days of storage, while grown under excess soil water with 50% shading compare to control. The differences in respiration rate of cultivar ‘Geockchima’ between the excess water with 50% shading and control were negligible. In contrast, Lee et al. (2005) reported no significant effects of growing conditions on postharvest respiration rates. The effects of treatment on respiration rates may be due to changes of morphology caused by excessive soil water with 50% shading. We infer that an alteration of cell size due to growing environment causes difference in respiration rates. This putative relationship warrants further investigation. Moreover, the effect of cultivars are also be explored in more details.

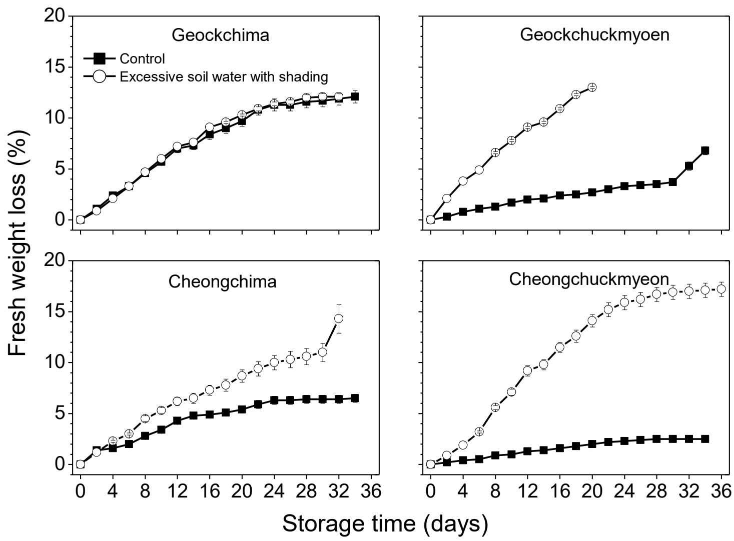

Figure 2. Effect of cultivation under excessive soil water and shading treatments, and control conditions, on fresh weight loss of the four lettuce cultivars stored at 5 °C. Values are means ± standard errors (n = 4).

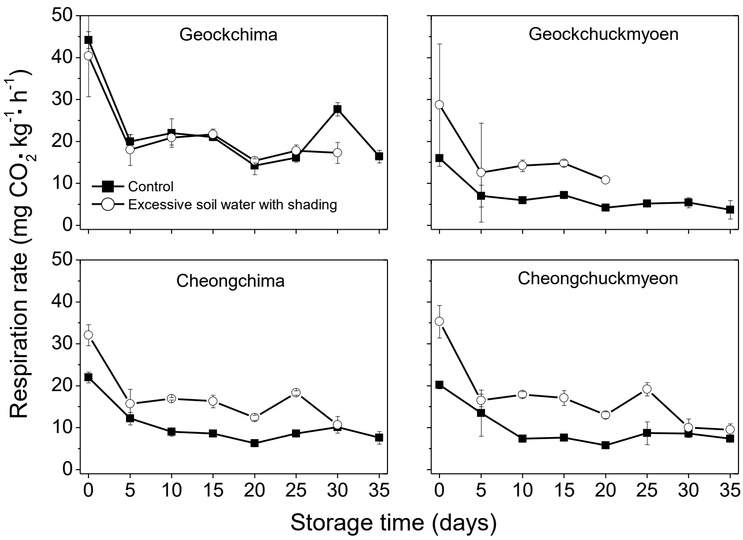

Figure 3. Postharvest respiration rates of four lettuce cultivars at 5 °C according to cultivation under excessive soil water, shading, or control conditions in a greenhouse. Values are means ± standard errors (n = 4).

The effects of growing conditions on changes in lettuce color during postharvest storage were assessed (Fig. 4) according to the method described in the manual Hunter Lab (2001). Total color change (ΔE) values of all lettuce samples were increased markedly with advancement of storage duration. Color changes were started apparently after 4days of storage. However, the effects of growing conditions were detectable in the 'Cheongchuckmyeon' and 'Cheongchima' cultivars. In these two cultivars, color changes were greater in control plants than those in the treatment groups. Total color change (ΔE) differed between the early and late periods of storage. However, color changes values had no significant difference between two growing conditions for the cultivars 'Geokchima' and 'Geockchuckmyeon' (Fig. 4). In this experiment, 'a' (greenness) values with excessive soil water and 50% shading was negative in compared to control. Although the differences of color change values was small. This experiment revealed that the postharvest quality of leaf lettuce could be reduced despite of grown under favorable control conditions. The degree of color change in the favorable control condition was larger than that resulting from excessive soil water with 50% shading, the absolute value of color change was remarkable in favorable control condition. Therefore, optimum storage conditions are necessary to ensure a good quality product, regardless of its initial condition. According to Lee et al. (2005), color changes in stored leaf lettuce are closely associated with changes in chlorophyll content, which is more influenced by cultivars than by growing conditions. The results reported here agree with this earlier work.

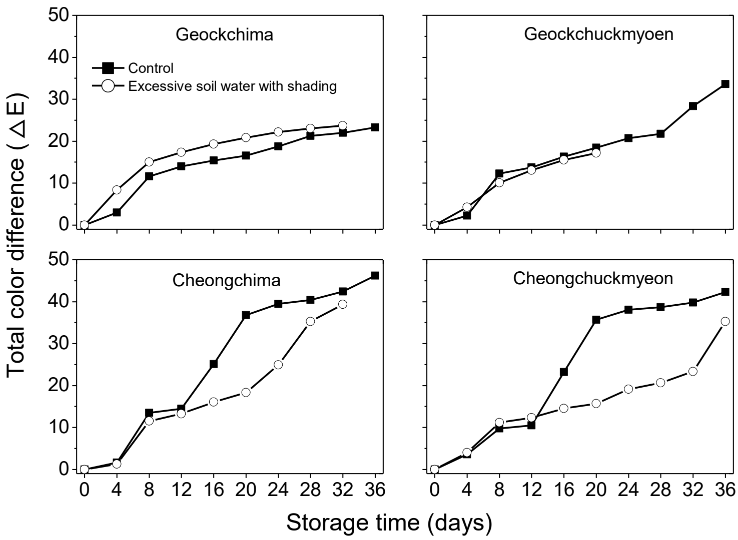

Figure 4. Effects of cultivation under excessive soil water, shading, or control conditions in a greenhouse on total color differences (ΔE) of the four lettuce cultivars stored at 5 °C. Total color difference: 0–1.5 = detectable, 1.6–6.0 = appreciable, > 6.1 = noticeable.

Qualitative measures of lettuce freshness judged by appearance and that was differed by cultivars and growing conditions (Fig. 5). Marketability represents by the time at up to which the product retain a value of 4. The marketability period for 'Geockchima' was 10 days for control condition growing product group, whereas it was 8 days for the product group grown under excess soil water with 50% shading condition.

The marketability period for ‘Geockchuckmyeon’ was 6 days in the control group and 4 days in the excess soil water with 50% shading group. The marketability period for ‘Cheongchima’ was 4 days in the control group and 8 days in the excessive soil water with 50% shading group. The marketability period for ‘Cheongchuckmyeon’ was 6 days in the control group and 12 days in the excess water with shading group. Hence, marketability was dependent on both cultivars and growing conditions. The marketability with large color changes was decreased more rapidly in ‘Cheongchima’ and ‘Cheongchuckmyeon’. The ‘Geockchima’ and ‘Geockchuckmyeon’ cultivars exhibited the least-marked color change, which was influenced by growing conditions (Fig. 4). Fig. 5 shows that the growing conditions had different effects on shelf-life depending upon the external appearance of cultivars. Cultivars ‘Geockchima’ and ‘Geockchuckmyeon’ had longer shelf-life when grown in control condition than those grown under excess soil water with 50% shading condition. Conversely, ‘Cheongchima’ and ‘Cheongchuckmyeon’ had shorter shelf-life when grown under excess water with 50% shading condition compared to control. It is assumed that there is a relationship of shelf-life with chlorophyll content of the cultivars (Agüero et al., 2008; Lee et al., 2008a). However, we cannot provide evidence of this due to lack of a pigment content analysis. The shelf-life of lettuce tended to be shorter in the presence of high chlorophyll (Lee et al., 2008a). However, broccoli florets tended to be increased in the presence of chlorophyll (Shi et al., 2016). This seems plausible, but requires further experimentation. These findings are in agreement with previous reports on vegetable quality is influenced by excess water supply, growing conditions, and combinations of other factors, including genetic factors and environmental conditions during storage (Beverly et al., 1993, Magwaza et al., 2017).

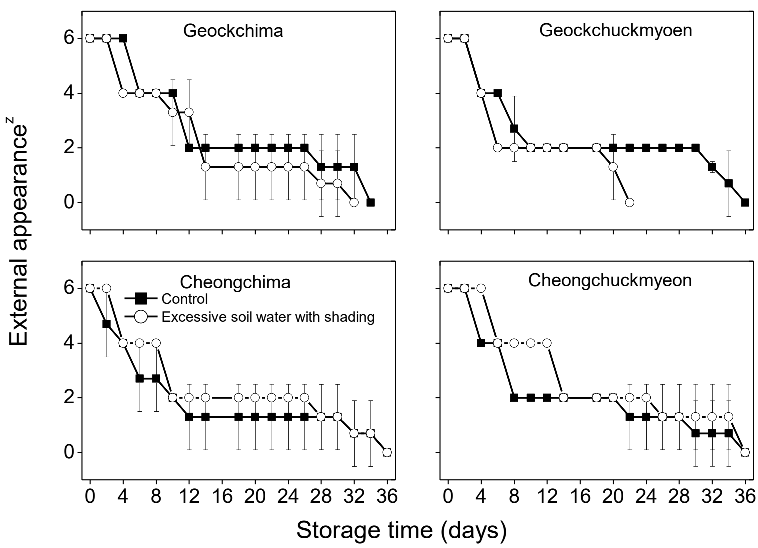

Figure 5. Effects of cultivation under excessive soil water, shading, and control conditions on the external appearance of the four lettuce cultivars grown in a greenhouse and stored at 5 °C. External appearance: 6 = very good, 4 = good, 2 = poor, 0 = very poor. Values are means ± standard errors (n = 4).

CONCLUSIONS

The aim of this study was to determine the effects of growing conditions, including excess soil water with 50% shading and cultivars on postharvest quality of leaf lettuce. The crossed design allowed separation of the effects of these two sets of factors and showed that conditions affected the postharvest qualities of the lettuce. Plant height, plant weight and number of leaves were affected by growing conditions and by cultivars; leaf thickness was affected by cultivars. Weight loss during storage was accelerated in three of the four cultivars (the exception was 'Geockchima'). Similarly, respiration rate was elevated in plants of three cultivars subjected to excess watering and 50% shading during cultivation. However, color of 'Cheongchima' and 'Cheongchuckmyeon' during storage was not markedly changed when plants grown in excess watering with 50% shaded compared to controls. It could be concluded that leaf lettuce should be grown in normal growing condition for getting higher yield, but for extending postharvest storage days with marketability; they should be grown under excess soil water with 50% shading conditions.

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Influence of chemical composition on the biochemical methane potential of agro-industrial substrates from Estonia

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Abstract. Batch trials were carried out to evaluate the Biochemical Methane Potential (BMP) of 61 different substrates collected from agricultural farms and industrial sites in Estonia. Tests were performed in 500 mL plasma bottles at 36°C. The highest methane yield from all tested substrates was obtained from unconsumed dairy products (557 ± 101 L kg^{-1} VS) while the lowest was obtained from animal slurries (238 L kg^{-1} VS $\pm$ 42). From tested energy crops, foxtail millet achieved the highest methane yield (320 L kg^{-1} VS). Silages from different crops presented methane yields from 296 ± 31 L CH_4 kg^{-1} VS to 319 ± 19 L CH_4 kg^{-1} VS. The influence of chemical composition and kinetic rate constants (k) on methane potential was analyzed. Anaerobic digestibility of selected agro-industrial substrates was markedly influenced by their organic content, i.e. total proteins and lignin concentrations. Rate constants were found to correlate negatively with hemicellulose, cellulose and lignin ($p < 0.05$). Results from this study suggest that an appropriate characterization of the chemical composition of the substrates is important not only for predicting BMP and the kinetics rates, but also for identifying possible inhibitors during the anaerobic digestion process. Results on the BMP and national availability of studied substrates indicate that herbal biomass and agro-industrial residues are promising substrates for biogas production in agricultural biogas facilities in Estonia.

Key words: Biogas, Biomass, Biochemical Methane Potential, Kinetic rate, Agro-industrial, Wastes.

INTRODUCTION

Climate change mitigation is a matter of great interest worldwide, in which renewable energy systems have gained high interest due to their low carbon footprint and high environmental sustainability. Energy from biomass stands at the fourth largest energy source in the world, due to resource availability in rural and urban areas and pollution reduction in the case of municipal solid waste (Frank et al., 2016). Data on the energy potential of different organic substrates have been widely studied, resulting in more efficient reactors with process and inhibition control (Munoz et al., 2015).

However, bioreactors with incorporated instrumentation, control and automation are very rare in developing countries, due to economic and training skills limitations.

Research projects in northern European countries have extensively study biomass availability, chemical characteristics and its influence on the energy potential to identify substrates with high potential for the energy industry. Among the most suitable substrates are forestry and agricultural residues, sewage and industrial organic wastewater, municipal solid wastes and livestock and poultry slurries (Gissen et al., 2014).

Several studies have been conducted on the assessment and comparison of biogas and methane potential of different substrates at different experimental scales (lab, bench, pilot and industrial scale). However, few studies have been conducted on the comparison of different biomass residues and its relation between the chemical composition and the bioenergy potential. Those studies usually report data on no more than 10 different substrates. Correlation of methane potential with different parameters of biomass composition have widely been studied. Results obtained have consolidated predictive models of specific methane yields from different sources of biomass. The most studied correlations between methane yield and chemical composition of biomass are cellulose, hemicellulose, crude fat, acid detergent lignin (ADL), acid detergent fiber (ADF) (Herrmann et al., 2016). However, results from those studies are limited to: 1) few datasets mainly from residual biomass and 2) correlations between methane yield of different substrates ratios or samples of the same crop species.

Studies from different protein-rich substrates have shown to have great potential for methane production. Unfortunately, high concentration of such materials may cause anaerobic digestion instability due to the release of ammonia nitrogen (NH_3-N). Ammonia, which is released from the degradation of amino acids during acidogenesis, has been identified as a common inhibitor for different microorganisms involved during the anaerobic digestion process, in which the most sensible are the methanogens (Yuan & Zhu, 2016). Low methane yields have been attributed to insufficient ammonia nitrogen (500 mg L^{-1}) due to low microbial activity and buffering capacity, while excessive concentration (> 5,000 mg L^{-1}) may result in biogas inhibition (Yenigün & Demirel, 2013).

Anaerobic degradation of biomass is reduced by enfolded cellulose and hemicellulose in lignin. This is explained by limited accessibility of particulate substrate by microorganisms during the hydrolytic phase (Herrmann et al., 2016). The synthesis of complex polymers is fundamental for enzyme penetration and efficient biodegradation during all steps of anaerobic digestion: hydrolysis, anaerobic fermentation and methanogenesis.

Nutrients are categorized as micronutrients (also known as trace elements) and macro-nutrients. It is very important to check nutrient concentration at first to avoid poor performance of anaerobic digestion or biogas inhibition. Trace elements are one of the significant factors for micro-organisms growth and activity as they play an essential role for many physiological and biochemical processes. Lack of understanding of metabolic behavior of trace element requirements of methanogens could result in low methane yields, acidification and process instability during anaerobic digestion of energy crops. Therefore, proper nutrient concentrations in the digester is important for enhancement of methane and biogas production and process stability. Poor management of process control could derivate in high concentration of trace elements in the digestate triggering possible toxicity risks during its disposal and/or utilization as biofertilizer.

Degradation rate of compounds can be described by a means of a differential kinetic equation. Methane prediction of a specific substrate can be achieved by knowing the biodegradation kinetics. The first order kinetic constant (k) represents a measure of biodegradability rate. The higher the k value, the higher the biodegradability of the substrate in the digestate.

In Estonia, there is estimated an area of around 286 thousand hectares of abandoned agricultural land that can be considered for cultivation of energy crops and around 128 thousand hectares of semi-natural grasslands (Astover et al., 2008). The calculated theoretical herbal biomass production is up to 2 billion tons per year (Roostalu & Melts, 2008). In Estonia, there are other agro-industrial sources of biomass that can also be considered for the production of biogas, such as fermentation slops from brewery industry, unconsumed milk products, grain mill residues, etc. Nowadays, Estonia has 18 biogas plants, in which 5 are based on agro residues.

The novelty of the present research study stands on the correlation analysis of dataset obtained from the chemical composition of 61 different agro-industrial residues and its relation with the methanogenic potential and the kinetic rate constants. The aim of this study is to evaluate methane yield of the main agro-industrial substrates of Estonia and to identify main chemical parameters that affect methane yield.

MATERIALS AND METHODS

Inoculum

The inoculum was collected from an anaerobic reactor from a facility located at Tallinn, Estonia. The reactor works as part of a wastewater treatment plant (Estonia). Sludge samples collected were gently stirred and filtered with a 2 mm mesh to remove large particles. For the trials, the sludge was previously incubated for one week at mesophilic temperature (36 °C) under a headspace of N_2/CO_2 (80:20) for degasification (consumption of residual organic matter). The main characteristics of the inoculum were as follows: total solids (TS) 22.1 g L^{-1}, suspended solids (SS) 15.7 g L^{-1}, volatile suspended solids (VSS) 598 g kg^{-1} SS.

Feedstock

Based on biomass availability, 61 substrates were collected from different locations (agricultural farms and industrial sites) in Estonia. The substrates selected were: energy crops (jerusalem artichoke with and without flowers, sunflower collected at 2 different periods, hemp collected at 2 different periods, Amur silvergrass, energygrass and millet), silages (grass, maize, alfalfa, timothy grass and red clover), hay, animal slurries (cattle and pig) and agro-industrial residues such as brewery residues (distillery slops) and grain mill residues (aspiration dust, bran and flour) and unconsumed milk products. For the case of energy crops, i.e. silage and hay samples, they were conditioned by milling to achieve particles size of 1 mm. All samples were stored in plastic boxes in a fridge at 4 °C before use.

Experimental procedure

The Biochemical Methane Potential (BMP) test performed in this study was based on a modified version of the guidelines described by Owen et al. (1979). The experiment was carried out in triplicate using 575 mL plasma bottles containing 150 mL of inoculum (in-reactor biomass concentration 7.26 g VSS L^{-1}) and 0.3 g TS of each substrate. Distilled water was added to reach an effective volume of 200 mL. A set of 3 bottles without substrate were prepared for each batch to study the methane production of inoculum (blank test). Previous work (Luna del-Risco et al., 2011) has indicated that inoculum collected from Tallinn wastewater treatment plant is sufficient in providing the nutrients necessary for operating a successful BMP test and thus no additional nutrient medium was added. The bottles were closed and the headspace was flushed with N_2/CO_2 (80:20). Test bottles were incubated at 36 °C in a set of Mermet isothermal chambers. Samples were incubated for up to 78 days, and stirred manually twice a day. Biogas production and gas composition were determined periodically. Cumulative methane yield was calculated as the sum of methane produced over the incubation period minus the methane yield in blank test. Biogas production was expressed at standard conditions (0 °C, 1 atm.) per kilogram of TS or VS of substrate added to the test.

The rate of degradation of substrates was assumed to follow the first–order kinetics as done by Gunaseelan (2009). Methane production was modeled by fitting the experimental data with the first-order decay rate model (Eq. 1) in GraphPad 5.0.

$$B = B_{max} \cdot [1 - \exp(-k \cdot t)], \quad (1)$$

where B is the cumulative methane yield (L kg^{-1} TS or L kg^{-1} VS) at time t (days); B_{max} is the maximum methane yield (L kg^{-1} TS or L kg^{-1} VS) and k is the first-order decay rate constant (1 d^{-1}).

Analytical methods

Substrates were analyzed for pH, total solids (TS), volatile solids (VS), total organic carbon (TOC), total nitrogen (TN), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (ADL), calcium (Ca), phosphorus (P), magnesium (Mg) and potassium (K). pH was measured by a Sentron 1001pH to check samples. TS was measured by drying substrates for 24 hours at 105 °C and VS by incineration at 550 °C for 2 hours. TOC was determined by catalytically-aided platinum 680 °C combustion technique (Shimadzu TOC-V), TN was determined by copper catalyst Kjeldhal method using a Kjekltec Auto 1030 and total proteins (TP) were calculated by multiplying total nitrogen values by a factor of 6.25 (TP = TN*6.25) in the case of plant biomass and by a factor of 6.38 for milk proteins (Merrill & Watt, 1955, Merrill & Watt, 1973). NDF and ADF were determined using a Foss Tecator Fibertec 1020. Lignin was determined as described by AOAC 973.18 method. On the basis of NDF, ADF and ADL analysis, hemicellulose (NDF-ADF) and cellulose (ADF-ADL) concentrations were calculated as proposed by Van Soest et al. (1991). Ca, P and Mg were determined using a Fiastar 5000 following the o-Cresolphthalein complexone method (Connerty & Briggs, 1966), the stannous chloride method (ISO/DIS 15681-1, 2001) and the titan yellow method (Heaton, 1960), respectively. Total fat and proteins concentrations of unconsumed milk products were taken from the manufacturer.

Biogas production was measured by pressure increase in test bottles using a calibrated pressure transducer (0–4 bar, Endress & Hauser), equipped with needle and a valve to avoid gas leakages during measurements.

Methane content was analyzed chromatographically using a gas chromatograph (CP-4900 MICRO-GC, Varian Inc., Palo Alto, CA 94304) that was equipped with an ultra-low volume thermal conductivity detector (TCD) and two columns (molecular sieve 5A and Porapak Q), with the former for analyzing gaseous hydrogen (H_2), oxygen (O_2), methane (CH_4), carbon monoxide (CO), and nitrogen (N_2), and the latter for carbon dioxide (CO_2). The operating conditions for the micro-GC were 10 s for stabilization, 100 ms for sample injection, 30 s for sampling, 120 s for running, and 8 s for backflushing. The temperatures for the sampling line, columns, and the injector were set at 50, 80, and 110 °C, respectively. Argon at a pressure of 4.2 kg cm^{-2} was used as the carrier gas and its flow rate was automatically controlled by the micro-GC. Methane yield was expressed as normal L (273 K and 1,013 mbar) per kg of VS (kg^{-1} VS).

Statistical analysis

The dependence of methane potential, (i.e. highest cumulative methane yield achieved in the BMP test, and rate constant values, k) on the chemical composition of substrates was studied by correlation analysis. Statistical analyses were performed with STATISTICA version 8.0.360.0 (Statsoft, Inc.) using the Shapiro–Wilk's test for normality. Correlation analysis was done by calculating Pearson's correlation coefficients (r) and their significance levels *p*. *p*-values below 0.05 were regarded as significant.

RESULTS AND DISCUSSION

Chemical composition of substrates

Results on the chemical composition of substrates analyzed are presented in Tables 1, 2 and 3. Due to a wide variety of substrates from different sources, a specific set of analyses were considered for each group independently. Lack of information for some parameters was due to errors in the chemical characterization.

Overall, results obtained in this study are consistent with the findings of other authors. Chemical composition of silages and hay (Tables 1 and 2) is very similar to that reported by Amon et al. (2007) and Dinuccio et al. (2010). Concentrations of macro and micro-nutrients found in this study (Table 1) are similar to the findings of Baležentienė & Mikulionienė (2006) for timothy silages (P: 2.8 g kg^{-1} TS; Ca: 2.1 g kg^{-1} TS; Mg: 0.4 g kg^{-1} TS; K: 27.1 g kg^{-1} TS). Organic content and fiber concentrations found in animal slurries (Table 2) appear to be consistent with the findings of Thygesen et al. (2014). Chemical composition of energy crops (Table 2) is within the same range of that found by other authors (Mursec et al., 2009; Klimiuk et al., 2010; Pakarinen et al., 2011; Uusitalo et al., 2014). Chemical composition of unconsumed dairy products and selected agro-industrial residues (Table 3) was similar to the results from Dubrovskis et al. (2009) and Dinuccio et al. (2010).

Table 1. Chemical composition of different silages from Estonia

	n	TS (%)	SD	VS (%)	SD	Total Proteins (g kg^{-1} TS)	SD	Hemicellulose (g kg^{-1} TS)	SD	Cellulose (g kg^{-1} TS)	SD	Lignin (g kg^{-1} TS)	SD	P (g kg^{-1} TS)	SD	Ca (g kg^{-1} TS)	SD	Mg (g kg^{-1} TS)	SD	K (g kg^{-1} TS)	SD
Grass silage	4	31.4	6.7	92.7	0.5	114	9.2	219	15.9	ND	-	ND	-	2.55	0.3	6.11	0.8	1.44	0.15	24.5	1.8
Maiz silage	3	17.4	0.6	95.2	0.5	98.5	8.5	266	37.7	ND	-	ND	-	1.97	0.4	4.82	0.3	1.59	0.19	14.8	0.9
Silage mix*	19	29.4	8.6	92.0	1.8	147	25	178	42	ND	-	ND	-	2.81	0.5	9.22	2.3	1.95	0.3	23.5	4.9
Hay	4	91.3	0.4	93.7	1.5	99.2	16	272	53.5	354.6	39	58	21	ND	-	ND	-	ND	-	ND	-

n: number of samples tested for same substrate (each sample was analyzed in triplicate); * Mixture of different ratios of grasses and legumes silages; Mix rate not specified; ND: Not Determined.

Table 2. Chemical composition of animal slurries, some energy crops and hay from Estonia

Substrate	n	TS (%)	SD	VS (%)	SD	Hemicellulose (g kg^{-1} TS)	SD	Cellulose (g kg^{-1} TS)	SD	Lignin (g kg^{-1} TS)	SD
Animal slurries											
Pig slurry	1	7.0	2.7	79.4	2.8	145	18	104	12.5	72	5.8
Cattle slurry*	9	7.8	2.8	78.2	3	107	13	167	7	112	10
Energy crops											
Jerusalem Artichock	2	21.4	1.3	95	4	49.8	7	234.6	36	53.8	5
Sunflower	2	25.8	1.5	89	2.4	62.4	15	307	47.1	80	3.9
Energy grass	1	27.2	1.6	93	3.1	273.3	20	378.5	32.1	96.5	4.9
Hemp	2	30.2	0.9	94	6	107	1.6	544	8	79.5	11.4
Amur Silvergrass	1	36.4	2.1	95	4.8	301	15	420	21.6	70	12.8
Foxtail millet	1	22	1.3	92	6.2	316	32	330	15.4	53.4	8.7

n: number of samples tested for same substrate (each sample was analyzed in triplicate); * TN = 4.32 (0.34) g kg^{-1} TS.

Biochemical Methane Potential (BMP) and kinetic rate constants

BMP and kinetics rates constants obtained in this study were compared with similar studies conducted in countries such as Finland, Denmark, Sweden, Hungary, Austria, among others.

BMP results are grouped according to their origin and presented in Table 4. Cumulative methane yields for grass silage, maize silage and mix silage were 319 L CH_4 kg^{-1} VS, 307 L CH_4 kg^{-1} VS and 296 L CH_4 kg^{-1} VS, respectively. In a study conducted by Lehtomäki et al. (2008), they obtained a methane yield of grass silage of 300-372 L CH_4 kg^{-1} VS. Those results are consistent with our findings.

For maize silage, Pobeheim et al. (2010) found methane potentials ranging from 295 to 370 L CH_4 kg^{-1} VS. Our results from methane yield of hay (286 L CH_4 kg^{-1} VS, Table 4) are similar to the result from Kaparaju et al. (2002) who found a value of 270 L CH_4 kg^{-1} VS. Cattle and pig slurry presented a methane potential of 238 ± 42 L CH_4 kg^{-1} VS and 317 L CH_4 kg^{-1} VS, respectively. Vedrenne et al. (2008) found methane potential for pig slurry of 175–350 L CH_4 kg^{-1} VS. For cattle slurry, a methane potential of 243 L CH_4 kg^{-1} VS was found in the study conducted by Steffen et al. (1998). Results on the methane potential of studied energy crops are presented in Table 4. Heiermann et al. (2009) found an average methane potential of 280 ± 30 L CH_4 kg^{-1} VS and 297 ± 108 L CH_4 kg^{-1} VS for hemp and Jerusalem artichoke, which are in agreement with the results of this study (289 L CH_4 kg^{-1} VS and 310 L CH_4 kg^{-1} VS, respectively). For sunflower, Antonopoulou et al. (2010) found a methane potential of 260 L kg^{-1} VS, slightly lower than the value measured in this study (296 L CH_4 kg^{-1} VS).

Pokój et al. (2010) studied amur silver grass and obtained a methane potential of 210 L kg^{-1} VS which is much lower than the result from this study (317 L CH_4 kg^{-1} VS). Similarly, the methane yield of millet (323 L CH_4 kg^{-1} VS) was lower than those observed by Mahamat et al. (1989) (257 L CH_4 kg^{-1} VS). This variation on the methane potential of sunflower, amur silver grass and millet could be explained by differences in harvesting time or chemical composition. For energy grass (Szarvasi-1), Janowszky & Janowszky (2002) have reported methane potential of 300–350 L CH_4 kg^{-1} VS, slightly higher than the value of this study (290 L CH_4 kg^{-1} VS).

To our knowledge, only few studies on dairy derived products have been conducted on the methane potential of unconsumed milk products (whey, expired milk, poor quality industrial leftovers). The authors have found a study conducted by Alkanok et al. (2013), in which results from dairy product wastes obtained was 350 L CH_4 kg^{-1} VS. Dinuccio et al. (2010) found a methanogenic potential of 501 L CH_4 kg^{-1} VS for whey. This result appears to be within the same range of our findings (480–660 L CH_4 kg^{-1} VS).

For grain mill residues, the methane yield observed in this study (328 L CH_4 kg^{-1} VS) was much higher than the results reported by Dubrovskis et al. (2009) who obtained a methane yield of 130 L CH_4 kg^{-1} VS from grain mill wastes. This variation can be explained by the difference in the chemical composition of the substrate. Methane potential of distillery slops (358 L CH_4 kg^{-1} VS, Table 4) was in the same range as the results obtained by Steffen et al. (1998) for fermentation slops (338 L CH_4 kg^{-1} VS).

To characterize the conversion rate of selected substrates during anaerobic digestion, kinetic rate constants k were calculated and the values obtained are shown in Table 4. Kinetic rate constants are key elements to quantify the speed of substrate biodegradation.

Table 3. Chemical composition of unconsumed milk products and selected agro-industrial residues

Substrate	n	TS (%)	SD	VS (%)	SD	TOC (g kg^{-1} TS)	SD	Hemicellulose (g kg^{-1} TS)	SD	Cellulose (g kg^{-1} TS)	SD	Lignin (g kg^{-1} TS)	SD	Total Proteins (g kg^{-1} TS)	SD	Fats (g kg^{-1} TS)	SD
Unconsumed Cheese*	3	36.4	17.1	98	1.6	ND	-	ND	-	ND	-	ND	-	334	200	495	234
Unconsumed Milk	4	11.7	9	99	0.2	ND	-	ND	-	ND	-	ND	-	295	53	277	63
Grain mill residues	3	86	6	92	2.2	415	41	313.1	96	140	64	50.7	10	ND	-	ND	-
Distillery slops	2	75	2.8	92	1.4	455	50	ND	-	ND	-	ND	-	ND	-	ND	-

n: number of samples tested for same substrate (each sample was analyzed in triplicate); ND: Not Determined; *: includes sour cream.

Table 4. Kinetic rate constants are key elements to quantify the speed of substrate biodegradation

Substrate	n	CH_4 kg^{-1} TS	SD	CH_4 L kg^{-1} VS	SD	k d^{-1}	SD
Grass silage	4	296	19	319	19	0.172	0.02
Maiz silage	3	292	21	307	21	0.150	0.02
Silage mix*	19	272	31	296	31	0.230	0.05
Hay	4	268	33	286	33	0.086	0.01
Pig slurry	1	252	21	317	21	0.139	0.13
Cattle slurry	9	186	42	238	41	0.092	0.04
Jerusalem Artichocke	2	294	4	310	7	0.179	0.02
Sunflower	2	262	8	296	15	0.154	0.04
Energy grass	1	270	17	290	19	0.061	0.06
Hemp	2	272	9	289	11	0.095	0.01
Amur Silvergrass	1	300	32	317	38	0.064	0.06
Foxtail millet	1	296	27	323	29	0.101	0.10
Unconsumed Cheese**	3	644	60	658	56	0.260	0.07
Unconsumed Milk	4	478	24	481	24	0.344	0.03
Grain mill residues	3	300	38	328	49	0.160	0.03
Distillery slops	2	331	35	358	33	0.131	0.03

n: number of samples tested for same substrate (each sample was analyzed in triplicate); * Mixture of different ratios of grasses and legumes silages. Mix rate not specified; ** Includes sour cream.

The fastest kinetic rate constant was found for unconsumed milk products (0.344 ± 0.03 d^{-1}) while the slowest was found for energy grass (0.061 d^{-1}). As for agricultural biomass, k for grass silage, maize silage, silage mix and hay varied between 0.086 and 0.230 d^{-1}. Chynoweth et al. (1993) found conversion rate constants for different ensiled substrates (millet, energycane, napiergrass) ranging from 0.072 to 0.106 d^{-1}. In the case of animal slurries, k values for pig slurry were higher than for cattle slurry. Conversion rate constant for cattle manure (0.092 d^{-1}, Table 4) is similar to the result from Sánchez et al. (2000) who found a value of 0.086 ± 0.004 d^{-1}.

As for energy crops, the highest k value was found for Jerusalem artichoke (0.179 ± 0.02 d^{-1}). This variation between the kinetic rates obtained could be explained by the concentration of the lignocellulosic fraction of the substrates. For agro-industrial substrates, the lowest rate was found for distillery slops (0.131 ± 0.03 d^{-1}). In a study conducted by Jiménez et al. (2004) on the anaerobic digestion of untreated molasses, a conversion rate constant of 0.14 d^{-1} (9g COD added) was found. Conversion rates of unconsumed dairy products (0.260–0.344 d^{-1}, Table 4) were slightly lower than results obtained by Najafpour et al. (2009) for cheese whey (0.358 d^{-1}). Different chemical composition of the substrates could explain the difference in the rates. All results are explained by the influence of biomass composition with the biodegradation rate. Substrates with high concentrations of compounds such as lignin will result in process slow-down. In our results, chemical composition variance between cattle and pig slurry can explain the difference on the kinetic rates.

Correlations between chemical composition and BMP

Correlations between the cumulative methane production (in L CH_4 kg^{-1} TS) and the methane production rate constant with the chemical characteristics of substrates are presented in Table 5 and Figs 1 to 4.

Table 5. Pearson's correlation of cumulative methane yields and kinetic rate constants with the chemical composition of agro-industrial substrates

Variable	n	Cumulative methane yield		Kinetic rate constant	
		r	*p*	r	*p*
TS	60	-0.168	0.221	-0.109	0.413
VS	60	0.785	< 0.001*	0.033	0.8
TOC	7	0.36	0.427	0.425	0.401
Total Proteins	37	0.767	< 0.001*	0.249	0.136
Fats	7	0.365	0.421	-0.139	0.765
Hemicellulose	45	0.343	0.029*	-0.514	< 0.001*
Cellulose	20	-0.1	0.722	-0.505	0.023*
Lignin	18	-0.917	< 0.001*	-0.789	0.008*
P	26	-0.473	0.016*	0.741	< 0.001*
Ca	26	-0.563	0.002*	0.702	< 0.001*
Mg	26	0.059	0.771	0.513	0.007*
K	26	-0.613	<0.001*	0.764	<0.001*

* Statistically significant correlations ($p < 0.05$).

Among the different chemical parameters, only VS, total proteins, hemicellulose, lignin, P, Ca and K showed significant influence on the methane yield as single independent variables (Table 5). As expected, one of the main parameters influencing methane yield was organic matter, i.e. VS content, whose correlation with methane production was significantly positive. Proteins, at optimal concentrations, are also known to stimulate methane formation positively and therefore high methane yield can be attained from substrates rich in proteins (Nielfa et al., 2015).

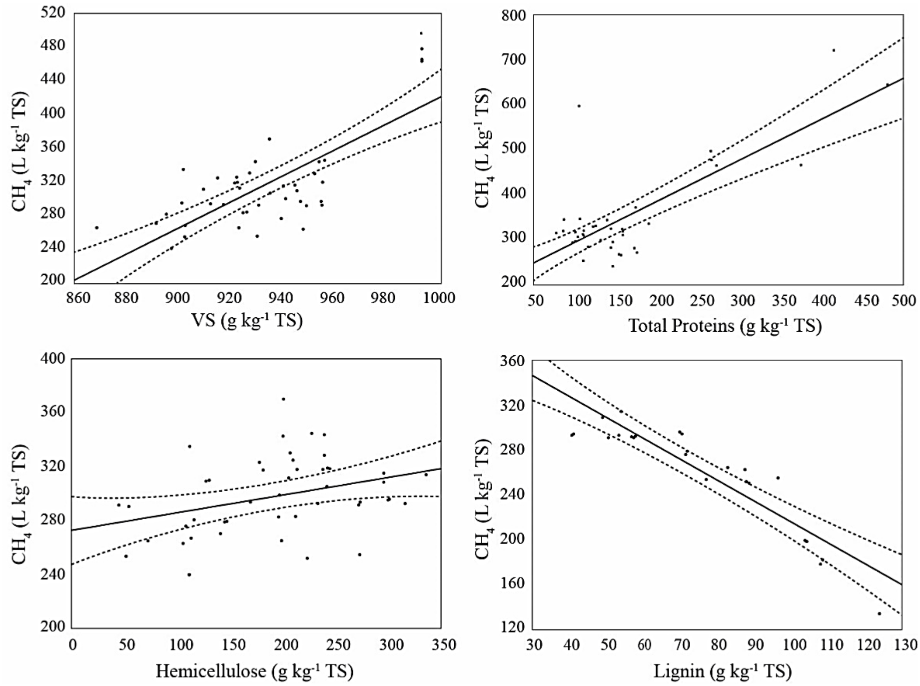

Figure 1. Pearson's correlation between methane yield and chemical parameters ($p < 0.05$). 95% confidence intervals are presented in dash lines. Y-axis values may vary compared with others.

In the case of biomass fiber composition, hemicellulose correlated positively with methane production ($p < 0.05$), although the correlation was poor. For cellulose, no significant correlation was found. Previous studies confirm that cellulose and hemicellulose can be bioconverted into methane and carbon dioxide during anaerobic digestion. However, degradation rate of cellulose depends mainly on whether it is lignin-incrusted or in a crystalline form (Klimiuk et al., 2010). Lignin content presented a strong negative correlation with methane production. Our results appear to be consistent with the findings of other authors (Klimiuk et al., 2010; Triolo et al., 2011; Pecorini et al., 2016).

Macronutrients (P, Ca, and K) were only measured for silages and their Pearson's correlations with methane yield were found negative and statistically significant. P and Ca are known for being essential for metabolic reactions and growth of anaerobic bacteria, but they can become toxic when present in high concentrations (Van Langerak et al., 1998; Chen et al., 2008). In our study, concentrations of these elements in the biomass were not excessively high to provoke a negative effect on methane production. So, it can be assumed that variations on the chemical composition of crops samples such as grasses, silages and hay and its different ratios affected the methane yield and therefore attributed for the negative correlation obtained.

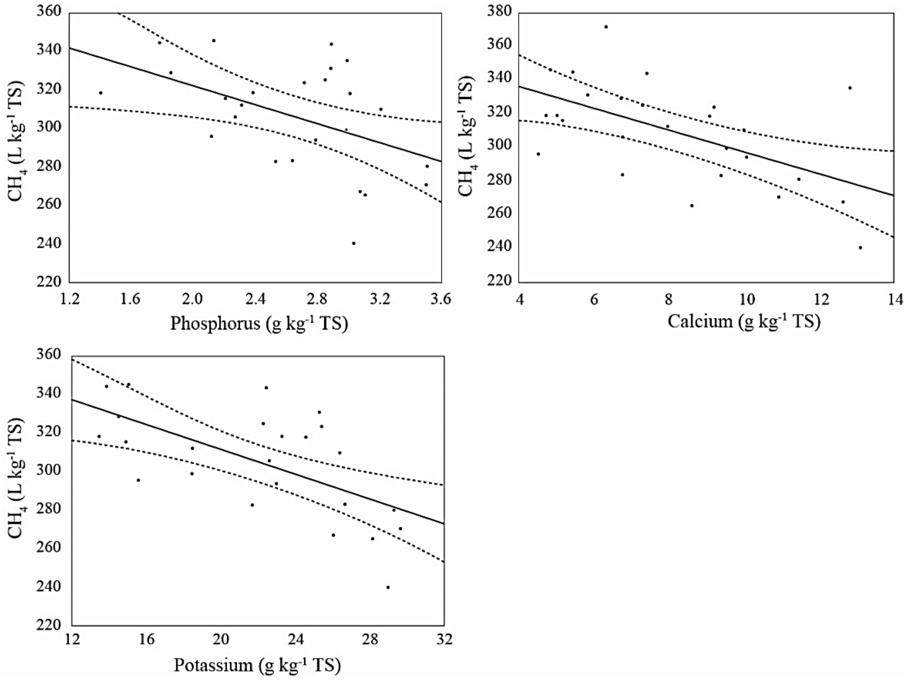

Figure 2. Pearson's correlation between methane yield and chemical parameters ($p < 0.05$). 95% confidence intervals are presented in dash lines. Y-axis values may vary compared with others.

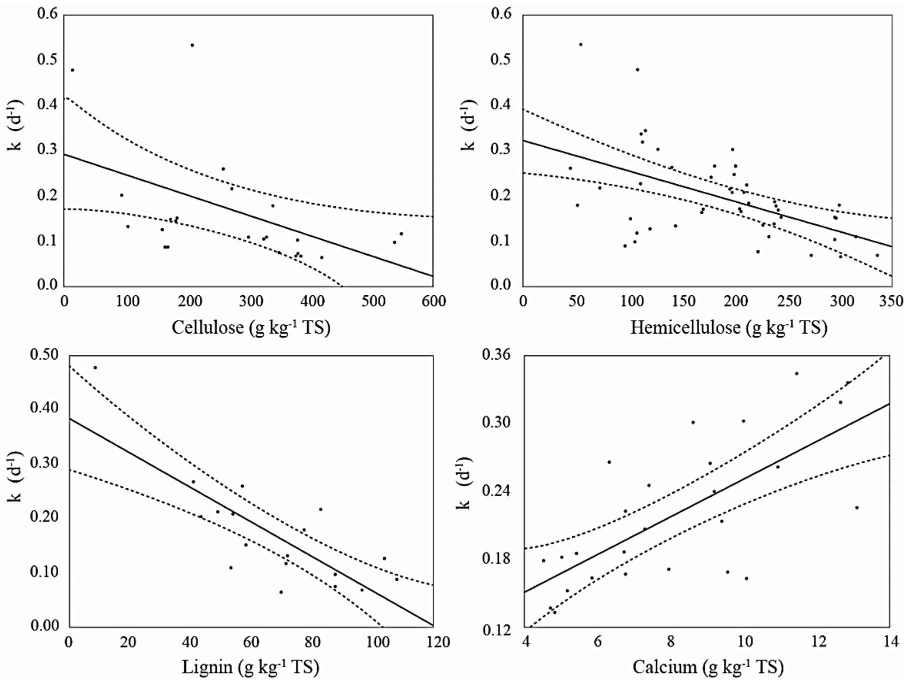

Figure 3. Pearson's correlation between methane production rate constant and chemical parameters ($p < 0.05$). 95% confidence intervals are presented in dash lines. Y-axis values may vary compared with others.

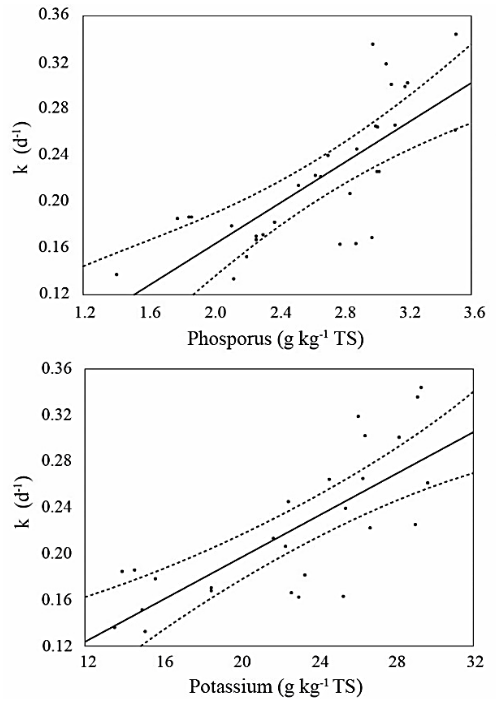

Figure 4. Pearson's correlation between methane production rate constant and chemical parameters ($p < 0.05$). 95% confidence intervals are presented in dash lines. Y-axis values may vary compared with others.

Accumulation of mineral elements in plants depends on soil properties, cultivation and fertilization, climate, harvesting time as well as plant properties (Juknevičius & Sabienė, 2007). Various plant species have a different ability to accumulate mineral elements, therefore content of Ca, P and K can differ significantly in different crops, especially between legume and grass species (Baležentienė & Mikulionienė, 2006). Concerning the methane production rate constant (k), positive correlations ($p < 0.05$) were only found for P, Ca, Mg and K (Table 5, Figs 3 and 4). These results suggest that P and light metal ions enhance the speed of the anaerobic biodegradation process. The most rapid bioconversion of studied substrates occurred in the tests with unconsumed milk products which contained high amount of proteins. In contrast, Figs 3 and 4 showed that high concentration of lignocellulosic material (hemicellulose, cellulose and lignin) in the substrate, resulted in low rate of methane production.

CONCLUSIONS

This study confirmed that studied Estonian substrates are suitable for bioenergy production by means of anaerobic digestion. Methane potential from unconsumed milk products should be considered for its integration into the Estonian energy market for bioenergy production. However, special attention on inhibitors control shall be considered from this kind of biomass to avoid process failure. Herbal biomass such as energy crops, silages, and hay presented also relatively high biochemical methane potential. Due to their high availability in Estonia, these substrates could be considered as potential source for biogas production in rural areas, and be considered as suitable co-substrates to animal slurries to increase biogas yield. As biogas is produced mainly from landfills and sewage sludge, methanisation of agro-industrial wastes could represent a potential effort to reach the established goal to cover 3% of transport energy use by 2020.

Correlation of chemical composition parameters identified lignin with the highest influence on specific methane yields. Methane yield decreases when lignin content and fibre fractions increases. Although, anaerobic digestion of agro-industrial wastes is extensively used in countries such Denmark, Germany, Austria, Sweden, in Estonia the utilization of such substrates in anaerobic digestion plants have not been widely applied. The results of this study positively highlight the bioenergy potential of studied substrates for the Estonian renewable energy mix.

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Soil organic carbon in long–term experiments: comparative analysis in Slovakia and Serbia

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Abstract. Soil organic carbon plays an important role in a long-term agroecosystem productivity, in the global C cycle, maintaining a soil nutrient pool and improving its availability. The objective of this study is the assess the impacts of long–term cropping practices on SOC dynamics in Slovakia and Serbia. Soil C sequestration is a complex process that is influenced by many factors, such as agricultural practice, climatic and soil conditions. For the both location the initial SOC decline was followed with the C stabilization and possible increase where proper practices were used. More intensive crop management systems that maintained residue cover provided the greatest benefit towards increasing the quantity of mineralizable nutrients within the active fraction of soil organic carbon (SOC), as well as increasing C sequestration as SOC. Long–term field experiments have contributed significantly to our current knowledge of soil quality and have been used to study the influence of crop management, fertilizer application and tillage practices on SOC content.

Key words: soil organic carbon, long–term experiment.

INTRODUCTION

Agricultural cultivation causes an immediate and rapid loss of soil organic carbon (SOC). The loss of SOC with tillage based on plowing could lasts for decades, reducing carbon (C) pools on agricultural soils up to 50% of their original levels on average (Mann, 1986). Examples of reduced soil organic matter have been observed worldwide regardless of climate, soil type, or original vegetation (Janzen, 2004). Generally, the rate of loss slows as SOC levels reach a new equilibrium that depends on tillage practices (West & Post, 2002) and the amount level of C inputs returned to the soil as crop residue or animal manures (Kirchmann et al., 2004). The quantity of existing soil C is controlled by a complex interaction of processes determined by C inputs and its decomposition rates. Returning crop residues to the field is highly recommended, in order to decrease chemical inputs, and promote soil C sequestration (Turmel et al., 2014). Tillage accelerate SOC oxidation to CO_2 by improving soil aeration, increasing contact between soil and crop residues and exposing aggregate-protected organic matter to microbial decomposition (Birkás et al., 2008). On the other hand, improved agricultural practices have great potential to increase C sequestered in soil (Follett, 2001; Lal, 2006; Dimassi, 2014), and some studies suggest that agricultural activities can elevate SOC content relative to natural systems (Buyanovsky & Wagner, 1998). Likewise, SOC stock is recommended indicators for evaluation of soil quality in EÚ (Michéli et al., 2008). Management practice that includes organic amendments can increase SOC, but the type of organic amendment, method of incorporation, and duration of application required for an elevation of SOC differ in relation to climate and cropping practices (Hoffmann et al., 2002; Woźniak et al., 2014; Rusu & Moraru, 2015; Šařec & Žemličková, 2016). Sequestration of C from plant biomass into organic matter is a key sequestration pathway in agriculture (Macák et al., 2010). Deficiency of macronutrients can be easily compensated with fertilizer application, however building the pool of SOC is a long–term and rather slow process. Similarly to that, physical soil properties, predominantly soil structure and compaction, mutually deteriorate together with SOC and commonly interact in lower production capacity of soil. Relative enrichment of the surface soil with organic matter results in an increase in microbial activity and a concomitant increase in the size and stability of soil aggregates (Carter & Stewart, 1996; Tamm et al., 2016). It is could be anticipated that the cropping technology cannot be fully transformed toward SOC conserving cultivation. However, some adjustments can notably influence SOC in soil, such as shallow plowing after wheat harvest, organic fertilizers, and soil loosening instead of plowing (Lal et al., 1998). For the region of the Central Europe, the critical level of SOC for a significant yield reduction has not been clearly established. Key and Angers (1999) argued that irrespective of soil type the with SOC content less than 1% it may not be possible to obtain potential yield. But generally it may be assumed that the critical limit of SOC in the temperate region is 2% (Loveland & Webb, 2003).

The objective of this study is the assess the impacts of long-term fertilization practices on SOC dynamics in Slovakia and Serbia.

MATERIALS AND METHODS

Experimental site in Slovakia

Field trial were conducted over 12 year at the experimental station of the Slovak University of Agricultural in Nitra in South–Western Slovakia in Dolná Malanta as a key side of monitoring web system for *Luvisols*. We refer results from two periods of trial 1996–2003 as a first period of research (Macák et al., 2010) and 2005–2007 (Candráková et al., 2011), as the second period of trial. The experimental site is located in a warm and moderate arid climatic region. The long-term average annual temperature of the site is 9.9 °C and 16.6 °C during the vegetation period (Table 1).

Table 1. Average monthly air temperatures and precipitation (1951–2000) at the experimental station of the Slovak University of Agricultural (Špánik et al., 2004) and Novi Sad (RHSS, 2017)

Months	Experimental station in Nitra		Experimental station Novi Sad	
	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)
January	-1.4	29.1	0.5	38.5
February	0.5	30.1	2.0	30.8
March	4.8	31.6	6.6	34.7
April	10.4	41.6	12.0	48.5
May	15.2	56.0	17,4	58.5
June	18.3	66.2	20,5	91.7
July	20.0	59.3	22,2	77.4
August	19.7	54.2	21.9	66.4
September	15.5	43.1	16.6	60,5
October	10.2	41.0	11.7	61.1
November	4.6	52.2	6.5	61.0
December	0.5	43.2	1.2	52.5
Average	9.9	-	1.6	-
Total		547.6		681.6

The average precipitation is 548 mm, including 323 mm during the vegetation period. Altitude of the site is 175 m. The experimental design was a split-plot with four replicates. The tillage was the main plot factor; the fertilization was the subplot factor. The subplots were 3 m wide by 10 m long with 0.5 m protective stripes on all sides with 2 × 9 m harvest plots. The plots were subjected to primary soil tillage treatments as follows: mouldboard ploughing (CT) to a depth of 22 cm (conventional tillage), and shallow loosening (RT) to a depth of 10 cm (reduced cultivation). Three fertilization treatments as follows: 0–without organic and inorganic fertilization, PH–mineral fertilizers (phosphorus and potassium) calculated to the 3 t ha^{-1} seed yield, PR–incorporation of all above-ground plant material and dose of phosphorus and potassium calculated to the 3 t ha^{-1} seed yield. The amount of preceding above-ground plant material was measures from harvested area of 18 m^2 at each PR treatment with four replication and values were calculated per one hectare. Nutrients were added on the base of balance method according to nutrient content in soil on yield level of 3 t ha^{-1} pea seeds under the normative nutrients with drawing per 1 ton of crop. The same method was used for all crops in crop rotation, nitrogen doses were calculated according to the content of N_{an} in soil samples at spring (Fecenko & Ložek, 2000). Common pea was growing after cereal preceding in spring barley (*Hordeum vulgare* L.) – common pea (*Pisum sativum* L.) – winter wheat (*Triticum aestivum* L.) – maize (*Zea mays* L.) crop rotation and from 2001 in winter wheat – common pea – maize – spring barley – red clever (*Trifolium pratense* L.) crop rotation. The soil samples for basal respiration and C sequestration were collected from the 20 cm topsoil layer by soil auger three times per year (in spring, summer and autumn). The soil samples were incubated at 28 °C and soil respiration was measured 17–18 days according Bernát and Seifert method. SOC content was determined by the Tyrin method. We considered the average values of soil basal respiration from three samples. For organic carbon stock (kg ha^{-1}) calculation, the soil bulk density was determined three times per year in each treatment with four replication from the soil layer from a depth of 5–10 cm by soil core Eijkelkamp sampling kit. The results were subjected to ANOVA analysis and Tukey HSD test by using software STATISTICA.

Experimental site in Serbia

A long–term experiment (LTE) titled 'Plodoredi' (Crop rotation) is situated at the Rimski Šančevi experimental field of the Institute of Field and Vegetable Crops in Novi Sad (N 45° 19`, E 19° 50`) on the southern border of the Chernozem zone of the Pannonian Basin. During the study period, the average annual temperature of 11.6 °C and 681 mm of precipitation was observed (Table 1). The experiment started in 1946/47, to conceptually correspond with the prevailing cropping technology in agricultural area, and to employ the achieved results in yield improvement. (Milošev et al., 2010). The unfertilized treatments were established 1946/47, and fertilized in 1969/70. The following treatments were analyzed: fertilized 3–year crop rotation: winter wheat, maize and soybean (MSWF); fertilized 2–year crop rotation winter wheat and maize (MWF); fertilized wheat monoculture (WWF); unfertilized 3–year rotation winter wheat, maize and soybean (MSW), and unfertilized 2–year rotation winter wheat and maize (MW). Crop rotation was arranged as single crop sequence in which all crops were grown each year according to the experimental design, and plots were divided into three subplots (90 × 30 m) representing the repetitions. Conventional tillage with mouldboard plough (30 cm), harrow disc, and cultivator (Compactor) were performed. Harvest residues were incorporated by ploughing. Winter wheat sowing was done in October, 20, while maize and soybean in April, 10. SOC content was determined by the Tyrin method. In our study the 0–60 cm layer of soil was analyzed as the significant changes were expected in that layer that was divided into 3 sub-layers (0–20 cm, 20–40 cm and 40–60 cm). The samples were collected after crop harvest in a disturbed state and were kept in the laboratory air-dried until the analysis. The average soil sample consisted of 5 drillings. Bulk density sampling for each treatment was carried out after harvest by the core method using cylinders of 100 cm^3 volume by Kopecky. Each year, samples were taken from three soil layers (0–60 cm) in three replicates per experimental plot. Data reported for SOC stock and yield were analyzed by the analysis of variance, ANOVA was used to separate treatment means when there was a significant difference at the $P < 0.05$ level. Replication across treatments and effects of year was considered a random effect and cropping systems were considered a fixed effect. In order to analyze temporal interdependencies of soil organic matter regression analysis was conducted. The independent variable (x) was time and SOM content (%) was dependent variable (y).The data were statistically processed by using the program STATISTICA series 12.6.

RESULTS AND DISCUSSION

From the starting point in 1996 the tillage treatments via different level of soil disturbance influenced the potential flux of CO_2 expressed as soil basal respiration of the soil and input output balance of soil C stock.

The average values for eight years significantly indicated higher soil basal respiration in reduced tillage treatments represented by shallow loosening (Fig. 1). Soil basal respiration is defined as rate of respiration in soil, which originates from the mineralization of organic matter (Creamer et al., 2014). In our experiment, reduced tillage create better precondition for basal respiration, which indicated higher potential of biological activity mainly due to the higher content of organic substrate of topsoil.

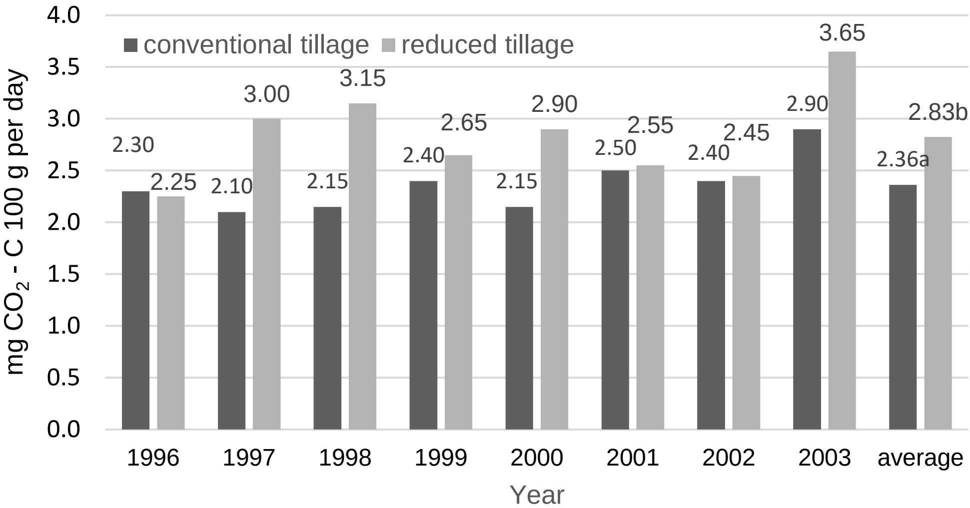

Figure 1. Soil basal respiration in conventional and reduced tillage treatment from 1996–2003, Slovak University of Agriculture in Nitra, Experimental Station Dolná Malanta, the average means at different letter are significant at the $P < 0.5$ probability level.

Mineral fertilizers may support the basal respiration via increasing of roots biomass nutrient availability and strongly correlated with soil organic carbon (Dimassi et al. 2014) but in oure fertilization treatments average soil basal respiration ranged only in narrow interval of 2.52–2.65 mg CO_2–C 100 g per day without statistical differences at the $P < 0.5$ probability level. The reduced tillage has positive influence on soil carbon sequestration with comparison to conventional mouldboard ploughing (Table 2).

Table 2. Soil carbon stock (t ha^{-1}) in each tillage and fertilization treatments. The means between fertilization treatments (small letters) and tillage treatments (capital letters) followed by the same letter are not significant at $P < 0.05$ probability level

Conventional tillage/fertilization treatments	1996–2003	2005–2007	Reduced tillage/fertilization treatments	1996–2003	2005–2007
CT-0	30.9a	29.1a	CT-0	32.2a	31.1a
CT-PH	31.2b	30.2a	CT-PH	32.9a	33.4b
CT-PZ	32.1b	33.4b	CT-PZ	32.6a	33.9b
Conventional tillage	31.4A	30.9A	Reduced tillage	32.5B	32.8B

Both fertilization treatments reached the higher soil C stock with comparison to treatments without any form of fertilization. In control treatment without fertilization 31.4 t ha^{-1} of C in 20 cm soil layer was stored with comparison to 32.5 t ha^{-1} in treatments with forecrop biomass incorporation. After another four year treatment history in 2007 the significant influence of tillage on soil C stock was confirm. Temporal change in SOC can be defined in two ways as an absolute change in stored C which provides an estimate of the actual C exchange between soil and atmosphere or as a net change in storage among treatments (Ellert et al., 2008). Some differences between the treatments with application of mineral fertilizers (PH) or organic and mineral fertilizers (PZ) with control treatments (RT-0) are associated with the decomposition of incorporated plant residues and great amount of biomass production (roots, exudates and post-harvest residues) (Table 2, Figs 2–4).

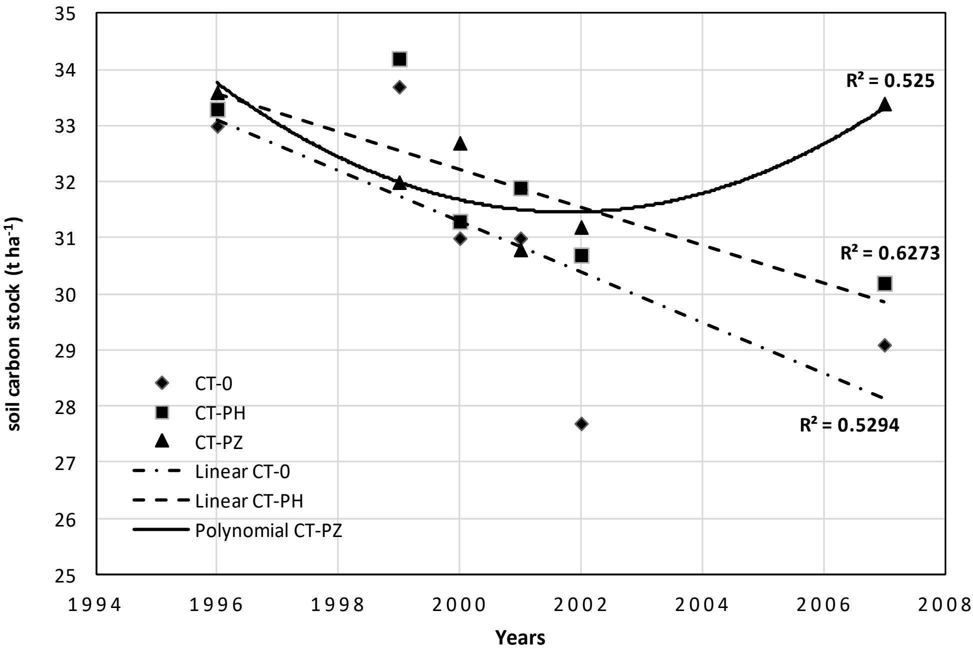

Figure 2. The changes of the soil carbon stock (t ha^{-1}) in the upper soil layer of 20 cm at different fertilization treatments in conventional tillage. SAU Nitra, Experimental station Dolná Malanta, Slovakia.

In treatments with incorporation of aboveground plant material (PZ) average input of aboveground dry matter calculated for whole rotation cycles was 5.15 t ha^{-1}. Non significant differences between treatments with application of mineral fertilizers (PH) and fertilizers plus incorporation of all aboveground plant biomass (PZ) of growing crops indicated that for increase of stocked SOC in the soil profile another input of SOM is needed. Similarly Hernanz et al. (2009) for active sequestration recommended adoption of another measure as crop rotation, tillage treatment and also input of organic matter.

The results of the long–term field trials on chernozem soils showed that properly applied agronomic practices such as crop rotation, fertilization, and tillage are the most appropriate method for preservation of SOC content in soil. The application of N fertilizers resulted in greater aboveground and belowground biomass in the fertilized treatments, which was beneficial for maintaining SOC in the soil but not sufficient for C enrichment, while mouldboard plowing resulted with SOC depletion (Manojlović et al., 2008).

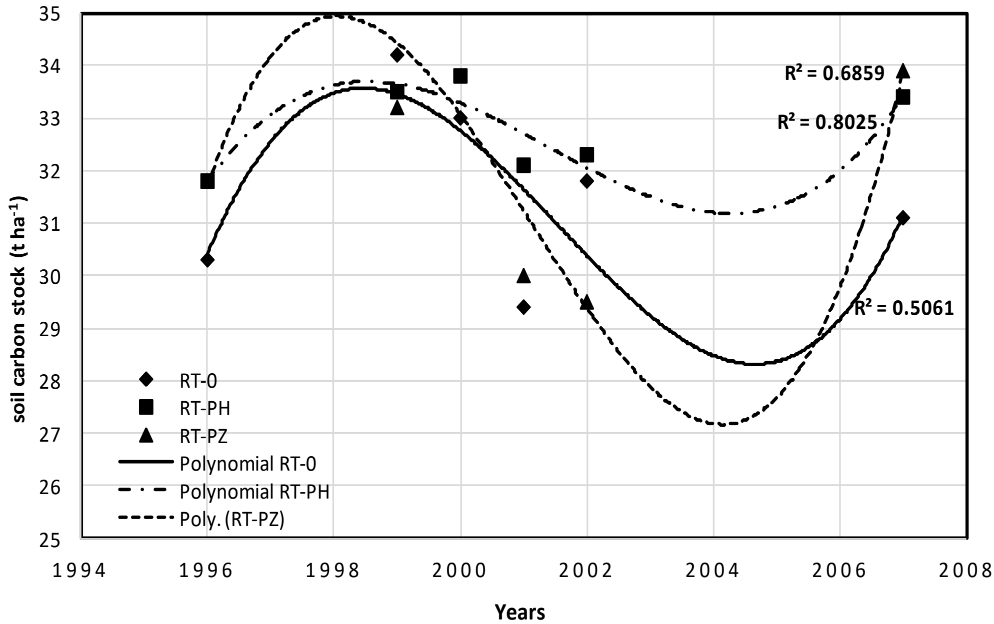

Figure 3. The changes of the soil carbon stock (t ha^{-1}) in the upper soil layer of 20 cm at different fertilization treatments in reduced tillage. SAU Nitra, Experimental station Dolná Malanta, Slovakia.

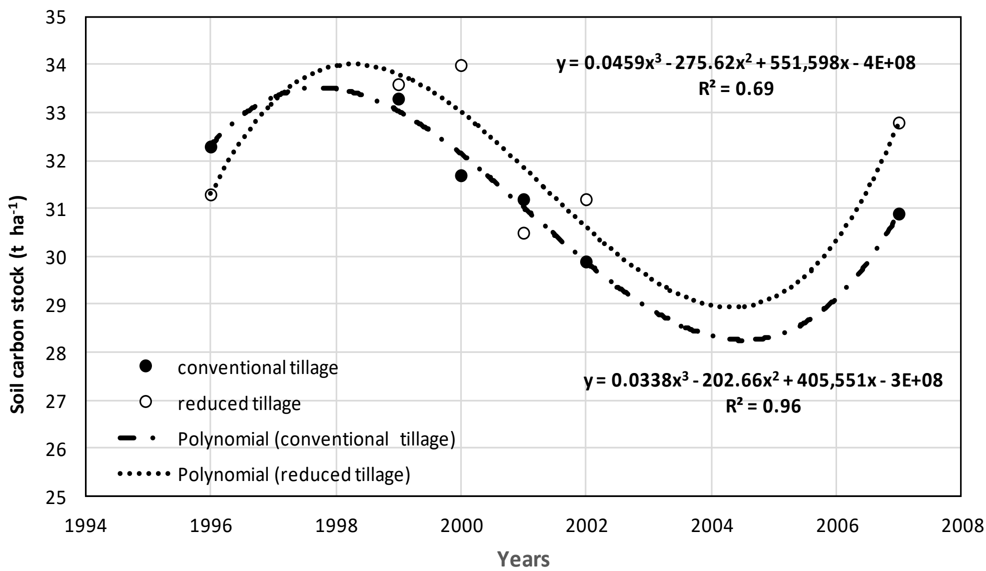

Figure 4. The changes of the soil carbon stock (t ha^{-1}) in the upper soil layer of 20 cm at conventional and reduced tillage treatments. SAU Nitra, Experimental station Dolná Malanta, Slovakia.

Regression coefficients showed significant SOC change over time that can be explained with the quadratic equation. Higher correlation was found at 3–year fertilized rotation whereas 3-year unfertilized rotation can not be explained with quadratic function (Fig. 5). This indicate stabile trend of SOC change with less variation. Generally, for the most cropping system SOC stabilization occurred after 2000 that could be attributed with the improved crop residue management in relation with advanced machinery. The preservation of SOC in wheat monoculture, found in the LTE, coincided with findings reported by Lithourgidis et al. (2006) that under continuous wheat cropping particular soil properties (such as SOC) could be preserved. By contrast, the higher content of SOC at WWF in this study did not correspond with higher yields of winter wheat, which indicates that long–term monoculture is not a sustainable cropping alternative for wheat production (Miloševet al., 2014). Although the soil under permanent wheat monoculture exerts some favorable physical and chemical soil properties, it cannot be recommended for wide adoption as a management option, since significant yield variation could be expected as well as and pathogen proliferation. Defining a clear relationship between the agricultural yields of wheat and the OM is not always possible to determine, because the grain refers to the plot, and the amount of returned C is conditioned by the effect of a number of factors which can have a significant impact on OM (infestation level, rainfall, pest attack, the time of sowing, fertilising etc). The fertilized two–year and 3-year rotations can be considered as potential alternatives for sustaining yield and preserving soil properties with improved cropping technology (Šeremešić et al., 2011).

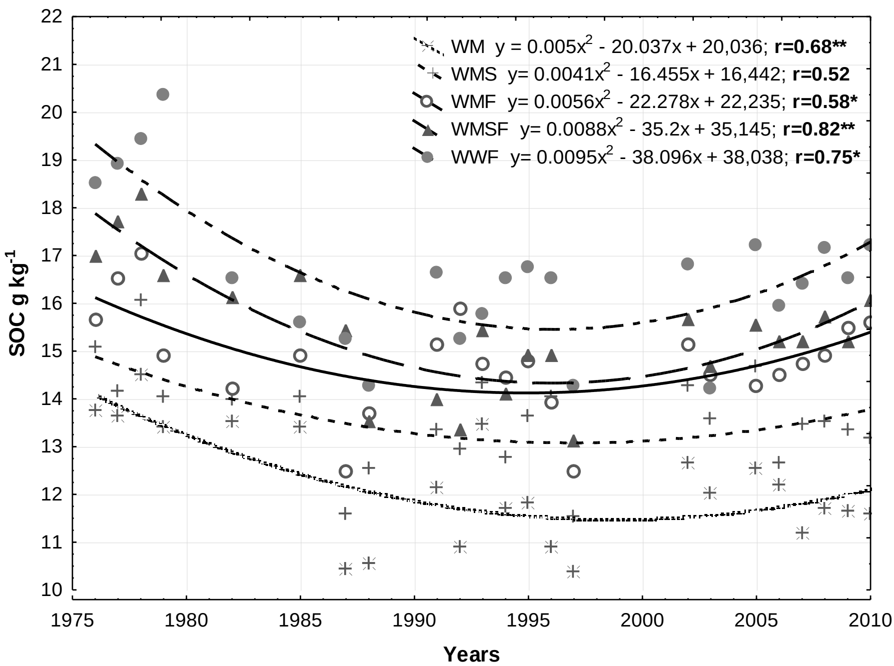

Figure 5. Regression fit of soil organic carbon (SOC) from the LTE experiment (1975–2010). **significant at $P < 0.01$ probability level; *significant at $P < 0.05$ probability level; fertilized 3-year rotation (MSWF); fertilized 2-year rotation (MWF); fertilized wheat monoculture (WWF); unfertilized 3-year rotation (MSW) and unfertilized 2-year rotation (MW).

The average SOC stock for selected cropping systems (0–20 cm) showed significant differences for the analysed period. Significantely higher SOC stock was observed in winter wheat monoculture compared with WMF, WMS and WM (Fig. 6). Significantely lower value was found at the unfertilized 2-year rotation comapred to other systems. Besides that, the unfertilized crop rotation contain 80% of the SOC found at the fertilized rotation. The study conducted at the same long-term experiment showed that additional C input did not increase the SOC pool, suggesting that the invetsigated cropping systems had a limited ability to increase SOC (Šeremešić et al., 2017).

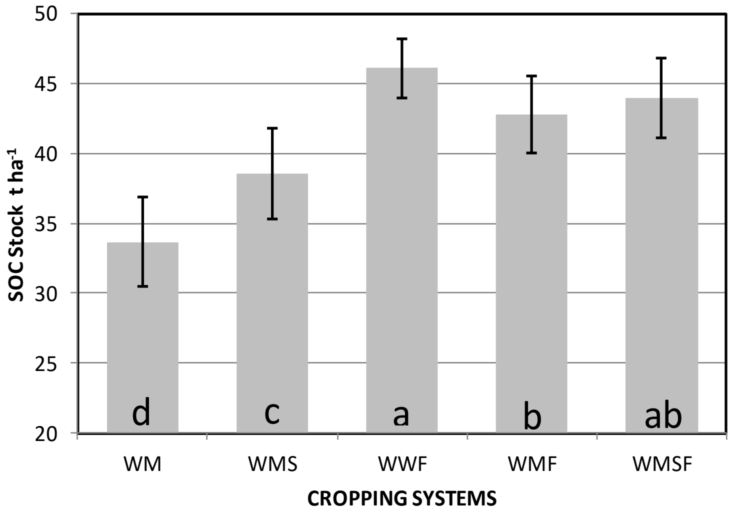

Figure 6. Average soil carbon stock (t ha^{-1}) of different cropping systems in LTE calculated from 1975–2010 (0–20 cm). The column followed by the same letter are not significant at $P < 0.05$ probability level; fertilized 3-year rotation (MSWF), fertilized 2-year rotation (MWF), fertilized wheat monoculture (WWF), unfertilized 3-year rotation (MSW) and unfertilized 2-year rotation (MW).

Further on, explaining the relationship between the crop residues C input and SOC must implicate on the fact that crop are grown in rotation. Therefore, in our study C inputs were accounted as average for the entire rotation. On the basis of the dispersion diagram (Fig. 7) the amount of C in plant residues and SOC content in the soil can be displayed using the regression curve, covering the average values of SOC content on different systems of farming for 2 layer of soil and the amount of C remaining in the plant residues inputs. The values of the coefficient of reggresion shows that their relationship is largely explained and that there is a strong positive correlation between the total average C incorporated with crop residue and SOC content in the soil. For the 0–20 cm soil depth higher regresion was observed.

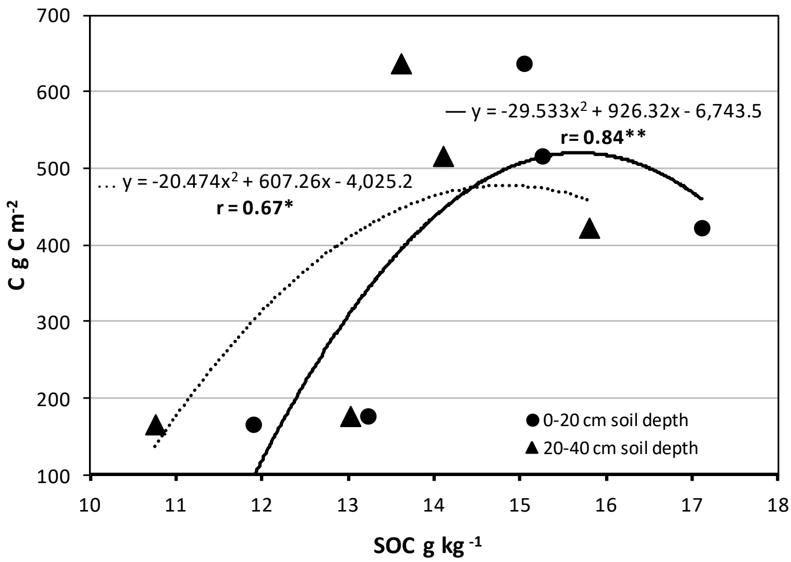

Figure 7. Relationship of C input and soil organic matter (SOC) for different depths.

CONCLUSIONS

The fertilization treatments do not revealed the significant differences in soil basal respiration in an average. Higher soil basal respiration and more CO_2 was realised from reduced-till compared to conventional tillage despite there being increased levels of soil C. The reduced tillage has positive influence on soil C sequestration with comparison to conventional mouldboard ploughing. The NPK treatment was important for increasing crop yields, organic material inputs, and soil C fractions, so it could increase the sustainability of cropping system in the long–term experiments in Slovakia and Serbia. The recommended fresh residues C necessary for SOC maintenance is estimated at approximately 500 g C m^{-2} per year. In addition to that, soil tillage was found to be significantly related to changes in SOC.

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Anaerobic co-digestion of oil refinery wastewater and chicken manure to produce biogas, and kinetic parameters determination in batch reactors

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Abstract. In order to improve the anaerobic fermentation of oil refinery wastewater (ORWW) via an appropriate nutrients pool for microbial and buffer capacity growth, a study was carried out on related anaerobic co-digestion (AcoD) with a rich organic carbon source, namely chicken manure (CM). The kinetic parameters were investigated (including cumulative biogas production, bio-methane content, retention time, and soluble chemical oxygen demand stabilisation rate) of batch AcoD experiments related to six ORWW:CM-ratio treatments (5:0, 4:1, 3:2, 2:3, 1:4, and 0:5) under mesophilic conditions. The highest soluble chemical oxygen demand removal rate was obtained for the 4:1-ratio treatment. However, the highest biogas production and bio-methane contents were achieved for the 1:4-ratio treatment. When taking into consideration the highest oil refinery wastewater portion in the AcoD mixtures and the statistical test results ($LSD_{0.05}$) for the kinetic parameters, it can be seen that the 4:1-ratio treatment provided the maximum biogas production levels.

Key words: anaerobic co-fermentation, bio-methane production, oil refinery wastewater, ammonia accumulation.

INTRODUCTION[1]

With the remarkable growth in energy demand and the large amount of wastewater production from industrial areas, today's world has been facing these two challenging problems for some time (Tommasi et al., 2012). The refining process for crude oil consumes a bulk quantity of water. As a consequence, a significant volume of wastewater is generated. The volume of petroleum refinery effluents generated during processing is between 0.4–1.6 times the volume of the crude oil processed (Diya'uddeen et al., 2011). This means that when taking into consideration the importance of crude oil products in today's word, millions of barrels of oily sludge and effluents are being generated each day. Therefore, an efficient and economical approach should be developed in order to make it possible to manage petroleum refinery effluents, and to try to introduce new areas of technology for oil refinery wastewater (ORWW) recycling. Treatments for ORWW have been the focus of a good many studies, and several areas of technology have been proposed such as absorption, microwave-assisted catalytic wet air oxidation, photo degradation, ultrasound, photo-Fenton oxidation, and biodegradation (Diya'uddeen et al., 2011; Rastegar et al., 2011; Siddique et al., 2014; Siddique et al., 2015; Choromanski et al., 2016; Haak et al., 2016; Roy et al., 2016; Wang et al., 2016).

As is known, anaerobic digestion (AD) is a biological process which is used to biodegrade organic substrates in the absence of oxygen (Zhang et al., 2016), although the petroleum derivatives can be used as an additional carbon source in the fermentation process. However, the presence of heavy metals, ammonia, sulphides, and chlorophenols has a negative effect on AD process efficiency. On the other hand, previous researchers illustrated the fact that the negative effects of petroleum products on tested bacterial groups can be decreased by means of providing proper nutrients in the bio-digester (Choromanski et al., 2016). Therefore, providing a nourished-enriched substrate to carry out co-digestion with ORWW can provide the supporting conditions for microbial adaptation.

As a reliable natural methodology, anaerobic co-digestion (AcoD) has been used extensively for the treatment of large amounts of waste materials (Siddique et al., 2015). The main achievement of AcoD for substrates with manure is that it shows an increase in bio-methane yields through the means of creating an appropriate C:N ratio, robust pH levels, and consistent nutrient contents (Abouelenien et al., 2014; Li et al., 2014).

As one of the main livestock manure products in China when it comes to biogas production, chicken manure (CM) has a higher fraction of biodegradable organic material than other livestock manures. However, due to an accumulation of ammonia and volatile fatty acids (VFAs) which lead to fermentation inhibition, CM has not been fully utilised so far (Abouelenien et al., 2014).

[1] CM: chicken manure; ORWW: oil refinery wastewater; AcoD: anaerobic co-digestion; TS: total solid; VS: volatile solid; TOC: total organic carbon; TN: total nitrogen; C:N: carbon: nitrogen ratio; TCOD: total chemical oxygen demand; CODs: soluble chemical oxygen demand; LSD: least significant difference test; BGP: cumulative biogas production; BMP: cumulative bio-methane production; TAN: Total ammonia nitrogen; FAN: free ammonia nitrogen.

Although AD technology had been applied to treat ORWW in several recent research studies (Choromanski et al., 2016; Haak et al., 2016; Roy et al., 2016; Wang et al., 2016; Mehryar et al., 2017), very limited studies have been reported on AcoD in ORWW with agricultural and organic wastes. Three examples that can be given are reports on AcoD in petrochemical wastewater with activated manure in a continuously-stirred tank reactor (Siddique et al., 2014; Siddique et al., 2015), AcoD in ORWW with molasses in an up-flow anaerobic sludge blanket reactor (Rastegar et al., 2011), and AcoD in ORWW with sugarcane bagasse in a batch digester (Mehryar et al., 2017). The majority of past studies have focused on the effects of toxin elements in ORWW on the AD process or microbial community analysis during the fermentation period. That is an area that can be evaluated by surveying the AcoD process performance.

In this study, the areas that proved to be assets were providing an appropriate environment for a microbial community to self-adapt in petroleum derivatives, and increasing the stability and performance of the AcoD process for ORWW (as a nutrient-low substrate) with CM (as a nutrient-rich substrate and one of the most widely-available products to be generated by livestock in China). To this end, the main objective of this study was to evaluate the anaerobic co-digestion process of oil refinery wastewater and chicken manure. In order to investigate the optimum ORWW:CM ratio for treatment, four different kinetic parameters (KPs) including cumulative biogas production (BGP), bio-methane content of the BGP, retention time, and a soluble chemical oxygen demand (CODs) stabilisation rate were experimentally measured and pertinently discussed.

MATERIALS AND METHODS

Utilising substrates and seed sludge for anaerobic co-digestion processes

The ORWW was collected from the Jinlin SINOPEC oil refinery factory, Nanjing, Jiangsu Province, China. The CM was obtained from a local livestock farm located near the College of Engineering, Nanjing Agricultural University (NJAU), which itself is located in Pukou District, Nanjing, Jiangsu Province, China. The inoculum of anaerobically-digested sewage sludge was taken from a wastewater treatment plant at Yangzi Petrochemical Co Ltd, Nanjing, China. In order to remove the dissolved methane and easily-degradable organic matter from the collected sewage sludge, the activation stage set out by Xi et al. (2014) was utilised, and was further used as an inoculum (Xi et al., 2014).

Experimental set-up for bio-methane production (BMP)

Batch lab-scale experiments of simulated practical anaerobic digestions were conducted under mesophilic conditions (37.0 ± 1.0) °C (Xi et al., 2014; Hassan et al., 2016; Mehryar et al., 2017) using lab-scale anaerobic digesters fabricated from 1 L glass bottles (Fig. 1). Batch reactors were used to survey the AcoD in ORWW which had been mixed with CM, as an external organic carbon source. The working volume of each digester was kept at 800 mL, including 400 mL inoculum, which was an optimised amount of the inoculum required to co-digest oil refinery wastewater with activated manure (Siddique et al., 2015).

The contents of each digester were smoothly shaken manually for two minutes a day before biogas volume measurements were taken. In order to be able to obtain the optimum portion of ORWW and CM from the AcoD process, six different mixing ratios of ORWW:CM were designed; including 5:0, 4:1, 3:2, 2:3, 1:4, and 0:5-ratio treatments. Each treatment was conducted in triplicate. The CM with inoculum (the 0:5-ratio treatment), and ORWW with inoculum (the 5:0-ratio treatment) were also anaerobically digested as controls. To be able to estimate the background biogas production from inoculum, three digesters containing unmixed inoculum were used as blank assay digesters.

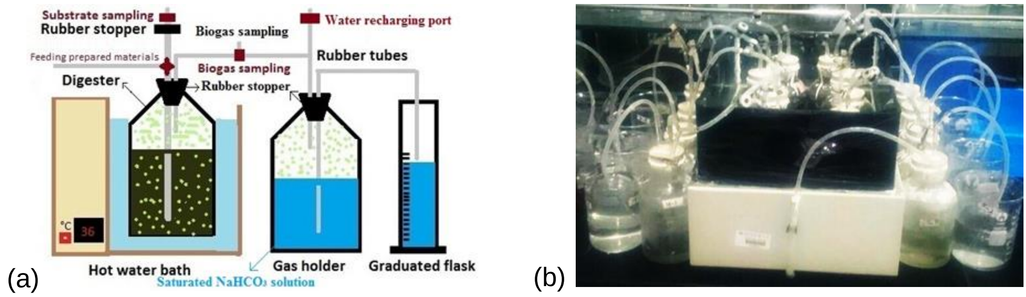

Figure 1. (a) Schematic description of batch digesters (b) manufactured batch digesters used in the anaerobic co-digestion experiments.

Analytical methods

According to the Standard Methods of the American Public Health Association, the characteristics of the substrates and inoculum, including pH, total solids (TS), volatile solids (VS), total organic carbon (TOC), total nitrogen (TN), carbon: nitrogen ratio (C:N ratio), total chemical oxygen demand (TCOD), and CODs were determined prior to the experiments (APHA, 2006). The pH value was directly measured from the liquid samples using a digital pH meter (FE20K, Mettler-Toledo, Switzerland). The total content of volatile fatty acids (TVFA) was determined via gas liquid chromatography (Model GC-2014, Shimadzu, Japan), equipped with a thermal conductivity detector (TCD), which had a column (DA, 30m × 0.53mm × µm Stabilwax) and flame ionisation detector, while injector and detector temperatures were set at 150 °C and 240 °C, respectively. The CODs stabilisation was calculated by Eq. (1) (Hassan et al., 2016; Mehryar et al., 2017):

$$\mathrm{CODs}\ (\%) = \frac{\mathrm{CODs_i}(mg \cdot L^{-1}) - \mathrm{CODs_f}\,(mg \cdot L^{-1})}{\mathrm{CODs_i}(mg \cdot L^{-1})} \times 100 \qquad (1)$$

where *i* and *f* are the initial and the final CODs (mg L^{-1}) values respectively during the AcoD process. The total ammonia nitrogen (TAN) content was also measured by means of an ammonia meter (Lianhua Tech Co, China) (Hassan et al., 2016). The free ammonia concentration figure (FAN) was calculated by Eq. (2) (Chen et al., 2014):

$$FAN\ (mg \cdot L^{-1}) = \frac{TAN(mg \cdot L^{-1}) \times K_a}{C_H \times (\frac{K_a}{C_H} + 1)} \qquad (2)$$

where *FAN* is the free ammonia nitrogen concentration (mg L^{-1}); *TAN* is the total ammonia nitrogen content (mg L^{-1}); K_a is the temperature-dependent dissociation constant (1.097×10^{-9} for the mesophilic condition); and C_H is the concentration of hydrogen ions.

During the digestion process, the volume of biogas production from each digester was recorded daily by using the liquid displacement method, for which the saturated $NaHCO_3$ solution was utilised as a displacement liquid (Xi et al., 2014). Biogas composition was determined by means of gas chromatograph (GC 9890A, Renhua, China), equipped with the TCD and a column (Φ4 mm × 1 μm, Shimadzu, Japan) and with hydrogen gas being used as a carrier gas. The oven, injector, and TCD temperature were recorded at 150 °C, 100 °C, and 120 °C respectively. The flow rate of carrier gas and the injected sample were at 50 mL min^{-1} and 0.5 mL respectively.

Statistical analysis procedure

To be able to examine the effect of various mixing ratio treatments for ORWW with CM on the KPs for the AcoD process, a completely randomised design was utilised. All of the data obtained were analysed by looking at the variance procedure (ANOVA) using the *Statistix 8.1* software (Tallahassee, Florida, USA). When the *F-test* indicated a statistical significance at the $P = 0.05$ probability level, the treatment means were separated by using the least significant difference test ($LSD_{0.05}$).

RESULTS AND DISCUSSION

Substrate characteristics

The estimated characterisations of the applied substrates are shown in Table 1. The experimental determined characteristics for ORWW were in line with previous studies (Hu et al., 2013; Siddique et al., 2014; Siddique et al., 2015). And the characteristics of the CM conferred its fermentation ability, which was in agreement with previous reports (Abouelenien et al., 2014; Li et al., 2014). As Table 1 demonstrates, the initial C:N ratios of substrates were 10.41:1, 151.40:1, and 18.37:1 for the CM, ORWW, and inoculum respectively. While the reported range for a preferred C:N ratio in previous research lies between 15–30 (Abouelenien et al., 2014), the C:N ratio for ORWW and CM are outside this range. One of the main purposes of applying the AcoD technique in several studies was the C:N ratio adjustment in the bio-digesters (Ahn et al., 2010; Zhang et al., 2013; Abouelenien et al., 2014), which is one of the objectives of this fresh research exercise. Through experimentally utilising ORWW:CM AcoD mixtures, the C:N ratio and nutrient content of the feed substrate were improved. The physicochemical characteristics of the different AcoD treatments are presented in Table 2.

Table 1. Characteristics of the applied substrates and inoculum

Characteristics	Substrates		
	CM	ORWW	Inoculum
pH	7.89 ± 0.10*	7.49 ± 0.10	7.55 ± 0.10
TS (%)	33.86 ± 2.50	1.73 ± 0.03	2.51 ± 0.10
VS (% of TS)	65.35 ± 0.60	2.69 ± 0.70	49.35 ± 1.00
TOC (g.kg of TS^{-1})	440.22 ± 3.50	30.28 ± 2.10	368.13 ± 4.30
TN (g.kg of TS^{-1})	42.27 ± 0.10	0.20 ± 0.01	20.04 ± 0.20
TCOD (mg L^{-1})	530 ± 180	18,500 ± 300	5,200 ± 100
C:N ratio	10.41:1	151.40:1	18.37:1

* mean ± standard deviation.

Table 2. Physicochemical characteristics of the different ratio treatments' substrates

Characteristics	Treatments *					
	5:0	4:1	3:2	2:3	1:4	0:5
pH	7.75 ± 0.00	8.17 ± 0.11	8.52 ± 0.04	8.21 ± 0.02	8.03 ± 0.02	8.12 ± 0.25**
CODs (g L^{-1})	1.60 ± 0.20	4.96 ± 0.15	5.20 ± 0.10	5.60 ± 0.23	10.00 ± 0.50	11.84 ± 0.40
TAN (g L^{-1})	2.26 ± 0.01	1.15 ± 0.11	1.38 ± 0.08	1.88 ± 0.07	2.17 ± 0.14	2.34 ± 0.10
TN (g kg of TS^{-1})	0.19 ± 0.08	0.63 ± 0.17	1.07 ± 0.31	1.51 ± 0.16	1.98 ± 0.24	2.24 ± 0.43
TOC (g kg of TS^{-1})	3.81 ± 0.32	8.29 ± 1.01	12.79 ± 0.1	17.28 ± 1.16	22.22 ± 3.15	25.03 ± 3.71
C:N ratio	19.5:1	13.5:1	12:1	11.5:1	11.25:1	11:1

* Oil refinery wastewater: chicken manure; ** mean ± standard deviation.

BMP

The daily BMP for different treatments are shown in Fig. 2 (a) which illustrated the success of AcoD for ORWW and CM. The BMP started on the first day after fermentation onset, and kept increasing until reaching a peak on the seventh to tenth days of the digestion period for different treatments. After the main peaks had been reached there was no continuous decrease in the BMP kinetic trends for the different AcoD treatments and, indeed, one or two more small BMP peaks were experience later on. In order to be able to explain this observation, the interaction between pH and TVFA may lead to an 'inhibited steady state' representing a lower methane yield (Zhang et al., 2013). The 5:0-ratio treatment (ORWW alone) did not produce any considerable amounts of biogas, although the presence of the different hydrocarbon profiles can lead to an uncertain level of influence on petroleum products in biological systems (Choromanski et al., 2016). However, the presence of sulphur compound as a highly toxic material which existed in the form of hydrogen sulphide (H_2S) (Diya'uddeen et al., 2011) could be the main reason for the current observation. On the other hand, a high concentration of petroleum hydrocarbons (PHCs) and malnourished bacteria could be other reasons for lower levels of performance. Similarly, Choromanski et al. (2016) demonstrated that the spent engine oil had a significant effect on the BGP performance levels during the initial days of the digestion period (Choromanski et al., 2016). They reported that, thanks to the adaptation by the micro-organisms to the components in the spent engine oil, the output BGP in the second week of the digestion period was increased (Choromanski et al., 2016). Although the 0:5-ratio treatment produced the highest BMP volume and the 5:0-ratio treatment did not produce any BGP; the present study discussion focuses on the different AcoD treatments and their KPs so that the different AcoD treatments can be evaluated and the optimum conditions discovered. The results obtained clarified the fact that the KPs could be improved by means of a dilution of toxic substances and the buffering capacity development of the feedstock, which were the benefits of AcoD using livestock manures (Siddique et al., 2014).

As shown in Table 3, the 1:4-ratio treatment produced the second highest BGP volume, this being (301.27 ± 4.02) mL g VS^{-1} BGP during a period of 35 days after the 0:5-ratio treatment (with CM alone). The 2:3-ratio treatment produced biogas with a (54.20 ± 2.02)% bio-methane content and the highest quality of BGP over the course of a period of 34 days. However, the most feasible treatment was the one which consumed the higher volumes of ORWW and produced biogas with a high bio-methane content and a shorter retention time, this being the 4:1-ratio treatment. During a period of 36 days, its BGP and BMP were at (194.02 ± 3.42) mL g VS^{-1} and (85.24 ± 4.92) mL g VS^{-1}

respectively. These experimental findings showed that by increasing the ORWW ratio in AcoD mixtures, the BGP and BMP levels were more stable. In order to explain this, focusing on the estimating pH, TAN, and FAN to evaluate the ammonia accumulation levels can be more beneficial.

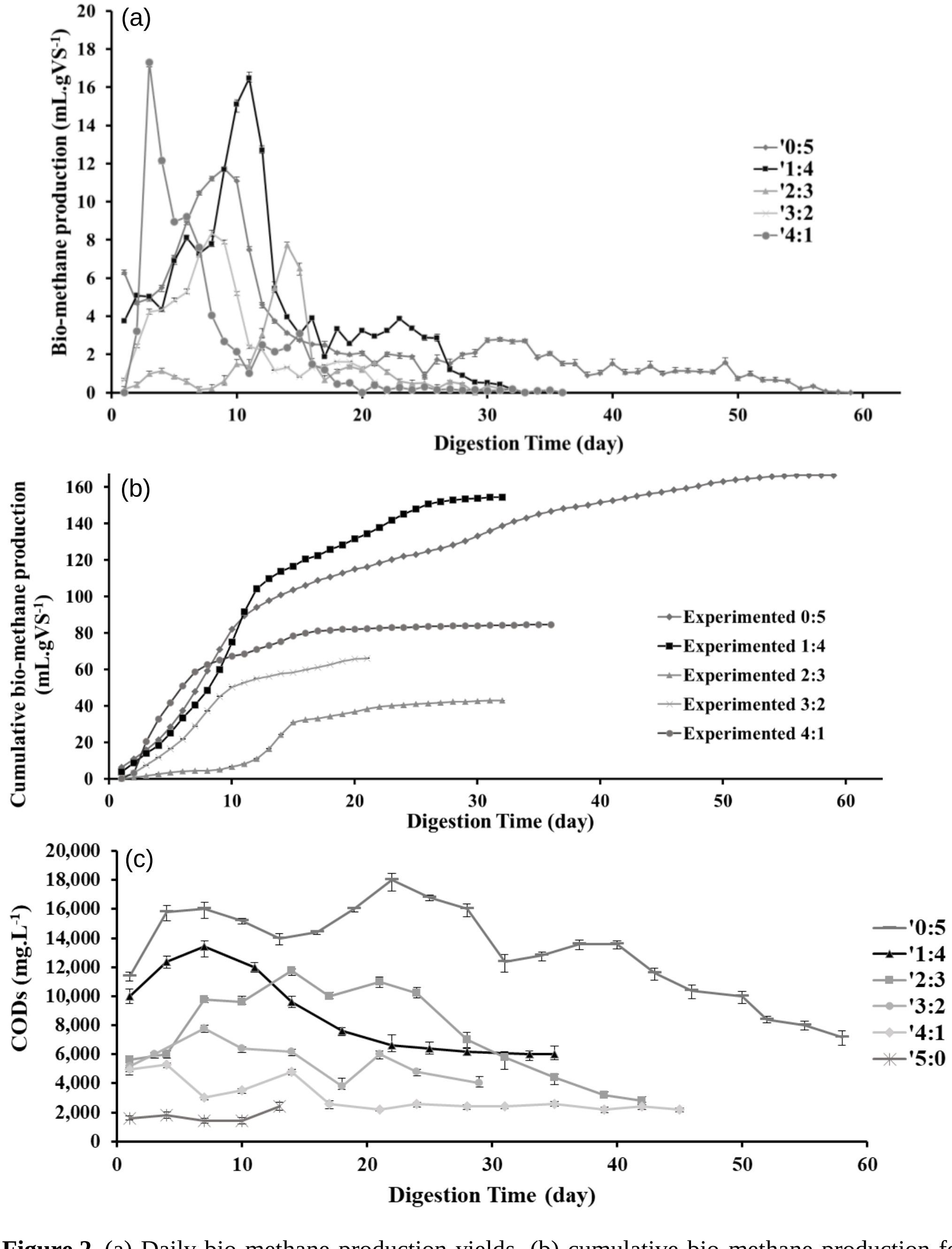

Figure 2. (a) Daily bio-methane production yields, (b) cumulative bio-methane production for the different AcoD treatments (ORWW:CM ratio), and (c) temporal CODs variation during digestion time.

CODs stabilisation profile during AcoD

The chemical oxygen demands a (COD) concentration of the wastewater, especially since CODs are one of the most important parameters when it comes to analysing the AD and AcoD process (Hassan et al., 2016), which was measured every three days in the present study. The CODs variation trends for various treatments are depicted in Fig. 2 (c). The first stage of the AD process is hydrolysis, which leads to CODs increasing in the initial days of the digestion period. During this stage, bacteria transform the particulate organic substrate into liquefied monomers and polymers, ie proteins, carbohydrates, and fats are transformed into amino acids, monosaccharides, and fatty acids respectively, which directly or indirectly takes part in the methane and carbon dioxide production process (Fang et al., 2014). By consuming the components produced, biogas will be produced and CODs will be reduced. The estimated CODs variation graphs depict the same trends. The estimated CODs variation graphs (Fig. 2 (c)) depicted the fact that, by decreasing the CM ratio in the ORWW:CM mixtures, the initial CODs were reduced, which is a factor that is related to the total levels of organic matter in the feedstock. Generally, the observed CODs variation trends in the present study showed that the results were consistent with what other investigators had previously observed (Ahn et al., 2010; Choromanski et al., 2016; Hassan et al., 2016).

To be able to evaluate the quality of the anaerobic fermentation process and discuss different effects upon it, various KPs have been mentioned by many authors such as BGP, bio-methane content, CODs stabilisation rate, ammonia removal, microbial communities' variations, VS removal, AD process retention time, TVFA removal, and so forth (Haak et al., 2016; Hassan et al., 2016; Li et al., 2016; Siddique et al., 2015). Based on the research objectives and research limitations, one or more of these parameters have been focused upon in different studies. The main parameters in previous research can be listed as including BGP, bio-methane content, CODs stabilisation rate, ammonia removal, TVFA stabilisation, and retention time (Abouelenien et al., 2014; Hassan et al., 2016; Xi et al., 2014). The results of a mean comparison (*$LSD_{0.05}$ test*) for the KPs – including the percentage of CODs stabilisation in all ORWW:CM mixtures with their AcoD process retention time, BGP and bio-methane content, and its percentage for different AcoD treatments – are represented in Table 3.

Table 3. Results of the $LSD_{0.05}$ analysis of the kinetic parameters for the different AcoD treatments

Treatments*	Parameters				
	Retention time (day)	CODs reduction (%)	Cumulative BGP (mL g VS^{-1})	Cumulative BMP (mL g VS^{-1})	Bio-methane content of BGP (%)
5:0	0.00^{d}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{d}
4:1	36.67 ± 4.51^{b}**	55.65 ± 3.00^{a}	194.02 ± 1.08^{b}	85.24 ± 1.08^{c}	43.95 ± 2.34^{c}
3:2	22.00 ± 1.53^{c}	16.67 ± 2.00^{d}	151.11 ± 3.42^{c}	81.44 ± 3.42^{c}	53.76 ± 4.92^{a}
2:3	34.00 ± 2.65^{b}	50.00 ± 2.00^{b}	77.82 ± 2.59^{d}	42.19 ± 2.59^{d}	54.20 ± 2.02^{a}
1:4	35.33 ± 1.53^{b}	40.00 ± 3.00^{c}	301.27 ± 4.02^{a}	143.15 ± 1.97^{b}	47.53 ± 1.75^{b}
0:5	58.66 ± 2.52^{a}	42.57 ± 1.50^{c}	302.87 ± 7.41^{a}	166.40 ± 5.39^{a}	54.98 ± 3.20^{a}

* Oil refinery wastewater: chicken manure; ** mean ± standard deviation. While different letters within the same column present the significantly different values at $P = 0.05$ probability level by *$LSD_{0.05}$ test.*

In general, a shorter retention time and higher values for the BGP, the bio-methane content, and the CODs stabilisation rate are preferred. As shown in Table 3, the shortest retention time was obtained (22.00 ± 1.53)d for the 3:2-ratio treatment, while the CODs stabilisation rate was (16.67 ± 2.00)%, as the lowest value in comparison with other AcoD treatments. The results of the $LSD_{0.05}$ analysis showed that during non-significantly different retention times, the 4:1-ratio treatment produced a lower BGP and its resultant bio-methane content than the 1:4-ratio treatment (which demonstrated the greatest KPs values). The highest CODs stabilisation rate was related to the 4:1-ratio treatment. On the other hand, the fraction of ORWW in the mixture for the 4:1-ratio treatment was four times higher than that of the 1:4-ratio treatment. Therefore the use of the 1:4-ratio treatment means treating a greater ORWW proportion in the mixture, which was one of the present study's main aims. Thanks to this discovery, the experimental results showed that the 4:1-ratio treatment is the optimum one.

pH and TVFA profiles

With regard to monitoring the AD process, pH and TVFA values can provide a proper and applicable dataset. The temporal variations in pH versus TVFA during the digestion period for the various AcoD treatments are presented in Fig. 3 (a and b). The estimated TVFA values demonstrated that the highest TVFA concentration observed was for the 0:5-ratio treatment, while the 5:0-ratio treatment had the lowest TVFA concentration levels (≤ 300 mg L^{-1}). This low performance was due to low ammonia nitrogen concentration levels (≤ 500 mg L^{-1}) which can cause a loss of biomass and a decrease in acetoclastic methanogenic activity (Rajagopal et al., 2013). Although TVFA production of the 0:5-ratio treatment produced the highest resultant figures of all AcoD treatments, its bio-methane production trends and volume during the first and second weeks of the digestion period was lower than the others. The estimated peaks of TVFA production led to a partial drop of pH levels and an increase in BMP, with these findings being similar to those of previous studies that presented evidently contrasting trends between TVFA and pH (Zhang et al., 2013; Hassan et al., 2016). The TVFA peak for the 4:1-ratio treatment was taking place sooner than for the others, which led to its higher BMP rate and volume during the first week of the digestion period (Fig. 2 (a and b)) when comparing it to other AcoD treatments. The main intermediate acid products during the AD process for organic wastes included volatile fatty acids such as acetic, propionic, butyric, and valeric acids (Zhang et al., 2014). These are not toxic – in fact they can be produced and normally consumed by bacteria in the form of nutrients. However, their rapid increase or accumulation can lead to lower pH levels and increase the acidogenic bacteria which can have an effect on methanogens (Kwietniewska & Jerzy, 2014; Liu et al., 2015). On the other hand, acetate degradation and the production of bio-methane will be reformed (Abouelenien et al., 2014) with the same trends being observed after the initial ten days of the digestion period. Then the pH values were stable to the end of the experiments. This pH stability confirms that the AcoD process reached the methanogenesis phase (Zhang et al., 2013). As an important index when it comes to showing the VFAs conversion to bio-methane, the TVFA stabilisation rate for different AcoD treatments ranged between (87.04–97.38)%. This confirmed that the successful fermentation systems were successfully carried out. The measured pH levels and TVFA results clarified the fact that AcoD in ORWW when combined with CM improved VFAs accumulation by balancing the C:N ratio and producing ammonia nitrogen, with these results being in line with those of previous studies (Abouelenien et al., 2009; Zhang et al., 2013; Choromanski et al., 2016). However, focusing on the TAN and FAN can provide a good description of BMP trends and the AcoD process.

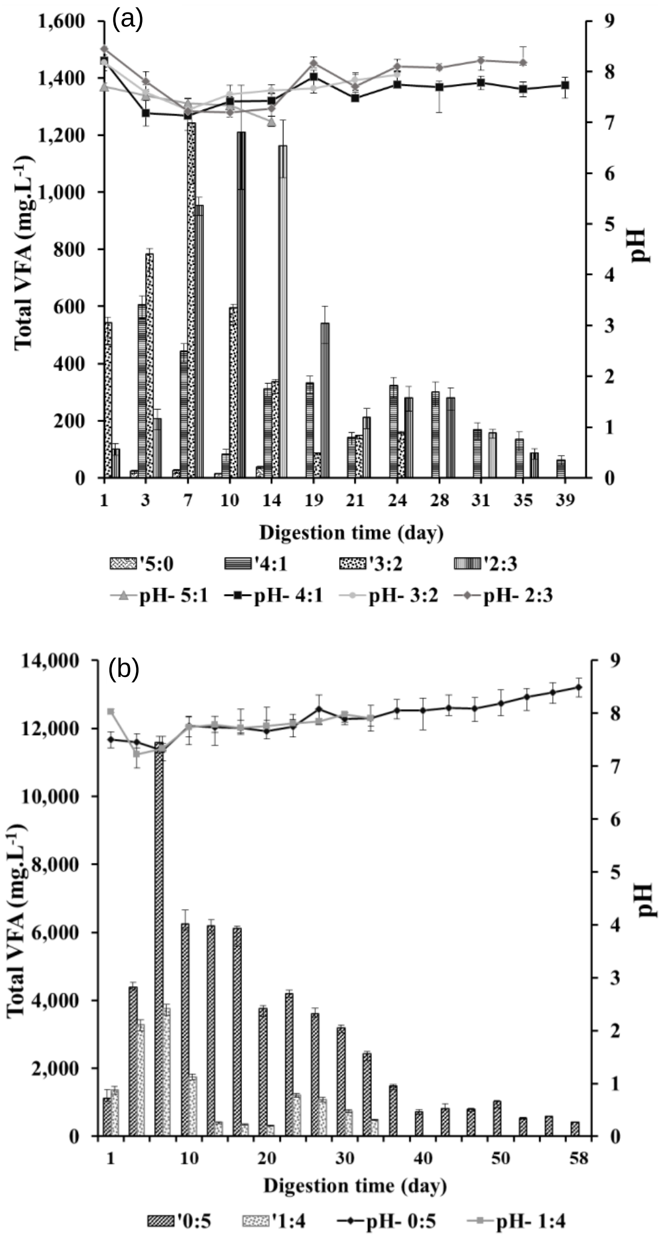

Figure 3. The temporal variations of the pH vs. TVFA during digestion period for the different AcoD treatments (ORWW:CM ratio) with TVFA values (a) less than 2,000 mg L^{-1}, and (b) more than 2,000 mg L^{-1}.

TAN and FAN

Ammonia is the end-product of the anaerobic digestion of proteins, urea, and nucleic acids (Rajagopal et al., 2013). The TAN and FAN have been considered as the effective agents in all bioprocesses, with their variation trends during the batch digestion period for different AcoD treatments being shown in Fig. 4 (a and b). The low TAN concentration levels (≤ 500 mg L^{-1}) can result in a low methane yield, a loss of biomass, and limiting aceticlastic methanogenic activity (Rajagopal et al., 2013). In contrast, a high concentration of TAN may inhibit the anaerobic process and decrease the BMP.

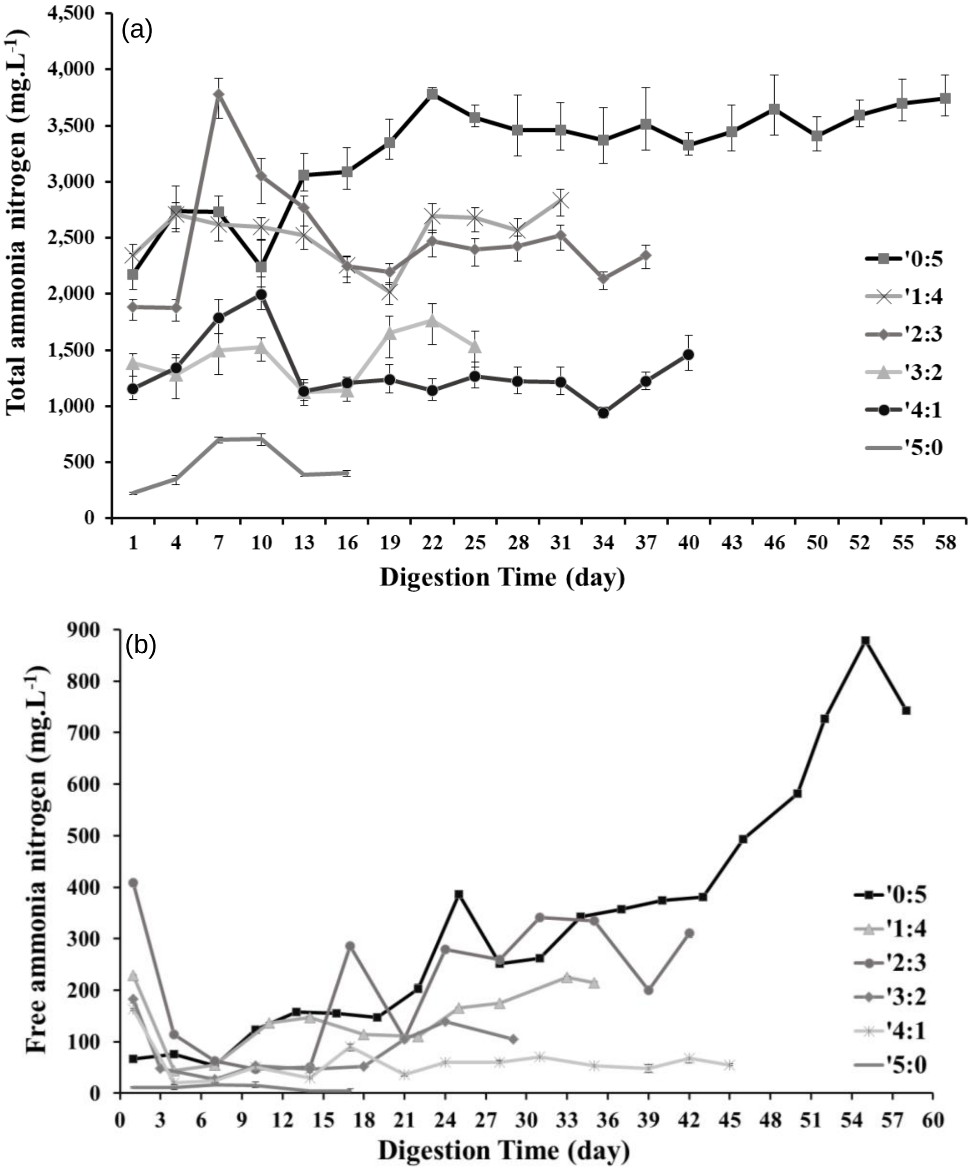

Figure 4. (a) total ammonia nitrogen (TAN), and (b) free ammonia nitrogen (FAN) variation trends for different AcoD treatments during fermentation process.

As the estimated results showed, the initial TAN content in the 5:0-ratio treatment (ORWW alone) was (407.5 ± 21.0) mg L^{-1}, which was insufficient when it came to preparing a good growth of bacterial agents for BMP. The results for AcoD treatments confirmed that when CM was added to ORWW, the TAN accumulation process was improved and the resultant values increased in comparison to that of the 5:0-ratio treatment. Generally in line with the reported previous studies, the experimental TAN data clarified the fact that bio-methane inhibition for various treatments occurred during the TAN production process at more than 2,000 mg L^{-1}. The previous studies explained the fact that AD inhibition can occur in a rather wide range between 1,700 mg L^{-1} and 14,000 mg L^{-1} for TAN, which is a function of the differences in feedstock, inoculums, operational conditions (such as temperature and pH), and acclimatisation periods (Zhang et al., 2014). In addition to TAN, the estimated results for FAN proved that higher levels of FAN concentration led to a reduction in the BMP. Just as for TAN, the wide range of between 200 mg L^{-1} and 800 mg L^{-1} was reported by previous researchers as being the critical range for FAN concentration (Chen et al., 2014). Between AcoD treatments, the 0:5 and 2:3-ratio treatments faced a high concentration of FAN (> 200 mg L^{-1}), with the resultant biogas and BMP output being inhibited. Chen et al. (2014) reported that releasing levels of FAN concentrations were the result of AD in livestock, which affected the activities of methanogenic bacteria and led to low levels of methane production (Chen et al., 2014). As may be seen in Fig. 4 (a and b), the FAN concentration levels increased along with a pH increase which expressed the fact that pH played the most effective role in FAN production and variation trends. Rajagopal et al. (2013) reported that with the pH value increasing from seven to eight, the free ammonia will increase by a factor of eight, which makes the biogas process a more sensitive one (Rajagopal et al., 2013). In this research project, the CM was a nutrient-rich substrate for bacterial growth. The results observed served to confirm the fact that decreasing FAN and TAN accumulation could improve the AcoD for ORWW:CM through the process of diluting toxic components in those treatments which had a lower CM ratio.

CONCLUSIONS

As part of the research for this study, evaluations were carried out on AcoD for ORWW with a rich organic carbon source, namely CM for bio-methane production. The significant and positive effect of AcoD for ORWW with CM in biogas production was confirmed by the experimental data that has been recorded. The data observed presented a picture of decreasing FAN and TAN accumulation due to a lower CM ratio which could improve AcoD for ORWW:CM. The improvement which was realised in terms of bio-methane production was due mainly to a dilution in toxic compounds for substrates, especially FAN. Besides this, it was also thanks to preventing ammonia accumulation in the bio-digester and ensuring the presence of proper nutrients to support microbial activities, although they had different areas of impact upon biogas production. It was the FAN values that had an impact upon biogas production rather than those from the TAN. In addition, in order to maximise ORWW usage and to produce acceptable bio-methane, a ratio treatment of 4:1 of ORWW:CM is recommended when designing the AcoD process for the continuous stirrer tank reactor (CSTR) digesters.

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Band structures for binding and holding of objects made from recycled metallic materials

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Abstract. The aim of the present research is the investigation of the possibility and effectiveness of using the band structures made from recycled metallic materials for binding and holding of objects (in particular, tubular objects as pipelines or shells). The using of band elements and structures as such is a perspective way to increase the safety and bearing capacity of the pipelines and vessels. Nowadays during repair works the outer surfaces of the mentioned objects are braided by the steel tapes, i.e. the objects are strengthened by the binding. The mentioned steel bands are specially produced for binding purpose. From the other hand after stamping of small-size details (like the elements of supply chains for different apparatus) the metallic waste in the shape of perforated metallic tapes are received and needs to be reused in compliance with the good practice in effective resource using and recycling. The band structures for binding and holding of tubular objects, produced from the perforated metallic tape by the longitudinal profiling, multilayer and spiral winding are presented. It is proposed to apply in industry the composite band structures made from perforated metallic materials and epoxy matrix for binding and holding of tubular objects as pipelines or shells, which allows simplifying and speeding up the repair works especially in the cases of the local damages.

Key words: perforated metallic waste, pipe repair bandages, environment.

INTRODUCTION

Perforated metallic materials (PMM) have a wide application in mechanical engineering and building including different solutions for ecological tasks (Lisicins et al., 2016). For these purposes the PPM of different shapes, for example, sheet, band, pipe and shell is used (Perforated metal, 2016).

During last years the structures, produced from the PPM, have been used in the modern buildings and constructions as well as in the systems for environmental control and technological facilities for aeration, ventilation, heating and filtration of the different gas and liquid substances as well as for absorption of the acoustic waves. Many of these and other applications are proposed by Mironovs & Lisicins (2015). From all the nomenclature of such structures the tubular perforated elements (Figs 1, 2) are of great importance not only due to functional characteristics, but due to decorative nature as well.

Figure 1. Composite sound dumper in perforated steel shell.

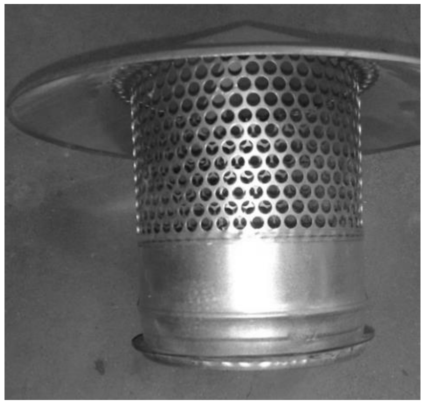
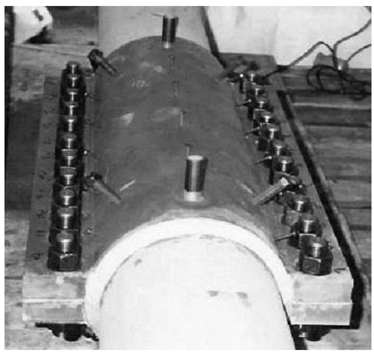


Figure 2. Perforated metallic roof ventilation lid.

One of the perspective way for applying the PMM is using the band structures made from recycled metallic materials for binding and holding of objects (in particular, tubular objects as high-pressure pipelines or shells) with the goal to increase the safety and bearing capacity of the pipelines and vessels. Nowadays during repair works the outer surfaces of the mentioned objects are braided by the steel tapes, i.e. the objects are strengthened by the binding. The mentioned steel bands are specially produced for binding purpose (Fig. 3). Using of such bands provides the safe transporting, storage, mounting of tubular objects and increases its strength.

a)



b)

Figure 3. View of the band clamp (a) and epoxy shell repair system (b) (Pipeline repair, 2017).

The aim of the present research is the investigation of the possibility and effectiveness of using the band structures made from recycled metallic materials for binding and holding of tubular objects. After stamping of small-size details (like the elements of supply chains for different apparatus) the metallic waste in the shape of perforated metallic tapes are constantly received and needs to be reused in compliance with the good practice in effective resource using and recycling.

The different methods for producing the band structures from PMM were proposed by Mironovs et al. (2013). Commonly the longitudinal profiling, multilayer and spiral winding are used. Joining of the elements of structures usually was done by soldering, welding or swaging of the band faces. Authors believe that the composite structure could be another one effective solution for binding and holding of tubular objects. It is proposed to use matrix from the epoxy resin reinforced by perforated steel band (Fig. 4). The advantages of proposed solution are the following:

- increased rigidity of the band structure;
- relatively light-weight structure;
- flexibility of the perforated reinforcement and polymer matrix;
- durability of the band;
- simplicity of the binding process;
- recycling of the technological waste.

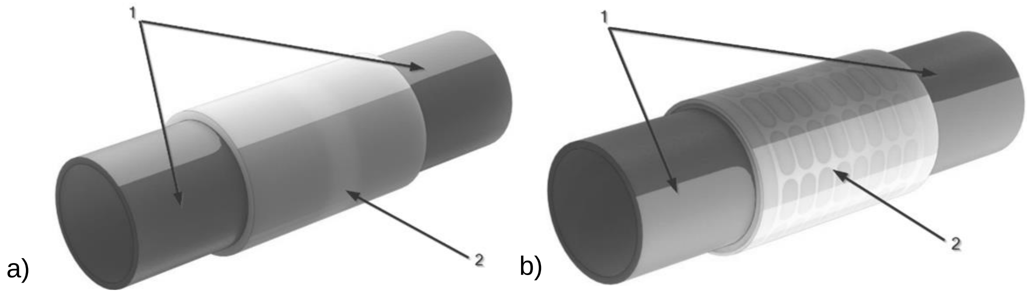


Figure 4. Schemes of pipe (1) repair band (2): a) without reinforcing; b) with reinforcing by perforated metallic band.

MATERIALS AND METHODS

Mechanical and geometrical parameters of PST-1 and PST-2 type perforated steel tape (trade mark of JSC 'DITTON Driving Chain Factory', Latvia), which was used for producing the repair bands for the tubular structures and perforated tubes are shown in Table 1.

For producing composite band with reinforcement from the perforated tape the matrix from the epoxy resin was used. The different composition of the epoxy compounds were used (Table 2) bearing in mind not only the physical and mechanical properties, but the shielding propeprties against electromagnetic fields as well (Mironovs et al., 2014).

It should be mentioned that the colour of compound differs according to material composition from yellow to dark grey. The addition of the basalt fibres allows changing the colour and strengthening the material.

From the mentioned materials the four type of samples with the thickness 8–10 mm and (other dimensions are 90 x 37 mm) were produced: without perforated reinforcement, with reinforcement by one, two and three layers of perforated material (Fig. 5). The thickness of the single perforated material layer is 1.5 mm.

Table 1. Mechanical and geometrical parameters of PST-1 type and PST-2 type perforated steel tape, which were used for producing of repair band

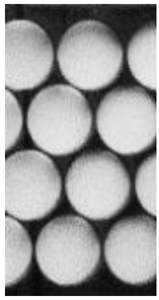
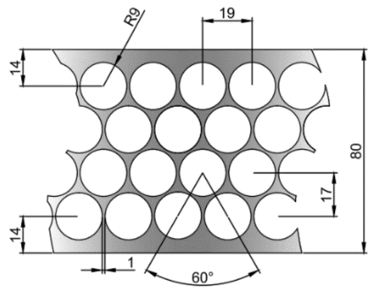
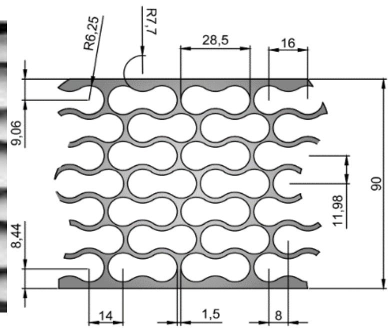
Parameter	Value	Tape representation	Tape geometry
Designation	PST-1		
Mark of steel	08пс-ОМ-Т-2-К		
Standard	GOST 503-81		
Thickness, mm	1.50		
Width, mm	80		
Permeable area, %	69.10		
Effective cross-sectional area, mm^2	25.13		
Tensile load bearing capacity, kN	5.54		
Tensile strength, MPa	220.65		
Displacement, mm	6.54		
Strain, %	3.93		
Designation	PST-2		
Mark of steel	50-Т-С-Н		
Standard	GOST 2284-79		
Thickness, mm	1.50		
Width, mm	90		
Permeable area, %	75.32		
Effective cross-sectional area, mm^2	26.43		
Tensile load bearing capacity, kn	10.01		
Tensile strength, MPa	406.81		
Displacement, mm	2.25		
Strain, %			

Table 2. Material composition of epoxy compounds

Material	Weight in %
Epoxy resin ED-20	46.41–47.70
Epoxy resin ED-181	32.59–33.50
Hardener	16.0
Calcium stearate	0.3
Montmorillonite	2.0
Basalt fiber	3.0

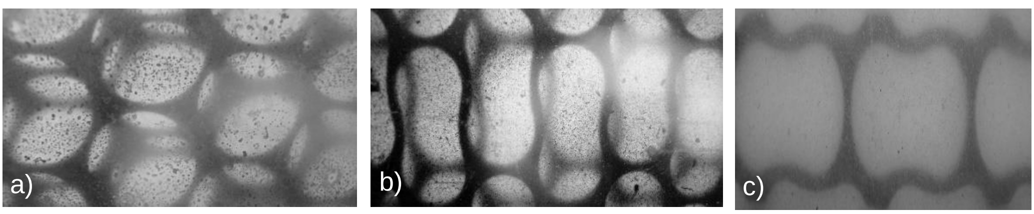
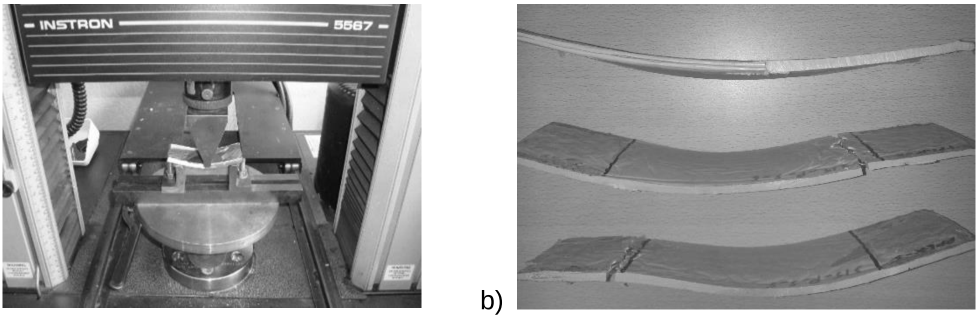


Figure 5. The view of the samples with the circular holes (a), oval holes (b) and complex holes (c).

The mechanical properties of the produced samples were tested by the Instron 5567 (USA) mechanical testing machine (Fig. 6): loadings rate was 30 mm min^{-1}, air temperature + 24 °C.



a) b)

Figure 6. The view of the testing process (a) and samples after testing (b).

RESULTS AND DISCUSSION

First of all, the flexural stress-strain state of the samples was evaluated. Fig. 7 shows the relationship between flexural stress and strain of composite sample with one-layer reinforcement from the perforated tape PST-2. This perforated tape is produced from the steel 50-T-C-H (Table 1) and is characterized by the high strength despite to the larger volume of perforated holes in comparison with the perforated tape PCT-1. As shown the three-point bending testing proves the possibility to use such composite structures for binding the tubular objects as high-pressure pipelines or shells.

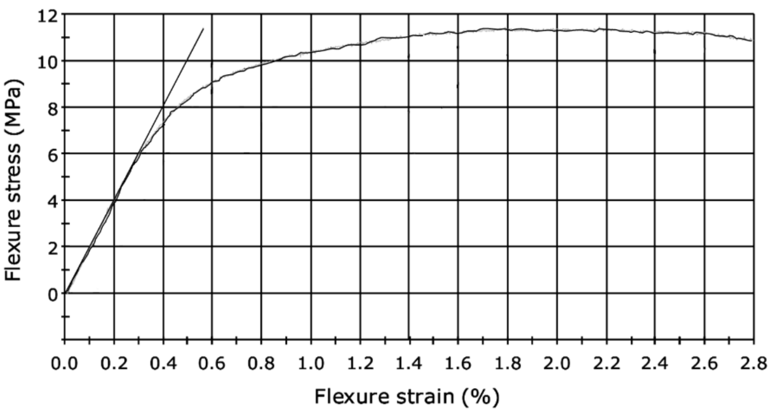


Figure 7. The relationship between flexure stress and flexure strain of composite sample, the straight line reflects the modulus of elasticity in shear.

After bending testing till destruction the different mechanism of destruction was revealed: commonly the breakage of the epoxy matrix was observed (Fig. 8, a), but in a number of cases firstly the perforated reinforcement (tape) was damaged or breaked (Fig. 8, b and c). It shold be noticed that the damage of the bands in all cases was starting with the delamination of the epoxy layer.

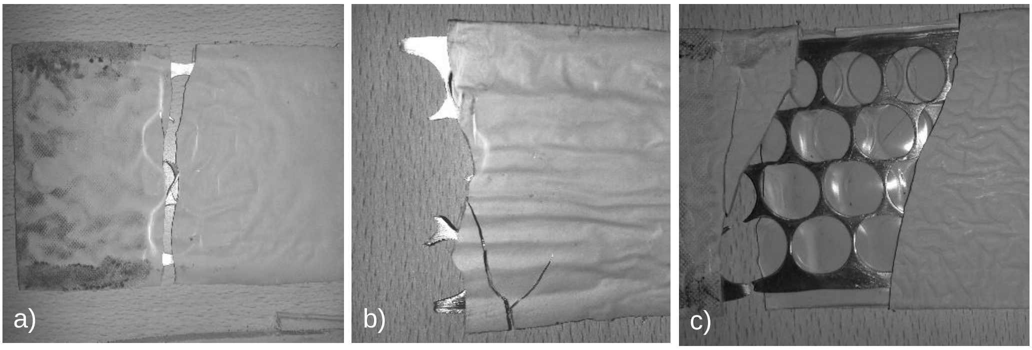


Figure 8. Macrostructure of the samples after breakage: a) breakage of the epoxy layer; b) breakage of the perforated reinforcement; c) damage of the perforated reinforcement.

In general the results of testing proves the high cohesive strength between the epoxy matrix and perforated reinforcement due to perforated holes filling by epoxy compound during the producing of the band. Taking into account the technological advantage (winding without complications) the application of perforated tape PST-1, which has lower strength, but higher plasticity than tape PST-2 seems more appropriate.

CONCLUSIONS

The using of the composite band structures made from perforated metallic materials and epoxy matrix for binding and holding of tubular objects as pipelines or shells allows simplifying and speeding up the repair works especially in the cases of the local damages. Because of perforation holes such binding decreases the weight of the band in comparison with the traditional band, produced specially from the solid metallic tapes. The existence of the perforation holes provides the strong cohesive strength between the epoxy matrix and perforated reinforcement, as well as sufficient mechanical properties of the bands, which were proven experimentally.

It was shown that for the producing the band it is recommended to use the more flexible perforated tape due to the better manufacturability during winding.

Thus, the application of the composite band structures allows eliminating possible ecological threats from the damages and breakdown of the pipelines etc. From the other hand using of such structures allows to recycle valuable perforated metallic waste, which at this time mainly not reused. For example, according to the information of the JSC 'Ditton Driving Chain Factory' (Ditton Driving Chain Factory, 2017) only this enterprise produces the few tons of the perforated steel waste per year. This company specializes in a wide range of roller-, bush-, leaf and other chains and produces not only mentioned products, but also the technological waste after cold stamping of the elements of driving chains of the motors. That's authors believe that the results of given research are useful and needed to be implemented.

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Effect of flame weed control on various weed species at various developmental stages

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Abstract. Physical methods of weed control as solarization, mulching, use of electricity, steam and flame are now an alternative in the organically grown crop. Flame weeder already has a wide range of practical use**,** particularly in the cultivation of vegetables in alternative form without any chemical treatment. Compared to chemical spraying, the use of flame weeder is more expensive, but we can compensate the costs by the added value of bioproducts. The issue of costs affects the wider use of the method in practice, but it may be offset by increased efficiency of weed control. The correct parameters of flame weeder, such as burner angle, burner height, the gas pressure, speed of weeder as well as the growth stage of the weed, weed species, climate conditions etc., can increase the effectiveness of weed control. Field and laboratory tests carried out in Canada and Slovakia were aimed at verifying the influence of parameters on the effectiveness of flame weed control.

Key words: flame weeder, weed control.

INTRODUCTION

A set of field and laboratory experiments are a continuation of long-term research, which started in 1994 in N.S.A.C. Nova Scotia (Canada) in collaboration with the Faculty of Engineering, SUA Nitra (Slovakia). Field tests, realized in 1994–1995 indicated a big difference in achieving effectiveness (Abrahám & Jablonický, 2007; Abrahám et al., 2011) of weed control with flame weeder Reinert-DA211. Influence of weather and different soil types on flame treatment was recorded. Subsequently, laboratory tests in Slovakia were made to confirm the influence of parameters (flame weeder driving speed, gas pressure, growth stage of the weeds) on flame treatment effectiveness in killing weeds. Different weed species at various developmental stages were tested. Consumption of gas on hectare M_H was established as a main parameter in order to compare effectiveness of the flame weeder.

MATERIALS AND METHODS

In the first experiment in N.S.A.C. (1994) with flame weeder Reinert-DA211 (Fig. 1), simulation of the passage of the flame weeder over the weeds were made to establish its thermal characteristics at different driving speed and different gas pressure.

Thermocouples Omega 5TC-GG-K-30 connected to a PC through a converter was used to measure change of weed temperature by simulated treatment (Bolla et al., 2003). The ends of thermocouples were placed close to the ground, in the middle of the path between the burners in wood skeleton to simulate weed. The flame weeder Reinert-DA211 repeatedly passed over the thermocouples and the change of temperature was recorded. The number of repetitions for one treatment was five. The changing parameters were driving speed of the burner and gas pressure.

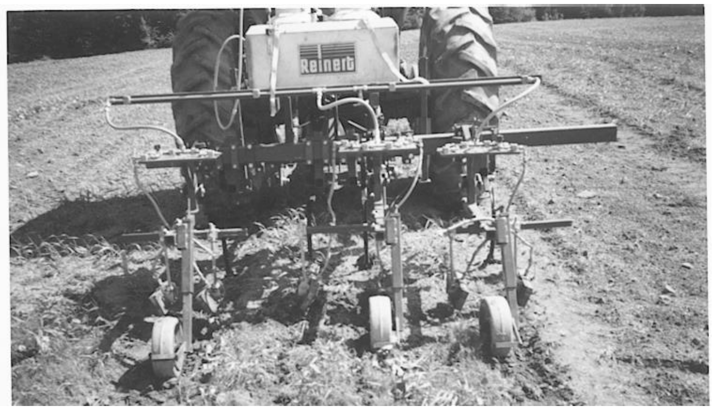

Figure 1. Flame weeder Reinert – DA211.

The flame weeder Reinert-DA211 is a three-row machine with six burners and two 25 kg LPG bottles. The bottles are placed in a bath filled with water with an anti-cloud heating; gas flow scheme is shown on Fig. 2. The gas supply to the burners is provided by hoses equipped with thermal sensors and a gas controller. The distribution of the flame to the weeds is modified by setting the driving speed of the flame weeder from 1 to 5 km h^{-1} and by setting the gas pressure from 0.05 to 0.25 MPa. The burners are turned into a row of plants at 45° angle, which allows the weeds to be treated in the crop line as well. The support wheels provide both directional control and adjustment of the height of the burners above the ground.

In subsequent laboratory experiments on Faculty of Engineering, Slovakia, verification of the effect of combinations of burner parameters in weeder driving speed v_p, gas pressure p_p, and weed growth stage (Lorenz et al., 1997) on weeds was made. A combination of the first two parameters results in a parameter of hourly gas consumption M_p. and a parameter of gas consumption M_H, which was converted by the time of treatment t_{tr} to consumption per hectare of treated surface (Majdan et al., 2011). The gas consumption M_p was determined by the measurement of the difference in LPG bottle weight in 25-minute intervals, and subsequently, recording of the treatment time of an area of 600 m^2 was verified with conversion to 1 ha.

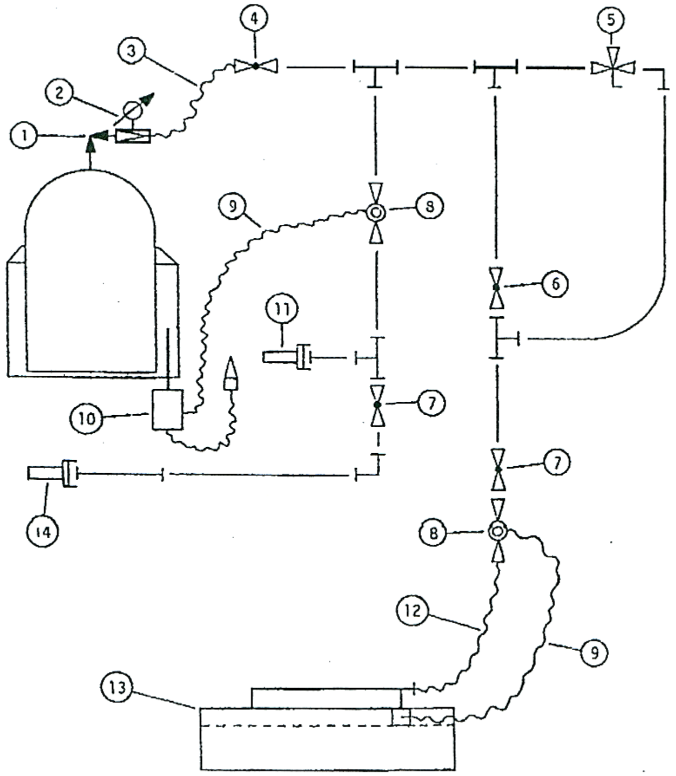

Figure 2. Gas flow scheme Reinert – DA211: 1 – gas bottle; 2 – pressure regulator; 3 – connecting hoses; 4 – throttle valve; 5 – starter; 6 – quick shutter; 7 – shut-off valve; 8 – safety valve; 9 – thermal fuse; 10 – thermal sensor; 11 – heating bottles; 12 – supply hoses; 13 – burner; 14 – ignition burner.

Laboratory experiments were carried out in 2013–2015 for Chenopodium album L. and Avena fatua L., which were pre-cultivated in containers 30 x 20 x 10 cm (L x W x H) in the minimum quantity of 15 pieces per container. The same burner DA-2011 connected to bottle of 25 kg LPG was used in a position of 10 cm above the passing of weeds at 45° angle. The containers with pre-cultivated were placed on a car pulled by rail with a small traction member enabling the setting of movement speed using an adjustable transformer 12 V. The treatment parameters are listed in Table 1. The effectiveness of treatment was monitored by counting the weeds before and after the treatment. Degree of plant damage was evaluated as completely (100%), partially (60%) or minimally (40%) damaged weeds, based on selected coefficients. Each treatment O01–O91 was performed in three growth stages of weeds, 3, 5 and 8 cotyledons for Chenopodium album L., and 3, 6 and 10 cm for Avena fatua L., and had four repetitions, which represented 120 treatments per weed species in total. For statistical evaluation the 'Analysis of Variance' was used to evaluate the impact of each factor separately. The calculated values P (Fisher test) were compared with table P_x value and when $P > 0.05^-$ (difference is not probable), $P > 0.05^+$ (difference is probable), and $P > 0.01^{++}$ (difference is highly probable).

Table 1. Treatment parameters at laboratory trial

Treatment	Speed v_p (km h^{-1})	Pressure p_p (MPa)	Consumption M_p (kg h^{-1})	Time of treatment t_{tr} (h)	Consumption M_H (kg ha^{-1})
O01	2	0.05	2.34	5	11.7
O11	2	0.1	9.69	5	48.45
O21	2	0.15	7.24	5	36.2
O31	2	0.25	12.1	5	60.5
O41	3	0.05	2.34	3.33	7.79
O51	3	0.15	7.24	3.33	24.1
O61	3	0.25	12.1	3.33	40.2
O71	4	0.05	2.34	2.25	5.26
O81	4	0.15	7.24	2.25	16.29
O91	4	0.25	12.1	2.25	27.23

RESULTS AND DISCUSSION

Experiments carried out with the three-row flame weeder Reinert – DA211 in Canada 1994 were used to determine the intensity of heat effect. Field trials with weeder passages over heat sensors at variable driving speed v_p and gas pressure p_p indicate a real time of heat application and its real value (Figs 3 and 4). While the increase of pressure p_p at speed 4 km h^{-1} leads to a temperature increase from approx. 280 °C up to 490 °C, and the time of application increase from 0.1 to 0.2 seconds, at speed 2 km h^{-1} and pressure 0.25 MPa a temperature of 760 °C was recorded, and the time of exposure extended to 0.7 seconds.

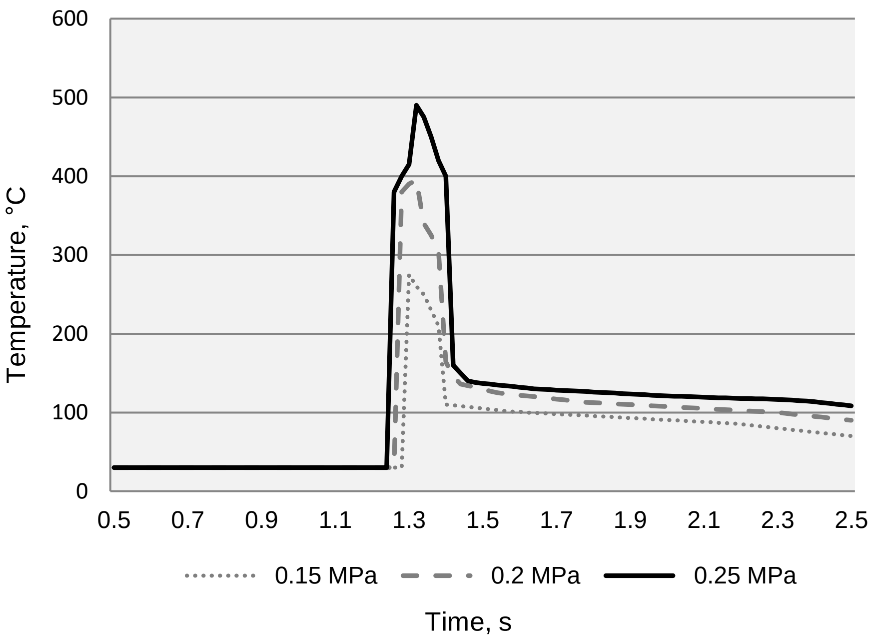

Figure 3. Measurement of thermal characteristics of flame weeder Reinert – DA211 at speed of 4 km h^{-1}.

The results help us to identify the effect of flame treatment on a given weeds accurately. Other aspect is the absorption area of weeds, which is represented by its gradual development and the number of cotyledons. An important finding is the fact that driving speed v_p has a more significant effect ($P > 0.01^{++}$) on the killing weeds than pressure change p_p ($P > 0,05^{+}$).

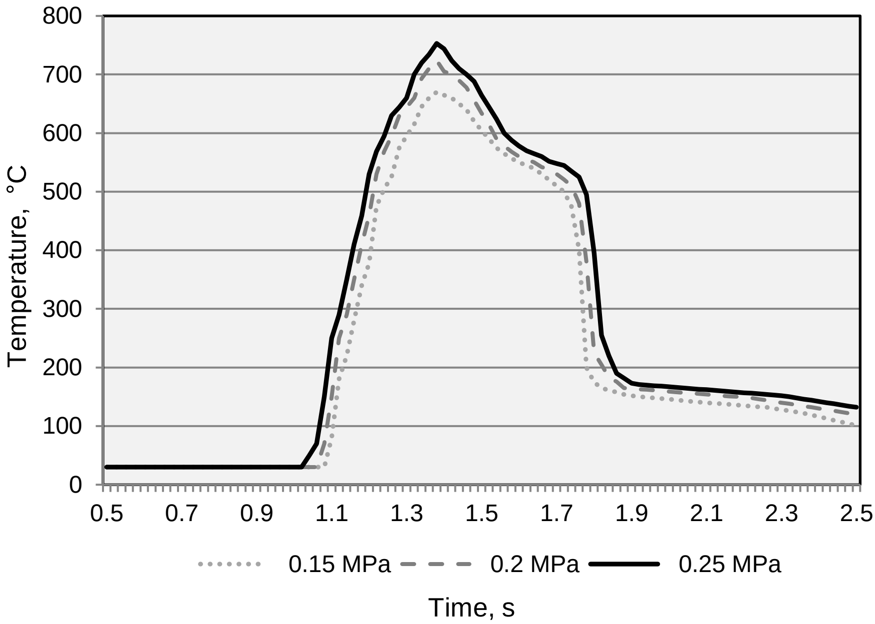

Figure 4. Measurement of thermal characteristics of flame weeder Reinert – DA211at speed of 2 km h^{-1}.

Laboratory experiments with pre-cultivated dicotyledonous and monocotyledonous weeds have been carried out mainly to ascertain the impact of changes in driving speed v_p and gas pressure changes p_p at different developmental stages of weeds. The results of control in Chenopodium album L. are shown in Fig. 5. Change of gas consumption M_H caused a significant effect ($P > 0.5^{+}$) on weeds control change in all growth stages. When gas consumption exceeded 50 km h^{-1}, the effect in all growing stages of Chenopodium album L. was more than 80%. On the other hand, in the case of growth stage under 3 cotyledons, only a half of the gas amount is needed to obtain the same results on weeds. Several experiments were performed even at later growth stages of weeds; however, the efficiency of flame application did not reach practically applicable results. Effect on Chenopodium album L. in 10 cotyledons at consumption 45 kg ha^{-1} was under 50%, which may be considered as financially unprofitable. Many weeds in later developmental stage regenerate after the treatment from its root system (Davis, 1975; Lorenz, 1997). The evaluation of damage extent was made according to the established methodology.

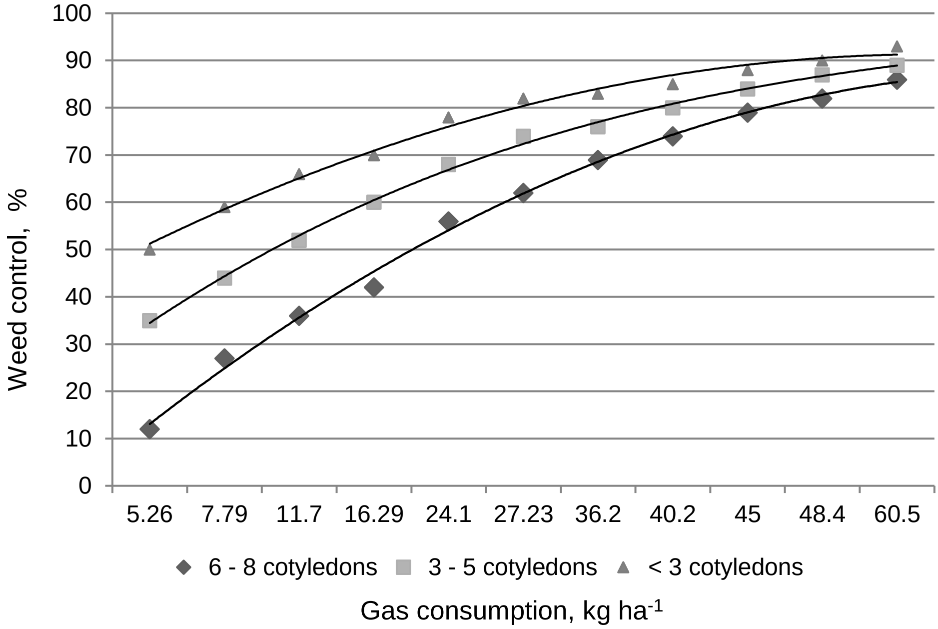

Figure 5. Effect of parameter changes on the flame treatment effectiveness in Chenopodium album L.

The results of weed control effectiveness in Avena fatua L. are shown in Fig. 6. Changes in gas consumption M_H caused only a minimal effect ($P > 0.5^-$) on weed control change in all growth stages. After the treatment of Avena fatua L., tops of the weeds were damaged by the flame, but quickly regenerated and continued to grow. The previous experiments indicate that for the control of Avena fatua L. it is important to set the flame from the burner at a proper angle so that the whole weeds are reached (Atkinson, 1995) and especially to place the burner as close as possible to the monocotyledonous weed (Bond & Grundy, 2001). At burner angle of 60° to the ground, the effectiveness of killing weeds significantly increases ($P > 0.5^+$). It is also very important to keep the height of the burner above the soil in the range from 10 to 15 cm, because even small deviations cause significantly lower effect on killing weeds ($P > 0.5^+$) – mainly to the lower part of these monocotyledonous weeds. An important factor is also the time of treatment and labour input. Moreover, weed thermal sensitivity depends on their developmental stage (Šniauka & Pocius, 2008). However, in the field of alternative, non-chemical growing of bio-products, this method can find a wider application, particularly when eliminating a high labour cost. The difference in the price of products with a higher added value can eliminate higher inputs in the usage of flame weeder (Birkett et al., 2001). Nowadays big greenhouses in Slovakia use track rollers with mounted burners for the weed control. The accuracy of these pathways considerably increases the efficiency and facilitates its application.

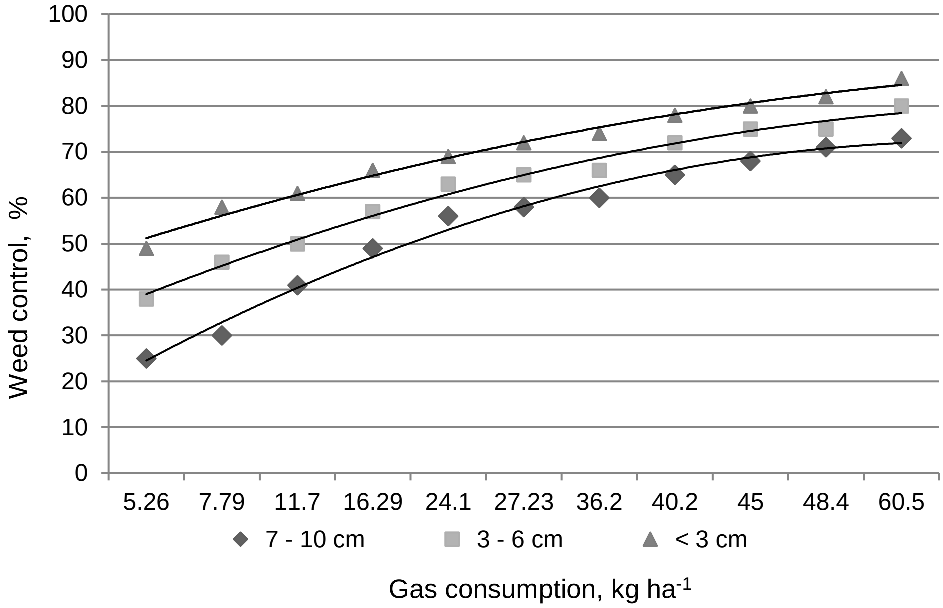

Figure 6. Effect of parameter changes on the flame treatment effectiveness in Avena fatua L.

CONCLUSION

The obtained results show that for practical use of flame weeder it seems to be more relevant to change the driving speed v_p rather than to change the gas pressure p_p. Similar results were recorded in testing rice and mustard (Parish, 1989; Ascard, 1997; Rifai et al., 2002). The flame weeder cannot compete with chemical or mechanical cultivators in terms of the costs (Abu-Hamdeh & Abu-Qudais, 2001). However, there are also other parameters in question, such as driving speed and time of treatment, which limitate the use of this method e.g. only for crops grown in the rows.

Moreover, setting a higher pressure p_p is limited with respect to the possibility of crop damage by distributed heat from burners. At higher pressure p_p, there is an overlapping of heat flow, which affects not only the weeds but also the crop. With a selective treatment, this is not a problem because the crop grows with a certain timing advance before weeds and has greater resistance (Virbickaite et al., 2006). In early stages of application, this can cause slow-down or even discontinuance of crop growth. On the other hand, as for the change in driving speed v_p, practical application is demanding in terms of accuracy due to the burner distance from the crop row, either horizontally or vertically, which is difficult to ensure at high speeds. Laboratory experiments have shown that the parameters of flame treatment must be adapted to the particular weed species and the developmental stage of the weeds, and the treatment has to be performed under favourable weather conditions.

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The nitrogen role in vegetables irrigated with treated municipal wastewater

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Abstract. The reuse of treated municipal wastewater for irrigation is an established alternative to conventional water, in many countries of the world, particularly where or when water resources are extremely limited. Wastewater reuse could represent a double benefit when used in agriculture, helping overcome any lack of water resources and additionally, enriching the soil with nutrients - especially nitrogen and phosphorus.

In the experimental site of Castellana Grotte (Apulia region, Southern Italy) during the 2012/13 and 2013/14 growing seasons, vegetable crops (fennel and lettuce) in succession were drip-irrigated with three different water sources. Two reclaimed water streams, obtained by applying different treatment schemes to the same municipal wastewater (an effluent from the full-scale treatment plant and an effluent from the Integrated Fixed-film Activated Sludge – Membrane BioReactor pilot plant) and a conventional source, to verify the crops response and nutrient contribution through wastewater supply.

Both lettuce and fennel yields were enhanced by the high content of nutrients in the effluent of one of the treatment plants, which had been operated for partial nitrogen removal. For Fennel 2013/14, wastewater-reuse led to a 54% reduction of nitrogen supply in relation to the other plots normally fertilized. In this way, an estimated saving of about 98.00 € ha^{-1} was achieved.

Crops irrigated with treated wastewater operated for partial nitrogen removal (IMBR) showed early ripening (8 days for lettuce and 35 days for fennel 2013/14) and better quality than others not similarly-treated. However, the wastewater presented a nitrate content in excess of legal limits (35 mg L^{-1}, D.M. 185/2003). Therefore, the contribution of nutrients increased production (47 vs 32 t ha^{-1} in IMBR and WELL 2012/13 fennel theses, 53 vs 31 t ha^{-1} in IMBR and WELL 2013 lettuce theses and 40 vs 31 t ha^{-1} in IMBR and WELL 2013/14 fennel theses respectively) and improved product quality, while simultaneously saving money for chemical fertilizers not supplied, producing less environmental impact.

Key words: irrigation reuse, nitrate, soil, nutrients, treated municipal wastewater, vegetables.

INTRODUCTION

Reclaimed wastewater and fertilizers

In recent years, the use of treated municipal wastewater for irrigation has become a very common practice in many countries of the world, especially in those with a dry climate where the water resource is extremely limited (Meli et al., 2002; FAO, 2010;

FAO, 2011). In many water-scarce countries such as Pakistan, Vietnam, Ghana and Mexico, wastewater is widely used for vegetable production (Pedrero et al., 2010).

The potential health risks and environmental impacts resulting from wastewater reuse in agriculture have already been studied (Angelakis et al., 2003). Sheikh et al. (1990) reported in Monterrey Wastewater Reclamation Study for Agriculture, food crops for raw consumption can be successfully irrigated with treated wastewater without adverse environmental or health effects. Furthermore, York et al. (2008), Lonigro et al. (2016) also demonstrated the safety and the suitability of reclaimed water use for agricultural irrigation.

Treated municipal wastewater reuse in agriculture may represent not only a resource to meet the growing water demand but also a cheap source of nitrogen and phosphorus nutrients (Chen et al., 2008; Disciglio et al., 2015).

Wastewater, containing macro and micro nutrients (e.g. nitrogen, phosphorus, potassium, calcium, and magnesium) that plants need to grow, can be considered a new and additional source of fertilizers leading to savings for an external supply. In addition, in some areas, it may be the only affordable source of fertilizers for poor farmers (Mateo–Sagasta & Burke, 2010).

Fertilizers are required for sustained food production, but their widespread and not rational use has aroused concern about resulting environmental pollution.

The nitrogen role

Nitrogen is essential to every living being. Nitrate (NO_3^-) is a naturally occurring form of nitrogen. Most crops require large nitrogen quantities to sustain high yields; it plays an essential role in plant biochemistry, participating in the formation of compounds essential to plant life such as amino acids, proteins, and nucleic acids. Nitrogen fertilization is an agronomic practice essential to meet the nutritional needs of crops. Nevertheless, the plant does not use all the nitrogen. In particular, nitrate is a soluble compound that, not being retained by the solid phase of the soil, can be easily leached from the soil by deep percolation to underground aquifers. Normally present in drinking water (World Health Organization (WHO) standards 50 mg L^{-1} and Italian Legislative Decree 31/2001), nitrate reaches high concentrations in plants (EC, 2011) and has always been considered potentially hazardous to human health.

Nitrate is absorbed in the blood, and hemoglobin is converted into methemoglobin that does not carry oxygen efficiently to important vital tissues such as the brain. Severe methemoglobinemia can result in brain damage and death (Self & Waskom, 2013). This outcome is directly related to the intensive and improper use of mineral fertilizer and manure for agriculture, sometimes exceeding crop–nitrogen demand (Mateo-Sagasta & Burke, 2010). Most of the nitrate we consume is from our diets, particularly from raw or cooked vegetables. In fact, vegetables constitute the major dietary source of nitrate, generally providing from 30 to 94% of the dietary intake (Di Gioia et al., 2013). The leafy vegetables (especially lettuce and spinach), fennel, celery and rocket (Sagratella et al., 2011) are capable of holding the largest concentrations of nitrates (Gonnella et al., 2002).

Nitrate levels can also vary within species, cultivars, and even genotypes with different ploidy (Blom–Zandstra, 1989). An accumulation of nitrate in vegetables occurs when crops absorb more than they require for their sustainable growth (Anjana et al., 2007). The accumulation of nitrate in crops and their edible parts can depend on several factors such as species and cultivars, amounts, timing and source of fertilizers used, the weather conditions (temperature, intensity, and duration of exposure to light), the physical-chemical nature of the soil and the presence of water.

The recent revision of EU Regulation (EC, 2011) redefines and – compared to the previous EC Regulations (EC, 2006a; EC, 2006b) – raises the levels of nitrates in certain leafy vegetables (lettuce, fresh spinach) because of climatic differences found among the Member States. Some areas with low temperatures favor the presence and accumulation of nitrate in vegetables. The EC Regulation takes into consideration that nitrate accumulation in vegetables is higher when solar radiation is lower (Di Gioia et al., 2013).

In order to evaluate a strategy to reduce the excessive nitrogen fertilizer use and related negative environmental impact, the aim of this study was to compare the effects of two different types of treated municipal wastewater: 1) a traditional municipal treated effluent and 2) an effluent from a pilot treatment plant, respectively, with different nutrients content (NO_3^-) on fennel and lettuce crop-performance. In particular, the qualitative and quantitative aspects of fennel and lettuce crop productions and the level and accumulation of nitrate in vegetables were investigated. The results reported in this paper refer to a two years trial of irrigation on vegetable crops in succession.

MATERIAL AND METHODS

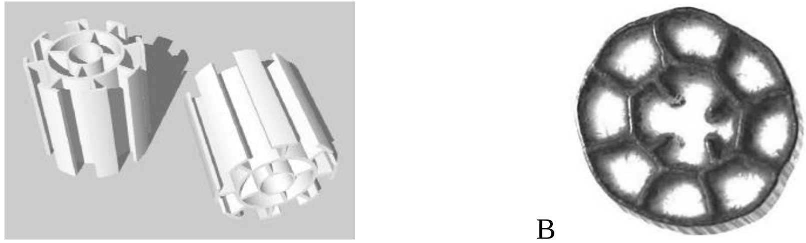
Treatments

The experimental trials were carried out in the countryside of Castellana Grotte (Bari, Southern Italy), near the municipal wastewater treatment plant. Three types of waters were compared in irrigation: the effluent from the full–scale treatment plant (EFF), the effluent from the IFAS-MBR pilot plant (IMBR) and conventional water drawn from a local well (WELL).

The full-scale municipal wastewater treatment plant is based on a pre-denitrification process scheme. The sewage, after pre-screening and primary settling, is sent to the first anoxic reactor where the nitrate recirculated from the following aerobic tank is removed from the liquid phase through biological denitrification. Subsequently, in the aerated reactor, oxidation of the organic fractions and nitrification occur. In the final settling tank, the produced sludge is separated from the liquid phase and partly recirculated to maintain the required biomass concentration. The secondary effluent is further treated through granular media sand filtration and chlorine disinfection, before being discharged on soil. During the experimental activities, a fraction of this effluent was split and used for irrigation at the test field located immediately outside the treatment plant.

The pilot-scale plant is based on the IFAS–MBR technology (Integrated Fixed–film Activated Sludge – Membrane BioReactor), it treats sewage after preliminary screening, where nitrates were intentionally not removed, to verify the effect on crops. The IFAS technology is based on the presence of suspended plastic carriers in the aerobic bioreactor (Fig. 1).

These carriers promote biomass accumulation in the form of a biofilm, and biological processes are carried out synergistically by the suspended biomass and the biofilm, resulting in limited biomass growth.



A B

Figure 1. Schematic illustration of a plastic carrier (A) and particular of biofilm (B).

The combination between IFAS and MBR has further potential benefits, since the membrane bioreactor allows optimal control of suspended biomass in terms of sludge retention time, possibly resulting in reduced production of partially stabilized sludge. Furthermore, membrane separation results in high-quality effluent in terms of suspended solids, favoring the adoption of UV disinfection technologies ‘on demand’. The end pipe of this plant is connected to a UV disinfection system that is activated when the irrigation line is switched on.

Field characteristics, agronomic conditions, and experimental design

The experimental field was located adjacent to the municipal wastewater treatment plant of Castellana Grotte (40°53’20”N 17°11’51”E; altitude 305 m a.s.l.) (Fig. 2). The trials were carried out in a loam soil, (USDA classification), with a field capacity (-0.02 MPa) of 24.4% dry weight (dw), a wilting point (-1.5 MPa) of 6.7% dw and a bulk density of 1.7 t m^{-3}. The main characteristics of the soil layer of the experimental site (0–0.4 m) are as follow: sand 44.4%; silt 44.1%; clay 11.5%; organic matter 1.50%; P_2O_5 (Olsen) 19 mg kg^{-1}; extractable K_2O ($BaCl_2$) 70 mg kg^{-1}; total N 1.11 g kg^{-1} (Kjeldahl); pH 8.1; electrical conductivity (1:2.5 w/v) 0.22 dS m^{-1}.

Figure 2. Satellite view of the municipal wastewater treatment plant of Castellana Grotte (Bari, Italy) and experimental field (red circle) (https://earth.google.com.

Three types of water were compared to irrigation: the effluent from the full–scale treatment plant (EFF), the effluent from the pilot IFAS–MBR plant (IMBR) and conventional water drawn from a local well (WELL). A localized low–pressure drip irrigation system with flow of 4 L h^{-1} was used for the irrigation of vegetable crops (fennel and lettuce) grown in succession. Lettuce and fennel are two of the most important leafy vegetables regarding their cultivation area and consumption rate in the world, characterized by high tendency to accumulate nitrates. The soil was tilled to a depth of 0.40 m, and then its surface was grinded before transplanting. During the cropping season, nutrient intakes and other management practices were estimated from local farmers. Pest and weed control were performed according to common management practices.

The efficiency of the irrigation method adopted was 90%. Evapotranspiration can be expressed by Eq. (1), where E = 'class A' pan evaporation (mm); K_c = crop coefficient; K_p = pan coefficient (0.8).

$$E_{Tc} = E \cdot K_p \cdot K_c \tag{1}$$

The three crops were irrigated when the soil water deficit (SWD) in the root zone was 35% of the total available water (TAW). Irrigation was scheduled based on evapotranspiration criterion providing water to the crops when the condition (2) for lettuce (a = 30 mm) and fennel (a = 25 mm) is met, where n = number of days required to reach the SWD_{lim} starting from the last watering; E_{tc} = crop evapotranspiration (mm); R_e = rainfall (mm); a = Readily Available Water

$$\sum_{1}^{n} (E_{Tc} - R_e) = a \tag{2}$$

The mean monthly main climate parameters recorded during the trial are reported in Table 1. These data were measured by a weather station located near the experimental area (ASSOCODIPUGLIA, http://www.agrometeopuglia.it/opencms/opencms/Agrometeo/Meteo/Osservazioni/dati Rilevati).

The experimental scheme adopted was the randomized block with four replicates realizing 12 large plot of the size of 20 x 20 m. During two years (2012/13 and 2013/14) of trials, three crops were grown in succession: fennel, lettuce, and fennel. Fennel 2012/13 (*Foeniculum vulgare* Mill) cv. Archimede was transplanted on September 29th, 2012 in single rows, spaced 0.2 m with plants 0.5 apart from each other, realizing a plant density of 10 plants m^{-2}, and was hand harvested on March 19th, 2013 in each plot. Pre-transplanting fertilization was applied to the soil by distributing 40 kg ha^{-1} N. Throughout the crop cycle, 110 kg ha^{-1} N were added through fertigation.

Lettuce (*Lactuca sativa* L.) cv. Iceberg, was transplanted in succession to fennel, on the same plots on April 18th, 2013 in single rows, spaced 0.5 m with plants 0.3 apart from each other, realizing a plant density of 6.7 plants m^{-2}, and was hand harvested on June 17th, 2013 in plots irrigated with IMBR water; after three days (June 20th) in plots irrigated with EFF and after eight days (June 25th) in plots irrigated with WELL water. Pre-transplanting fertilization was applied to the soil by distributing 40 kg ha^{-1}N. Throughout the crop cycle, 80 kg ha^{-1}N were added through fertigation. Fennel 2013/14 cv. Archimede, was transplanted, in succession to lettuce, on the same plots on August

30th, 2013 with the same modality of previous fennel and was hand harvested starting from December 10th 2013 (IMBR).

Table 1. Main climatic parameters recorded during the growing season of the three vegetable crops

Month	[a] Climatic Parameters							T_{max}	T_{min}	P
	T_{max} (°C)	T_{min} (°C)	RH_{max} (%)	RH_{min} (%)	Ev (mm)	Ws (m s^{-1})	P (mm)	Long term average (°C)	Long term average (°C)	Long term average (mm)
September 2012	27.80	16.10	95.30	34.80	3.73	2.51	8.60	24.13	15.07	59.60
October 2012	22.48	12.11	99.37	48.40	2.25	2.35	55.60	18.87	10.87	66.90
November 2012	16.77	9.83	98.56	67.70	1.17	2.92	151.60	15.00	7.87	80.10
December 2012	10.50	3.87	99.19	60.61	0.78	3.69	64.30	10.83	4.90	77.10
January 2013	10.49	3.28	99.45	62.84	0.91	3.62	96.80	9.43	3.47	72.80
February 2013	10.33	1.79	99.78	61.00	1.28	3.03	89.30	10.57	3.60	63.00
March 2013	13.44	5.68	99.23	57.87	1.93	4.42	62.20	12.57	5.03	63.00
Growing season Fennel	**15.97**	**7.52**	**98.70**	**56.17**	**1.72**	**3.22**	**528.40**	**14.49**	**7.26**	**68.93**
April 2013	19.31	8.48	89.13	37.23	3.29	3.33	11.00	16.53	7.63	44.70
May 2013	23.56	11.35	92.30	28.44	4.51	2.91	17.00	21.57	11.63	37.50
June 2013	26.20	14.50	82.50	31.46	5.07	2.44	11.20	25.57	15.23	31.00
Growing season Lettuce	**23.02**	**11.44**	**87.98**	**32.38**	**4.29**	**2.89**	**39.20**	**21.22**	**11.50**	**37.73**
August 2013	30.60	18.19	83.00	32.42	5.00	2.18	47.20	28.53	17.80	24.70
September 2013	26.20	14.45	92.54	35.07	3.64	2.01	9.60	24.13	15.07	59.60
October 2013	21.64	12.40	97.35	55.45	2.13	2.34	112.90	18.68	10.87	66.90
November 2013	15.13	7.99	98.17	67.20	1.19	2.55	145.60	15.00	7.87	80.10
December 2013	11.56	2.71	98.36	63.42	0.90	2.20	114.00	10.83	4.90	77.10
January 2014	11.36	4.96	98.37	70.29	0.87	3.38	58.60	9.43	3.47	72.80
Growing season Fennel	**19.42**	**10.12**	**94.63**	**53.98**	**2.29**	**2.44**	**487.90**	**17.77**	**10.00**	**381.2**

[a]T_{min}, T_{max}, monthly minimum, maximum air temperature; RH_{min}, RH_{max}, monthly minimum, maximum relative air umidity; P, total precipitation; W_s, monthly mean wind speed; Ev, total 'class A' pan evaporation.

Differently from the first fennel crop, the IMBR plots did not receive any dose of fertilizer in fertigation to evaluate the efficacy of the contribution of nutrient uptakes and fertilizer practices in more detail. Three harvestings were performed from December 2013 to January 2014, on the days after transplanting of 102, 107, 137 for IMBR, EFF and WELL plots, respectively. Marketable yield (t ha^{-1}), average weight (g) and clumps dry matter (%) were measured at harvesting time.

Water, soil, vegetable sampling and analysis

WELL, IMBR and EFF water samples were collected under the dripper at every watering throughout the crop irrigation period to quantify the main physicochemical parameters according to standard methods (APHA, 2012). The water samples were collected in triplicate in 1,000 mL PE bottles and transported to the laboratory in a refrigerated box. The samples were then kept in a refrigerator at +4 °C and examined within 24 h of their collection. The measured parameters were: pH, electrical conductivity (ECw, dS m^{-1}), BOD_5 (mg L^{-1}), COD (mg L^{-1}), ammonium-nitrogen (NH_4–N, mg L^{-1}), nitrate-nitrogen (NO_3–N, mg L^{-1}), phosphorus (PO_4–P, mg L^{-1}), sodium (Na^+, mg L^{-1}), calcium (Ca^{2+}, mg L^{-1}), magnesium (Mg^{2+}, mg L^{-1}), potassium (K^+, mg L^{-1}), sulphate (SO_4^-, mg L^{-1}), Sodium Adsorption Ratio (SAR). The anions and cations content were determined by ion-exchange chromatography (Metrohm mod. 883 Basic IC plus, Switzerland).

Soil samples were collected under the dripper in triplicate from each plot before and after every crop cycle (harvesting time) at depths decreasing from 0 to 0.4 m, every 0.2 m and they were air-dried, crushed, and passed through a 2 mm sieve before the chemical analysis. Nitrogen Kjeldahl (N), phosphorus available (P_2O_5), potassium exchangeable (K_2O), organic matter (O.M.), pH and electrical conductivity were routinely analyzed according to standard procedures (Spark, 1996).

Lettuce and fennel samples were collected at harvesting time, in triplicate from each treatment plot by picking all of the marketable size plants. The freshly collected plant samples were introduced in PE bags and immediately chilled to +4 °C and kept as such during transport to the laboratory for chemical analyses. On the marketable edible parts of vegetable crops were counted the number of plants and weighted to estimate total yield (TY, t ha^{-1}). On marketable samples from each plot, dry matter content (DM, % fresh matter) (AOAC, 1995) and an average weight of plants were also measured.

Nitrogen content of vegetables

Therefore, each sample consisted of a pool of 10 plants of a commercial size. In order not to affect the analytical determination, from each plant non-edible and damaged outer leaves were removed. The samples were not subjected to washing as this might result in the reduction of the levels of nitrates. Fresh weight was detected on the edible portion of the samples and then, after drying in a thermo-ventilated stove at 65 °C until the constant weight (dry weight) was reached. The dry substance thus obtained was finely ground with the micrometric mill and then subjected to quantitative analysis of nitrate using the method reported by Parente et al. (2002). For the determination of nitrate an ionic chromatograph Metrohm (Switzerland) mod. 883 Basic IC plus was used.

The determination of nitrate was carried out on the dry matter, while the analytical data was expressed in fresh weight.

Statistical analysis

Before to processing data with the analysis of variance, normal distribution was verified on all the experimental data, the Bartlett test was applied to verify the homogeneity of the error variance. When the data were normal and the Bartlett test was significant, the analysis of variance was performed with a nonparametric test to one classification criterion (The Kruskal-Wallis test). For multiple comparisons, it was applied the Nemenyi-Damico-Wolfe-Dunn test. In other cases, the F-test was performed for the analysis of variance (ANOVA) and the SNK test to compare the means.

RESULTS AND DISCUSSION

Irrigation water quality

In Table 2 the chemical–physical characteristics of the water used during the experimental irrigation period are reported.

Table 2. Means of the main chemical-physical parameters measured during the experimental period of trials, for the well water (WELL), full scale municipal wastewater treatment plant (EFF) and MBR pilot plant (IMBR) used for vegetable crops irrigation

		FENNEL 2012/13				LETTUCE 2013				FENNEL 2013/14				
Water parameters	U.M.	WELL	EFF	IMBR	p-value	WELL	EFF	IMBR	p-value	WELL	EFF	IMBR	p-value	Italia Law Limit Values (D.M. 185/2003) (R.R. 8/2012)
EC	(dS m^{-1})	0.94 ± 0.006	1.60 ± 0.37	0.99 ± 0.11	0.12	1.17 ± 0.14	1.13 ± 0.14	0.99 ± 0.02	0.27	0.66 ± 0.14	0.88 ± 0.03	0.94 ± 0.03	0.09	3.00
pH		7.71 ± 0.054	7.85 ± 0.03	7.79 ± 0.16	0.60	7.48 ± 0.07	7.67 ± 0.02	7.51 ± 0.05	0.09	7.30 ± 0.14	7.62 ± 0.05	7.36 ± 0.09	0.26	6–9.5
BOD_5	(mgO_2 L^{-1})	0 ± 0 **C**	10.3 ± 0.33 **B**	19.3 ± 2.4 **A**	0.001	1.35 ± 0.77 **B**	4.35 ± 0.32 **A**	4.30 ± 0 **A**	0.009	5.25 ± 0.72	7.27 ± 1.82	8.17 ± 2.51	0.51	20
COD	(mgO_2 L^{-1})	0 ± 0 **C**	13.5 ± 3.17 **B**	37.3 ± 4.3 **A**	< 0.001	2 ± 1.15 **B**	18 ± 0.58 **A**	16.5 ± 0.29 **A**	<0.001	8.5 ± 0.29	21.7 ± 2.70	22 ± 5.57	0.05	100
Na^+	(mg L^{-1})	55 ± 21.4	175 ± 61.2	66.3 ± 4.4	0.09	42 ± 13.8	74.5 ± 2.02	189.5 ± 68.4	0.16	12.5 ± 0.29 **B**	74.7 ± 3.33 **A**	62.7 ± 6.67 **A**	0.0007	
K^+	(mg L^{-1})	0 ± 0 **b**	3.5 ± 2.02 **b**	13.6 ± 3.2 **a**	0.03	1.5 ± 0.86 **b**	17.5 ± 0.29 **ab**	38 ± 11.5 **a**	0.05	0.5 ± 0.29 **B**	12 ± 1.52 **A**	13.3 ± 1.76 **A**	0.0047	
Ca^{2+}	(mg L^{-1})	45.8± 20.3	85.5 ± 3.17	78.3 ± 26.9	0.35	83.5 ± 5.48	48 ± 1.15	348 ± 169.1	0.18	60.5 ± 19.3	69.3 ± 13.2	95.7 ± 50.7	0.62	
Mg^{2+}	(mg L^{-1})	25 ± 5.2	27 ± 6.35	10.3 ± 3.75	0.23	39.5 ± 3.17**AB**	5.5 ± 1.44 **B**	59.5 ± 13.5 **A**	0.034	20 ± 11.6	8 ± 4.62	23.3 ± 19.1	0.47	
NH_4^+	(mg L^{-1})	3 1 ± 1.73 **B**	5 ± 2.89 **B**	19 ± 1.15 **A**	0.01	0 ± 0	0 ± 0	0 ± 0		0 ± 0	0 ± 0	11.3 ± 6.64	0.16	2 (15)[a]
Cl^-	(mg L^{-1})	79 ± 25.4	357 ± 144.9	92.6 ± 10.3	0.09	106.5 ± 41.3	122.5 ± 7.21	120.5 ± 14.7	0.91	16.9 ± 2.39 **B**	91.3 ± 12.3 **A**	91.3 ± 5.36 **A**	0.0017	250
NO_3^-	(mg L^{-1})	6.7 ± 3.75 **c**	33 ± 4.04 **b**	85 ± 9.45 **a**	0.02	13 ± 0 **B**	33.5 ± 3.17 **B**	73 ± 19.1 **A**	0.001	7.85 ± 0.09	34.7 ± 18.6	99.3 ± 36.2	0.06	35*(15)[a]
$P_2O_4^{3-}$	(mg L^{-1})	1.3 ± 1.3	0.66 ± 0.67	8.7 ± 4.7	0.23	0.01 ± 0.01 **b**	21 ± 6.24 **a**	15.3 ± 2.9 **a**	0.02	0 ± 0	15 ± 13	12.7 ± 7.22	0.34	10 (2)[b]
SO_4^{2-}	(mg L^{-1})	50 ± 24.2 **B**	129 ± 5.19 **A**	3.3 ± 3.3 **B**	0.008	6 ± 1.73 **B**	61 ± 11.5 **A**	31.5 ± 7.8 **B**	0.01	4.5 ± 2.59 **b**	61 ± 11.1 **a**	19.3 ± 13.4 **b**	0.034	500
SAR		1.79 ± 0.7	4.1 ± 1.27	1.93 ± 0.2	0.16	0.93 ± 0.28 **b**	2.71 ± 0.04 **a**	2.45 ± 0.35 **a**	0.03	0.44 ± 0.12 **b**	2.41 ± 0.43 **a**	1.96 ± 0.55 **a**	0.03	10

* Limit related to total nitrogen; [a]limit concentration for ammonium can be raised to the value in brackets upon special permission (R.R. 8/2012); [b]limit concentrations for total nitrogen and total phosphorus (in brackets the limit concentrations for areas declared vulnerable to nitrate and phosphate pollution); data are means ± standard error for each water analysed between September 2012 and January 2014; capital letters (A, B and C) represent significant differences at $P < 0.01$; lower case letters differences at $P < 0.05$.

In fennel 2013, the organic matter content expressed as BOD and COD, and the nitrate content present in IMBR water were doubled compared to EFF. For potassium, the value was just higher than EFF. Whereas lettuce 2013 showed in IMBR a content of nitrate and potassium twice compared with EFF. Regarding fennel 2013/14, the content of nitrate of IMBR was three times the EFF.

The higher NO_3^- levels in IMBR respect to EFF and WELL indicate that IMBR represents the major source of nutrient for the plants and the soil, and can contribute to crop growth (Gatta et al., 2014) (Generally it is not taken into account by farmers when applying fertilizer).

As reported in Jensen et al. (2006), from an agronomic perspective wastewater irrigation represents an opportunity for accessing 'free' nutrients which if realized contribute towards the inter–related objectives of productivity maximization, nutrient capture and wastewater reclamation and reuse. The resulting nitrogen excess in the soil is then particularly vulnerable to the risk of leaching, thus increasing the environmental problem of nitrate pollution (Gatta et al., 2014). Consequently, the use of wastewater for irrigation helps to reduce downstream health and environmental impacts that would otherwise result if wastewater was discharged directly into surface bodies (Mateo–Sagasta & Burke, 2010).

Table 3 shows main parameters of soil irrigated during the trial. No significant differences were found.

Table 3. Average values of main soil chemical parameters measured over the research period along the soil profile. EC and pH were measured on 1:2 (w/v) and 1:2.5 (w/v), respectively

Soil parameters	Fennel 2012/13			Lettuce 2013			Fennel 2013/14		
	WELL	EFF	IMBR	WELL	EFF	IMBR	WELL	EFF	IMBR
EC	0.22	0.20	0.21	0.38	0.35	0.43	0.16	0.25	0.26
pH	8.10	8.07	8.04	8.03	8.15	8.05	7.88	8.09	8.02
O.M. (%)	1.61	1.77	2.02	1.72	1.84	2.03	1.69	1.76	1.91
N (g kg^{-1})	1.11	1.11	1.14	1.08	1.21	1.39	1.11	1.25	1.40
P_2O_5 (mg kg^{-1})	82	87	133	43	47	77	36	38	43
K_2O (mg kg^{-1})	132	134	148	111	116	120	73	82	84

Harvest and yields

The harvest of fennel 2012/13 was performed after 171 days at once for all treatments (IMBR, EFF, and WELL). Data collected were a marketable yield (t ha^{-1}), average weight (g), dry matter of clumps (%) and nitrate concentration (mg kg^{-1}fw). The results obtained for the nitrates are respectively 169 mg kg^{-1} for WELL, 754 mg kg^{-1} for EFF and 1,040 mg kg^{-1} for IMBR.

The harvest of lettuce took place after 60, 63 and 68 days respectively for the theses IMBR, EFF and WELL. Data collected were a marketable yield (t ha^{-1}), average weight (g), dry matter of heads (%) and nitrate concentration (mg kg^{-1} fw). The data obtained show an average concentration of nitrate content of 244 mg kg^{-1} in the thesis irrigated with WELL, of 477 mg kg^{-1} in the thesis EFF and 804 mg kg^{-1} for the IMBR. The harvest of fennel 2013/14 was performed after 102, 109 and 137 days respectively for the thesis IMBR, EFF and WELL. Data collected were the same as the previous year trial. In this case, the results obtained for the nitrates are respectively 140 mg kg^{-1} for WELL, of 743 mg kg^{-1} for EFF and 245 mg kg^{-1} for IMBR. The levels of nitrate (mg kg^{-1} fresh weight) in the considered plants, the yield data (t ha^{-1}), the average weight (g), dry matter (%), the number of days from transplanting to harvesting and inputs of nitrogen (mg ha^{-1}) are reported in Table 4. The result is given as the average of 4 replicates.

The results show that, even for breeding crops particularly prone to a high accumulation of nitrates, the values obtained are well below the limits permitted by law. In fact, in none of the samples analyzed the nitrate concentration found exceeded the limits set by EU Regulation No. 1258/2011 (EC, 2011) (Fig. 3).

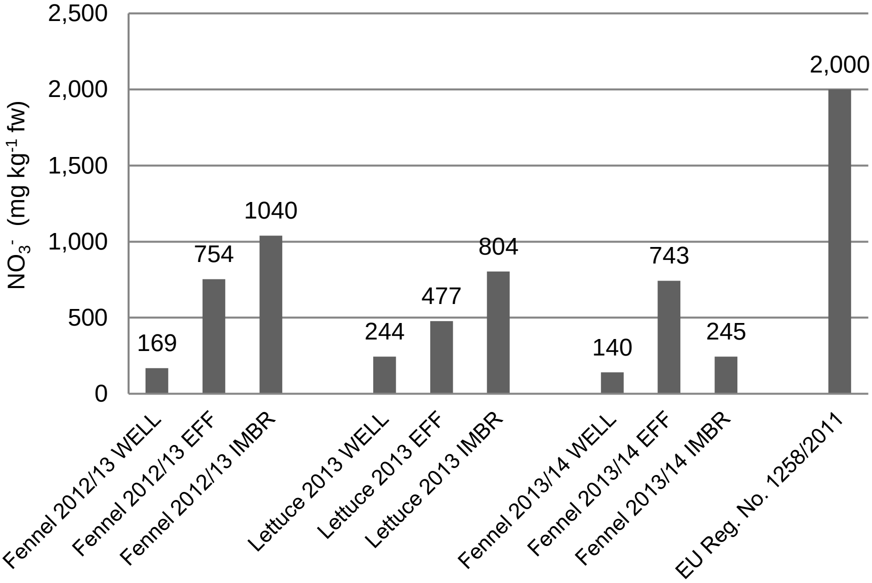


Figure 3. Comparison of average values of nitrates found in all the theses and limit defined by EU Regulation No. 1258/2011.

The use of wastewater to fertigate fennel and lettuce had positive effects on fertilizer management. Although excessive doses of nitrogen (mineral fertilization plus IMBR nitrate intake) were tested, the nitrate content of lettuce was found to be well below the limits allowed by law (2,000 mg kg^{-1}). Perhaps this is due to the effects of the climatic conditions of the experimental site located in Southern Italy, which may contribute to a content of nitrates lower compared to northern regions. Fennel 2013/14, grown in the same IMBR plots of lettuce and fennel 2012/13, did not get any doses of fertilizer. The only input of nitrates was from wastewater (74.8 kg ha^{-1}), and N supplied at transplanting.

In this case, wastewater reuse led to a reduction of 54% of nitrogen fertilizer in relation to the other plots normally fertilized. All this resulted in an advance of maturity (harvest made 35 days before conventional), better quality (marked green color of the leaves and more resilient post-harvest), a lower nitrate content than the average reported in the literature and a significant savings of chemical nitrogen fertilizers.

Table 4. Effects of water irrigation treatments (WELL = conventional water; EFF = effluent from full scale treatment plant; IMBR = effluent from pilot treatment plant) on yield, average plant weight, dry matter, levels of nitrate (on fresh weight), number of days from transplanting to harvest and nitrogen inputs at harvesting time

							N supply (kg ha^{-1})				
Crop	Treatments	Yield (t ha^{-1})	Average weight (g)	Dry matter (%)	NO_3^- (mg kg^{-1} fw)	Harv. time	Seasonal Irrigation Volume (m^3 ha^{-1})	Pre-transplanting fertilization $HPO_4(NH_4)_2$	Fertirrig. NH_4NO_3	N in the water	N TOTAL
	WELL	32.16 B	441 B	7.95 a	169 B	171	800	40	110	0	150.0
	EFF	46.09 A	656 A	7.43 a	754 A	171	800	40	110	4.8	154.8
	IMBR	47.80 A	668 A	7.38 a	1040 A	171	800	40	110	6	156.0
	WELL	31.28 B	556.41 B	4.93 a	244 B	68	1,800	40	80	4.1	124.1
	EFF	38.49 B	591.84 B	4.80 a	477 AB	63	1,800	40	80	15.5	135.5
	IMBR	53.64 A	826.01 A	4.01 b	804 A	60	1,800	40	80	69.2	189.2
	WELL	31.63 B	373.80 b	8.98 a	140 b	137	1,100	40	110	1.7	151.7
	EFF	29.82 C	380.01 b	8.70 a	743 a	109	1,100	40	110	12.4	162.4
	IMBR	39.97 A	487.02 a	7.67 b	245 b	102	1,100	40	0	34.8	74.8

Capital letters represent significant differences at *P < 0.01*; lower case letters differences at *P < 0.05*.

Usually, wastewater–watering protracts the crop development pattern, prolonging ripening, delaying flowering and reducing the economic fraction (marketable yield) (Jensen et al., 2006). In the case of leafy vegetables, high N–availability in wastewater promoting vegetative growth (= increasing production), indeed represents an important benefit. Moreover, the continuous supply of nitrogen with irrigation is, without doubt, the most important factor – especially in autumn–winter seasons – limiting nitrogen losses from gasification and leaching thereby improving the efficiency of fertilization.

Wastewater contains nutrients in many forms (ammonia, phosphates, nitrate, etc.), with a daily and seasonal variation in concentrations. Therefore, wastewater irrigation could contribute to reduced nutrients from the environment being high enough to at least partly fulfill crop nutrient requirements. Nonetheless, a total application of organic and mineral fertilizers is excessive.

Farmers frequently oversupply nutrients (Evers et al., 2006). Wastewater nutrient content is sufficient to partly meet crop nutrient requirements per growing season, but farmers often use wastewater only as a source of water and do not consider it as a source of nutrients. This conduct is frequently due to a lack of information on nutrient management and wastewater-quality from institutional organizations. With careful planning and management, the use of wastewater for agriculture can be beneficial to farmers, cities and the environment (Mateo–Sagasta & Burke, 2010).

CONCLUSIONS

This study focuses on agronomic aspects of wastewater and its nutrient opportunity, a resource still very undervalued and unexploited in Italy. According to the data obtained, it is possible to conclude that treated municipal wastewater without nutrient-removal influences the crop cycle and represents an alternate and relevant source of nutrients intake. In particular, the high nitrogen content enhances vegetative growth, promotes crop development and sustains both economic and environmental benefits. By the current market price of nitrogen fertilizer, the estimated savings is about 98.00 € ha^{-1}.

These results should encourage achievement of a more-sustainable agriculture through the use of treated municipal wastewater not deprived of nutrients, thereby limiting the use of higher-quality water, thus saving fertilizers and money. All this can be accomplished while respecting and protecting the environment.

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Peas and beans as a protein feed for dairy cows

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Abstract. The need for alternative protein sources to soybean meal, partially or fully substituted in the diets of dairy cows, is an urgent problem in farming nowadays. Soybean meal is the most common protein source included in feed concentrate for dairy cows in Latvia and in other European countries as well. Among possible alternatives, grain legumes seem interesting for dairy cow diets because of their rapid degradation in the rumen and readily available energy. Peas and beans will be an important source of proteins in feed. Biochemical tests were done on eight samples of domestically grown dried peas of average size, 11 samples of dried beans of average size and some samples of soybean meal to examine the chemical composition of the peas and beans. Peas and beans were included in the feed ration during a feeding trial on dairy cows. Milk yields and milk quality parameters were examined in the trial. The digestibility of peas of most varieties and breeding lines examined was considerably higher than that of soybean meal, while the digestibility of beans of all the varieties and breeding lines examined and of soybean meal was the same. The peas contained more reducing sugars, starches and had a higher value of NEL than the tested beans, which meant the peas had a higher nutritional value. The diets comprising beans and peas fed to the dairy cows increased the fat and protein contents of milk, compared with the control group and the beginning of the trial. The total amount of amino acids increased in the bulk milk samples of all the trial groups during the feeding trial.

Key words: peas, beans, dairy cows, nutritional value, productivity, milk quality.

INTRODUCTION

The need for alternative protein sources to soybean meal, partially or fully substituted in the diets of dairy cows, is an urgent problem in farming nowadays. The use of alternative sources of plant protein to soybean meal in diets for agricultural animals aims to reduce soybean imports into the EU and partially substitute genetically modified organisms in the food chain. Among possible alternatives, grain legume seem interesting for dairy cow diets because of their rapid degradation in the rumen and readily available energy (Wilkins & Jones, 2000; Volpelli et al., 2012). Pulses (peas, chickpeas, and beans) are an important source of proteins in food and feed. The protein contents of pulses are high, and the essential amino acid profiles of pulses are well-balanced.

Research studies indicate that some functional properties of pulse proteins may be comparable to those of other frequently used proteins such as soya (Boye et al., 2010). Protein is the source of amino acids and nitrogen in feeds. Livestock need it for growth and milk production. Protein is also needed by rumen bacteria, which digest much of the feed for ruminant animals like cattle, sheep and goats (Rayburn, 1996).

Legumes are not only a rich source of protein; they also contain fibre, which is essential for normal functioning of the digestive tract. Legumes are a rich source of vitamin B_6 that is required for normal amino acid metabolism and contain vitamin B_2, or riboflavin, which ensures energy exchange in cells, as well as fat and protein metabolism. Legumes also contain sugar and starch, which are the sources of energy and minerals such as magnesium that is important for normal cardiac function, manganese that is necessary for enzymes, such as transferases, and other elements that improve the metabolic processes of animals. Unlike protein products of animal origin, legumes contain much less fat (Mokoboki et al., 2000; Savadogo et al., 2000; Wilkins & Jones, 2000; Tessema & Baars, 2004; Huhtanen, 2005).

Information on the digestibility of nutrients is of great importance when identifying the nutritional quality of feeds. Digestibility is a measure of the biological availability of nutrients, and it is important in formulating a balanced ration in order to have maximum productivity in animals (Forejtova et al., 2005; Homolka et al., 2012).

Forage quality affects the potential of livestock to produce milk from the forage through the utilization of its nutrients. The level of animal productivity is controlled nutritionally through the daily intake of digestible nutrients and depends on the pace at which such nutrients can be metabolized and used for body processes (Bush et al., 1980; Karsli & Russell, 2002; Tessema & Baars, 2004; Căpriţă et al., 2012) The digestibility of a feedstuff and the fermentation pattern influence the daily dry matter intake (DMI), which is important for today's highly productive dairy cows. (Allen, 2000; Savadogo et al., 2000; Froidmont & Bartiaux-Thill, 2004).

Dietary factors can greatly affect the composition of milk of dairy cows, and nutrition offers the most effective ways for rapidly altering the composition of milk. Among milk components (fat, protein, lactose, minerals and vitamins), fat and protein are the two being most subjected to changes due to dietary manipulation (Santos, 2002). It is well accepted that amino acids, as building blocks of protein, play an essential role in the nutritional composition of a feedstuff (Haffner et al., 2000). The supply of amino acids by the mammary gland of dairy cows is elevated due to feeding higher amounts of rumen-undegradable protein.

The aim of the present research was to evaluate beans and peas as a protein-rich feed for dairy cows as well as the productivity of the dairy cows and milk quality indicators.

MATERIALS AND METHODS

Biochemical tests were done on eight samples of domestically grown peas of average size (n = 5), 11 samples of faba beans of average size (n = 5) and some samples of soybean meal (n = 5). Crude protein (LVS EN ISO 5983-2:2009) and digestibility (cellulase method) were identified in the present research. The average results were summarised and analysed for the tests carried out in the years 2014 and 2015. Table 1 presents the varieties and breeding lines of peas and faba beans used for the biochemical tests.

The forage tests were done at the accredited Research Laboratory of Agronomic Analyses of Latvia University of Agriculture (LLU) according to the following standards: dry matter – Feed Analyses met.2.2.1.1: 1993, crude protein – LVS EN ISO 5983-2: 2009, fibre – ISO 5498: 1981, NDF% – LVS EN ISO 16472: 2006, ADF%, NEL, MJ kg^{-1} – LVS EN ISO 13906: 2008, calcium – LVS EN ISO 6869: 2002, phosphorus – ISO 6491: 1998.

Table 1. Varieties and breeding lines of peas and faba beans investigated

No	Pea varieties	Bean varieties	Fodder
1	'Bruno'	'Ada'	Soybean cake
2	'Vitra'	'Lielplatone'	-
3	'Zaiga'	'Jogevas'	-
4	'Lasma'	'Fuego'	-
5	'Alma'	'Scirocco'	-
6	'Selga'	'Tolea'	-
7	Breeding line H-06-04-4	'Priekulu'	-
8	Breeding line H-86-19-3	'Priekulu 32'	-
9	-	'Bauskas'	-
10	-	'Valmieras'	-
11	-	Breeding line H-10-10-10	-

In vitro digestibility was estimated for totally 15 feed samples: peas 'Bruno', peas 'Capella', peas 'Looming', fodder beans (2 samples), fodder peas, soybean meal (2 samples), rapeseed meal, silage (grass+legume) (2 samples), hay (grass+legume) (2 samples), feed concentrate (meal) and feed concentrate (pellets). *In vitro* enzymatic digestibility was estimated at the accredited Research Laboratory of Agronomical Analyses of Latvia University of Agriculture employing the enzymatic method and procedure (De Boever et al., 1988). The *in vitro* digestibility method and procedure is as follows.

A small quantity (0.300 g) sample is weighed in a tube and 30 mL pepsin HCl solution is added (De Boever et al., 1988). The tube is closed with an overpressure cap, incubated at 38 °C for 24 hours and shaken twice a day. After 24 h the tubes are put in a warm water bath at 80 °C for 45 minutes. The solution is sucked out and washed three times with water of 60 °C and 30 mL of buffered cellulose solution is added. It is incubated at 39 °C for 24 hours and shaken twice a day, filtrated in a sintered glass crucible and washed 3 times with water of 60 °C. Then it is dried at 103 °C until a constant weight is obtained. Afterwards it is cooled in an exsiccator and weighed with a 0.1 mg precision. Then it is reduced to ash for at least 2 hours at 550 °C until a constant weight is obtained, cooled in an exsiccator and weighed again with a 0.1 mg precision. An equation for calculating the enzymatic activity is as follows:

$$\mathrm{DCom} = 100\times\left(1 - \frac{(A - B) \times 1{,}000}{(C - D) \times 0.300}\right)$$

where DCom – in vitro enzymatic digestibility; A – weight of the crucible+residu after drying; B – weight of the crucible+residu after ashing; C – absolute dry matter in g kg^{-1}; D – ash in g kg^{-1}.

In each test, three standard samples were used to correct for fluctuations in enzyme activity. The digestibility of dry matter and protein in feed rations was calculated in terms of the amount of feed consumed by cows per day, the chemical content of forage and *in vitro* digestibility indices of feedstuffs.

For the determination of the amino acid content of feed and milk, samples were hydrolysed according to the procedures described in Commission Regulation

No 152/2009 (2009). Amino acid tests were performed by means of AccQ Tag technology (Waters Corp., Miliford, MA) and quantified by means of Shimadzu HPLC (low pressure gradient system) consisting of a solvent delivery module LC–10AT$_{VP}$, an automatic injector SIL–10AD$_{VP}$, a column oven CTO–10AC$_{VP}$, a spectrofluorometric detector RF–10A$_{XL}$, a system controller SCL–10A$_{VP}$, and an on–line degasser DGU–14A. Amino acid separation was performed using a Nova–Pak C18, 4 μm, 150 × 3.9 mm (Waters Corp., Miliford, MA) chromatography column at 37 °C.

The scheme of cow diets is shown in Tables 2 and 3. During the trial, the dairy cows received the basic feed ration, which consisted of the following components measured per cow per day: 40 kg of silage (grass+legume), 3 kg of hay (grass+legume), 4 kg of fodder (grains), 4 kg of complementary and 0.15 kg of mineral additives.

Table 2. Scheme of cow diets

Group	1st group – trial	2nd group – trial	3rd group – trial	4th group –control
Feed ration	CF + 10–12% Pisum sativum ‘Bruno’ + 10–12% Vicia faba variety minora	CF + 20–24% Pisum sativum ‘Bruno’	CF + 20–24% Vicia faba variety minora	CF + soybean cake

CF – conventional feed (different grains and rapeseed cake).

Table 3. Dairy cow diets during the trials

Feedstuffs	Amount, kg	1st group	2nd group	3rd group	4th control group
Silage	40	40	40	40	40
Hay	3	3	3	3	3
Feed concentrate	4	4	4	4	4
Complementary additive	4	4	4	4	4
Peas+beans	-	1.82	-	-	-
Peas	-	-	1.9	-	-
Beans	-	-	-	1.7	-
Soybean meals	-	-	-	-	1
Mineral additive	0.15	0.15	0.15	0.15	0.15
Feed ration contains:					
Dry matter, kg	-	21.60	21.70	21.50	20.80
Crude protein, g	-	3,266	3,261	3,76	3,258
NEL, MJ	-	142.7	143.8	139	137.20
Calcium, g	-	153	157	155	162
Phosphorus, g	-	82.0	83.0	85.0	82.0

The difference in diet between the trial groups and the control group was that the trial groups of cows were fed diets composed of 1.82 kg of peas+beans (0.85 kg + 0.97 kg, respectively) (1st trial group), 1.9 kg of peas (2nd trial group) and 1.7 kg of beans (3rd trial group), while the control group received 1 kg of soybean meal (4th group).

The feed ration varied according to each cow’s milk yield and physiological state and was monthly corrected according to the lactation cycle.

The parameters of the feed ration corresponded to the NRC (2001), these dietary norms were set for cows with a live weight of 650 kg, a milk yield of 30 kg per day, a 4.10% fat and a 3.20% protein content of milk and a lactation period of 60–100.

The obtained results were statistically processed and analysed. To identify the magnitude of difference in the indicators of faba beans, peas and soybean meal, the data were analysed employing a nonparametric method – a Mann-Whitney U–criteria test. To identify cow productivity differences in comparison with the control group, the data were analysed by a Mann–Whitney test, and a Wilcoxon signed–rank test was done to identify differences in data between the beginning and the end of the experiment at a confidence interval of 95% ($\alpha = 0.05$). The data processing was performed using the data processing program *SPSS 16.0*.

RESULTS AND DISCUSSION

Biochemical composition of peas and beans

Data on the crude protein (CP) contents and the digestibility of peas and faba beans of the varieties and breeding lines examined, compared with soybean meal, are presented in Tables 3 and 4. After processing the data, one can see that the CP contents of peas and faba beans of the varieties and breeding lines examined show mainly significant differences ($p < 0.05$). The varieties and breeding lines that showed significant differences in the indicators were assigned the same number as presented in Table 5.

Table 4. Crude protein contents and digestibility of peas of the varieties and breeding lines examined (2014–2016 average data)

No	Varieties and breeding lines	Crude protein, % in DM	Digestibility, %
1	‘Bruno’	26.37 ± 0.055 ^{2,3,4,5,6,7,8}	82.0 ± 0.141 ^{2,3,4,5,6,7,8}
2	‘Vitra’	25.06 ± 0.120 ^{1,3,4,5,6,7,8}	82.5 ± 0.071 ^{1,3,4,5,6,7,8}
3	‘Zaiga’	21.93 ± 0.040 ^{1,2,4,5,6,7,8}	83.2 ± 0.141 ^{1,2,4,5,6,7,8}
4	‘Lasma’	20.11 ± 0.083 ^{1,2,3,5,6,7,8}	83.4 ± 0.071 ^{1,2,3,5,6,7,8}
5	‘Alma’	22.67 ± 0.066 ^{1,2,3,4,6,7,8}	81.4 ± 0.071 ^{1,2,3,4,6,7,8}
6	H-06-04-4	22.37 ± 0.221 ^{1,2,3,4,5,7,8}	83.7 ± 0.100 ^{1,2,3,4,5,7,8}
7	H-86-19-3	23.21 ± 0.160 ^{1,2,3,4,5,6,7}	82.9 ± 0.072 ^{1,2,3,4,5,6,8}
8	‘Selga’	18.59 ± 0.144 ^{1,2,3,4,5,6,7,}	82.5 ± 0.069 ^{1,2,3,4,5,6,7}
9	Soybean cake	50.42 ± 2.94 ^{1,2,3,4,5,6,7,8}	81.7 ± 0.774 ^{2,3,4,6,7,8}

Data are presented as means ± SD (n = 5 in each group). Means with different superscript numbers (^{1,2,3,4,5,6,7,8,9,10,11}) are significantly different among varieties ($p < 0.05$).

Overall, in the experiment, the highest CP content was identified in the pea variety ‘Bruno’ (26.37%) (used in the feeding trial), while the best digestibility (83.7%) was specific to the pea breeding line H-06-04-4, compared with the other pea varieties. The highest CP content of faba beans was identified in the variety ‘Priekulu’ (31.36%), while the faba bean variety ‘Jogevas’ had the best digestibility (81.50%), compared with the other faba bean varieties and breeding lines. The CP content of soybean meal was higher than that of peas and faba beans (51.31%), but the digestibility of it was lower (81.65%) and showed no trend towards a significant difference, compared with the pea varieties and breeding lines.

Table 5. Crude protein contents and digestibility of faba bean of the varieties and breeding lines examined (2014–2016 average data)

No	Varieties and breeding lines	Crude protein, % in DM	Digestibility %
1	'Ada'	30.75 ± 0.106 ^{2,3,4,5,6,7,8,10,11}	79.5 ± 0.071 ^{2,3,4,6,7,8,9,10,11}
2	'Lielplatones'	29.41 ± 0.444 $^{1\ 3\ 4\ 5\ 6\ 7}$	79.2 ± 0.068 ^{1,3,4,5,6,7,8,10,11}
3	'Jogevas'	29.68 ± 0.014 $^{1\ 2\ 4\ 5\ 6\ 7\ 8\ 10\ 11}$	81.5 ± 0.100 ^{1,2,4,6,7,9,10,11}
4	'Fuego'	26.66 ± 0.379 ^{1,2,3,5,6,7,8,9,10,11}	77.5 ± 0.076 ^{1,2,3,5,6,7,8,9,10,11}
5	'Scirocco'	28.42 ± 0.015 ^{1,2,3,4,6,7,8,9,10,11}	79.1 ± 0.505 ^{2,4,6,9}
6	'Tolea'	31.68 ± 0.92 ^{1,2,3,4,5,8,9,10,11}	80.1 ± 0.070 ^{1,2,3,4,5,7,8,9,10,11}
7	'Priekulu'	31.36 ± 0.485 ^{1,2,3,4,5,8,9,10,11}	78.9 ± 0.071 ^{1,2,3,4,6,8,9,10,11}
8	'Piekulu 32'	28.78 ± 0.072 ^{1,3,4,5,6,7,9,11}	79.1 ± 0.105 ^{1,2,4,6,7,9,10,11}
9	'Bauskas'	30.20 ± 0.704 ^{4,5,6,7,8,}	78.6 ± 0.08 ^{1,3,4,5,6,7,8,10,11}
10	'Valmieras'	29.18 ± 0.416 ^{1,3,4,5,6,7,11}	79.8 ± 0.09 ^{1,2,3,4,6,7,8,9,11}
11	H-10-10-10	29.35 ± 0.212 ^{1,3,4,5,6,7,8,10}	79.8 ± 0.149 ^{1,2,3,4,6,7,8,9,10}
12	Soybean cake	50.42 ± 2.940 ^{1,2,3,4,5,6,7,8,9,10,11}	81.7 ± 0.774 ^{1,2,4,5,6,7,8,9,10,11}

Data are presented as means ± SD (n = 5 in each group). Means with different superscript numbers (^{1,2,3,4,5,6,7,8,9,10,11}) are significantly different among varieties ($p < 0.05$).

A comparison of digestibility between soybean meal and beans shows significant ($p < 0.05$) differences for a number of bean varieties and the breeding line. The digestibility of soybean meal is better.

The indicator of feed digestibility is as important as the composition of feed. A high feed digestion rate increases the amount of nutrients in an animal's organism, thus providing a high overall productivity level. The digestibility of peas of the breeding line H-06-04-4 (83.70%) was considerably ($p < 0.05$) better than that of the pea variety 'Lasma' (83.40%) and soybean meal (81.65%). The bean varieties and the breeding line, compared with soybean meal, on average, had the same or slightly worse digestibility.

The contents of crude fibre and its fractions ADF, % and NDF, % were higher in beans, while NEL MJ kg^{-1} was higher in peas than in beans (Fig. 1). Table 6 shows that the highest NEL MJ kg^{-1} was found in the pea breeding line H-06-04-4, while among pea varieties with white flowers the best performers were 'Lasma' and 'Zaiga' and among pea varieties with pink flowers – 'Selga' and 'Vitra'.Among the faba bean varieties, the highest NEL MJ kg^{-1} (Table 6) was found in the variety 'Jogeva'.

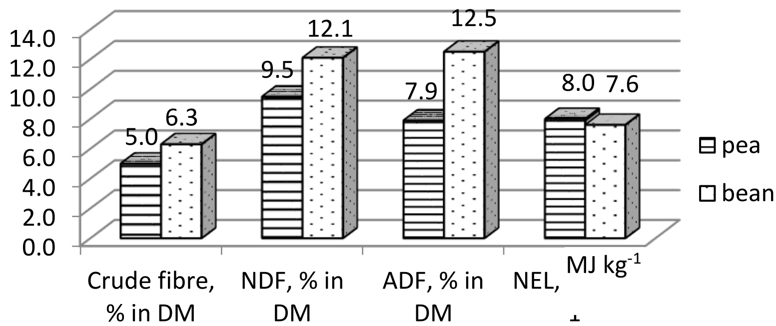

Figure 1. Fibre contents of peas (n = 16) and beans (n = 22) and NEL – average data.

Table 6. Biochemical composition of peas and beans (average data)

Indicators	DM, %	Fat, %	Reduced sugar, %	Starch, %	Crude fibre, %	NDF, %	ADF, %	NEL, MJ kg^{-1}	Ca, %	P, %
Peas										
Bruno	87.97	1.24	0.57	51.65	7.08	13.98	8.8	7.91	0.08	0.42
Vitra	88.33	1.26	0.27	53.53	6.87	9.9	8.24	7.95	0.08	0.4
Zaiga	89.71	1.53	0.23	52.65	4.06	8.58	7.33	8.03	0.12	0.37
Lasma	90.3	1.31	0.21	56.9	3.91	8.07	6.98	8.05	0.09	0.33
Alma	90.47	1.37	0.26	49.89	5.65	11.31	9.68	7.84	0.12	0.35
H-06-04-4	89.73	1.39	0.21	54.25	3.62	7.38	6.65	8.08	0.12	0.38
H-86-19-3	89.76	1.39	0.21	51.4	4.47	8.35	7.65	8.0	0.15	0.54
Selga	90.27	1.44	0.26	56.48	4.09	8.75	8.15	7.96	0.09	0.37
Faba Beans										
Ada	89.15	1.18	0.18	46.06	6.04	13.63	12.0	7.65	0.12	0.62
Lielplatones	88.46	1.01	0.16	47.15	5.86	14.77	12.4	7.62	0.13	0.6
Jogeva	89.01	1.12	0.15	46.9	5.4	9.73	9.41	7.86	0.12	0.62
Fuego	89.72	1.06	0.13	47.99	7.15	12.44	14.62	7.44	0.13	0.58
Scirocco	89.38	1.21	0.12	46.32	6.42	12.38	12.81	7.59	0.13	0.66
Tolea	89.75	0.92	0.15	44.79	5.83	12.04	11.27	7.71	0.11	0.55
Priekulu	90.28	1.05	0.16	44.22	6.67	11.42	12.81	7.59	0.12	0.69
Priekulu 32	90.08	0.99	0.16	45.77	6.89	11.41	15.53	7.61	0.11	0.69
Bauskas	89.97	0.94	0.1	46.07	6.0	10.68	13.61	7.56	0.12	0.62
Valmieras	90.56	1.03	0.1	45.47	6,45	11.52	11.58	7.69	0.12	0.78
XXX	88.92	0.77	0.11	44.61	6.48	12.75	11.65	7.68	0.11	0.58
Soybean										
Soybean 1	88.23	2.27	0.31	7.95	3.41	13.74	10.3	7.8	0.41	0.72
Soybean 2	87.41	2.42	0.22	7.62	3.57	13.98	9.02	7.89	0.42	0.71

In comparison with the bean varieties, all of the pea varieties and breeding lines had higher reducing sugar contents. The highest sugar content was found in 'Bruno' (0.57%) – the pea variety with pink flowers. Among the bean varieties, the highest sugar content was found in the breed 'Ada' (0.18%).

Compared with the bean varieties, all the varieties of peas had higher starch contents. Among the pea varieties with white flowers, the highest starch content was found in 'Lasma', while among the pea varieties with pink flowers – in 'Selga'.

The average calcium (Ca) content in the varieties of peas was 0.11% (n = 8) and 0.12% (n = 11) in the varieties of beans. The average phosphorus (P) content was higher in beans 0.64% (n = 11) than in peas – 0.40% (n = 8).

The obtained results of biochemical composition tests showed that the varieties and breeding lines of beans and peas grown in Latvia contained the proteins necessary in feed and may be used in cow diets, replacing an equivalent amount of soybean protein.

The analysis of feed rations fed to cows during the trials, in terms of chemical composition, proved that, in general, the rations met the standards. Slight differences were found in the provision of mineral elements to all the groups of cows. The feed rations satisfied the need of cows for dry matter, dietary energy, crude protein, calcium and phosphorus (Wilkins & Jones, 2000).

Voluntary intake and digestibility of forages are characteristics that affect the animal's performance. Under those management conditions, the herd's productivity relied heavily on the quality of feedstuffs produced, measured either in terms of composition and digestibility or in terms of fermentation characteristics. Therefore, it was important to determine feed quality characteristics, such as digestibility of dry matter and crude protein content for the formulation of balanced rations for ruminants (Mould, 2003; Huhtanen, 2005).

The results of a nutrient digestibility test on dairy cows are presented in Table 7. The research results show that dry matter digestibility in 2015 in the control group (4) was 68.73%, while in the trial groups (1, 2 and 3) – 69.29%, 69.42% and 69.06%, which was higher than in the control group by 0.56%, 0.69% and 0.33%, respectively.

Table 7. Digestibility of dry matter and protein in dairy cows in 2015–2016

Indices		1st trial group	2nd trial group	3rd trial group	4th control group
Dry matter digestibility, %	2016	69.29 ± 0.02	69.42 ± 0.02	69.06 ± 0.04	68.73 ± 0.07
	2015	69.02 ± 0.01	69.09 ± 0.07	68.75 ± 0.03	68.41 ± 0.007
Both years on average, %		69.16 ± 0.19	69.26 ± 0.23	68.91 ± 0.21	68.57 ± 0.22
Protein digestibility, %	2016	65.82 ± 0.10	65.97 ± 0.01	65.72 ± 0.01	65.67 ± 0.07
	2015	66.94 ± 0.007	67.04 ± 0.01	66.86 ± 0.08	66.82 ± 0.01
Both years on average, %		66.38 ± 0.79	66.51 ± 0.75	66.29 ± 0.80	66.25 ± 0.81

The results show that dry matter digestibility in the 1st and the 2nd trial groups of cows was similar – in the range of 69.02–69.09%, while in the 3rd trial and the 4th control groups – in the range of 68.75–68.41%. The digestibility test results show that in both trial years, on average, higher dry matter digestibility was found in the 2nd group, in the feed ration of which 'Bruno' peas were included, and in the 1st group whose ration comprised peas + beans.

The results show that in 2015 protein digestibility in the groups of cows was similar in the range of 65.67–65.97%. Protein digestibility in the control group of cows was 65.6s7%, which was lower than in the trial groups of cows by 0.15%, 0.30% and 0.05%, respectively. The highest protein digestibility was demonstrated by the 2nd trial group of cows – 65.97% and the 1st trial group of cows – 65.82%.

In 2016, protein digestibility increased in all the groups of cows, in comparison to the previous year. The highest protein digestibility was demonstrated by the 2nd trial group of cows – 67.04%, which was higher than in the other trial groups of cows by 0.10%, 0.18% and 0.22%, respectively. The research results show that in both trial years, on average, protein digestibility in the groups of cows was in the range of 66.25–66.51%.

The digestibility test results show that on average in both years higher protein digestibility was demonstrated by the groups of cows that were fed 'Bruno' peas and peas + faba beans. The analysis of feed rations fed to cows during the trials showed that, in general, the rations met the requirements of standards. Slight differences were found in the provision of mineral elements to all the groups of cows. The dry matter digestibility test results show that dry matter digestibility in the 1st and the 2nd trial group of cows was similar – in the range of 69.02–69.09%, while in 3rd trial and the control group was in the range of 68.75–68.41%. Protein digestibility was the highest in the 2nd trial group of cows – 67.04%, which was higher than in the other groups of cows by 0.10%, 0.18% and 0.22%, respectively.

The research results show that in both trial years, on average, protein digestibility in the groups of cows was in the range of 66.25–66.51%. The digestibility test results showed that on average dry matter and protein digestibility was higher in the 2nd trial group, the feed ration of which included 'Bruno' peas, and 1st trial group that received peas + beans.

Productivity and milk quality during the dietary experiment

The indicators of cow productivity and milk quality are presented in Tables 8, 9 and 10. The greatest decrease in milk yields was observed for the control group – by 3.98 kg of energy corrected milk (ECM); a smaller decrease was observed for the 3rd group – by 0.26 kg of ECM, compared with the initial stage of the experiment.

Table 8. Average data on the productivity of experiment cows

Experimental group	Average milk yield per day (kg)				Comparison between initial and final values (kg)
	Nov*, 2014	Beginning of xperiment	Middle of experiment	End of experiment	
1st group	22.68	23.52	22.24	20.46	-2.22
2nd group	23.48	21.58	21.76	21.38	-2.10
3rd group	20.74	19.70	20.28	20.48	-0.26
4th group (control)	24.62	24.92	21.96	20.64	-3.98[S]
	p–value (relative to control)				
	Nov, 2014	Beginning of experiment	Middle of experiment	End of experiment	
1st group	0.465	0.600	0.917	0.917	0.225
2nd group	0.917	0.347	0.917	0.754	0.138
3rd group	0.251	0.251	0.917	0.916	0.893
4th group (control)	-	-	-	-	0.043[S]

[S] – significant differences ($p < 0.05$); *–initial stage.

However, changes in productivity are mainly associated with the cows' physiological processes during their lactation and pregnancy cycle (Volpelli et al., 2012; Anderson et al., 2002). Even though the daily milk yields decreased in all the cow groups during the experiment, which was normal during the lactation period, yet the milk yield decreases in the experimental groups (1st, 2nd and 3rd) were smaller – 2.22, 2.10 and 0.26 kg, respectively, compared with the initial stage of the experiment ($p < 0.05$).

The milk chemical test results are presented in Tables 9 and 10. As the cows' productivity decreased during the experimental and lactation period, the fat and protein contents of milk increased in the 1st, 2nd and 3rd groups, compared with the control group (4th).

The fat content of milk slightly increased, on average, by 0.04%-points (0.82%) in the 3rd and 1st groups and by 0.01%-points (0.20%) in the 2nd group, compared with the control group, and from 0.33% to 0.37% ($p < 0.05$) compared with the initial stage of the experiment. The fat content of milk decreased by 0.36% in the control group, compared with the initial stage of the experiment. The diet comprising pulses made a positive effect on the protein content of milk during the experiment. The protein content of milk increased in all the experimental groups.

Table 9. Fat content of milk (average data)

Experimental group	Fat content of milk (%)				Comparison between initial and final values, %
	Nov*, 2014	Dec, 2014	Jan, 2015	Feb, 2015	
1st group	4.6	4.7	4.7	4.9	0.3
2nd group	4.74	4.6	4.5	4.9	0.2
3rd group	4.6	4.8	4.8	4.9	0.4
4th group (control)	5.3	4.9	4.4	4.9	-0.4
	p–value (relative to control)				
	Nov, 2014	Dec, 2014	Jan, 2015	Feb, 2015	
1st group	0.602	0.465	0.465	1.000	0.345
2nd group	0.465	0.251	0.917	0.917	0.686
3rd group	0.465	0.465	0.602	0.754	0.138
4th group (control)	-	-	-	-	0.500

^{S} – significant differences($p < 0.05$); *–initial stage.

Table 10. Protein content of milk (average data)

Experimental group	Protein content of milk (%)				Comparison between initial and final values, %
	Nov, 2014*	Dec, 2014	Jan, 2015	Feb, 2015	
1st group	3.1	3.4	3.3	3.7	0.6^{S}
2nd group	3.2	3.4	3.2	3.5	0.4^{S}
3rd group	3.2	3.3	3.2	3.6	0.4^{S}
4th group (control)	3.1	3.1	2.9	3.3	0.3
	p–value (relative to control)				
	Nov, 2014*	Dec, 2014	Jan, 2015	Feb, 2015	
1st group	0.917	0.251	0.117	0.074	0.043^{S}
2nd group	0.754	0.117	0.173	0.599	0.043^{S}
3rd group	0.60	0.35	0.120	0.300	0.043^{S}
4th group(control)	-	-	-	-	0.225

^{S} – significant differences($p < 0.05$); *–initial stage.

The protein content of milk increased by 0.31%-points or 9.28% in the 1st group, 0.17%-points or 5.09% in the 2nd group and 0.27%-points or 8.08% in the 3rd group, compared with the control group, and by 0.59, 0.36 and 0.44%-points, respectively, compared with the initial stage of the experiment. The fat content increased by 0.29%-points in the control group, compared with the initial stage of the experiment, yet the differences were insignificant ($p > 0.05$).

During the experiment, the milk quality indicators did not differ much from the results of other research studies and were within the normal range, which proved the positive effects of diets comprising beans and peas for dairy cows (Jemeljanovs et al., 2008; Tufarelli et al., 2012; Volpelli et al., 2012).

The total amount of amino acids increased in the milk samples of all the trial groups. The highest increase was found in the bulk milk samples of the 3rd and the 2nd trial group, 6.06 and 5.98 g kg^{-1}, respectively. The lowest increase was in the bulk milk samples of the 1st trial group (4.37 g kg^{-1}). However, an increase in the total amount of amino acids was observed in the control group, too (2.67 g kg^{-1}).

CONCLUSIONS

1.The obtained results of biochemical composition tests showed that the varieties and breeding lines of beans and peas grown in Latvia contained the proteins necessary in feed and may be used in cow diets, replacing an equivalent amount of soybean protein.

2.The analysis of feed rations fed to cows during the trials showed that, in general, the rations met the requirements of standards. Slight differences were found in the provision of mineral elements to all the groups of cows.

3.The dry matter digestibility test results showed that dry matter digestibility in the 1st trial (pea+bean+CF) and the 2nd trial (pea+CF) groups of cows was similar in the range of 69.02–69.09%, while in the 3rd trial (bean+CF) and control (soybean meal+CF) groups of cows – in the range of 68.75–68.41%.

4.The highest protein digestibility was demonstrated by the 2nd trial group of cows – 67.04%, which was higher than in the other trial groups of cows by 0.10%, 0.18% and 0.22%, respectively. The research results show that in both trial years, on average, protein digestibility in the groups of cows was in the range of 66.25–66.51%.

5.The digestibility test results showed that on average dry matter and protein digestibility was higher in the 2nd trial group, the feed ration of which included 'Bruno' peas, and 1st trial group that received peas + beans.

6.During the experiment, the cow productivity indicators decreased in all the groups, which was normal during the lactation period, yet the daily milk yield decreases in the experimental groups (diets comprising peas and beans) were smaller – 2.22, 2.10 and 0.26 kg, respectively, compared with the initial stage of the experiment and the control group (a diet comprising soybean meal).

7.The fat content of milk from the 3rd group (beans+CF) and the 1st group (peas+beans+CF) slightly increased, on average, by 0.04%-points or 0.82%, while that from the 2nd group (peas+CF) – by 0.01%-points or 0.20%, compared with the 4th (control) group (soybean meal+CF) ($p < 0.05$). The protein content of milk increased by 0.31%-points or 9.28% in the 1st group, 0.17%-points or 5.09% in the 2nd group and 0.27%-points or 8.08% in the 3rd group, compared with the control group.

8.The research results proved that the use of legumes as domestic feedstuffs for the purpose of raising the nutritional value of the feed and balancing protein in the feed ration for dairy cows is important and promising, as the legumes help better maintain the milk yield level during the lactation period and enhance the milk quality indicators.

9.The total amount of amino acids increased in the bulk milk samples of all the groups of cows.

10. The highest increase was found in the bulk milk samples of the 3rd and the 2nd trial group, 6.06 and 5.98 g kg^{-1}, respectively.

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Legumes in the diet of dairy cows from the economic perspective

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Abstract. Based on the experimental data, one can conclude that feed rations may comprise peas var. 'Bruno' and faba beans var. 'Lielplatone' grown in Latvia, thereby replacing the use of imported soybean cake. After summarising the results of trials, one can conclude that the diets comprising only one kind of legumes (peas or beans) were the most economically efficient, while the highest production efficiency was achieved if incorporating 22–24% 'Lielplatone' faba beans into the diet for dairy cows. In Europe and Latvia, foods of animal origin comprise, on average, 45% of the total agricultural output value; an essential role in the production of the foods is played by the supply of protein-rich feedstuffs to the livestock industry. An analysis of the factors influencing productivity in dairy farming shows that a diet is the most important factor that promote or hinder the functioning of the inherited genetic potential. In order to meet the dietary energy, protein and mineral requirements of cows, the cows have to be fed a diet according to their physiological condition. In recent years in many countries, research investigations into protein sources have been conducted, as a high protein content of feedstuff is the most expensive component of a feed ration. For this reason, their use in livestock diets might be economically inefficient and therefore the key focus has to be placed on opportunities to increase the content of protein in domestically produced feeds.

Key words: dairy feeding, legumes, economic assessment.

INTRODUCTION

The development of dairy farming is determined by a number of agro-ecological, social and economic factors (Fraser et al., 2007; Paulesich et al., 2007), which influence this industry's profitability, competitiveness as well as sustainability in the economic aspect. These factors may be attributed not only to the industry as a whole but also to individual processes taking place in livestock farming, including livestock feeding, as well as feedstuffs used.

Animal feeding and dietary components are one of the key factors influencing profitability in livestock farming. An analysis of the factors influencing productivity in dairy farming shows that a diet is the most important factor that promote or hinder the functioning of the inherited genetic potential. One of the factors limiting the absorption of feed by a cow is the cow's ability to absorb the dry matter of the feed. Adult cattle consume on average 1.8–2.6 kg of dry matter per 100 kg live weight, which, to a great extent, depends on feed quality (Ositis, 2005). Given the varying live weight of cattle, the dry matter requirement is a relatively constant value, yet the energy and protein contents of the dry matter vary depending on expected cattle productivity. To produce the quantity of milk reported in 2016 (978 thousand tonnes), 107–115 thousand tonnes of crude protein are necessary for dairy cows (producing 1 kg of milk requires 100–140 g of crude protein) (Report on Latvia's..., 2016).

According to an analysis of production costs, the cost of feed is the key component (Lawrence et al., 2008; Hansen & Gale, 2014), yet a detailed analysis of production costs indicates that the highest proportion of production costs relates to imported feed and its components, while the proportion of cost of domestic feed is insignificant. To meet the protein requirement, in addition to grain, by-products of food processing have been widely used in animal diets, adding feedstuffs suitable for a particular species to the feed ration. However, the production of by-products in Latvia cannot meet the need for protein in livestock farming. In contrast, imported protein-rich feedstuffs are expensive, and feeding such feedstuffs to livestock might be economically inefficient; therefore, the key focus has to be placed on increasing the protein content in domestic feed. According to studies by the Food and Agriculture Organisation (FAO) and the European Commission's responsible institutions, the use of regional (local) protein crops in the agriculture of EU Member States can provide the supply of feed at higher quality and more efficiently. For this reason, opportunities for the use of domestically grown protein crops for feed in dairy farming have to be assessed in order to minimise the cost of diets for agricultural animals.

The research aim was to assess the economic efficiency of domestic faba beans and peas used in diets of dairy cows.

MATERIALS AND METHODS

The economic efficiency of use of faba beans and peas grown in Latvia for dairy cow diets was assessed employing the experimental method, i.e. the faba beans, peas and their mixture were added to the diets, and the dietary component examined represented the key factor affecting the productivity of the dairy cows. The analytical research employed the monographic method, analysis and synthesis, data grouping etc. The research used findings of the research project 'Enhancing of Legumes Growing in Europe through Sustainable Cropping for Protein Supply for Food and Feed' (EUROLEGUME).

The feeding experiment on dairy cows was performed through two replications per treatment in the winter period (from November to February) in Malpils municipality (Latvia), on the farm 'Plakupi', in 2014–2015 and in Sigulda municipality (Latvia), on the farm 'Upites', in 2015–2016. The cows were kept under the tied-housing system, milked twice a day at an interval of 12 hours. By using the following indicators: the milk yield in the previous lactation, the lactation phase, the live weight, the average daily milk yield in the previous monitoring month and the milk fat and protein contents, four analogous cow groups were formed; each group comprised five experimental animals (n = 5).

The basic feed ration was the same for all the experimental groups. The control group (4th group) was fed a diet with soybean cake, while the experimental groups were fed diets comprising 20–24% 'Lielplatone' faba beans (3rd group), 20–24% 'Bruno' peas (2nd group) and a combination of 10–12% 'Lielplatone' faba beans and 10–12% 'Bruno' peas (1st group), reducing the amount of soybeans in their diets (Table 1).

Table 1. Experimental design of the dairy cow feeding trials conducted in 2014/2015 and in 2015/2016 (amount of peas and beans in the protein feed)

Experimental group	Conditioned feeds	Crude protein from soybean cake, g kg^{-1} feed	Crude protein from beans and peas, g kg^{-1} feed
1st group	CF + 10–12% *Pisum sativum* 'Bruno' + 10–12% *Vicia faba minora* 'Lielplatone'	-	66.8
2nd group	CF + 20–24% *Pisum sativum* 'Bruno'	-	63.3
3rd group	CF + 20–24% *Vicia faba minora* 'Lielplatone'	-	69.8
4th group[c]	CF + 14% soybean cake	72.1	-

CF – conventional feed (different grains and rapeseed cake); [c] – control group.

Milk yield as well as fat and protein contents of milk were measured, cost per unit of production (total cost divided by total production) and feed cost per kg of milk production, as well as average indicators for the experimental groups were calculated to perform the economic assessments. Differences in the average values of milk yield were identified by comparing the productivity indicators for the experimental and control groups, as well as by comparing the productivity indicators for the initial and final stages of the experiment.

The economical aspect of dairy cows is determined not only by their productivity but also their live weight. The higher a cow's weight, the more the cow consumes feed to maintain its living functions, which does not contribute to milk production but increases milk production cost. To compare the milk yields of cows relative to the live weights of the cows, relative milk yields were calculated, which represent the amount of energy corrected milk (ECM) a cow can produce if measured per 100 kg live weight.

The quantity of milk produced was identified by measuring productivity – standard litres of energy corrected milk, which was calculated by the following formula (Garcia et al., 2006):

$$ECM = Milk\ Yield \times \frac{0.383 \times Milk\ Fat, \% + 0.242 \times Milk\ Protein, \% + 0.7832}{3.14}, \quad (1)$$

where *ECM* – energy corrected milk.

The milk productivity of cows was calculated by the following formula:

$$\text{Milk productivity} = \frac{ECM, kg}{Body\ weight, kg} \times 100 \quad (2)$$

The breeds of dairy cows may be objectively compared only if the cows are kept on the same farm, under equal feeding, housing and exploitation conditions and if they have similar milk yields. Since the feeding experiment was carried out on different farms, the economic results of the feeding experiment were examined for each trial without summarising the experimental results.

The data were analysed by a Mann-Whitney test at the significance level α = 0.05 to identify differences in comparison with the control group (Montgomery, 2012). All statistical analyses were performed using SPSS for Windows version 20.0.

RESULTS AND DISCUSSION

It is known that the cow farming technology, including cow feeding, can considerably affect milk yield and, in its turn, change the cash flow. For this reason, it is important to analyse the economic implications derived of modifications of the average daily milk yield, the range of deviation, and the change in the milk yield caused by including peas and beans in the dairy cows' diets.

Dairy farms have built up experience in using legumes – faba beans and peas – in diets for cows in the form of both fresh biomass and dried seeds. For the purpose of the present research, the dairy cows were fed dry faba beans and peas as a component of the feed concentrate supplied. An important reason for incorporating legumes into the diet for dairy cows is that the legumes contain a considerable amount of energy in the form of starch and contribute to better protein absorption.

However, there are few research studies allowing determining the economic and protein absorption efficiency for feed rations comprising legumes. Thus, in cow diets, according to data available in the literature, faba beans might be up to 35% of the total feed concentrate ration (Tufarelli et al., 2012), although other information has suggested that dairy cow diets should not include more than 20% legumes. Latvian researchers Barbals & Brosova (2013) recommend incorporating 2 kg of faba beans into the daily feed ration for dairy cows; it causes no harm to the cow's health.

After examining the effects of different amounts of dietary legumes on the productivity of dairy cows, Vander Pol et al. (2008) demonstrated no significant changes when soybean flour was partially replaced by peas (150 g kg^{-1}). A similar finding was made by Tufarelli et al. (2012) who reported that replacing soybeans with faba beans (345 g kg^{-1}) in the diet for highly productive dairy cows (the average milk yield of 35 kg a day) did not influence the cows' productivity.

Regarding milk yield, to be efficient, dairy cows have to produce at least 1,000 kg of energy corrected milk (ECM) per 100 kg live weight per year; it is the milk with a 4.1% fat content and a 3.1% protein content (Garcia et al., 2006). An analysis of the available data on dairy farming allows concluding that in milk production, a crucial factor is the genetic potential of cows, though increasing milk yields and enhancing milk quality, to a great extent, depends on the cows' diet. As pointed out by Kureoja & Kaart (2002), the dairy cow farming technology (the kind of barn, the kind of housing, diets, animal welfare conditions etc.) as an exogenous factor makes a greater effect on cow productivity than the genetic parameters.

The milk yield changes are well seen in both trials (Table 2) if analysing relative monthly milk yields or monthly milk output per 100 kg cow live weight. No significant changes in relative milk yields were observed in any experimental group, compared with the control group, in the entire experimental period. The relative milk yields were similar in all the groups.

However, it has to be mentioned that the relative milk yield in the 3rd group (22–24% 'Lielplatone' faba beans) was the lowest at the initial stage (in November) of both the 1st trial (120.4 kg) and the 2nd one (128.0 kg). In contrast, at the final stage (in February) the relative milk yield in the 3rd group (104.2 kg) was higher than in the control group in the 1st trial, while in the 2nd trial it was the highest (128.8 kg), compared with the other experimental groups and the control group (Table 2).

Table 2. Milk production (milk productivity), kg per 100 kg live weight per month

First cow feeding trial 2014–2015				
Experimental group	Nov, 2014*	Dec, 2014	Jan, 2015	Feb, 2015
1st group	131.66 ± 27.9	141.08 ± 25.4	133.41 ± 20.4	110.85 ± 16.1
2nd group	126.51 ± 24.1	120.15 ± 25.2	121.15 ± 19.8	107.51 ± 19.3
3rd group	120.40 ± 22.9	110.96 ± 18.2	114.22 ± 20.8	104.19 ± 18.8
4th group[c]	132.22 ± 38.6	138.30 ± 40.7	121.87 ± 42.0	103.46 ± 23.7
p-value – to define differences in comparison of the control group				
1st group	0.917	0.917	0.754	0.465
2nd group	0.917	0.602	0.917	0.917
3rh group	0.347	0.251	0.917	0.917
Second cow feeding trial 2015–2016				
Experimental group	Nov, 2015*	Dec, 2015	Jan, 2016	Feb, 2016
1st group	130.2 ± 24.6	134.7 ± 19.4	134.1 ± 14.8	110.7 ± 15.4
2nd group	140.9 ± 23.1	141.6 ± 28.2	133.7 ± 29.7	119.2 ± 26.7
3rd group	128.00 ± 22.2	164.2 ± 10.5	152.0 ± 7.9	128.8 ± 6.8
4th group[c]	135.8 ± 27.4	148.7 ± 11.6	138.2 ± 10.2	117.6 ± 10.3
p-value – to define differences in comparison of the control group				
1st group	0.602	0.917	0.917	0.917
2nd group	0.917	0. 917	0.251	0.347
3rh group	0.917	0.347	0.465	0.251

Data are presented as means ± SD (standard deviation) (n = 5); * initial stage; [c] – control group.

The need for cheaper protein-rich feedstuffs has been referred to in a number of research studies owing to the problem of the high proportion of feed cost. Thus, as pointed out by Czuowska & Zekao (2016), the feed cost in dairy farming comprises 66% of total production cost. In the 1st and 2nd trials on the dairy cows, replacing soybean meal with legumes, the feed cost per cow per day for the experimental group decreased by 2.6–5.3% (Table 3). The greatest decrease in daily feed cost per cow (by 0.16 EUR), compared with the control group, was observed in the 3rd group, which was fed a diet in which soybean protein was replaced with 'Lielplatone' faba beans (22–24% of the total amount of protein feedstuffs).

Adding a combination of 'Lielplatone' faba beans (10–12%) and 'Bruno' peas (10–12%) (for the 1st group), the daily feed cost per cow decreased by 0.12 EUR, while the incorporation of only 'Bruno' peas (22–24%) into the diet (for the 2nd group), the daily feed cost per cow decreased by only 0.08 EUR (Table 3). This may be explained by the fact that the crude protein content of peas is lower, therefore the crude protein cost of this feedstuff is higher.

Table 3. Cost of dairy cow feed per day per cow 2014–2015

Indicators	1st group	2nd group	3rd group	4td group[c]
Haylage/silage EUR	0.60	0.60	0.60	0.60
Hay EUR	0.48	0.48	0.48	0.48
Grain EUR	0.75	0.75	0.75	0.75
Rapeseed oil cake, EUR	0.40	0.40	0.40	0.40
Peas, EUR	0.27	0.57	-	-
Beans, EUR	0.26	-	0.49	-
Soymeal, EUR	-	-	-	0.65
Mineral feed, EUR	0.16	0.16	0.16	0.16
Total, EUR	2.92	2.96	2.88	3.04
difference to control, EUR	-0.12	-0.08	-0.16	-
difference to control, %	-3.9	-2.6	-5.3	-

[c] – control group.

After summarising the data on feed costs and dairy cow production, feed costs per kg of milk produced were calculated. As shown in Table 4, this value differed significantly between the 1st trial and the 2nd one, which might relate to the different cow productivity levels on the experimental farms. Of course, higher productivity at the same feed cost resulted in a lower unit production cost. However, the results of both trials reveal trends in feed costs and production costs in the experimental period when soybeans were replaced with faba beans and peas as sources of dietary protein.

According to Silva et al. (2008), the performance of economic analyses by using production costs and an economic efficiency index, such as gross and net rates, contributes to decision-making. At the final stage of both trials, the productivity of cows (average milk yield per cow per day), the total milk production (kg) for each group and the total revenue from milk sales, as well as the total feed cost were calculated for the entire experimental period (December-February) (Table 4).

Table 4. Total feed costs, gross profit and average feed costs per 1 kg of milk yield in the trials (December-February)

First cow feeding trial 2014–2015				
Indicator	1st group	2nd group	3rd group	4th groupc
Milk yield per cow per day (kg)	22.07	21.57	20.48	22.51
Total milk yield per group per trial (kg)	9,957.20	9,710.90	9,064.10	10,156.00
Milk wholesale price (EUR kg^{-1})	0.21	0.21	0.21	0.21
Income from milk sales (EUR)	2,091.01	2,039.29	1,903.46	2,132.76
Total feed costs per group (EUR)	1,313.33	1,330.88	1,296.23	1,366.88
Difference to control (EUR)	-53.56	-36.01	-70.66	x
Difference to control (%)	-3.9	-2.6	-5.2	x
Income over feed costs (EUR)	777.69	708.41	607.24	765.89
Difference to control (EUR)	11.80	-57.48	-158.65	x
Difference to control (%)	1.5	-7.5	-20.7	x
Average feed costs per kg of milk yield (EUR kg^{-1})	0.132	0.137	0.143	0.135
Difference to control (EUR)	-0.003	0.002	0.008	x
Difference to control (%)	-2.3	1.5	5.9	x

Table 4 (continued)

Second cow feeding trial 2015–2016				
Indicator	1st group	2nd group	3rd group	4th groupc
Milk yield per cow per day (kg)	28.04	29.16	34.31^S	27.89
Total milk yield per group per trial (kg)	2,643.40	13,134.30	15,475.70	12,575.50
Milk wholesale price (EUR kg^{-1})	0.21	0.21	0.21	0.21
Income from milk sales (EUR)	2,655.11	2,758.20	3,249.90	2,640.86
Total feed costs per group (EUR)	1,313.33	1,330.88	1,296.23	1,366.88
Difference to control (EUR)	-53.56	-36.01	-70.66	x
Difference to control (%)	-3.9	-2.6	-5.2	x
Income over feed costs (EUR)	1,341.79	1,427.33	1,953.67	1,273.98
Difference to control (EUR)	67.81	153.35	679.69	x
Difference to control (%)	5.3	12.0	53.4	x
Average feed costs per kg of milk yield (EUR kg^{-1})	0.104	0.101	0.084	0.109
Difference to control (EUR)	-0.005	-0.007	-0.025	x
Difference to control (%)	-4.4	-6.8	-22.9	x

^{c} – control group; ^{S} –significant difference, $p < 0.05$

The feed components and therefore the feed costs were the same in both trials, both for the experimental groups and the control group. The feed costs for all the experimental groups (1st–3rd) were 2.6–5.2% lower, given the market price on faba beans and peas and the price on soybeans fed to the control group.

In the experimental period, compared with the control group, the cost savings (EUR 70.66) were found to be the greatest in the 3rd group (22–24% faba beans), EUR 53.56 were saved on the 1st group (10–12% peas and 10–12% beans), and EUR 36.01 on the 2nd one (22–24% peas).

From the economic perspective, it is important not only to reduce feed costs but also to provide sufficiently high productivity and to reduce production costs. However, to make an economic efficiency assessment for legumes, it is important to determine the unit production cost for the entire experimental period (December-February). In the first experimental period, the feed cost per kg milk produced was EUR 0.03–0.05 higher than in the 2nd trial.

A comparison of feed costs between the experimental groups and the control group revealed that the feed cost per kg milk produced for the 1st group (10–12% peas and 10–12% beans) was 2.3–4.4% lower. Consequently, compared with the control group, the difference between revenue from milk sales and feed costs (income over feed costs) was 1.5–5.3% greater in the 1st and 2nd trials.

The 3rd group that was fed a diet comprising faba beans showed a lower feed ration cost; however, in the 1st trial it showed a 5.9% higher feed cost per kg milk produced. In the 2nd trial, the 3rd group showed the best performance with a 22.9% or 2.5 cent lower feed cost per kg milk produced than the control group did. A similar trend was observed for the 2nd group; in the 1st trial the feed cost for this group was 1.5% higher, whereas in the 2nd trial it was 6.8% lower than for the control group.

The 1st trial (2014) showed that replacing soybean feedstuffs with peas and beans in the diet for cows of average milk productivity (20–24 kg per day) resulted in lower income over feed costs from the experimental groups than from the control group, or the profits were the same. However, in the 2nd trial, if replacing soybean feedstuffs with peas and beans in the diet for cows of higher milk productivity (28–31 kg per day), the income over feed costs from all the experimental groups were significantly higher than from the control group. In the experimental period, the average feed cost per kg milk produced followed a similar trend – replacing soybean feedstuffs with 220–240 g kg^{-1} faba beans (3^{rd} group) resulted in a 22.9% (2.5 cents) lower cost than for the control group.

Lima et al. (2015) assessed the economic efficiency of feedstuffs used in an experiment in conjunction with production costs and productivity, thereby identifying the most economically efficient diet providing the highest profitability. After performing a comprehensive assessment of the results of both trials, one can conclude that the highest production efficiency was achieved if incorporating 22–24% 'Lielplatone' faba beans into the dairy cow diet. The experimental diet comprising faba beans allowed reducing feed cost, whereas dairy cow productivity was constant. This means that replacing soybean feedstuffs with faba beans resulted in higher economic returns from the feedstuffs used in livestock farming, i.e. the same cow productivity at lower resource consumption. The most economically efficient diets were those comprising only one kind of legumes (peas or beans).

CONCLUSIONS

Based on the experimental data, one can conclude that feed rations may comprise peas and beans grown in Latvia, thereby reducing the consumption of imported soybeans. An analysis of the effects of the use of faba beans and peas **in the dairy cow diet** on feed cost and productivity in the experimental period revealed that:

- the daily feed ration cost decreased by EUR 0.16 for the 3^{rd} group (22–24% 'Lielplatone' faba beans), EUR 0.12 for the 1^{st} group (10–12% 'Bruno' peas and 10–12% 'Lielplatone' beans), and EUR 0.08 for the 2^{nd} group (22–24% 'Bruno' peas);
- compared with the control group, the total saving on feed was EUR 70.66 in the 3^{rd} group, EUR 53.56 in the 1^{st} group (10–12% peas and 10–12% beans) and EUR 36.01 in the 2^{nd} group (22–24% peas);
- in the 2^{nd} trial, the average milk yield in the 3^{rd} group (22–24% faba beans) was significantly higher than in the control group, whilst the 1^{st} and 2^{nd} groups showed no significant changes in the average daily milk yield, as well as in the milk fat content and the milk protein content;
- both trials showed that the feed cost per kg milk produced for dairy cows in the group fed with 10–12% peas and 10–12% beans was 2.3–4.4% lower than for the control group.

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Requirements for inserting intercropping in aquaponics system for sustainability in agricultural production system

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Abstract. In recent years, the recirculating aquaponics system has gained high attention and significant popularity for organic vegetables and fruits production which contributes to the sustainable aquaculture for tropical regions. This review aims to summarize the possibility for practicing intercropping in aquaponics to produce high-quality fruits, vegetables and fish without any chemical fertilizer and minimum ecological impact for a sustainable agriculture. Although many studies have addressed about aquaponics for producing high-value crops such as tomato, cucumber, and lettuce, there is still a lack of complete information to support the development of intercropping in aquaponics and limited focus on its commercial implementation. Moreover, this study will focus first on the requirements for inserting intercropping in aquaponics and technical improvements needed to adapt as potential for sustainable food production system to increase productivity around the world, especially in countries have deficiency in water and land resources as well as soil problem like salinity and reduce environmental emissions. Secondly, the insertion of intercropping in aquaponics must be for crops with high value and for crops that can complement together such as tomato with basil and tomato with lettuce. Thirdly, in technical improvement in this study will summarize the strategies and factors that affect the intercropping in aquaponics system such as the nutrients needed for crops under intercropping aquaponics, stocking density and feeding rate which are important to know the concentration of ammonia that is produced and converted to nitrate so that the plants can uptake it. Studying the requirements for inserting and improving intercropping in aquaponics will increase our understanding of needed for new agriculture technique that contributes to the sustainable aquaponics for tropical regions.

Key words: Intercropping, Aquaponics, feeding rate, feeding frequency, high crop value.

INTRODUCTION

Aquaponics is the integration or/and interlinking of conventional aquaculture with hydroponic plant production (Diver & Rinehart, 2010). Aquaponics can be considered as a sustainable food production method since it bridges the gap between production and consumption of fish around the world without depleting the non-renewable resources. The important characteristic of aquaponics designing systems is that it has closed nutrient cycles (Nelson, 2008) and can be designing as decoupled system consists of 2 independent recirculation systems connected unidirectionally from the fish component to the plant component (Kloas et al., 2015) and enables sustainable agricultural production through complete biological processes among fish, bacteria and the plant (Nelson, 2008). Aquaponics can be implemented as the medium in a wide range of suitable fish species and plants at the same time in the system (Nelson, 2007; Diver & Rinehart, 2010; Resh, 2012). Moreover, Aquaponics is composed of three main components such as fish, plants and microbes. All the three components must be available for an aquaponics system to function since the nutrients, absorbed by plants, is a product of nitrification process of wastes excreted by fish. Moreover microbes bridge the process between the other two components (Nelson, 2008; Klinger & Naylor, 2012; Somerville et al., 2014).

In aquaponics, fish physiology and the composition of fish feed from protein are the main factors affecting nutrient availability in solution inside the system. Further other factors such as fish species, growth stage, stocking density and the rates of inside microbial nitrification also play a role here (Rakocy et al., 2006). Fish feed waste is the primary nutrient source for plants followed by fertilizers, periodically. While most of the required nutrients such as nitrogen, potassium and other elements for plant growth are available in the fertilizers (Tyson et al., 2004; Rakocy et al., 2006).

TYPES OF AQUAPONICS SYSTEMS

In an aquaponics system, three components work together such as fish tank (aquaculture), bio-filter (for nitrification) and grow bed (hydroponics). Aquaponics types are classified on the basis of grow bed designs according to hydroponics and can also be classified as closed (coupled) and decoupled systems (Kloas et al., 2015). The first type according to hydroponics, floating raft (RAFT, deep water culture – DWC) is widely used for lettuce. The second type, Nutrient Film Technique (NFT) is used widely for lettuce. NFT is not only used in Lettuce production, but also in Garlic as in (Abou-Hadid et al., 1986), Tomato, Cucumber, Strawberry and Beans (Chrissy, 2017). The final type is media-based bed that uses substrates media such as peatmoss, tuff and perlite in (flood and drain or ebb-and flow) (Diver, 2006; Lennard & Leonard, 2006; Rakocy et al., 2006; Nelson & Pade, 2007; Love et al., 2014).

The media-based bed is widely used in small scale aquaponics since not only that it could act as a filtration unit but also it does not require separate biofilters as it contains media (e.g., pumice stones or clay beads) in the grow bed for nitrification (Zou et al., 2016). In addition, the substrate which is used in media based bed provides high surface area for microbial growth during biological processes in aquaponics. This is the most popular grow bed design among home gardeners (Bernstein et al., 2014). Moreover, media-based bed is suitable for different kinds of vegetables and fruit species, because the grow bed can support high root density due to potential blockage of recirculating flow while NFT bed design is suitable only for small vegetable species such as lettuce because the grow bed cannot support a high quantity of roots due to potential blockage of recirculating flow (Engle, 2015). Thus, efficient solid removal is critical for NFT to prevent clogging in grow bed channel. Floating-raft is the commonly adopted type in aquaponics system since it allows the plant roots to freely absorb the nutrients in the water without clogging water channel (Liang & Chien, 2013; Engle, 2015). NFT and floating-raft aquaponics systems require a biofilter for nitrification and a sedimentation tank for solid removal (Nelson, 2008; Engle, 2015). Recently, many researchers conducted experiments to determine the efficiency of applied Nutrient film technique and deep water culture in large scale commercial operations (Bernstein et al., 2014).

COMPONENTS OF AQUAPONICS SYSTEM

Aquaponics system works on the principle of connection between the components, hydroponics and recirculating aquaculture elements. This can be otherwise described as, a combined culture of fish, bacteria and plants. For this reason, any aquaponics system must be composed of basic components such as fish tank, aerator, pipes, lights, pump, biofilter, hydroponics components-media beds, NFT, DWC, RAFT or deep water and sump tank. In addition to this, half barrels, PVC media, buckets, plastic containers etc. may be used (Rakocy et al., 2006). Devices of sedimentation cover plate separators, tube or settling basins. Each component in aquaponics system plays a specific role. Fish excretes organic wastes as ammonia in the fish tank while the biofilter component has two specific processes for converting fish waste metabolites such as ammonia into nitrite and then to nitrate through nitrification. The hydroponics component in aquaponics system provides the required nutrients to plants. Moreover, aquaponics system must ensure processes such as aeration, water movement and the biological process to produce nitrate in the final stage. Finally, aquaponics system is the culture for symbiosis between fish, bacteria and plants, and considered as sustainable agriculture system using water and nutrients in coupled recirculation (Rakocy et al., 2006; Somerville et al., 2014).

Positive effects of aquaponics

Today’s competitive environment facing serious of issues includes changes of climate, degradation of soil, food security, and scarcity of water and rise of population. All these problems were possibly addressed by aquaponics through their closed loop system which consists of aquaculture and hydroponic elements (Goddek et al., 2015). It is the rapidly developing sector of agriculture which should be sustainable and also meet demands of biochemical aspect. Principally, aquaponics which is the combination of horticulture and aquaculture in a single recirculating aquaponic system (SRAPS) gives a sustainable approach (Kloas et al., 2015). Each part in aquaponics system can be controlled to maintain good environmental conditions through controlling water quality, temperature, pH, efficiency of the water used, improved waste management throw nitrification and nutrient recycling (Rakocy, 2007; McCarthy, 2011; Thorarinsdottir, 2015; Sallenave, 2016). Further water recirculation is the final outcome achieved (Lin et al., 2002; Hamlin et al., 2008; Endut et al., 2009; Martins et al., 2010). Moreover, aquaponics can protect crops from diseases and drought (Azad, 2015). Because of its unique, sustainable and environmental-friendly operational procedures, aquaponics has greater impact on sustainable food production (fish and vegetables), reduced environmental pollution and less water consumption which directly increases the profit of farmers in contrast to standalone RAS and hydroponics systems, aquaponics has a better economic benefit may include results from (McMurtry et al., 1997; Love et al., 2014; Love et al., 2015; Miličić et al., 2017).

IMPORTANT CRITERIA AND FACTORS THAT AFFECTS AQUAPONICS SYSTEMS

pH

In any aquatic system, pH stabilization is an important and critical phenomenon since the living organisms operate within a cycling system and it controls fish metabolism, microbial activities and affects the availability of nitrogen to plants (Kuhn et al., 2010; Zou et al., 2016). In aquaponics, it is important to know the optimal pH range for overall best performance from plant, bacteria and fish since it is necessary to achieve sustainability of all the biological interactions occurring in the system. The plant roots, bacteria, and fish must be able to absorb nutrients even at higher pH levels, because providing the optimal pH for every part in a system is challenging. The pH solution is the most important parameter in aquaponics systems because it controls fish metabolism, microbial activities and affects the availability of nitrogen to plants (Kuhn et al., 2010; Zou et al., 2016) and it must be balanced among fish, bacteria and plant demands at the same time. In general, the tolerance range for most plants is 5.5–7.5 (Somerville et al., 2014). So, during experiments period, ideal pH will be maintained for whole aquaponics system through adding calcium hydroxide and potassium hydroxide to the base of the addition tank, where it gets dissolved and slowly enters the system.

Moreover, the pH level for crops must be within the optimal range because if pH exceeds this range, plants are unable to absorb nutrients such as iron, calcium, and magnesium available in the water which becomes deficient in plants (Rakocy et al., 2006; Somerville et al., 2014). On the other hand, the optimal pH for nitrification in biological processes is in the range of 7.5–8.5 (Kim et al., 2007). The ideal pH for the nitrification process is 8.5 (Wortman & Dawson, 2015). Recent studies inferred that pH 6.0 is optimal for plant growth and efficient nitrogen utilization in aquaponics at the expense of increased N_2O emission due to high denitrification (Zou et al., 2016). In aquaponics systems, pH was usually managed in the range of 6–7.0 because this range is optimal for bacteria to function to its fullest capacity and the plant roots have full access to absorb all the essential micro and macronutrients (Wortman & Dawson, 2015). Tilapia can tolerate wide pH ranges and each tilapia species has different optimal pH (Lemarié et al., 2004; Arimoro, 2006). Due to higher pH, fish is usually affected due to toxicity of ammonia. Further, higher pH levels lead to higher toxicity in tilapia, but best performance can be achieved in the range of 6.5–8.5. Therefore, it is important to keep the pH value at possible stable level from (6–7.0) (Rakocy et al., 2006).

Dissolved Oxygen

Dissolved oxygen (DO) is one of the most important parameters in fish culture. This is also critical to the beneficial nitrifying bacteria that convert fish waste into nutrients so that the plants can absorb (Somerville et al., 2014). Dissolved oxygen (DO) level describes the amount of molecular oxygen within water in the aquaponics system. This is essential component for plants, fish and nitrifying bacteria to live and flourish in aquaponics system and measured in terms of milligrams per liter. In new aquaponics system, continuous monitoring of DO amount should be done until the system is standardized by determined feeding and stocking rate to provide sufficient aeration (Stark, 1996; Timmons & Ebeling, 2013; Somerville et al., 2014). In aquaponics, the addition of excess oxygen do not show any negative effects or risks because when the water gets saturated, the additional oxygen is simply dispersed into the atmosphere.

The water temperature must be taken care and checked frequently because it is strongly related to dissolved oxygen and when DO is insufficient at high water temperatures, the root rot symptoms may appear (Rakocy, 2007). Most plants need high levels of DO (> 3 mg L^{-1}), while for biofilters, it is recommended above 1.7 mg L^{-1} to maintain the activity of nitrifiers (Ruiz et al., 2003). On the other hand, tilapia is tolerant when the DO concentration is low, but growth rates will be affected. Tilapia, when there is low level of dissolved oxygen (1 mg L^{-1}), it comes to the surface for oxygen-rich surface water. Moreover, it is recommended that DO levels should be maintained at 5 mg L^{-1} or higher in aquaponics systems to prevent from stress to fish and plants (Bernstein, 2011; Rakocy, 2007).

Tank water temperature

Water temperature is a major influence factor on organisms inside the aquaponics culture systems. The water temperature not only affects the organisms in it but also show effects on growth of fish, feeding rate and fish size (Gardeur et al., 2007). Moreover, the water tank temperature influences the behavior and physiological process of fish in the aquaponics system and the performance of biofilter (Xia & Li, 2010). In addition, high temperature has a direct effect system processes in terms of reduced dissolved oxygen, increased unionized (toxic) ammonia and restricted absorption of calcium (Somerville et al., 2014). Several studies have been reported that each fish species has ideal range of water temperature as because it is affected directly by maximum growth (Person-Le Ruyet et al., 2006; Björnsson et al., 2007; Oyugi et al., 2011; Somerville et al., 2014). Tilapia in aquaponics system require warm temperatures (25–27 °C) (DeLong et al., 2009) for maximum growth (Rakocy et al., 2006; Savidov et al., 2007). When the ideal temperature is not maintained in fish tanks, the growth is drastically reduced and cause diseases which results in other criticalities such as reduced reproduction, sluggishness due to retarded digestion capacity of fishes (Bailey & Alanärä, 2006). The ideal temperature for vegetable growth is 20–25 °C and for biofilters (nitrifying bacteria) it ranges from 25 to 30 °C while tilapia dies when the temperature drops below 10 °C.

TYPES OF TILAPIA USED IN AQUAPONICS

Tilapia is one of the most popular fish species in aquaponics systems (Rakocy et al., 2006) and the basic requirements for successful biological process the aquaponics system (Love et al., 2014). Tilapia has a great attention because of its high availability, easily cultivable nature, fast growing, stress and disease-resistant and highly adaptable to a wide range of environmental conditions such as pH, water temperature, dissolved oxygen (DO), salinity, light intensity and photoperiods (El-Sayed & Kawanna, 2004; Hussain, 2004). Due to these characteristics, tilapia culture is being practiced in most of the tropical, subtropical and temperate regions to reduce the global rising demands for protein sources (Ng & Romano, 2013). Fish is important to produce ammonia which is the major end product in the breakdown of proteins which is converted to nitrate in next stage by bacteria (Rakocy et al., 2006). There are many species of tilapia such as Blue tilapia (Oreochromis aureus), Nile tilapia (*Oreochromis niloticus*), hybrid tilapia (*Oreochromis mossambicus* x *Oreochromis* *niloticus*), Nile tilapia ‘red strain’ hybrid (*Oreochromis niloticus* x *O. aureus*) and red tilapia (*Oreochromis* sp.) which are being used successfully in aquaponics system. In a study conducted by Palm et al. (2014), *Oreochromis niloticus* is used for better growth of lettuce (*Lactuca sativa*) and cucumber fruits (*Cucumis sativus*) in aquaponics system. Further, another study conducted by Knaus & Palm (2017) recorded better growth in basil (*Ocimum basilicum*) and parsley (*Petroselinum crispum*) when used *O. niloticus* in aquaponics system. Consequently, under identical aquaponics system design, optimal fish and plant choice govern the growth performance of the cultivated plants (Knaus & Palm, 2017).

BACTERIA IN AQUAPONICS

Bacteria is one of the three basic requirements to complete the biological processes (nitrification) in aquaponics system. Nitrification process is a major biological process in biofilter aquaponics and forms the basic process for removing ammonia, a metabolic waste excreted by fish. Ammonia is toxic to fish at concentrations above 0.05 mg L^{-1} (Rakocy et al., 2006; Reed et al., 2009). Nitrification process in aquaponics system provides elements for the plants which eliminates ammonia and nitrite (Gutierrez-Wing & Malone, 2006) through two types of bacteria of which the first type is composed of *Nitrobacter*, *Nitrospina* and *Nitrococcus*, a group of nitrifying bacteria that oxidize ammonia(NH_3 or NH_4^+) convert into nitrite (NO_2^-) which is also toxic to fish. The second type of nitrifying bacteria composed of *Nitrosomonas* and *Nitrosococcus* that oxidize nitrite converts the nitrite into nitrate (NO_3^-) (Somerville et al., 2014). In aquaponics, biofilters use sand, gravel, shells or various plastic media with large surface areas which is optimal to develop extensive colonies of nitrifying bacteria (Rakocy et al., 2006). The nitrification process results in the transformation of 93% to 96% of ammonia-nitrogen to nitrate, an end product of nitrification, in infiltration units (Prinsloo et al., 1999). Nitrate is the primary source of nitrogen for plants (Resh, 2012). Nitrite is the intermediate product of nitrification and toxic to both fish as well as plants while nitrate is not toxic to fish. The nitrifying bacteria in aquaponics system is affected by pH. The optimum pH range for nitrification is 7.0 to 9.0 although most studies indicate that the ideal pH for efficient activity of *Nitrosomonas* spec. is 7.8–8.0 and for *Nitrobacter* spec. it is 7.2–8.2. On the other hand, the optimal temperature range for nitrifying bacteria is 17–34 °C while the optimum levels of DO for nitrification process at 4–8 mg L^{-1}. (Somerville et al., 2014) This is the level required in the case of fish and the plants. Nitrification is affected negatively if the DO level is less than 2 mg L^{-1}. So it is mandatory to ensure adequate pH, water temperature and DO for successful biofiltration process (Rakocy et al., 2006).

INTERCROPPING IN AQUAPONICS CONDITIONS

Recently, intercropping has been recognized as cropping system and beneficial system for the production of two or more crops such as vegetables under greenhouse conditions in the same location and must be at least a part of production cycles. Intercropping has played important role in most countries in Asia, Africa and Latin America for food security under conventional conditions (soil conditions). Soil degradation, desertification and water pollution (Gregory et al., 2002) are the main reasons for innovation and adapting to intercropping techniques combined with new technologies such as hydroponics and aquaponics. Nowadays, it is urgent to study and develop the practices for applying intercropping under hydroponics and aquaponics systems, because it remains the potential strategy in sustainable food systems (Malézieux et al., 2009; Li, 2011; Lithourgidis et al., 2011).

Advantages and disadvantages of intercropping

Intercropping has many advantages over single crop cultivation according to recent and past studies. Most of the studies about intercropping application are under soil conditions. Inserted or combined intercropping in aquaponics will bring greater attention for many reasons such as production of vegetables in a biological way meeting high quality standard without compromising the quantity. The main reason to adopt intercropping, besides these advantages is that food security can be achieved through sustainable agriculture system. As mentioned above, intercropping has many advantages over single cropping (Gebru, 2015) such as efficient water usage, increased total productivity per unit land, per unit time, and increased efficiency in using space among different species and thus reducing the cost of production, labor and environmental impacts (Anitha et al., 2001; Li, 2011; Bedoussac et al., 2015).

Yu et al. (2015) reported that intercropping uses land up to 22% more efficiently than corresponding sole crops with same growth duration. In addition, intercropping can increase profitability, especially when the basic requirements such as water, light and nutrients are efficiently and effectively used than single cropping systems which leads in less time and energy consumption in food production (Barros Júnior et al., 2009; Filho et al., 2010).

FACTORS AFFECTING INTERCROPPING IN AQUAPONICS SYSTEMS

Crop properties and aquaponics types

Various researchers have proved that wide varieties of crops especially vegetables such as lettuce and tomato (Rakocy et al., 1993) or basil (Adler et al., 2003), cucumber and other herbs (Savidov et al., 2007) have been grown in the different types of aquaponics systems. However, crop properties varied on the basis of types of aquaponics. For instance, Cormier (2016) reported that tomatoes and other fruiting plants are not better in floating raft systems. Likewise, deep flow technique (DFT) is generally utilized for herbs (oregano, thyme and basil) and salad greens (include arugula, chicory, spinach and lettuce). Lettuce grow in raft and other media with the ideal temperature of 15–27 °C and temperature tolerate over 27 °C lettuce bolts; tomatoes grow in only very popular hydroponic varieties and aquaponics with the ideal temperature of 26 °C and temperature tolerate over 7.2 °C (Sawyer, 2013). Richard Tyson (2012) examined that the aquaponics fish called tilapia was commonly grown in good quality water with yellow perch, largemouth bass, rainbow trout, koi and bluegills. Brook (2017) reported that salad greens, lettuce, peppers, tomatoes and herbs like low-water thyme and basil grown in media bed.

It was recommended by the researchers to practice intercropping of two or more crops in aquaponics. But prior to implementing dual crops, in this instance, tomato and lettuce, care must be taken with aquaponics system since there are many factors that could possible affect the success of intercropping.

First, the crop's complete characteristics, properties and suitable type of aquaponics system must be studied followed by the investigation of factors that affect each crop over the other in terms of botanical characteristics (Beets, 1982). The other agronomic factors, especially population density must be studied because it is very important to know the optimal density for each crop and size of the bed to ensure that each crop has optimal aeration and light to complete photosynthesis process. For example, tomato and lettuce can be intercropped in aquaponics but it is must to know the planting density for both crops and crops arrangement is critical such that the crops have no effect over each other. Further nutrient availability is a critical factor because the study must ensure that aquaponics can produce efficient amount of nitrate and other macro and micro elements which the crop needed to grow. In addition, researchers such as (Alves et al., 2010; Filho et al., 2013) reported that strategy for managing the time for intercropping is important because, the competition between the crops for nutrient uptake needs to be minimized and the period of coexistence of species influenced the crop productivities, for example, tomato and lettuce, and cucumber and lettuce, respectively.

Nutrients needed for crops under intercropping aquaponics

The main challenge for success in intercropping under aquaponics is how to achieve and assess the optimum amount of elements needed from nutrient concentration for both crops from transplant stage to growth until the harvesting period by calculating the nutrients needed for both crops in intercropping to prevent nutrient deficiency. A deeper understanding of needs from the nutrient solution for both crops in intercropping is important to manage fish stocking density, feeding rate, frequency of fish, feed composition and bacterial surface area to achieve the elements needed. Moreover, it is important to determine the ratio of elements uptake by both crops in each stage in order to manage the fish culture composed of the above factors. The other factors that are to be considered are pH, water temperature for both crops and all other elements of the aquaponics system such as the fish tank, bacteria tank and beds. In addition, management of fruit crops like tomato is difficult than leafy crops such as lettuce, because tomato has different nutrient demands based on the different developmental stages from first flower to start setting fruits. Fruit crops, when start setting fruits, need more specific nutrients such as Ca, Mg and K (Nelson, 2008). It denotes that knowledge on both the crops and its stage of growth is a must and its timely application is mandated. Most intercropping crops are chosen as fruits and leafy crops because it is highly profitable and has value.

Role of foliar application in intercropping aquaponics

A crucial point for aquaponics in general, and for intercropping in specific, is the nutrient balance in system culture, as it is critical for both crops during growth until harvesting stage. According to previous studies (Adler et al., 1996; Seawright et al., 1998; Graber & Junge, 2009), it is reported that aquaponics system produces lower levels of potassium (K), iron (Fe), manganese (Mn) and sulfur (S). Therefore, the optimal nutrient balance, during the growth cycle for both crops, can be achieved by considering all the factors inserted in the above title (i.e., nutrients needed for crops under intercropping aquaponics) and by supplementation of the required elements throw foliar application such as potassium and iron, because these elements are not released from fish feeds (Rakocy et al., 1997; Rakocy et al., 2006; Roosta & Hamidpour, 2011). Foliar application is one of the efficient methods of plant nutrition in case of nutrient deficiency for Mg, Zn, and Mn during growth stage (Roosta & Hamidpour, 2011).

Stocking density of tilapia under intercropping aquaponics

Stocking density is considered as one of the important factors in aquaponics, besides feeding rate and frequency since it varies according to fish type. In aquaponics systems, especially for intercropping, stocking density must be ideal and optimum to ensure that the waste is converted to ammonia and nitrate in the final phase. Through optimum stocking density, one can obtain maximum production without effects on environment, optimum health, economic benefits (Rahman & Marimuthu, 2010) and minimum occurrence of physiological and behavioral disorders (Ashley, 2007; Ayyat et al., 2011). Tilapia is the fish species which is commonly cultured in aquaponics system. In order to recover the operating expenses and huge capital cost of aquaponics systems and earn a profit, both the hydroponic vegetable components and fish rearing should be continuously operated near maximum production capacity (Rakocy et al., 2006). Working a framework close to its critical standing crop utilizes space proficiently, expands production and decreases variation in the daily feed input to the system, a critical factor in estimating the hydroponic segment.

There are three stocking methods that can keep up fish biomass close to the critical standing crop: multiple rearing units, stock splitting, and sequential rearing. Sequential rearing includes the culture of various age groups of fish in a similar rearing tank. When one age group achieves marketable size, it is specifically collected with nets and a grading system, and an equivalent number of fingerlings are quickly restocked in a similar tank. Stock splitting includes stocking high densities of fingerlings and intermittently splitting the populace in half as the critical standing crop of the rearing tank is attained. This technique keeps away from the vestige issue of stunted fish and enhances stock inventory. With different rearing units, the whole populace is moved to bigger rearing tanks when the critical standing crop of the underlying rearing tank is reached. Multiple rearing units, for the most part, come in modules of two to four tanks and are associated with a typical filtration framework. After the biggest tank is gathered, the greater part of the rest of the gatherings of fish is moved to the following biggest tank and the smallest tank is restocked with fingerlings. A variety of the idea of multiple rearing units is the division of a long raceway into compartments with portable screens. As the fish develop, their compartment is expanded in size and drew nearer to one end of the raceway where they will, in the long run, be collected. Each rearing tank contains an alternate age group of fish, yet they are not moved amid the production cycle. This system does not utilize space proficiently in the beginning periods of development, however, the fish are never exasperated and the work associated with moving the fish is killed. Cropping systems showed that batch and staggered production of basil in an aquaponic system were comparable and both were approximately three times more productive than field production.

In addition, stocking density has direct effects on growth, survival, water quality and fish behavior (de Oliveira et al., 2012). On the other hand, if a farmer stock fishes in the fish tank at less optimal conditions, the efficiency and profitability of the culture system gets reduced. For this reason, Ahmed & Hamad (2013) reported that increasing stocking density from 100 to 200 fish m^{-3} in the fish tank results in negative impact by reduced survival, growth and benefits. On the other hand, another study conducted by

El-Saidy & Hussein (2015) about an effect of low stocking density (50 fish m^{-3}) inferred that there is a positive effect on growth performance and feed utilization parameters. However farmers and commercial producers always look for the optimum stocking density to achieve maximum profits. Finally, according to (El-Salam et al., 2014), it could be concluded that the optimum stocking density is 100 fish m^{-3} and the dietary protein levels is 25%, so that the maximum growth is achieved with highest profit analysis.

Feeding rate of tilapia in intercropping aquaponics

Feeding rate is an important factor that affects fish growth as well as the crops in intercropping system under aquaponics. Optimized feeding rate is important for a fish to growth healthy and this can be determined measuring correct balance of composition of feeding like proteins, vitamins, carbohydrates, fats, and minerals. The amount of feeding rate for each aquaponics system will be g m^{-1} per square meter of plant growing area and this feeding rate will be calculated for daily amount of feed per square meter of plant growing area. Table 1 composition (%) of the fish feed in aquaponics systems especially tilapia fish.

Table 1. Proximate composition (%) of the fish feed

Nutrient	(%Content)
Crude Protein	Around 30%
Fat	4%–6%
Cellulose	5%–20%
Mixed Inorganic	3%–5%
Phosphorous	0.54%–1.14

Feeding rate calculation

The feeding rate will be ranged for example between 50–80 g of fish feed per square meter per day. If the plant growing area is 1.125 m^2 per replicate and the total area will be (3*1.125 m^2) and then the total area for one treatment 3.375 m^2 and the feed ration would be 60 g per day per m^2

80 g $\longrightarrow$ day per m^2
x g $\longrightarrow$ day per 3.375 m^2

The amount of feed must be 270 g per day per 3.375 m^2 of area of growing plant whereas the fish biomass of tilapia in a system eats 1–2% from the total weight of body fish per day. i.e., the mean value of feed for fish is that, for each 100 g fish, 2 g must be fed.

Protein is the basic composition in fish meal as it is the building block in fish body and it is also necessary for muscle formation, enzymatic functions, and partially helps in energy maintenance (Bahnasawy, 2009). Further, proteins form the basic structures and enzymes in all living organisms. The optimum protein requirement of tilapia depends on size, age, and water temperature. In general, younger fish (fry and fingerlings) require a diet which is richer than what is fed during the grow-out stage. In their grow-out stage, tilapia needs 25–35 percent of protein in its diet for optimal growth using essential and non-essential amino acids. Several studies estimated that the protein requirement for juvenile tilapia varies from 32 to 50% whereas, for larger tilapia it is between 25 to 30% (Hafedh, 1999; El-Saidy & Gaber, 2005; Ali, Al-Ogaily et al., 2007; Nguyen, Davis & Saoud, 2009; Abdel-Tawwab et al., 2010). In aquaponic system, the feeding fish type it is one of the largest variable costs (Naylor et al., 2009). Feeding rate of tilapia does not treat the possible feeding rate of tilapia in coupled aquaponics but only the protein requirement. However, since the feeding rate ratio of Rakocy et al. (2006) is an important factor in coupled aquaponics. Rakocy pointed out the NFT and gravel systems must have a feed ratio is approximately 25 percent of suggested ratio for Raft hydroponics. Some factors which determine the optimum feeding ratio are the exchange rate of water, level of nutrient in source water, type of plant and degree etc. Decreasing rate of exchange of water, increasing source-water nutrient levels, slow solids removal (as a result in release of more dissolved nutrients) and slow growing plants would permit a lesser feeding rate ratio. Floating and sinking pellet these are the two types of commercial fish feed that can be classified into floating (extruded) or traditional sinking (pressure-pelleted) pellets. Both floating and sinking feed can produce satisfactory growth, but some fish species prefer floating and others sinking (Craig & Helfrich, 2009). Tilapia can utilize both the floating pellets and sinking pellets very efficiently (Santiago, 1987). Kawser et al. (2016) reported that tilapia O. niloticus fed with floating pellet was better than sinking pellet in terms of growth response, feed utilization and nutrient retention. Feeding must be done by hand very slowly and carefully to ensure ingestion of feed completely.

Avoid overfeeding in aquaponics system

In aquaponics system, it is important and advantageous to determine the optimal feeding rate to avoid overfeeding. Moreover, overfeeding has negative effects on the success of aquaponics since overfeeding overloads fish stomach and intestine, leading to decreased digestive efficiency and reduced feed utilization. In biofilter process, heterotrophic bacteria feeds extreme waste from overfeeding which consumes substantial amounts of oxygen. As a result of food decomposition, the level of ammonia and nitrite rise to toxic levels in a relatively short period. In addition, the unconsumed feed pellets can block the mechanical filters, leading to decreased water flow and anoxic areas. Finally, protein is the most expensive component in a diet (Somerville et al., 2014). Therefore, the quantity of protein in the diet should be ideal for fish growth where the excess protein in fish diets may turn unused and cause unnecessary expenses (Ahmad et al., 2004).

MANAGEMENT OF THE NUTRIENT SOLUTION IN INTERCROPPING UNDER AQUAPONICS VS. HYDROPONICS

In hydroponics system, nutrient solution is prepared according to the plant needs and growth stages. Moreover, the pH, Electrical conductivity (EC) and nutrient composition solution can be modified during the life cycle of crops. Intercropping in hydroponics system is easier to apply when compared to aquaponics, because in hydroponics, nutrients can be applied to tank nutrient solution with ideal concentration, while in aquaponics, the nutrients are produced only from the fish waste through nitrification process i.e., conversion of ammonia to nitrate to nitrite along with the production of a low amount of potassium and calcium. According to Table 2, the total nitrogen needs for intercropping between tomato and lettuce is 341 mg L^{-1}, while in aquaponics, the total nitrogen for intercropping the same crops is 172 mg L^{-1}. In addition, the total calcium (300 mg L^{-1}) required for intercropping under hydroponics for tomato and lettuce is higher than aquaponics (214 mg L^{-1}). Moreover, the total concentration of

Table 2. The differences between total nutrient concentrations in intercropping in aquaponics and hydroponics for lettuce and tomato, all nutrients reported in mg L^{-1}

Plant species	System	NO3-N	Ca	PO4-P	Mg	SO4S	Na	K	CL	Fe	Mn	Cu	Zn	B	Mo	Source
Lettuce (*Lactuca sativa*)	Hydroponics	190	200	50	50	66	50–90	210	65–235	5	0.5	0.15	0.15	0.3	0.05	Resh, 2012
Tomato (*Solanum lycopersicum*)	Hydroponics	151	110	39	24	48	–	254	–	0.8	0.6	0.05	0.3	0.2	0.05	Sonneveld & Voogt, 2009
Total Nutrient concentrations		341	300	89	74	114	50	464	65	5.8	1.1	0.2	0.45	0.5	0.1	
Lettuce (*Lactuca sativa*)	Aquaponic	137	180	9	44	–	17	106	–	–	–	–	–	–	–	Pantanella et al., 2012
Tomato (*Solanum lycopersicum*)	Aquaponic	35	34	8	–	–		27	–	0.2	–	0.04	0.37	–	–	Roosta & Hamidpour, 2011
Total Nutrient concentrations		172	214	17	44	–	17	133		0.2	–	0.04	0.37	–	–	

Mg, P, Na, K and microelements under hydroponics for intercropping is higher than in aquaponics for the same crops. According to the information mentioned above, it can be concluded that the aquaponics concentration for macro and micro elements are below the recommended hydroponics level. Also, the other differences between the nutrient solutions between both the systems is the total dissolved solid. In aquaponics, the dissolved solid is organic molecules, while in hydroponics, it is mineral compounds.

CONCLUSION AND RECOMMENDATIONS

The current study provided some valid insights in terms of how to choose crops for intercropping in aquaponics in specific and how to execute aquaponics successfully in general. In aquaponics, the most important point to achieve success is to understand the characteristics of individual living and non-living components such as fish, crop, nitrification biofilter systems and how to combine all the above. The study would like to recommend the future researchers to obtain complete information about the systems being used before venturing into aquaponics since managing aquaponics system is critical and care must be taken to avoid any mistakes during the production cycle in getting the expected output. Prior to adapting intercropping under aquaponics, more knowledge should be gained in various areas such as follows:

- While selecting crops for intercropping in aquaponics, care must be taken such that one crop is leafy and other is a fruit type (for example, tomato and lettuce; tomato and parsley; cucumber and lettuce; strawberry and lettuce, etc.);
- It is important to determine the planting density of both crops (tomato and lettuce) in order to increase the efficiency in using space and also the growing area for beds;
- Selection of aquaponics system for plants that suits and meets the requirements For example, NFT bed design is suitable only for small vegetable species such as lettuce (Engle, 2015);
- The stocking density of fish needs to be determined since this is important for the plant to be provided with nutrients required by both crops, thus integrating the nutrient flow;
- The fish feed composition and the response of fish to the feed are important to be determined prior to beginning the aquaponics process;
- It is important to determine the amount of ammonia that could possibly be produced by feed;
- The amount of biofilter media needed for nitrifying bacteria is critical to be measured prior to beginning the setup;
- The dissolved oxygen and the temperature in tank must be daily checked whereas the pH should be checked frequently;
- Plants selected for aquaponics must have the ability to act as biofilter that takes up waste generated in the system.

The success of intercropping under aquaponics will reduce the gap between crop consumption and fish needs that are grown under organic conditions. There are practical challenges in intercropping high value crops such as tomato and lettuce under aquaponics. According to report Statista (2014), 24.98 Million metric tons of Lettuce was produced globally in the year 2014. It is especially important as a commercial crop in Asia, North and Central America, and Europe. These challenges need to be resolved to increase the production of crops under organic conditions and reduce the usage of fertilizers. For the above to happen, the current study suggests the future researchers to develop full information about the crops of high economical value in terms of its growth under controlled and standardized aquaponics systems so that it becomes possible to achieve the sustainable agricultural production that is viable both in terms of quality and quantity.

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Evaluation of smart economy development in the RIGA planning region (LATVIA)

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Abstract. *Aim of the study: to evaluate the indicators of smart economy development and the interaction with other indicators for the Riga planning region.*
Methods: analysis of documentation and statistical data, the Analytic Hierarchy Process (AHP) method.
To evaluate the potential development of smart economy in the Riga planning region (henceforth – RPR), it is important to understand the present situation in the region, its specificity, and role in the context of provisional future trends. Two approaches have been employed in the present study to evaluate the situation on a regional level. One includes the collection and comparison of the basic economic development indicators, whereas AHP method has been used in the second approach, where 5 experts expressed their opinion on the options of potential development of smart specialisation in the RPR.
RPR as a capital city region is pronouncedly monocentric, with Riga city as its socioeconomic core that develops a wide network of functional ties, and creates a home for the part of the society that works in Riga, but lives in the adjacent suburban territories – Pieriga.
Traditionally the basic indicators of development include only the demographic and economic indicators. Sometimes these results are not objective, do not describe the potential for development, but clearly show the inequal social and economic situation in the region. To characterise the economic development in the RPR, we will include the economic profile data, statistical data and expert opinions on the population, regional government, state and EU influence on the development of the Pieriga region smart specialisation.

Key words: development of smart economy, smart specialisation.

INTRODUCTION

According to the classification of regions in the ESPON study 'EDORA – European Development Opportunities for Rural Areas' (2013), Riga planning region (RPR) can be characterised as a mostly urban region. This region, just like similar regions in other Eastern European countries has a high density of high added value manufacturers and service providers which are specialised in the knowledge economy, mostly in the capital city Riga and the adjacent Pieriga region.

Modern economy is pushed by globalisation, characterised by market liberalisation and ever increasing activity in the knowledge and creative economic fields. All regions need to adapt to the new situation by diversifying their economies and promoting manufacturing of higher added value products. Experience of many European regions suggests that in 21st century every region can experience positive development trends by successfully understanding their competitive strengths and developing them. Inclusion in global knowledge networks by merging global knowledge with local resources is very important. Information and communication technologies play a significant role in this process, by allowing to create wide cooperation networks and reach the critical mass digitally.

The aim of the study is evaluation of smart economy development indicators in the Riga planning region. To reach the aim, we proposed the following **tasks:** 1) evaluate the indicators of the RPR economic profile; 2) compare them with the data gathered during the project 5.2.3. 'Latvian rural and regional development processes and opportunities of the knowledge economy in the context of smart economy' research methods: Analysis of documentation and statistical data, the Analytic Hierarchy Process (AHP) method. In the context of ECOSOC-LV 5.2.3. project objectives, the key task of the study is to see for ways how to establish and develop smart regions, using the capacity of all the resources affecting regional development.

The research object: Smart economy development indicators and their interaction with other indicators of the RPR. This research has been supported by the State research programme 5.2. 'Transformation of national economy, Smart development, government and legal framework for sustainable development of the state and society - new approaches for creation of sustainable knowledge society' (EKOSOC-LV). The author is using data gathered during the project 5.2.3. 'Latvian rural and regional development processes and opportunities of the knowledge economy in the context of smart economy'.

MATERIALS AND METHODS

During this process of the economic transformation significant role is played by the analysis of smart specialisation resources, strategy development, and bringing smart specialisation to life. Smart specialisation is focused on the development of smart economy within a certain territory, taking into account specific development priorities, unique needs and challenges. Per regulation No. 1301/2013 of the European Parliament and the Council 'Smart specialisation strategy is a strategy of national or regional level innovation, with priority for development of competitive advantage, by promoting business investment in research and innovation'.

To develop the knowledge economy, it is necessary to establish the strengths and competitive advantage of a certain region, which would allow for further innovation and increase the added value of the manufactured produce. It is important to note that smart specialisation applies not only to highly technological innovation, but also non-technological innovation. For example, an innovative tourism product or designer goods are equally significant part of the knowledge economy as high technology products, because the most significant trait of knowledge economy is the use of intellectual property to increase value of a product.

Comparative advantages of a certain region are revealed by evaluation of its competitiveness. Competitiveness of a region can be defined as the ability to fulfil the needs of the local population, and sustain a high quality of life via an efficient use of local and also imported resources. Efficient use of locally available resources and import of other necessary resources, increase the ability of local businesses to create and develop new products, increase the competitiveness of existing products, which is one of the most significant prerequisites for regional development and a core task of smart specialisation strategy (see Table 1).

Table 1. Indicators of RPR economic development (RPR of the current situation description and analysis 2014

GDP (2011)	12, 858 EUR per capita
Income tax (2013)	590 EUR per capita
Unemployment (2012)	5.3%
Average wage (2013)	785 EUR
Economically active market statistic units (2012)	85,124
Startups (2012)	9,553
Worforce education	30% higher
	35% professional
	25% general

Location and territory of the region

The geographical location of the Riga region (Fig. 1) is unique in the sense that it is simultaneously situated on the shore and deep inland the continent. This is an excellent prerequisite to develop contacts and emerge as a national and international mediator. Riga region is easily accessible from many European countries. It is located right in the middle of Latvia and the Baltic states, it is right on the border the Eastern- and Western European cultural space, belongs to the central part of the Baltic sea area, which is one of the potential centres the rapid development of the modern world. Geographical location in the centre of the Baltic states, being on the borders of Eastern and Western European cultures has created Riga region as a bridge between different countries and their inhabitants.

Riga planning region consists of two NUTS III level statistical regions – Riga (includes Riga city) and Pieriga (includes the rest of the RPR territory). In the economic profile, data analysis is carried out not only of the Riga planning region, but also the Riga and Pieriga statistical regions. The total area of the RPR is 10,435 km^2, covering 16,2% of the state territory (RPR Economic Profile 2010).

Administrative division of the region

Riga region consists of two republic cities – Riga and Jurmala – together with regional governments of 28 further districts, including 10 cities. Average population density if 105 residents per km^2, but in the central parts of the region (Riga, Jurmala, Pieriga) – up to 280 residents per km^2 – tripling the national average (Fig. 2). The central parts of the region can be comparable with other European and Scandinavian metropolitan areas in terms of population density.

Population structure and variable placement in the regional area can be considered as a potential for development, an advantage, that creates positive prerequisites for development of various economic, social, and cultural activities and intensive exchange of values within the region. The last decades have seen development of highly populated areas just adjacent to the Riga city, thus giving rise to a Riga metro area – Pieriga. The cities of Riga and Jurmala, and the Pieriga region make up a regional core with the highest concentration of population, manufacturing and services, accompanied by concentration of development-related problems (RPR Economic Profile 2010)

Figure 1. Riga planning region within Europe.

Figure 2. Administrative territories of the Riga planning region.

Human resources

Riga planning region is home to 1.1 million people – nearly half of the total Latvian population. This is significant in the Latvian scale, but might not be enough to be competitive internationally. Here, knowledge and skills that may translate to a competitive product, are the most important. RPR concentrates the absolute majority of higher education opportunities and scientific potential. Workforce skill levels, influenced by the accessibility and offer of higher education as well as the work ethic, is important. Multinational makeup of the population can be considered an advantage. The highest concentration of the workforce is in the Pieriga region – whereas other regions are affected by depopulation (see. Table 2).

Table 2. Characterisation of RPR smart population and smart resources (data from project 5.2.3. 'Latvian rural and regional development processes and opportunities of the knowledge economy in the context of smart economy')

RPR regions	Higher Ed.	Employment in the primary sector	Long term unemployment, %	Agricultural land, %	Forests, %	Mineral-resources, m^3 x 1,000	Total density of motorway network
Ādaži region	31.9	2.09	11.52	0.89	0.46	0	1,175
Aloja region	11.4	20.77	43.43	0.79	0.52	886,95	0,645
Babīte region	34.7	2.52	11.43	0.59	0.40	206,44	0,944
Baldone region	22.5	4.77	11.46	0.73	0.57	110,24	0,909
Carnikava region	34.4	2.13	17.19	0.70	0.58	0	1,976

Table 2 (continued)

Engure region	18.8	6.67	16.57	0.82	0.56	1,290,76	0,809
Garkalne region	41	1.21	14.29	0.36	0.73	6,466,91	1,347
Ikšķile region	35.2	2.46	8.09	0.66	0.47	1,858,82	1,419
Inčukalns region	20.1	4.26	35.4	0.65	0.63	594,21	1,093
Jaunpils region	11.9	27.25	23.46	0.96	0.36	52,85	1,015
Kandava region	13.8	13.08	41.01	0.88	0.44	14,048,8	0,915
Krimulda region	18.1	11.67	31.79	0.90	0.42	2,975,42	0,910
Ķegums region	18.4	9.60	25.9	0.79	0.56	2,856,3	0,830
Ķekava region	30.1	2.21	9.2	0.60	0.53	3,522,65	1,031
Lielvārde region	18.8	7.22	16.67	0.86	0.32	522,88	1,316
Limbaži region	16.4	9.92	42.3	0.78	0.47	1,234,72	1,051
Mālpils region	17.6	8.09	20.15	0.91	0.47	147,3	1,049
Mārupe region	38.8	3.54	8.33	0.87	0.26	2,014,27	1,781
Ogre region	21.9	4.70	13.11	0.87	0.48	5,941,3	1,117
Olaine region	20.9	1.66	19.45	0.80	0.59	304,9	0,677
Ropaži region	19.3	4.17	12.58	0.70	0.64	15,744,7	0,906
Salacgrīva region	14.2	9.29	28.34	0.72	0.59	2,060,81	0,899
Salaspils region	25	1.26	15.49	0.53	0.35	6,662,05	1,355
Saulkrasti region	25.6	2.81	13.97	0.58	0.59	0	4,761
Sēja region	18.7	8.36	28.57	0.83	0.55	0	0,799
Sigulda region	25	4.14	22.42	0.82	0.50	2,121,53	1,062
Stopiņi region	29.3	1.03	17.09	0.52	0.45	141,44	1,683
Tukums region	16.7	9.18	24.39	0.92	0.35	14,526,1	0,907

There are pronounced problems with the negative population growth, aging population and external emigration, that decrease the perspective workforce resources for future growth. In the following years, a sharp decline in number of children and young people in education or training is expected. The relatively healthy demographic situation, when compared to other regions can be lost in the near future, which poses a huge burden on the future education and migration policies (RPR Economic Profile 2010).

Available natural resources, guarantees for the development of the region

RPR has the main vital and commercial resources. There are sufficient ground waters for communal needs, large areas of agricultural land (1/3 of the RPR territory), and forests (1/2 of the RPR territory). The unique water system of the Riga Gulf, 3 large rivers, numerous lakes and water reservoirs allow for creation of internal waterways. Numerous minerals, such as gypsum, peat, sand, gravel, dolomite, therapeutic mud and water are fit for commercial use (see Table 1).

Availability of financial resources and associated factors

The main types of RPR financial resources are regional government budgets, state support, incl. EU funding and private investments. Investment opportunities are dictated by many circumstances – state investments are dependent on the overall national financial situation, balance of payments, external debt, international commitments; for regional governments, it may be specifics of budget income, borrowing potential, loan payments; private investments are dependent on the crediting possibilities and availability of personal investment capital; EU – the availability of programmes and EU support priorities (RPR Economic Profile 2010).

Within Latvia and even within RPR there are significant differences in the regional government income per capita. Regional governments mostly make up their budget on income from taxation. Most of RPR regional governments have the best parameters of income from taxation per capita nationally (see Table 3).

Table 3. Characterisation of the RPR smart government **(**data from project 5.3.2. 'Latvian rural and regional development processes and opportunities of the knowledge economy in the context of smart economy')

RPR regions	EU funding (ERAF_ESF_KF) EUR per 1,000 inhabitants	EU support (ELGF_ELFLF_ZF) EUR per 1,000 inhabitants	Voter activity	Changes in the regional e-index, %	NGA zone
Ādaži region	915,549	366,316	45	16%	2
Aloja region	1,227,372	3,331,471	49	115%	0.5
Babīte region	3,159,306	374,574	44	47%	1
Baldone region	840,036	314,484	45	21%	1
Carnikava region	908,007	128,766	52	26%	1
Engure region	444,278	993,499	44	17%	1
Garkalne region	113,019	48,675	52	19%	1
Ikšķile region	913,792	2,501,82	51	18%	1
Inčukalns region	3,924,477	129,402	45	-22%	1
Jaunpils region	152,316	7,261,915	45	28%	0
Kandava region	1,331,141	1,884,321	44	-7%	0.5
Krimulda region	166,119	2,427,684	52	35%	1
Ķegums region	386,088	1,388,203	47	-18%	1
Ķekava region	772,894	173,841	49	9%	1
Lielvārde region	409,321	837,121	46	25%	1
Limbaži region	979,490	1,726,744	40	-5%	1
Mālpils region	145,078	1,768,176	37	-11%	1
Mārupe region	2,951,158	369,938	48	-1%	1
Ogre region	1,403,267	758,001	42	5%	0.5
Olaine region	949,094	172,058	48	1%	1
Ropaži region	1,008,109	311,698	50	95%	1
Salacgrīva region	822,287	1,709,465	39	24%	1
Salaspils region	1,445,939	219,718	48	38%	1
Saulkrasti region	1,529,361	951,836	46	12%	1
Sēja region	376,907	1,989,269	51	0%	0
Sigulda region	727,982	469,338	46	39%	0.5
Stopiņi region	2,076,750	247,563	50	0%	2
Tukums region	1,308,391	1,934,721	35	3%	0.5

Economic development

Riga region is the driving force of Latvian economy. A report on the business environment by the Ministry of Economics of Latvia (henceforth – LR EM) states that, based on provisional data for 2014, there were 109,626 economically active individual merchants and commercial companies in Latvia (excluding farmer or fishermen companies and self-employed persons). 99,6% of the above can be categorised as small or medium-sized businesses (henceforth SMB). Division of SMBs in Latvia is as follows: microcompanies – 89.6%, small businesses – 8.9%, medium-sized businesses – 1.5%. A significant indicator of economic activity is the number of businesses per 1,000 people. In the last 10 years, this number has steadily increase from 17 in 2001 to 83 in 2014 (LR Ministry of Economy 2016) (see Table 4).

Table 4. Characterisation of RPR smart economy (data from the project 5.2.3. 'Latvian rural and regional development processes and opportunities of the knowledge economy in the context of smart economy')

RPR regions	Innovative businesses	Innov. business turnover	Innovative businesses employees	Turnover per employee	Self-employed per 1,000 inhabitants
Ādaži region	31.5	1.13	20.2	95	19,1
Aloja region	18.63	2.28	11.58	1,776	40,52
Babīte region	29.93	8.66	13.89	32,589	20,85
Baldone region	22.54	4.33	11.88	13,494	16,49
Carnikava region	33.22	2.,24	26.05	29,748	21,21
Engure region	12.56	2.38	5.58	18,930	21,35
Garkalne region	34.82	6.54	21.73	22,306	24,04
Ikšķile region	31.06	10.48	20.11	17,847	31,91
Inčukalns region	21.89	1.19	6.56	25,681	12,23
Jaunpils region	13.64	9.95	9.03	64,350	17,79
Kandava region	11.92	1.2	3.78	8,275	26,44
Krimulda region	18.13	2.22	7.35	11,963	24,6
Ķegums region	26.28	5.65	12.22	16,688	21,71
Ķekava region	32.25	2.41	7.45	25,810	19,86
Lielvārde region	17.87	14.99	17.1	39,049	22,1
Limbaži region	18.06	6.63	11.08	5,020	41,07
Mālpils region	19.09	3.64	8.91	9,016	22,05
Mārupe region	31.38	11.43	20.62	912	20
Ogre region	27.82	11.92	19.9	24,168	23,4
Olaine region	26.85	30.93	32.85	59,929	11,37
Ropaži region	29.33	7.52	14.78	28,545	11,9
Salacgrīva region	15.34	2.74	9.91	12,907	30,48
Salaspils region	23	8.6	17.83	25,136	13,79
Saulkrasti region	26.36	3.73	19.52	10,384	21,52
Sēja region	17.44	3.89	12.85	15,335	18,35
Sigulda region	28.55	6.66	17.49	17,709	24,21
Stopiņi region	24.42	2.03	8.26	28,936	16,49
Tukums region	16.38	2.83	10.7	16,319	20,31

Economic structure and businesses

The economic structure of Riga region is dominated by the service businesses with associated fields – sales, professional services, real estate businesses. Each of these fields takes up more than 10% of the total economy in the Riga region based of the amount of economically active market sector statistical units, with sales reaching nearly 25%. By this measure, agriculture, construction, transport and storage also make up a significant portion of the total economy. Manufacturing takes up 6% of the total Riga region economy.

The share of total added value in Riga planning region makes up 67% nationally. In certain fields this share is more than 70% nationally (construction, sales, hospitality and catering, transport and logistics, real estate operations), which is a sign of concentration of these types of business activity in the region, mostly in Riga and Pieriga. In the banking and finance sector, Riga region takes an 85% share of the national economy. In all these fields, Riga region can be considered the main player in Latvia. This situation characterises the situation in the country quite well. (RPR Economic Profile 2010).

RESULTS AND DISCUSSION

Data from the project 'Latvian rural and regional development processes and opportunities of the knowledge economy in the context of smart economy' were divided into 4 main groups from all 28 regions of the RPR – smart population, smart resources (see Table 2), smart government (see Table 3), and smart economy (see Table 4). The studies were summarized in 28 Riga Planning Regional development fundamentals that were used to evaluate the smart economic indicators used in 16 of all 19 indicators for each county (EKOSOC- LV 5.2.3 Project data, the SCP data, 2015). Situation display, were processed Riga Planning Region (except the republic cities are Riga and Jurmala) regions data, the results shown in Rotated Component Matrix (see Table 6). In the calculation was based on smart economic indicators (see Table 5).

Table 5. Description of smart economy indicators (data from the project 5.2.3. 'Latvian rural and regional development processes and opportunities of the knowledge economy in the context of smart economy')

Smart resources
Agricultural areas, %
Forests, %
Mineral resources, m^3 x 1,000 (2013)
RSS expenses (2001–2015), EUR
Total density of motorway network (km/km^2, RAIM calc.)
Smart population
Population percentage with higher education, %
Percentage of workforce in the primary sector, %
Percentage of long-term unemployed amongst all jobseekers, %
NGOs per 1,000 pers.
Smart economy
Percentage of innovative businesses, %
Percentage of the turnover of innovative businesses, from total turnover, %
Percentage of the workforce employed by innovative businesses, %
Innovative business turnover per 1 employee, EUR
The number of self-employed persons, per 1,000 inhabitants
Smart governing
The amount of EU funding (ERAF_ESF_KF) EUR per 1,000 inhabitants, 2009–2014
EU support (ELGF_ELFLF_ZF) EUR per 1,000 inhabitants, 2009–2014
Voter activity %, Regional government elections 2013
Changes in the regional E-Index (2015–2014)
NGA zone

The data acquired during the project 'Latvian rural and regional development processes and opportunities of the knowledge economy in the context of smart economy' were processed with factor and cluster analysis.

During the factor analysis, we obtained four independent groups. It was shown that most characteristics that are corresponding to smart economy are in the group 'smart population', followed by 'smart economy' and 'smart government', with 'smart resources' being the least appropriate of the pack. Factor analysis showed that smart economy is influenced by the turnover of innovative businesses and the number of employees as well as turnover per employee and the number of self-employed individuals per 1,000 inhabitants. But smart economy is also influenced by smart population, with the main factors being the percentage of workforce with higher education, number of innovative businesses, availability of EU funding, EU support in EUR per 1,000 inhabitants., NGA zone etc. (see Table 6).

Table 6. Distribution of RPR district economic profile by factor analysis (by the author)

Rotated Component Matrix[a]

	Component			
	1	2	3	4
Mineralresources, m3 (in thousands)	.041	-.153	.078	-.797
Total density of motorway network	.276	-.087	.260	.580
Higher ed.	.730	.140	.483	.254
Long-term unemployment,%	-.608	-.400	-.289	-.063
NGO per 1,000 inhabitants	-.003	-.265	.744	.250
Innovative businesses	.668	.266	.562	.121
Innov. business turnover	.022	.871	.118	.161
Innov. business employees	.311	.640	.392	.261
Turnover per employee	-.203	.842	-.080	-.069
Self-employed per 1,000 employees	-.412	-.551	.242	.256
ERAF_ESF_KF, EUR per 1,000 inhabitants	.460	-.129	-.162	.036
ELGF_ELFLF_ZF, EUR per 1,000 inhabitants	-.907	-.049	-.068	.059
Voter activity	.149	.282	.645	.008
Changes in the region e-index,%	-.275	-.013	.625	-.396
NGA zone	.705	-.111	-.061	.170
Employed in the primary sector	-.938	-.169	-.068	-.046
	Smart population	Smart economy	Smart government	Smart resources

During the project, the Analytic Hierarchy Process (AHP) method was employed to study the creation and development of Pieriga region smart specialisation. The aim of the study was to establish the creation and development of smart specialisation of Pieriga region. A total of 6 experts from the Pieriga region took part in the study – 1 entrepreneur, 1 manager of the regional government, 1 scientist, 1 manager of a commercial farm and 2 members of a business support institution. Results showed that in the evaluation process, the largest share went to the smart economy – 0.42, a close second being smart population – 0.25. Experts deem smart resources (0.18) and smart government (0.15) to be the least influential on the smart economy (see Fig. 3). This shows that, according to experts, high indicators of smart economy are a prerequisitie for creation and development of smart specialisation in the regions. Smart economy is the cornerstone of development and future specialisation of all RPR regions.

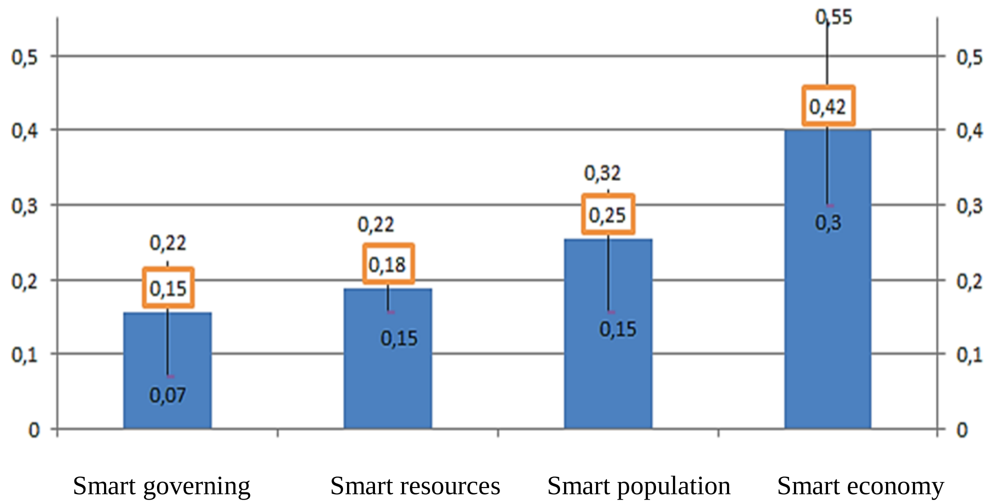

Figure 3. Evaluation of smart economy indicators in development of Pieriga region smart specialisation, 2016 (data from the project 5.2.3. 'Latvian rural and regional development processes and opportunities of the knowledge economy in the context of smart economy').

Cluster analysis divided 28 regions of RPR in 4 groups. The highest indicators are for group 3 followed sequentially by groups 4, 2 and, lastly, 1. Cluster analysis selects the most significant indicators – agricultural land, percentage of population with higher education, workers employed in the primary sector, long-term jobseekers, innovative businesses, employees working in innovative businesses, turnover per employee, self-employed individuals per 1,000 inhabitants, EU support and co-financing in EUR per 1,000 inhabitants, NGA zone (see Table 7).

Table 7. Distribution of RPR regional economic profile data clusters (by the author)

Final Cluster Centers				
	Cluster			
	1	2	3	4
Number of Cases in each Cluster	1	9	4	14
Higher Ed.	11.90	16.14	30.73	26.46
Innovative businesses	14	18	27	27
Innov. bus. turnover	9.95	3.45	5,83	9.49
Innov. bus. employees	9	10	12	18
Turnover per employee	64350	10811	22030	23797
Self-employed per 1,000 inhabitants	18	27	17	20
ERAF_ESF_KF, EUR per 1,000 inhabitants	152316.28	749208.19	3027922.64	884332.04
ELGF_ELFLF_ZF, EUR per 1,000 inhabitants	7261914.66	2017783.86	280369.36	428252.46

CONCLUSIONS

1. RPR further development, it is necessary to plan the structural reforms in order to attract new technologies and promote innovation, improve the region's resource base, as these factors form the basis for regional economic development and defines smart specialization.

2. For counties happen smart specialization formation and development, it should be based on high intelligent Economic Indicators (Innovative businesses, Innovative businesses in the turnover, Innovative business employees, Turnover per 1 employee and Self-employed per 1,000 inhabitants) indicators, because they affect the entire RPR county's future development and possible specialization.

3. Expert assessment of regional smart specialization formation and development must be based on intelligent high economic performance, since they form the basis of all the RPR county's future development and possible specialization.

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Through Economic Growth to the Viability of Rural Space

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Abstract. Rural areas as a living space for the population has been increasingly explored in official documents of various EU institutions and in research topics. Both the documents and the research papers stress the necessity to enhance and maintain the viability of rural areas. The viability of rural areas is ensured by employment opportunities and readiness of residents for active and innovative economic activities. The paper presents an analysis of vertical and horizontal changes in entrepreneurship in the period of 2009–2015 and their effect on changes in the living space of the analysed territories in Latvia's regions. The processes in administrative territories of regions, municipalities were analysed, as the life of residents is influenced not only by national policies but also by on-going processes in the administrative territories of local governments. The data of LURSOFT for the period of 2009–2015 and the Central Statistical Bureau for the period of 2013–2015 were used as the sources of information. The data were processed by quantitative (growth) and qualitative (structural change) statistical analysis methods. The Eurostat methodology and the methodology developed by the authors for classification of industries were employed for the analysis of structural changes in the national economy. The development level-rate matrix method was used for an in-depth examination of the research results. The research results showed that, in spite of the global economic crisis, both vertical growth and positive horizontal change took place in the national economy of all five regions of Latvia, nine cities of national significance as well as all 110 municipalities that composed the rural areas of Latvia. The authors arrived at the conclusion that, first, performance trends contributing to economic growth were observed in the rural space; second, there was no direct causal relationship between the population density and economic activity in the rural territories; third, the economic growth in the rural territories was greatly affected by the quality of local governance and local community residents' readiness for active, innovative and inclusive activities.

Key words: living space, structural changes, knowledge-based economy, local governance, local community.
JEL codes: P25, R11

INTRODUCTION

In the last decade, prospects for national development of Latvia have been in the focus of attention for several times. On June 10, 2010, the Saeima approved the Sustainable Development Strategy of Latvia until 2030 'Latvia 2030' (Latvia 2030, 2010). Two years later, a new policy document, the National Development Plan 2014–2020, was adopted by the Saeima on December 20, 2012 (NAP, 2012). One of the most essential objectives was to reach the level of the EU Member States in all the areas of life, primarily, in the economic development, which functions as an important tangible factor for smart growth (Bacon & Brewin, 2008). The question remains: what is the progress in achieving the aims?

In terms of an area and the population of the rural area, Latvia is actually close to the averages of the European Union (EU-28). In the EU, rural territories occupy 44.1% and intermediary territories – 44.4% of the total area, while in Latvia the rural area accounts for 40.2% and intermediary territories account for 43.6%. A similar situation is observed with regard to the distribution of the population. In the EU, 19.2% of the total population live in rural territories and 36.4% in intermediary territories; in Latvia the numbers are 22.2% and 27.0% (CAP Context…, 2016 update), respectively. At the same time, labour productivity in Latvia is less than 75% of the EU–28 average and, consequently, GDP per inhabitant in Latvia is less than 75% of the EU–28 average. The data for Latvia in Global Competitiveness Index do not show any improvement. On the contrary, a drop was observed (49th place in 2016/2017 instead of 44th in 2015/2016) which is the worst result among the three Baltic States (The Global Competitiveness…, (2016). For this reason, since 49.2% of the total population of Latvia live in 110 municipalities of rural areas, a topical problem for researchers is the promotion of viability of the rural space through smart growth and forming vital rural areas, as the role of rural space in the wellbeing of the population increases (Making Europe…, 2016).

The **theoretical framework of the present research** involves the understanding of viability of rural areas and the role of a knowledge-based economy in the mentioned processes. Rural vitality and viability have become an important research problem in the beginning of the 21st century. First of all, the meanings of the concepts have to be explained. Vital rural territories are the territories where strong, active and inclusive relationships among residents, the private sector, the public sector and civil society organisations function in the economic, social and environmental spaces. Vital communities are those that are able to cultivate and enhance these relationships in order to create, adapt and thrive in the changing world (Sott, 2010). Vitality is increasingly portrayed as a complex, multi-dimensional concept that increased the use of the skills, knowledge and ability of local people, strengthened relationships and communication, improved community initiative, responsibility and adaptability, sustainable, healthy ecosystems with multiple community benefits, appropriately diverse and healthy economies (Grigsby, 2001) Besides, rural viability is explained as the ability of a local community to succeed by using available physical and human resources of this territory. Particularly effective leadership within the community is necessary in order to assert successful community action, encourage social well-being, and improve community viability (Bearden et al. (without a year); Ricketts & Place, 2009). Economic activities pay a significant role in both vitality and viability. The health of the local economy is viewed as one of the key factors for maintaining the viability of a territory inhabited by a community (Grigsby, 2001; Sott, 2010). Creative and diversified economic activities have to be promoted, because they contribute to employment and make a territory more populated. Integrated economic sectors and strong local economies are necessary (Bacon & Brewin, 2008; The Rural…, 2010; Naldi et al., 2015). There are three priorities in the field of economic development: developing an economy based on knowledge and innovation, promoting a more resource efficient, greener and more competitive economy and fostering a high-employment economy delivering economic, social and territorial cohesion (European Commission, 2010). As Latvia joined the European Union in 2004 and integrated into the OECD country group in 2016, the formation and development of a knowledge-based economy have become a practical task and an object of research. 'The knowledge based economy' is an expression coined to describe trends in advanced economies towards greater dependence on knowledge, information and high skill levels, and the increasing need for ready access to all of these by the business and public sectors (OECD, The Measurement..., 2005).

The aim of the research: to assess vertical and horizontal changes in entrepreneurship in the period of 2009–2015 with a special focus on trends in the changes in the knowledge-based economic segment. The research performed an assessment of the changes in: a/ Latvia as a whole; b/ five regions of Latvia; c/ rural municipalities, local administrative units of the regions of Latvia.

LURSOFT data for the period of 2009–2015 and the Central Statistical Bureau data for the period of 2013–2015 were used as information sources. The data were processed by quantitative (growth) and qualitative (structural change) statistical analyses and development level-rate matrix methods.

RESULTS AND DISCUSSION

Vertical changes in entrepreneurship in the period of 2009–2015

The analysed period was complicated. It involved both the economic crisis and the post-crisis period. Since knowledge-based economic growth has been prioritised in the 21st century, the research simultaneously analysed economic growth both in the entire economy and in the knowledge-based economic segment, which was the focus of the research. Such an approach is in line with the OECD strategy stating that technology is bringing unprecedented chances in rural areas (Innovative Rural Regions). According to the EUROSTAT methodology, the knowledge-based economic segment consists of high-tech (HT), medium high-tech (MHT) factories and knowledge intensive services (HT, MHT, KIS) (European Commission, 2008).

The comparison of the key indicators of entrepreneurship (the number of enterprises, the number of employees and the net turnover) both in the cities of national significance and in 110 municipalities shows trends in entrepreneurship in the period of six years (Table 1).

Table 1. Growth of entrepreneurship in the period of 2009–2015 (vertical growth, % change)

Indicators	Cities of national significance 9 cities)		Rural territory of 110 municipalities (incl. towns in municipalities)	
	All enterprises	KBE segment	All enterprises	KBE segment
Number of enterprises	152.9%	185.1%	179.6%	236.6%
Number of employees	114.8%	129.7%	128.5%	143.1%
Total net turnover	143.7%	146.9%	169.2%	133.1%
Net turnover per employee	125.2%	113.3%	131.8%	133.8%

Source: the authors' calculations based on LURSOFT data.

The number of enterprises increased at a faster rate than the number of employees, total net turnover and, particularly, net turnover per employee. This trend could be observed both at the national level and in rural territories with regard to entrepreneurship as a whole and the KBE ('Knowledge-based entrepreneurship') segment. However, the growth of the KBE segment both in the cities and in rural territories considerably exceeded an increase of the number of all enterprises. This means that innovative economic activity strengthened, as the growth of the KBE segment contributes to the new knowledge and skills of beginners in entrepreneurship. Not a less important finding is that rural areas as a space, in terms of entrepreneurship, moved at least a step towards the level of cities, as growth rates were higher in the rural territories (municipalities) than in the cities, which decreased disparities between the cities and the rural areas.

The overall situation in Latvia is important, but only for the comparison with its neighbouring countries, first of all, Estonia and Lithuania, and the country's internal territorial units, which can reveal similarities and differences in development processes of the territorial units or reveal how successfully the spatial aspect of cohesion is being implemented.

The processing of the LURSOFT data showed that the growth of entrepreneurship as a whole and the vertical growth of its KBE segment were observed in all the regions, as well as in the country's nine cities of national significance. However, the growth of entrepreneurship as a whole in all the regions was faster than that in the nine cities of national significance, even though the growth rates in the regions were different (Table 2). It is necessary to stress the growth of the KBE segment in particular, which outpaced that of entrepreneurship as a whole both in terms of number of enterprises and in terms of number of employees. The mentioned faster growth took place not only in the cities but also in all the regions. Two regions, Vidzeme and Kurzeme, should be particularly highlighted, as the net turnover per employee in the KBE segment exceeded that in the regional economy.

Table 2. Growth of entrepreneurship in the regions in the period of 2009–2015 (vertical growth, % change)

	Growth of entrepreneurship as a whole					
	Zemgale	Pieriga	Vidzeme	Latgale	Kurzeme	9 cities
Number of enterprises	177.1	198.0	165.1	160.8	158.7	152.9
Number of employees	123.8	137.3	124.4	110.7	121.0	114.8
Total net turnover	164.1	171.6	175.4	151.0	165.9	143.8
Net turnover per employee	132.5	124.9	132.8	136.4	137.2	125.2
	Growth of knowledge–based entrepreneurship (KBE)					
	Zemgale	Pieriga	Vidzeme	Latgale	Kurzeme	9 cities
Number of enterprises	236.8	255.3	202.5	177.6	207.8	185.1
Number of employees	125.2	159.8	127.3	122.4	129.8	129.7
Total net turnover	135.0	128.4	186.9	144.8	146.9	146.9
Net turnover per employee	107.6	119.1	134.8	116.8	227.1	112.3

Source: the authors' calculations based on LURSOFT data.

Horizontal changes in entrepreneurship in the period of 2009–2015

Horizontal changes in entrepreneurship as a whole reflect not only the size of any particular segment but also its influence. The increasing number of enterprises in a segment provide additional work places for people. In addition, if a significantly greater proportion of net turnover of the segment is in the total net turnover, the segment's problems receives additional attention in the economic development strategy. The trends may be positive and negative.

Table 3. Similarities and differences in segmental restructuring processes in the regions in the period of 2009–2015 (structural change in %-points)

	Manufacturing segment					
Indicators	Zemgale	Pieriga	Vidzeme	Latgale	Kurzeme	9 cities
Number of enterprises	-1.77	-1.07	-0.79	-1.93	-0.34	-0.60
Number of employees	+1.21	-1.49	+0.85	-0.53	+0.29	-1.89
Net turnover	+7.16	+0.50	-1.76	+6.01	-0.65	-0.65
	Segment of agriculture, forestry and fisheries					
Number of enterprises	+1.41	-0.31	+5.61	+10.75	+7.11	+0.1
Number of employees	+1.29	-0.45	+1.72	+5.53	+3.20	+0.10
Net turnover	-0.74	+1.72	+5.36	+9.32	+7.67	+0.24
Services segment						
Number of enterprises	-1.44	+2.39	-4.83	-7.70	-5.81	+1.9
Number of employees	-3.32	+1.25	-2.98	-2.65	-3.10	+2.74
Net turnover	-6.95	-1.70	-5.50	-2.59	-7.36	+6.20
Segment of other industries (construction, environmental and communal services, mining)						
Number of enterprises	+1.78	-0.93	+0.01	-8.12	-0.9	-1.33
Number of employees	+0.82	+0.69	+0.41	-2.35	-0.39	-1.09
Net turnover	+0.53	+1.34	+2.08	-2.76	+0.4	-5.79
	Knowledge-based economic segment					
Number of enterprises	+4.08	+6.54	+2.72	+0.99	+2.9	+5.47
Number of employees	+0.14	+2.28	+0.26	+1.2	+5.4	+3.30
Net turnover	-1.10	-3.77	+0.29	-0.26	-0.6	+0.21

Source: the authors' calculations based on LURSOFT data.

The data of Table 3 reveal these trends. There are two positive ones. First, the influence of agriculture, forestry and fisheries rose, as this segment's proportion increased in terms of numbers of enterprises and employees in four regions, which led to an increase in the segment's proportion of net turnover in the total net turnover. The greatest growth of this segment was observed in Latgale region where the preservation of rural vitality is of great importance due to the decrease of the population, the long distance from the capital city of Riga and the region's location close to the border. Second, the growth of the KBE segment was quite noticeable. In all the regions and cities, an increase in the proportion of this segment took the form of an increase in both the number of enterprises and the number of employees. Unfortunately, the proportion of the net turnover increased only in Vidzeme region and the cities. The maximum decrease in the proportion of net turnover of the KBE segment in the total net turnover was observed in Pieriga region, which could be explained by an increase in the proportions of net turnover in a number of other economic segments and a minimum increase in net turnover (18.2%-points) in the segment of knowledge-based services in the six-year period of analysis, as well as by the fact that the mentioned services dominated (96.3%) particularly in Pieriga region. There is a global trend that the so-called gentrification process intensifies due to the movement of competent and wealthy individuals to peri-urban territories with the purpose to live in the favourable natural environment and do distance work or provide knowledge-intensive services on the Internet at the place of residence (Kruzmetra, Z., 2011).

A negative trend is a decrease in the proportion of manufacturing, although it is an economic segment that considerably contributes to the added value during production. The number of this segment's enterprises decreased in all the regions and cities. The proportion of individuals employed in this segment decreased in two regions and the cities. According to the results of the survey, manufacturing took the 2nd place in providing jobs (21.86% of the total employees) right behind the segment of services (53.8%); it should be noted that employment and incomes are among the key factors contributing to retaining population in rural areas (Bacon & Brewin, 2008; The Rural..., 2010). Progress in this segment could be expected if the processing of biological products increases, which is among the strategic objectives of the bioeconomy (Making Bioeconomy..., 2015). Furthermore, an increase in the proportion of the knowledge-based economic segment in terms of numbers of both enterprises and employees has not yet resulted in an adequate increase in the net turnover, although a maximum increase in this particular indicator may be expected.

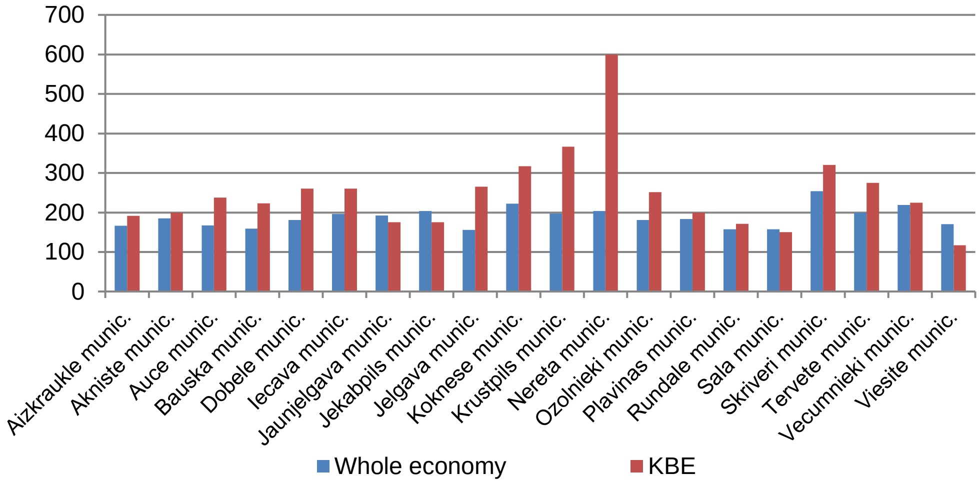
Vertical and horizontal changes in entrepreneurship in Zemgale region in the period of 2009–2015

Since official EU documents and research papers increasingly stress the local territory approach (Grigsby, 2001; Janvry & Sadoulet, 2007; Sott, 2010; Making Europe…, 2016), the present research also performed a vertical and horizontal analysis at the regional level. Zemgale region consisting of 20 municipalities was chosen as an example. The research results convincingly showed that disparities of the municipalities in both vertical growth and segmental distribution within the regions are even more pronounced than regional differences. Therefore, an analysis of spatial viability problems in these local territories, which make up the regions, is needed.

The comparison of quantitative growth in the whole economy and the knowledge-based economic segment revealed that the growth of the KBE segment in terms of numbers of enterprises and employees and, particularly, in terms of net turnover in 16 municipalities of the region exceeded that in the remaining four municipalities (Jaunjelgava, Jekabpils, Sala and Viesite), convincingly proving the role of the KBE segment in preserving the vitality of rural areas and, to a greater extent, their viability, which is significantly affected by economic growth (Fig. 1).

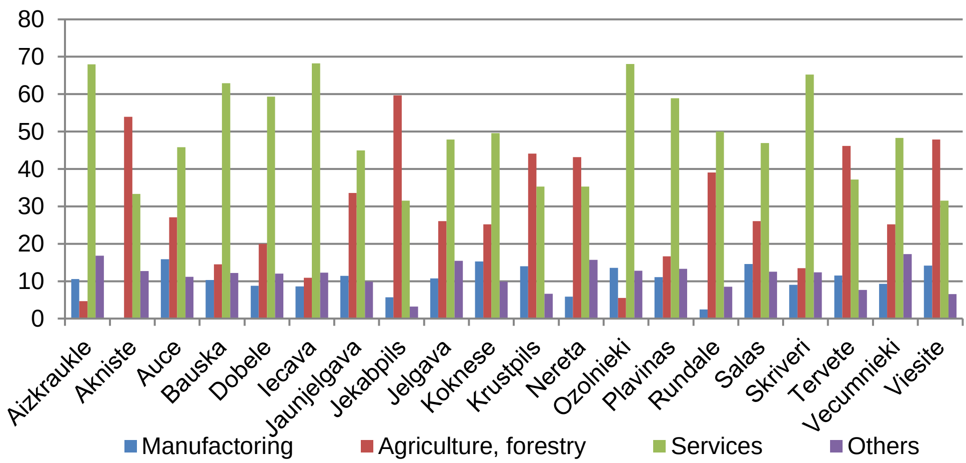
The data also show that the key factor of disparities was not the location of a municipality. Thus the municipalities of Nereta (with the greatest increase in the number of employees and the second greatest increase in the net turnover) and Viesite (with decreases in the number of employees and net turnover) are neighbouring municipalities, both are situated far away from the capital city and both lie close to the border of Latvia and Lithuania. Consequently, such performance must have been affected by other entrepreneurship influencing factors.

Disparities across municipalities within a region are also indicated by the sizes of segments of entrepreneurship (Fig. 2). Agriculture and forestry is the dominant segment in six municipalities out of the twenty municipalities of Zemgale region: 59.68% in Jekabpils, 53.97% in Akniste, 47.83% in Viesite, 46.15% in Tervete, 44.12% in Krustpils and 43.14% in Nereta. Manufacturing ranged from 15.95% in Auce municipality to 0.0% in Akniste municipality. Both municipalities lie close to the border with Lithuania, and the only difference is that they are not neighbouring ones. This means that the location is not the key influencing factor. The segment of services was specific to the majority of market sector statistical units in the municipalities, and it was the dominant segment in 14 municipalities. It is useful to remember that retaining rural vitality also involves meeting the needs of residents for various services, which contributes to maintaining population in the rural space.



Source: the authors' calculations based on LURSOFT data

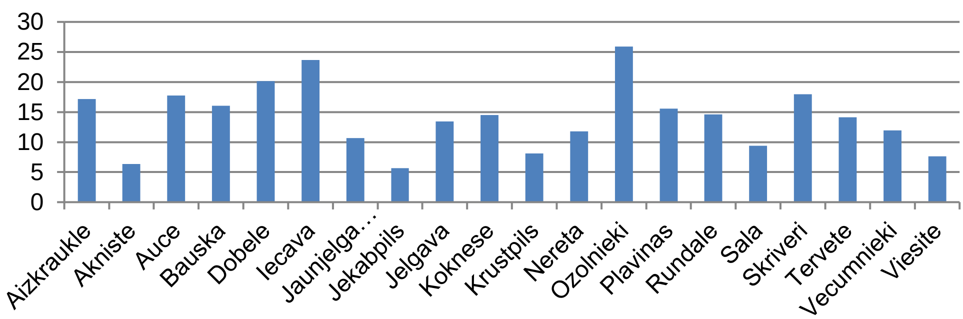
Figure 1. Vertical growth of entrepreneurship in the municipalities of Zemgale region in the period of 2009–2015.



Source: the authors' calculations based on LURSOFT data

Figure 2. Percentage of segments of entrepreneurship in the municipalities of Zemgale region in 2015.

The knowledge-based segment could be singled out from the list of registered enterprises in the LURSOFT database to assess its position and role in the economy of a municipality. The share of the knowledge-based segment ranged from 5.65% (Jekabpils municipality) to 25.9% (Ozolnieki municipality). A share of more than 20.0% was observed in three municipalities, the share in the range of 15.1–20.0% was in five municipalities, the share in the range of 10.0–15.0% was in seven municipalities and the share was less than 10.0% in five municipalities (Fig. 3). Each municipality provided such knowledge-intensive services as educational, health, cultural and sport services. Another point is that recording HT and MHT manufacturing is not easy. Entrepreneurs have to identify niche products that will be competitive in the international market, since domestic demand for innovative products is very insignificant. The research results reveals the complicated nature of this process. In seven municipalities, a component of this segment existed already before 2009, and it remained during the entire period of analysis. In four municipalities, HT and MHT enterprises started operating in the period of analysis, which means that a segment has emerged that has been able to survive. In two municipalities, there were activities aimed at establishing this component of the segment, yet stability lacked there (it vanished after it had appeared). Finally, there were seven municipalities where only knowledge-based services were provided. This means that the establishment of a knowledge-based segment in the economy of a municipality requires both the understanding of the need for such a segment and competence in forming the segment practically, and it particularly relates to the foundation and maintenance of HT and MHT enterprises, as well as the assessment of their performance.



Source: the authors' calculations based on LURSOFT data

Figure 3. Percentage of the knowledge-based economic segment in the economy of Zemgale region municipalities in 2015.

Prerequisites for the successful entry of the KBE segment are the availability of both tangible and intangible capital. Intangible capital, more generally, knowledge capital, should be an important driver of modern economic growth (Corrado et al., 2006). The category of 'positive local development' was introduced to avoid depopulation (Bacon & Brewin, 2008). An analysis of the indicators of Zemgale region's municipalities showed that depopulation did not directly correlate with a decline in economic activity; the situation was just opposite. The population in a municipality decreased, while economic activity in it increased. It was observed in most of the region's municipalities (Table 4).

Table 4. Comparison of changes in the population and the number of market sector's statistical units per 1,000 capita in the period of2009–2015 (in %-points)

1. Significant increase in the population Ozolnieki (+3.9) Significant increase in economically active statistical units Bauska (+88.5), Iecava (+107.69), Jaunjelgava (+74.36), Nereta (+65.12), Ozolnieki (+64.3), Plavinas (+76.5), Rundale (+114.3), Skriveri (+116.13), Vecumnieki (+117.2)	**2.** Above-average decrease in the population Iecava (-4.5) Above-average increase in economically active statistical units Aizkraukle (+40.4), Auce (+47.4), Dobele (+42.2), Jekabpils (+11.7), Jelgava (+29.5), Koknese (+47.8), Tervete (+40.4), Viesite (+32.8)
3. Below-average decrease in the population Aizkraukle (-9.56), Akniste (-8.85), Bauska (-8.48), Dobele (-8.0), Jaunjelgava (-6.3), Jelgava (-7.6), Koknese (-5.5), Krustpils (-6.6), Rundale (-9.5), Sala (-8.8), Skriveri (-7.4), Vecumnieki (-8.4) Below-average increase in economically active statistical units Krustpils (+10.8), Salas (+1.26)	**4.** Significant decrease in the population Auce (-11.6), Jekabpils (-11.7), Nereta (-10.1), Plavinas (-10.7), Tervete (-10.4), Viesite (-10.6) Insignificant increase in economically active statistical units Akniste (-2.9)

Source: the authors' calculations based on LURSOFT data.

The survey of experts representing the regions focused on the skills of local governments to perform not only administrative functions but also actively implement the role of a leader of a community mobilising residents for the multifaceted enhancement of their common life space. Smart growth is possible only if local residents are ready for change in their economic and social life and in the surrounding environment (Rivza et al., 2016). Consequently, there is a need to perform a further in-depth examination of the entire range and variations of local government activities done to maximally contribute to the viability of the local space engaging residents in the formation of the smart territory. The public has to accept the truth that the 21st century is a period of fast change, besides, it equally refers to both urban and rural territories (Kruzmetra, M., 2015).

CONCLUSIONS

1. Upward trends in economic processes were observed in Latvia on the whole in the period of the research. The growth of entrepreneurship took place both in the cities of national significance and in rural areas consisting of 110 municipalities; besides, the growth was faster in the rural areas than in the cities. This is, of course, a positive trend. The knowledge-based economic segment grew faster than the total economy did. If taking into consideration the drop of the Global Competitiveness Index for Latvia and the fact that the country lagged behind the other Baltic States, the growth pace has to be regarded as insufficient.

2. At a regional level, economic growth in the national economy as a whole was observed in all the regions, and the regional growth was higher than that in the cities. However, the growth trends began to differ. Higher growth rates both in the total economy and in the knowledge-based economic segment in terms of numbers of enterprises and employees were reported in Pieriga region, which were higher than those

in the cities. Knowledge-based services contributed to this trend in the region, as the proportion of the services in the KBE segment in Pieriga region was the highest among the regions. However, the comparison of increases in the net turnover per employee in the total economy and in the knowledge-based economic segment in the regions revealed that the highest increase was reported in Kurzeme region, which makes us consider that the new economic pattern in this part of Latvia yields higher returns. This implies that when promoting an increase in the knowledge-based economic segment, the focus has to be placed on quality instead of quantity.

3. The research clearly showed that an analysis of progress in smart growth and the viability of rural space at a regional level does not yet provide real implementation of the local approach strategy, as municipalities within a region differed in a number of essential indicators. First, there were differences in the proportion of economic segments among manufacturing or agriculture and forestry, as the segment of services dominated in any municipality. Second, there were internal differences in the KBE segment, which was represented only by knowledge-based services or by both the mentioned services and products produced by HT and MHT enterprises that made a greater financial contribution than service providers. Municipalities currently focus on knowledge-intensive services, less focus is placed on high-tech and medium-high-tech manufacturing industries. A logical question arises – how to solve this problem.

4. The research findings made during the present research make the authors focus on the effects of intangible capital in relation to the vitality and viability of rural areas in their future research in order to make progress towards the formation of a smart space, as communities build capacity for smart growth with the public, private and non-profit sectors.

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Work strain predictors in construction work

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Abstract. The aim of this study was to predict the work strain indicators for construction workers and to work out measures for prevention of strain at work. Subjective and objective research methods were utilized in the research, including survey, work intensity measurements (Borg Scale), work strain index, fatigue index and work ability index determination, objective blood pressure and pulse measurements. Statistical analysis with significance calculations was carried out. The limitation of this study is the small number of subjects involved in the research. Subjective evaluation of fatigue index and strain index resulted in moderate and somewhat hard work heaviness categories accordingly to Borg Scale. Measurements of heart rate, blood pressure approved work strain of employees of both professions does not exceed the admissible heart rate limit during physical load. The research proved that the strongest work strain predictors were connected with psycho-social risks rather than with physical ones.

Key words: fatigue, work heaviness, auxiliary workers, road workers, heart-rate, blood pressure.

INTRODUCTION

One of the leading branches of national economy in Latvia is construction, in which the greatest number of economically active population is employed. The employed, irrespective of wider and more dynamic introduction of new technologies in a lot of branches of Latvian national economy, incl. construction, are still subjected to increased strain at work. Work related musculoskeletal disorders (MSDs) and injuries are among the most frequently reported causes of lost or restricted work time (Occupational Safety and Health Administration, 2015) in construction, comparing to other branches. There is the highest number of accidents and the employed mostly suffer from musculoskeletal and connective tissue diseases, there have also been lethal cases at work places. Data from literature prove that construction workers' work is characterized by the following strain indicators: physically heavy work, forced work postures in bent or turned aside positions, long working hours, restricted time for task completion, fast speed of work, different microclimate (Kaukiainen et al., 2002; Kalkis et al., 2016). Construction works require a good physical condition from an employee as well as the ability to control diversity of work situations. The restricted time limit and work at increased rate promote physical and mental fatigue (Leino-Arjas et al., 1999; Roja et al., 2016), which in a long-term period affect the development of work related musculoskeletal disorders (WRMSD) (Wiker et al., 1989). Data from literature reveals that WRMSD and other health problems are related with employees' age, and that older employees suffer more often from these disorders than younger ones (Higgset et al., 1993, Okunribido & Wynn, 2010). The cases have been described when construction workers, lifting and moving heavy loads, quite often exceed the admissible lifting limit, which, without doubt, affects employees' health and work ability (Koningsveld & Molen, 1997; Van den Berg et al., 2008). Strain can also result in changes of arterial blood pressure and heart rate in employees. According to research of European and American scientists dynamic exercise of high intensity in normal conditions can cause the maximum value of systolic blood pressure to increase up to 250 mm/Hg and that of diastolic pressure up to 110 mm/Hg (Astrand, 1960).

The aim of this study was to predict the work strain indicators for construction workers and to work out measures for prevention of strain at work. The study was approved by the Human Ethics and Institutional Review Board of Riga Stradiņš University, Latvia in 2015.

MATERIALS AND METHODS

The study was carried out in a medium size construction enterprise in Latvia. It involved 15 auxiliary workers, employed in construction of buildings, and 5 workers, employed in road construction. All participants were male right-handers. Selection criteria were: all participants were clinically healthy without acute or chronic musculoskeletal or cardiovascular diseases. All the employees had agreed to participate in the study.

Duties of workers employed in building construction include: loading and unloading of building refuse with hands, its pushing, participation in processes of preparation for construction works, etc., which are related with physical effort. In general it is manual work. Road workers are employed in levelling of asphalt or road fractions, which involves hands mainly. Representatives of both studied professions work in forced postures.

The survey of employees was carried out with specially worked out questionnaire in order to find out their opinion on existing work conditions, work strain and factors, affecting workability. The following questions were included in this questionnaire: age, length of service, height, weight, smoking status, MSDs after work, physical activity in the leisure time, supervisor support at the work, colleagues support, requirements for work, work intensity, work ability. Smoking status was determined by the question: 'do you smoke or have you ever smoked?' with the four response alternatives: no, never (0), yes, but not anymore (1), yes, occasionally (2) and yes, every day (3). Musculoskeletal disorders after work in neck, shoulders, back, elbow, hip, knee and foot/ankle were evaluated by assessing pain/discomfort intensity after the work. Pain/discomfort intensity was classified by participants to be no pain/discomfort, mild pain/discomfort, moderate pain/discomfort or severe pain/discomfort.

About leisure-time physical activity the participants reported which of the following activities levels that corresponded best to their own level: inactive (e.g., reading, watching TV, movies); some physical activity (e.g., bicycling, walking,); regular activity (e.g., running, gymnastics).

Borg Scale of Ratings of Perceived Exertion (RPE) was used to measure work intensity (strain) and work heaviness category (WHC) (Borg, 1982). The RPE or Borg Scale measures a performer's rate of perceived exertion – that is, how hard workers think they are working. It is a scale from 6 to 20, where 6 means – no intensity (strain) at all, and 20 is maximum intensity. The RPE measurements were done during the work process, not just after they had stopped the work.

In the studied groups, blood pressure (mm/Hg) and heart rate checking by the pulse (beats per minute, beats min^{-1}) were measured by the device Omron HEM-780. The measurements were performed in the following order: before starting work, during the rest pauses and at the end of the work cycle. Length of the work cycle was 40 minutes (for auxiliary workers – loading building refuse, for road construction workers – levelling of road fractions.) Before measurements the workers had a 5-minute rest. Heart rate by checking the pulse, was evaluated at rest after 5-minute break, workers being in a quiet room in comfort temperature.

Work strain was evaluated applying a special computer program ErgoIntelligenceTM Upper Extremity Assessment (UEA) (NexGen Ergonomics Inc., 2015). The Strain Index (SI) is a score value based on a multiple of six variables: intensity of exertion, duration of exertion, efforts per minute, hand/wrist posture, rate of performing the work and duration of task. The variables and score in the SI are derived from physiological, biomechanical, and epidemiological principles. Moore & Garg (1995) conducted a study in the poultry industry using the SI and found that with the increase of the SI score the mean incidence rate for distal upper extremity disorders also increased. Based on these findings, they recommended a cut-off score of 7 for the SI, as an identification criterion to determine high-risk jobs.

Degree of fatigue. In order to determine tiredness of the employees, the fatigue index (FI) was calculated and computer programme HSE Fatigue Index was utilized, which according to fatigue index determines degree of tiredness: 0...20 – low, 21...40 – medium, 41...60 – high, 61...80 – very high, 81...100 – extremely high (see the Table 1) (Calculator of fatigue index, 2015).

Table 1. Fatigue index (FI) value scale points and fatigue level

FI	Degree of tiredness
0...20	Low
21...40	Medium
41...60	High
61...80	Very high
81...100	Extremely high

Work ability was evaluated calculating Work ability index (WAI) (Ilmarinen J., 2007).

The WAI is an instrument used in research to assess work ability during workplace surveys. The index is determined basing on the answers to a series of questions which take into consideration the demands of work, the worker's health status and resources. WAI is a summary measure of seven items (range 7–49) (Table 2).

Table 2. Items of the Work Ability Index

	Items	Range
1	Current work ability compared with the lifetime best	0–10
2	Work ability in relation to the demands of the job	2–10
3	Number of current diseases diagnosed by a physician	1–7
4	Estimated work impairment due to diseases	1–6
5	Sick leave during the past year (12 months)	1–5
6	Own prognosis of work ability 2 years from now	1–7
7	Mental resources	1–4

Statistical analysis. The results acquired were entered into the computer and processed using *MS Excel* software and statistical data processing program *SPSS.20.0* according to popular descriptive statistical methods, including statistical significance calculations with ANOVA and Student t-test ($p < 0.05$). Reliability interval (inter-rater agreement) was also calculated determining Cohen's Kappa coefficient (k) (Landis & Koch, 1977). This coefficient identifies connectivity of the experimental data, the number of participants and the proportion or correlation of the participants' acceptance of the experimental data:

$$k = (P_O - P_C) / (1 - P_C), \qquad (1)$$

where P_O – correspondence proportion of objective experimental data with respondents' responses ('yes' or 'no'), P_C – correspondence proportion of data with number of participants ($P_C = \Sigma p_i^2$, where p_i is acceptance of each participant expressed in percent or as fractional number).

RESULTS AND DISCUSSION

The results reveal that workers from building construction fall in different age groups: 46.6% – age group from 18–30, 20.0% – 40 to 50, 33.3% – 60 to 72 years of age. Length of service in the profession: 53.3% – from 0 to 5 years, 26.6% – from 6 to 10 years and 20.0% – more than 11 years. It should be noted that older employees in the studied enterprise had worked longer than 11 years. Road construction workers were at the age of 18 to 65 years, length of service in the profession of three older workers was from 14–16 years. Both categories of workers had increased BMI. Heart rate by checking the pulse during the rest period was in norm. The background factors with mean differences in analysed groups with statistical significance of the research group and analysed aspects are shown in Table 3.

Obtained data and analysis of mean differences with t-test indicate that there are no statistically significant differences between the analysed groups (auxiliary construction workers and road workers).

Education: 11 construction workers had primary school education, 7 – secondary education, 2 – secondary education specialized in construction. All the studied employees smoked. Participants don't have physical activity in the leisure time.

Work conditions were evaluated by the respondents as follows: representatives of both studied professions considered their work as very intensive, at increased rate. The work is dynamic, it involves mainly hands. The respondents – road workers (90%) mention also load on the lower back, as the body takes bent posture during the work, but

80% of auxiliary workers note additional load on shoulder girdle. All participants indicated that the job requires high demands, lack of the management support and low control of the work process.

Table 3. Background factors of the research groups: mean age and range, length of service, mean height, mean weight, mean body mass index (BMI), heart rate at rest (beats min^{-1}), standard deviation (± SD), t – test statistics and *p* – probability

Profession	n	Age (years)	Range of age (years)	Length of service (years)	Height, cm	Weight, kg	BMI, kg m-2	Resting heart rate (beats min^{-1})
Auxiliary construction workers	15	41.9 ± 20.9	18–72	7.1 ± 5.1	170.3 ± 0.5	76.0 ± 10.3	26.2 ± 2.9	68 ± 8
Road workers	5	42.2 ± 19.5	18–65	9.8 ± 7.2	172.8 ± 0.8	79.6 ± 7.4	26.8 ± 3.1	64 ± 7
Mean difference		-0.333		-2.733	-2.53	-3.60	-0.582	
t		-0.031		0.942	-0.817	-0.715	-0.376	
p		0.975		0.358	0.425	0.484	0.711	

Applying Borg Scale in order to determine work strain it was found out that 80% of older auxiliary workers in building construction recognized that their work corresponds to very heavy work category (17–18 points), but 20% – heavy (15–16 points). At the same time all the road workers recognized their work as very, very heavy (19–20 points). It allows us to consider that the employees, involved in the study work with intensive work load and increased strain. To ascertain the correspondence of the employees' subjective opinion with the performed work, measurements of heart rate by counting the pulse were done. Results are revealed in Tables 4 and 5.

Table 4. Results of the measurements of heart rate by counting the pulse (beats min^{-1}), blood pressure (mm/Hg) (systolic – Sys, diastolic – Dias, mm/Hg) for road workers, mean values, standard deviation (± SD), standard error of the mean (± SEM), statistical significance – *p* (t-test)

Indicator	Age, years	Before the work			During the rest			After the work		
		Sys, mm/Hg	Dias, mm/Hg	Pulse rate, beats min^{-1}	Sys, mm/Hg	Dias, mm/Hg	Pulse rate, beats min^{-1}	Sys, mm/Hg	Dias, mm/Hg	Pulse rate, beats min^{-1}
	18	110	70	70	122	72	68	140	96	76
	26	112	74	72	130	70	74	142	90	80
	51	140	90	80	150	94	80	160	98	84
	65	138	80	62	140	78	74	150	80	78
	51	130	70	82	138	74	80	148	100	86
Ave-rage	42.20	126.00	76.80	73.20	136.00	77.60	75.20	148.00	92.80	80.80
± SD	19.51	14.21	8.44	8.07	10.58	9.63	5.02	7.87	8.07	4.15
± SEM		6.36	3.77	3.61	4.73	4.31	2.24	3.52	3.61	1.85
t-value		19.82	20.35	20.27	28.34	18.01	33.50	42.03	25.70	43.56
p		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table 5. Results of the measurements of heart rate by counting the pulse (beats min^{-1}), blood pressure (systolic – sys, diastolic – dias, mm/Hg) for auxiliary workers in building construction, mean values, standard deviation (SD), statistical significance *(p)* (ANOVA)

		Before the work			During the rest			After the work		
Age group	Age, years	Sys, mm/Hg	Dias, mm/Hg	Pulse rate, beats min^{-1}	Sys, mm/Hg	Dias, mm/Hg	Pulse rate, beats min^{-1}	Sys, mm/Hg	Dias, mm/Hg	Pulse rate, beats min^{-1}
	18	120	90	62	122	90	66	130	86	70
	19	120	80	78	128	86	82	135	90	86
	20	110	90	90	116	80	94	130	90	94
	20	110	70	80	115	78	84	128	80	88
	22	130	80	82	138	90	84	144	92	90
Mean	19.80	118.00	82.00	78.40	123.80	84.80	82.00	133.40	87.60	85.60
± SD	1.48	8.37	8.37	10.24	9.50	5.59	10.10	6.47	4.77	9.21
	27	130	90	70	140	92	74	156	94	78
	28	140	90	76	142	90	78	148	96	82
	40	120	82	56	130	80	60	136	82	66
	50	136	72	60	138	74	62	146	92	68
	50	148	90	80	150	86	82	156	90	88
Mean	39.00	134.80	84.80	68.40	140.00	84.40	71.20	148.40	90.80	76.40
± SD	11.27	10.55	7.95	10.24	7.21	7.40	9.76	8.29	5.40	9.32
	62	138	88	78	140	90	80	154	92	86
	65	130	80	80	148	84	82	150	88	84
	65	140	80	82	144	88	84	150	90	86
	70	140	90	70	144	92	76	152	98	80
	72	136	90	74	140	90	78	152	92	82
mean	66.80	136.80	85.60	76.80	143.20	88.80	80.00	151.60	92.00	83.60
± SD	4.09	4.15	5.18	4.82	3.35	3.03	3.16	1.67	3.74	2.61
ANOVA F-value	8.060	8.443	0.335	1.859	10.578	0.933	2.390	12.489	0.450	1.069
ANOVA p	0.06	0.05	0.722	0.198	0.02	0.420	0.134	0.01	0.648	0.162

Results of the measurements of heart rate by counting the pulse (beats min^{-1}), blood preassure (mm/Hg) (systolic – sys, diastolic – dias) for road workers does not show statistically significant differences by all analysed aspects for all road workers – confirmed by t-test ($p \leq 0.001$).

Analysis of results by all indicated age groups by ANOVA has indicated that there are no significant differences of the measurements of heart rate by counting the pulse (beats min^{-1}), blood pressure (mm/Hg) (before the work systolic – sys) with significance level 0.05; for the measurements of heart rate by counting the pulse (beats min^{-1}), blood pressure (mm/Hg) (during the rest systolic – sys) with significance level 0.02 and the measurements of heart rate by counting the pulse (beats min^{-1}), blood pressure (mm/Hg) (after the work systolic – sys) with significance level $p \leq 0.01$. For all other analysed aspects differences by age groups are statistically significant.

The average blood pressure measurements reveal that during intensive work systolic and diastolic pressure increases in older participants of both professions, and the mean values are: in building construction auxiliary workers 151.60 ± 1.67 and 92 ± 3.74, but in the oldest road construction worker 150.00 ± 1.54 and 91.78 ± 8.11. Analysing heart rate according to pulse it can be concluded that work strain of employees of both professions does not exceed the admissible heart rate limit during physical load, which shows that the load is appropriate for employees' age. For older (age group 51–75 years) building construction auxiliary workers, according to RPE evaluation, maximum Heart Rate should be 170 or 180 beats per minute, but actually performing work duties intensively for 40 minutes, the average heart rate was 83.60 ± 2.61, but the calculated admissible limit is from 101.40 to 116.9 beats min^{-1}, respectively. In road construction workers of different age heart rate also does not exceed the admissible limit: in 18 years old from 121.12 to 161.6, but in the older (65 years) from 93.0 to 124.0 beats min^{-1}.

The research results can be compared with other authors' investigations on the assessment of blood pressure. In several investigations it is pointed that systolic blood pressure increases significantly and proportionally to workload during exercise test in healthy adults (Wielemborek-Musial et al., 2016). Heart rate results acquired in our study do not correspond to above mentioned authors' results, since within the assessment of heart rate it was found that physical load in the studied employees, in fact, did not increase regardless their subjective evaluation. The present study clearly demonstrated that work stress is closely related to blood pressure *ie* blood pressure was higher in individuals reporting high job strain in combination of high job demand and low job control (Karasek et al., 1981). That is in accordance with our research survey results on job requirements and lack of management support.

To assess employees' work strain more profoundly, work strain index and fatigue index were determined. Calculation results are shown in Table 6.

Analysing the acquired results, it should be concluded that for building construction auxiliary workers SI = 4.9 ± 1.6 and it is higher than that for road construction workers (SI 4.2 ± 1.6), which could be related with different stress situations (heavy work load, increased requirements at work, lack of support from colleagues, restricted time limits, etc.). The same refers to fatigue index, for building construction auxiliary workers it is higher (37.5 ± 4.1) than that for road construction workers (31.2 ± 3.3). It could be explained by the fact that road construction workers have regulated rest pauses, whereas work of building construction auxiliary workers proceeds at increased rate and restricted time limits.

Table 6. Mean values of work strain index (SI) and fatigue index (FI), standard deviation (± SD) and Cohen's kappa (κ), the rate of perceived exertion (RPE, scale 6–20), work heaviness category (WHC)

Professions	Mean RPE ± SD	WHC	SI ± SN	κ	FI ± SD	κ
Road construction workers (n = 5)	14 ± 2	Moderate	4.2 ± 1.3	0.81	31.2 ± 3.3	0.81
Building construction auxiliary workers (n = 15)	16 ± 2	Somewhat hard	4.9 ± 1.6	0.85	37.5 ± 4.1	0.80

It should be noted that evaluating work strain and fatigue in the studied groups, their subjective evaluation corresponds to the calculated evaluation. At the same time the calculated stress index and fatigue index do not exceed admissible levels. It is explained in other findings that the cumulative fatigue in industry would likely to ensue if the heart rate exceeds 110 beats min^{-1} (Brouha, 1967).

Workability evaluation is shown in Table 7.

Table 7. Workability index and criteria (n = 20)

Workability index (WAI)	Scores	Rating scores ± SD
Road construction workers (n = 5)	7…49	40 ± 2.91
Building construction auxiliary workers (n = 15)	7…49	35 ± 3.32

Analysis of workability revealed the following results: building construction auxiliary workers evaluate their workability as moderate (WAI = 35 ± 3.32), but road construction workers – as good (WAI = 40 ± 2.91).

Analysing workability of the studied employees it should be concluded that though, in the survey, the employees noted increased work strain and physically heavy work, their workability is medium and good. In older employees it was even better than in younger ones, and 64% of older employees considered their workability as very good. Apart from that they did not mention any case of illness within recent years. This corresponds with other authors 'research conclusions that physical load at work is related to the age of employees rather than to employees' workability and subjective health evaluation, incl. musculoskeletal pain (Lunde et al., 2016.). All questioned employees consider that they will be able to work in future as well. 36% of younger building construction auxiliary workers noted that they were not sure of being able to work in future relating it with physically heavy work and excessive work load. The 'good' workability indices could be explained by big unemployment in Latvia and fear of work loss due to what employees do not give the true information.

The limitation of this study is the small number of subjects involved in the research (road construction workers n = 5, building construction auxiliary workers n = 15). Such small investigation group can increase the chance of assuming as true a false premise (Faber & Fonseca, 2014), but at the same time such research is quick to conduct with regard to enrolling subjects, reviewing subject records, performing analyses or asking subjects to complete study survey (Hackshaw, 2008).

CONCLUSIONS

The research proved that the strongest work strain predictors were associated with psychosocial risks rather than with physical ones. Survey results concludes that all employees in our research are subjected not only to physical, but also to psychosocial risk factors indicating high job demand, lack of management support, restricted control of the work process. The calculated BMI can be related to unhealthy life style of employees. Borg Scale results allowed to consider that older auxiliary workers and all road construction workers have highest work categories. Despite this fact, work strain index and fatigue index resulted in higher levels for building construction auxiliary workers than that for road construction workers. That can be explained with more regulated rest breaks and lower work pace. Objective measurements of heart rate, blood pressure approved work strain of employees of both professions does not exceed the admissible heart rate limit during physical load, which shows that the load is appropriate for employees' age. Statistical analysis with ANOVA and t-test showed that there are no significant differences of the measurements of heart rate and blood pressure in all indicated age groups, but the results can be explained with the limitation of this study that included small number of subjects. Both study groups in our research indicated moderate and good workability that was proved with WAI determination, but such results can be linked with false information from employees due to fear of work loss.

According to this study some management interventions in construction work organisation with effective management tools can significantly help to cope with Work strain predictors in the workplaces. Therefore, the research will be continued paying more attention to studies of psycho-social risks for auxiliary workers and road construction workers. Possibilities to improve work organisation will be studied.

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Results of fifteen-year monitoring of winter oilseed rape (*Brassica napus L.*) production in selected farm businesses of the Czech Republic from the viewpoint of technological and economic parameters

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Abstract. The paper presents field trials focused on technological and economic comparison of conventional tillage (CT) and reduced tillage (RT) technologies of soil cultivation and drilling of winter oilseed rape (*Brassica napus L.*). During fifteen production years starting in 2001/02, trials were set up in 520 fields of around 40 farm businesses located in all of the districts of the Czech Republic. With respect to average seed yields, no significant differences were proved with respect to tillage systems, to the application of organic fertilizers and to the fertilization during sowing. Irregular distribution of trial fields into the individual production areas influenced the outcomes thou. Concerning winter rape seed yields, costs per production unit, and earnings per hectare, the most suitable production area proved still to be the potatoes one, but particularly over the recent period also beet production area. The corn production area produced, despite some exceptions, worst results. Over the fifteen-year time, the average oilseed rape yield of all 520 monitored fields was 3.72 t ha^{-1}. Reduced tillage attained average yield of 3.73 t ha^{-1}, i.e. matched almost exactly the one of 3.70 t ha^{-1} attained by conventional tillage. Unit production costs realized by conventional tillage surpassed by 4.1% those gained by reduced tillage. Related earnings per hectare were on the other hand lower by 17.0%. With respect to fuel and labour consumption, reduced tillage brought significant savings reaching in average 20.2%, respectively 24.0%. In terms of yields, reduced tillage with deeper soil loosening proved repeatedly favourable results.

Key words: Brassica napus L., tillage system, ploughing, costs, fuel consumption, labour consumption.

INTRODUCTION

Over the recent decades, various soil tillage systems have emerged alternative to conventional tillage (CT) comprising ploughing. These systems, i.e. reduced tillage (RT) or conservation tillage, generally do not invert soil and leave significant portion of crop residue on the soil surface (at least 30% to be entitled to naming ‘conservation tillage’). Reduced tillage is primarily used as a means to conserve soil moisture, to reduce production costs and to protect soils from erosion and compaction (Holland, 2004). Soil erosion is, also in Europe, a major environmental problem. According to Verheijen et al. (2009), soil erosion rates for tilled, arable land in Europe are, on average, 3 to 40 times greater than the upper limit of tolerable soil erosion. For conditions prevalent in Europe, this limit is, as equal to soil formation, ca. 1.4 t ha^{-1} yr^{-1}. In the intensive agricultural systems generally used in Europe, the effects of erosion on crop yields mainly occur due to the reduction of the amount of water the soil can store and make available to plants. As long as soil depth is sufficient, yield losses may be minor, as the nutrient losses due to erosion can be compensated for by the raised doses of fertilizers (Bakker et al., 2004, 2007). According to many authors (Holland, 2004; Lahmar, 2010; Wauters et al., 2010), the implementation of conservation agriculture and conservation tillage is clearly lagging in Europe in comparison to other continents. According to the results of the study on a soil loss done by Kisić et al. (2016), RT and tillage across the slope are recommended as tillage which preserves soil. The results of Novák et al. (2016) confirmed the importance of soil conservation technologies in reduction of risk of land degradation by water erosion. Another research (Kroulík et al., 2009) focused on compaction and field traffic intensity suggested that 145.6% of covered area can be run-over repeatedly for conventional tillage, 44.8% for minimum tillage and 18.4% only for direct seeding.

There has been considerable research on the effects of conservation tillage on crop yield in many areas in Europe over the last three decades. Often, detailed reports were published both on the economic and environmental effects of conservation agriculture (e.g. Lopez & Arrue, 1997; Tebrügge & During, 1999; Hocking et al., 2003; Kisić et al., 2010; Răus et al., 2016). However, the suggestions from different studies often seem contradictory and are therefore difficult to interpret (e.g. Cantero-Martinez et al., 2003; Lopez & Arrue, 1997). This is to be expected: both the agro-environmental conditions as well as the form of reduced tillage applied vary seriously between individual studies. The recent study of Madarász et al. (2016) however suggested that over the ten trial years, tillage type was a more important factor in the question of yields than the highly variable climate of the studied years. During the first three years of technological changeover to RT, a decrease of 8.7% was measured, respective to CT. However, the next seven years brought a 12.7% increase of RT yields of all the crops grown.

According to the analysis of 563 observations carried out by van den Putte et al. (2010), no significant yield effect of soil tillage practices was observed for potatoes, sugar beet, spring cereals and fodder maize. A significant yield reduction occurred under conservation agriculture only for grain maize and winter cereals.

Soil tillage systems must be adapted to plant requirements in accordance with crop rotation and to the pedoclimatic conditions of the area (Răus et al., 2016). In the conditions of the Czech Republic and also at large, the most suitable conditions for tillage intensity and depth reduction are in drier conditions of maize and beet production regions on medium-textured soils with higher natural fertility (Procházková & Dovrtěl, 2000; Horák et al., 2007). According to Šařec et al. (2010), RT brings the highest advantage on heavier soils in drier and warmer climatic regions. There, soil environment frequently even impede quality stand establishment using conventional soil cultivation technology including ploughing. In such case, RT is practically the only way of stand establishment. According to Hůla et al. (2008), replacing ploughing with a shallow soil loosening followed by sowing using no-till drills is a suitable alternative. Bednář et al. (2013) suggested an increase in between-the rows spacing (to 37.5 cm), and a decrease in sowings and the number of plants per m^2 (35 and fewer) both of which have a positive influence on the decrease of competition among individual oilseed rape plants.

Moreover, deeper soil loosening was proposed in order to ensure the disruption of compacted layers, and to ensure the balance of water regimen in soil profile.

A comparison of the different components of the total costs revealed that reduced-tillage required herbicide costs and larger machinery, but these costs were largely offset by reduced operating costs (Sanchez-Giron et al., 2004; 2007). In various other studies, it was concluded that slightly lower crop yields can be offset by the reduced fuel inputs and labour consumption (Gemtos et al., 1998; Bonciarelli & Archetti, 2000; Tebrügge, 2000). The advantage should be given to systems with lower level of tillage intensity, not only to reduce costs but also because of the possibility of simpler production organization due to less machine and labour requirement (Grubor et al., 2015). However, this may be dependent on particular situation and farm-specific properties such as cropping system, farm size etc. (Sanchez-Giron et al., 2007).

The purpose of this study was to evaluate conventional tillage (CT) and reduced tillage (RT) systems of winter oilseed rape production mainly in terms of yields, costs, labour and fuel consumption in the farming conditions of the Czech Republic. The evaluation was carried out by means of long-term operational monitoring of around 40 agricultural businesses that started in 2001. The monitoring followed field trials established by the authors in Opařany in 1998 (Šařec et al., 2002).

MATERIALS AND METHODS

Since the production year 2001/02, operational monitoring and measurements were carried out in the Czech Republic where around 40 agricultural businesses growing winter oilseed rape participated in. The businesses were selected in order to represent various production areas, i.e. pedoclimatic conditions, and different production technologies. According to the production system used, observations were sorted into one of the two key groups, i.e. conventional tillage (CT) and reduced tillage (RT) group. Other sorting criteria, besides production year, were:

- production area: forage, potato, cereal, beet, maize;
- winter oilseed rape variety: conventional, hybrid, mixed (both types of varieties used in a field);
- application of organic fertilizers (manure, slurry, compost, sugar cane boiling residues etc.);
- application of fertilizers at sowing.

Each production year, at least one field was examined in a particular business. If a business employed different tillage systems simultaneously, more fields representing those systems were observed. Especially the following values were monitored or measured:

- characteristics of individual fields: size, system of soil tillage and stand establishment, previous crop, manner of crop residue management, year of previous application of farmyard manure;
- characteristics of soil: bulk density (Kopecky's cylinders of a volume of 100 cm^3), gravimetric moisture, cone index (registered penetrometer PEN 70 developed at the CULS Prague);

- characteristics of crop stand: the number of plants per m^2, the weight of roots, hybrid / conventional variety, yield;
- data on conducted filed operations: machinery used, fuel and labour consumption, material applied and its rate, costs and other supplementary information.

The measurements concerning relevant soil and stand characteristics were completed by the authors in early spring each year. After the completion of terrain experiments, evaluation of monitored data followed each year. The authors processed relevant production records of plant cultivation specialists and work records of machinery operators at each agricultural business. Machinery costs were calculated in a common way and consisted of ownership (depreciation, financing costs, insurance and taxes, housing) and operation costs (repair and maintenance costs, fuel and oil costs, labour costs). With every business, the amount of expenses spent, i.e. machinery and material costs, was evaluated compared to the achieved seed yield, respectively revenues. Earnings from one hectare were calculated as total costs deducted from revenues, i.e. average annual farm price one ton of oilseeds multiplied by seed yield. Costs related to agricultural land were not included. The results were assessed using the sorting criteria mentioned above, and allowed thus to draw conclusions with a subsequent proposal for a suitable technology of effective winter oilseed rape production in particular conditions.

RESULTS AND DISCUSSION

During fifteen production years starting in 2001/02, trials were established in 531 fields located in all of the regions of the Czech Republic, but 11 fields were due to drought over the period of oilseed rape sowing or due to adverse winter climatic conditions sowed with another crop in spring. Reduced tillage (RT) system of oilseed rape production was employed in 290 cases, conventional tillage (CT) in 230 cases only. This imbalance developed over the monitored period, when some of the farm businesses swapped their system from CT to RT.

Fig. 1 documents the overall weather conditions in the Czech Republic over the period of the experiment along with the long-term norms. During some of the production years, weather development was more favourable at highlands in terms of rape growth and yields, during others on the contrary in lower placed regions. In some years, e.g. 2008 and 2009, crop stands may have been also damaged by hail storms. Concerning oilseed rape stand establishment, substantial difficulties due to droughts in autumn occurred particularly in the year 2015, and to some extent also in 2003 and 2008. Major dry frost came during winter of 2002/03, and some of the stands had to be ploughed down in spring. Generally, winters have become milder lately, and consequently springs have been often coming earlier. An extreme weather occurred principally during the year 2015, when spring and summer were exceptionally both hot and dry. It did not reduce rape yields substantially in 2015, but lowered the level of ground water to such extent that it influenced the yields of the year 2016 (Fig. 3). Since RT generally manage better soil moisture in drier conditions, it reached higher rape yields mostly in the latter mentioned year.

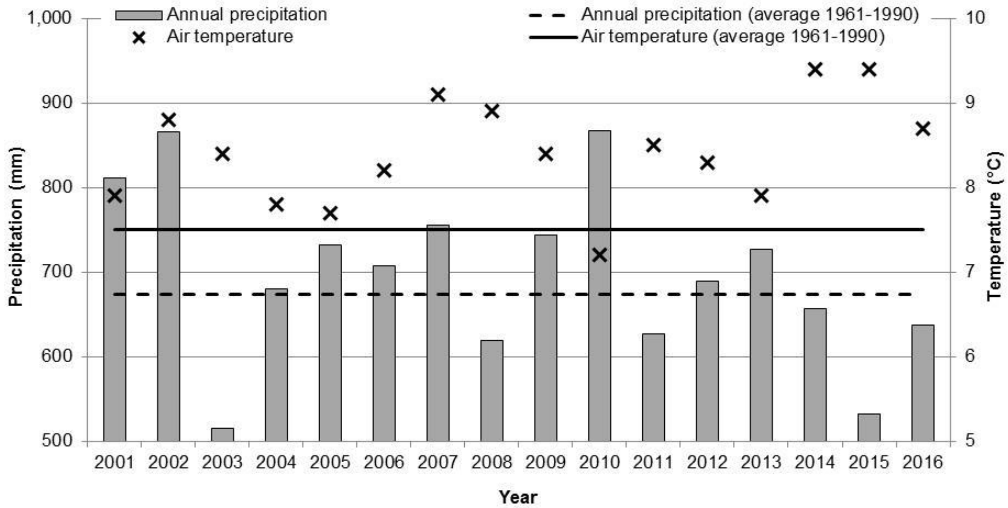

Figure 1. Graph of annual precipitation and mean temperatures in the Czech Republic in the years 2001–2016 and the long-term norms.

Oilseed rape production system characteristics

RT prevailed on heavy-textured soils in arid regions, i.e. in maize production area and in most of the beet production area. CT was used predominantly by farms with lighter soils and higher annual precipitation rates that could be found in potato and marginally beet production area. Choice of the tillage system was influenced also by the equipment that a particular agricultural business owned.

The most frequent tillage operations within RT consisted of two soil cultivations, followed in some cases by a seedbed preparation. Within CT, the common tillage procedures consisted of a stubble cultivation followed by ploughing, and a seedbed preparation done once or twice.

Disc cultivators prevailed within CT, whereas within RT, where two stubble cultivations were usual, tine cultivators were common, particularly for the second cultivation. Under RT, deeper (20 cm and more) soil loosening became more frequent in the course of time (Fig. 2).

Prior to oilseed rape sowing, manure was applied mainly in forage and potato production areas (30%, resp. 36%, of the cases), where the production of manure was adequate and potatoes production decreasing. Therefore, manure could be applied prior to oilseed rape. On the other hand in cereal, beet and maize production areas, where manure was applied primarily prior to sugar beet or corn maize, the application prior to oilseed rape displayed lower frequencies (only 16%, 10%, resp. 6% of the cases).

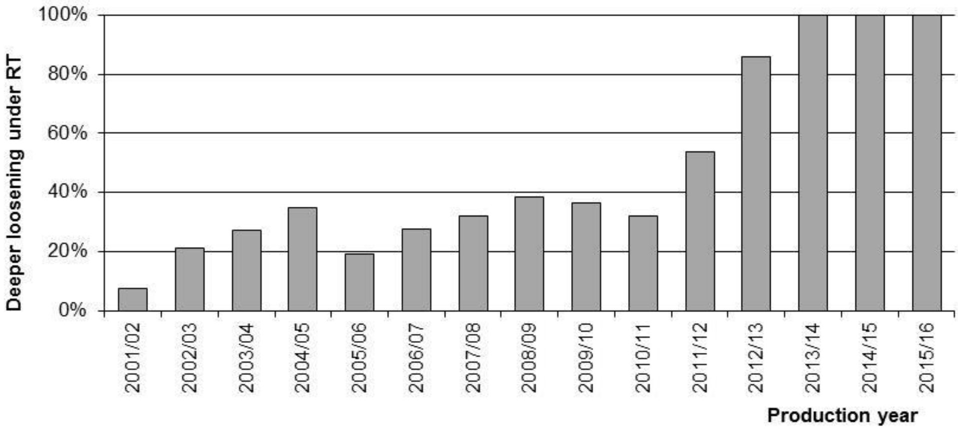

Figure 2. Graph of development of relative frequency of deeper (20 cm and more) soil cultivation employed under reduced tillage (RT) over the monitored period.

Yield results

Over the monitored period of fifteen production years, the average oilseed rape yield from all 520 fields was 3.72 t ha^{-1}. Table 1 shows average seed yields according to several sorting criteria. Average yield attained by CT matched almost exactly the one attained by RT.

Table 1. Average oilseed rape yields and frequencies of cases according to the tillage system and other sorting criteria over the whole monitored period of fifteen years

	Tillage system				Aggregate	
	RT Yield (t ha^{-1})	Frequency	CT Yield (t ha^{-1})	Frequency		
Production area						
Forage	3.45	32	3.62	12	3.49	44
Potato	4.18	16	3.81	67	3.88	83
Cereal	3.56	91	3.50	47	3.54	138
Beet	3.91	135	3.74	104	3.84	239
Maize	3.33	16	-	-	3.33	16
Variety						
Conventional	3.66	119	3.61	86	3.64	205
Hybrid	3.81	169	3.76	136	3.79	305
Mixed	2.33	2	3.58	8	3.33	10
Fertilizers at sowing						
No	3.72	174	3.69	225	3.70	399
Yes	3.76	116	4.07	5	3.77	121
Organic fertilizers						
No	3.70	213	3.69	129	3.70	342
Yes	3.83	77	3.71	101	3.76	178
Aggregate						
	3.73	290	3.70	230	3.72	520

Over the first five years of the monitoring, CT yields generally surpassed RT yields (Fig. 3). But gradually, this trend turned over and RT reached higher yields. One of the reasons might be that farmers got used to the specific requirements and opportunities of RT system and may have improved it over time, e.g. by employing the deeper soil loosening (Fig. 2). Another reason might be that favourable effect of RT was gradual and needed time to evolve.

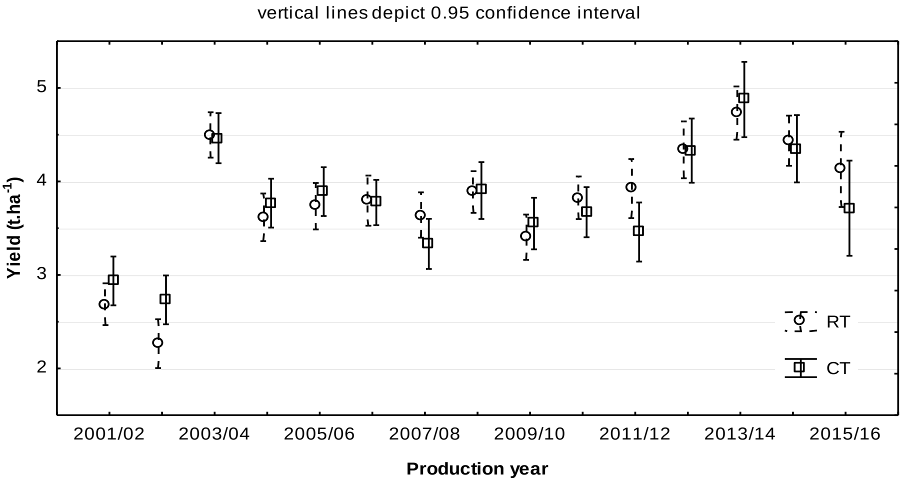

Figure 3. Graph of development of average oilseed yields attained by reduced tillage (RT) and conventional (CT) systems over the monitored period.

Concerning regionalization, potato production area demonstrated the highest average yield, followed by beet production area, while maize production area, where only RT was used, proved inferior results. In all of the production areas except the forage one, average seed yields attained by RT surpassed those produced using CT.

The average yield of more expensive hybrid varieties surpassed by 4.1% the one given by conventional varieties. With fertilizer application during rape sowing, which was mainly the case of RT, the average yield exceeded the yield produced when no fertilizers were applied while sowing by 1.9%. If organic fertilizers were applied, the average yield attained by merely 1.8% higher value. Relatively small frequencies and uneven distribution of cases into individual categories may have influenced the results. For example, in maize production area, RT was the only tillage system employed. Therefore, results of CT were not harmed due to unsuitability of maize production area in terms of winter oilseed rape growing.

Statistical analysis of seed yields showed no significant differences with regard to the tillage system used, to fertilizer application at sowing, and to organic fertilizer application. Oilseed rape variety type (*t*-Test, $n = 508$ – mixed varieties excluded, $p = 0.04583$), and production area (Table 2) were the two sorting criteria where significant differences were demonstrated between the average rape yields. Average yield attained in the cereal production area differed significantly compared to the beet and potato production areas (Table 2).

Table 2. Results of *Turkey HSD* test (homogenous groups) of oilseed rape yields according to production area over the whole monitored period of fifteen years

Production area	Average yield ($t.ha^{-1}$)	1	2
Maize	3.328	****	****
Forage	3.493	****	****
Cereal	3.540		****
Beet	3.837	****	
Potato	3.880	****	

The trials thus correspond only partly with what Madarász et al. (2016) proved, i.e. by 12.6& significantly higher rape yield of conservation compared to ploughing technology over ten-year period. One reason might be the monitoring and operational character of the trials, another one the differences in local climatic and other conditions. The latter reason may be reduced by the following example.

In three cases in the production year 2014/15 and in two cases in 2015/16, an agricultural business employed CT and RT with deeper soil loosening in the same field (Table 3). Pedoclimatic conditions, material and machinery (except some tillage or drilling implements) used did not differ. Table 2 shows repeated by up to 0.5 $t\ ha^{-1}$ higher yields gained by RT with deeper loosening. This outcome complies with the suggestions of Bednář et al. (2013).

Table 3. Seed yields of three agricultural businesses employing simultaneously different tillage systems in the years 2014/15 and 2015/16 (RT always with deeper soil loosening)

Farm/ Area	Tillage system	Stand establishment operations	Yield ($t\ ha^{-1}$) 2014/15	2015/16	Total
	CT	stubble cultivation; ploughing; seedbed preparation; sowing	4.30	4.20	4.25
	RT	stubble cultivation; deeper loosening (Simba); sowing	4.80	4.60	4.70
	CT	stubble cultivation; ploughing; sowing with seedbed preparation (Lemken – power harrow)	4.30	3.60	3.95
		stubble cultivation; deeper loosening (Horsch); sowing (Horsch)	4.50	3.90	4.20
		stubble cultivation; sowing with deeper loosening (Simba)	4.70	4.30	4.50
	CT	stubble cultivation; ploughing; seedbed preparation; sowing	4.10	—	4.10
		stubble cultivation; deeper loosening;sowing	4.37	—	4.37
		stubble cultivation; deeper loosening with fertilizer application to 0.25 m (150 kg PK per ha); sowing	4.60	—	4.60

According to *Dependent (Paired) t*-Test, the difference between yields of RT and CT, i.e. in average 0.41 $t\ ha^{-1}$, resp. 10.1% (Fig. 4), was statistically significant (p = 0.00016).

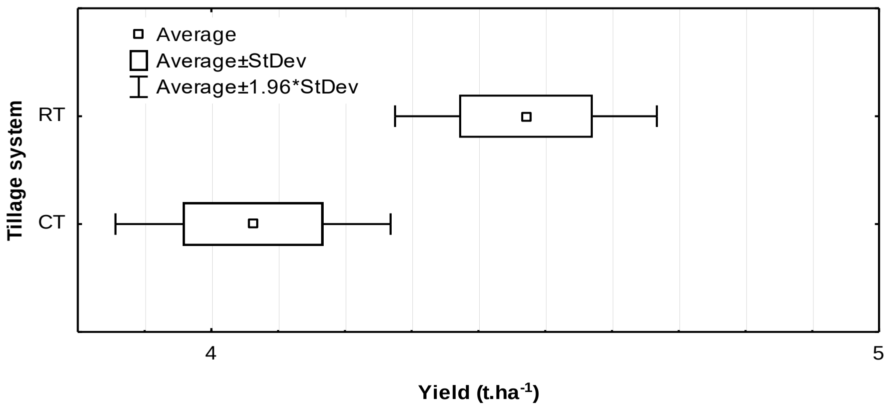

Figure 4. Graph of difference between yields provided by CT and RT with deeper soil loosening at three agricultural business that employed both systems simultaneously in the same field in 2014/15 and 2015/16 (*Dependent (Paired) t*-Test, n = 8, p = 0.00016).

Technological and economic indicators

The following technological and economic indicators were monitored or calculated (Table 4): length of vegetative period, fuel consumption, labour consumption, machinery, material and total costs, unit costs per ton of production and earnings per hectare.

Table 4. Average duration of vegetative period, fuel and labour consumption, averages of individual cost components, average costs per ton of oilseed rape production, and earnings per hectare according to the tillage technology and other criteria over the whole monitored period

	Veget. period (days)	Consumption Fuel ($l\ ha^{-1}$)	Labour ($hrs\ ha^{-1}$)	Average costs Machinery ($CZK\ ha^{-1}$)	Material ($CZK\ ha^{-1}$)	Total ($CZK\ ha^{-1}$)	Unit. ($CZK\ t^{-1}$)	Earnings ($CZK\ ha^{-1}$)
Tillage system								
RT	345	71.75	3.70	6,126.67	12,655.11	18,913.16	5,384.13	12,825.55
CT	343	89.92	4.87	6,922.86	12,794.58	19,880.92	5,616.29	10,959.83
Production area								
Forage	359	90.21	5.40	6,419.52	12,513.23	19,214.57	5,686.99	8,370.13
Potato	350	88.72	4.97	6,949.16	12,570.60	19,699.28	5,200.77	13,788.03
Cereal	348	75.95	3.88	6,213.71	11,441.60	17,737.92	5,333.94	12,328.05
Beet	338	77.70	3.97	6,508.64	13,469.70	20,129.80	5,567.97	12,113.09
Maize	334	68.99	3.63	6,043.58	13,787.19	19,880.77	6,526.41	8,198.60
Variety								
Conv.	345	77.04	3.98	6,300.66	11,687.68	18,131.26	5,342.56	12,011.22
Hybrid	344	81.36	4.37	6,605.73	13,416.39	20,167.04	5,574.63	12,219.73
Mixed	332	87.89	4.57	6,261.10	12,476.24	18,957.34	5,765.54	5,085.19
Fertilizers at sowing								
No	343	82.98	4.45	6,664.56	12,757.95	19,568.13	5,554.00	12,006.75
Yes	348	69.25	3.47	5,866.39	12,581.09	18,592.94	5,265.28	11,979.14
Organic fertilizers								
No	343	70.13	3.58	6,068.47	11,889.06	18,147.88	5,207.44	12,811.79
Yes	347	98.33	5.44	7,267.28	14,307.18	21,634.01	6,023.59	10,441.22
Aggregate								
	344	79.78	4.22	6,478.83	12,716.80	19,341.21	5,486.81	12,000.33

With respect to the tillage system, the average fuel consumption of RT was by 20.2% lower than the one of CT, and the labour consumption lower again by 24.0%. The difference may have been stressed by an uneven distribution of application of organic fertilizer between the groups. If those were used, the fuel consumption would rise in average by 28.2%. As well the total costs were lower with RT, namely by 4.9%. In detail, machinery costs were lower with RT by 11.5%, material costs by mere 1.1%. Together with the slightly higher rape yield, costs per ton of seed produced using RT were by 232 CZK t^{-1}, i.e. by 4.1%, lower than those generated by CT. Related earnings per hectare reached by RT were therefore higher by 17.0%. Mainly thanks to its highest average rape yield, the potato production area demonstrated the lowest unit costs per ton of production and highest earnings per one hectare. Evaluation of the results according to the other criteria, such as organic fertilizer application etc., is only informative due to uneven distribution of cases in individual categories.

The fuel and labour consumption as well as the value of costs were increased by organic fertilizer application. Taking into account similar average yields, the unit cost per ton of seed production exceeded by 15.7% the average of the cases where no organic fertilizers were applied. Other benefits, such as an increase in soil carbon, of organic fertilizer than the immediate influence on yield must be taken into account, but they are difficult to quantify. Average length of vegetative period did not vary much except for production areas. It was slightly longer in production areas located at higher altitudes.

With respect to the costs per unit of production (Fig. 5), the best results were reached in potato production area with RT followed by CT there, and in cereal production area with both CT and RT. Beet production area with RT showed also very good results, particularly in recent years.

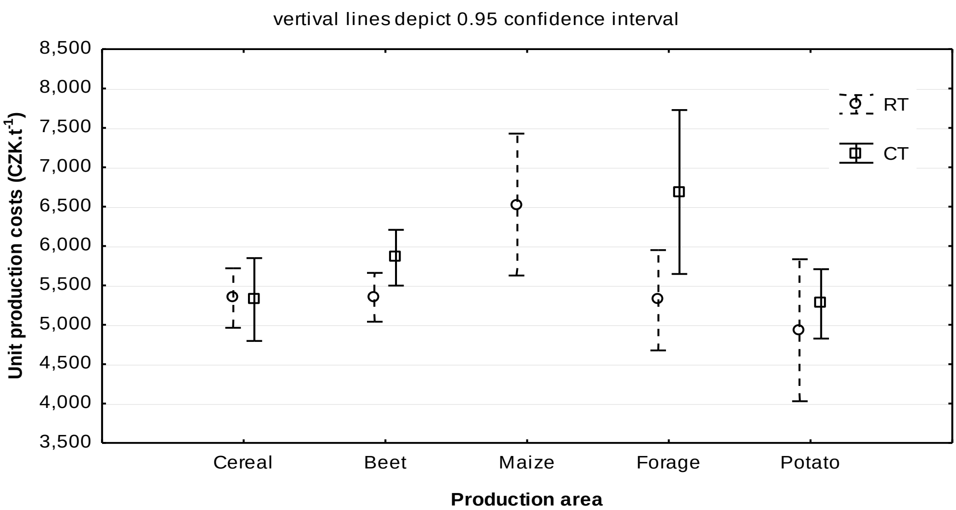

Figure 5. Graph of costs per one ton of produced rapeseed with respect to the production area and soil tillage system over the whole monitored period of fifteen years.

From the viewpoint of tillage system, fertilizer application at sowing and organic fertilizer application, the following variables proved statistically significant differences: fuel and labour consumptions, machinery costs and total costs (*t*-Test, $n = 520$, $\alpha = 0.05$). Earnings per hectare differed significantly regarding tillage system and organic fertiliser application. Material and unit costs differed significantly only with respect to organic fertilizer application. The conclusion of Sanchez-Giron et al. (2004; 2007) on higher herbicide costs of reduced-tillage was thus not confirmed, in opposite to the conclusion on lower machinery costs. Decrease in fuel and labour consumption (Gemtos et al., 1998; Bonciarelli & Archetti, 2000; Tebrügge, 2000; Grubor et al., 2015) was validated entirely.

CONCLUSIONS

The average fuel consumption of RT was by 20.2% lower than that of the CT, the overall labour consumption again lower by 24.0%. The total costs were lower by 4.9% as well. On the other hand, yields reached by RT were slightly higher, i.e. by 0.9%, and therefore the resulting unit costs lower by 4.1%. The potatoes production area proved to be the most favourable in terms of oilseed rape yields. Beet production area demonstrated also good results, namely over the recent years. In all of the production areas except the forage one, average seed yields reached by RT surpassed those produced using CT. Concerning earnings per hectare, RT results proved superior even in all of the production areas.

From the viewpoint of oilseed yields, of economics as well as of labour and fuel consumption, RT proved to be more than an adequate alternative to CT, particularly when employed on purpose and systematically. Lately, RT with deeper soil loosening has spread more and more, namely in order to ensure the disruption of compacted layers, and proved favourable results.

The operational monitoring and measurement conclusions were limited by an uneven distribution of cases into individual categories that prevented to adhere to the ceteris paribus rule of standard filed trials. On the other hand, the monitoring and measurements brought benefits of broader statistical survey that mirrored real conditions of the Czech agriculture. Since large collection of data was gathered, further on, the research will focus on analysis of particular details, e.g. of depth of soil tillage, specific material cost components etc.

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Juvenile growth and frost damages of poplar clone OP42 in Latvia

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Abstract. Short rotation plantations in the northern Europe are commonly established using poplar clone OP42 (*Populus maximowiczii* Henry × *P. trichocarpa* Torr. and Gray). We assessed its growth and suitability to the climate in central part of Latvia at juvenile age. Trees that had formed single stem were significantly higher (121 ± 2.5 cm), thicker (7.1 ± 0.48 mm) and had longer branches (32 ± 1.5 cm) than trees that had formed multiple stems. In beginning of the second growing season all trees had died stems and 19.6% of them formed new shoots from the ground level. The sprouting trees had random spatial distribution in the field. Regardless of the number of stems, the sprouting trees were significantly lower (110 ± 3.9 cm) than the dead trees (119 ± 2.0 cm). During the repeated assessment about one month later, proportion of the sprouting trees increased up to 44%, but the detected relations between measured traits of sprouting and dead trees remained. Clone OP42 had serious frost induced damages also in autumn phenology experiment (96% trees with severely damaged leaves). Our results suggest that frost prone sites are not suitable for establishment of plantations of OP42 clone.

Key words: *Populus maximowiczii* × *P. trichocarpa*, clone OP42, sprouting, freeze-thaw, wood dysfunction.

INTRODUCTION

Bioeconomy concept is rapidly gaining popularity in both policy and science (Staffas et al., 2013; Pülzl et al., 2014), and it is closely linked to European Commission goal to develop a resource-efficient and low carbon economy by 2050 (EC, 2011). Bioeconomy emphases use of renewable raw materials (Scarlat et al., 2015), including necessity to increase biomass production in sustainably managed industrial crops (EC, 2012). Woody biomass is one of the largest sources of renewable energy in EU and its consumption is expected to increase in the following decades (Openshaw, 2011; Bentsen & Felby, 2012; Lauri et al., 2014). However native tree species in the Northern Europe (with exception of grey alder) have relatively long rotation period – from ca. 40 years for birch and aspen up to more than 100 years for pine and oak (Rytter et al., 2013); and therefore have limited flexibility to respond to changes in wood demand in a short period of time. Hence, during the last decades fast growing tree species are increasingly used (Gailis & Jansons, 2010; Uri et al., 2010; Jansons et al., 2013), and currently ca. 50,000 ha of short rotation plantations is established in Europe (Don et al., 2012). *Populus* spp. are among the most energy-efficient perennial energy crops (Djomo et al., 2015), and are commonly used in the Northern Europe (Don et al., 2012) as well as in the Baltic States (Tullus & Vares, 2005; Bardule et al., 2016; Zeps et al., 2016). Poplars are mainly grown for bioenergy under rotation of 8–10 years (Djomo et al., 2015), but could also be grown for solid wood and veneer production in a rotation 20 years (Christersson, 2010). In Latvia, results from poplar trials indicate superior yield than the most productive native species (Jansons et al., 2014); yet results from other Northern Europe countries indicate that even higher yields could be gained (Karačić et al., 2003; Tullus et al., 2013). However, poplars in the Northern Europe are reported to suffer from frost damage at different severity (Ferm et al., 1989; Christersson, 1996; Ilstedt, 1996; Karačić et al., 2003; Christersson, 2006; Pliura et al., 2014). Pliura et al. (2014) studying poplar hybrids in two juvenile clonal trials in Lithuania found out that clones with late growth cessation were of the worse survival, possibly due to delayed shoot maturation and winter damage. In Sweden, two poplar clones had shown high biomass in one of the two experimental sites, while were completely eliminated by frost at the other (Karačić, 2005). One of the most widely used poplar clone worldwide and also in the Northern Europe is hybrid *Populus maximowiczii* (Henry) × *P. trichocarpa* (Torr. and Gray) clone OP42 (Taeroe et al., 2015). It is included in trials as far north as 60°N (Johansson & Karačić, 2011; Johansson & Hjelm, 2012b; Hjelm et al., 2015), indicating its suitability for climate at northern latitudes. Clone OP42 is also included in several trials representing different site conditions in Latvia and considered to be perspective (unpublished data). The hypothesis of the study is that poplar clone OP42 does not suffer from frost damages in Latvia. The objectives are to characterize the meteorological conditions, juvenile growth and frost damages for clone OP42, and compare the autumn phenology of OP42 to other poplar clones as a potential cause of autumn frost damages.

MATERIAL AND METHODS

The study was done in poplar trial located in central part of Latvia (56°34`N, 24°31`E), near Vecumnieki.

Vecumnieki trial was established in spring 2015 on drained peat soil with pH 6,0 using 30 cm long cuttings of clone OP42 (*Populus maximowiczii* (Henry) × *P. trichocarpa* (Torr. and Gray)) obtained from trees grown in western part of Latvia. Trees were planted in 17 rows in a density of 4 × 2 m, total number of planted cuttings 3,393. Weed control (ploughing and herbicide) was carried out once in autumn 2014 (before planting) and manual weed control (ca. 25 cm around the tree) during the growing season of 2015.

In the trial, at the end of the growing season (27/08/2015–18/11/2015) temperature measurements once per hour were done at three different heights – (1) air temperature was measured 20 cm above the ground; (2) soil temperature was measured at the surface level and (3) at the 20 cm depth (Fig. 1). Air temperature first dropped below 0 °C in September 11 and until October 6 negative air temperature recurred occasionally – in nine out of 22 days. During this period, the longest frost (temperature below 0 °C) occurred in September 27 (9 hours, minimal hourly temperature -2 °C) and September 30 (7 hours, minimal hourly temperature -3 °C). In October 7 frost became more intense – air temperature was below 0 °C for 14 hours and dropped down to -12.5 °C; similar temperatures remained for 10 days – mean minimal hourly temperature was from -9.5 to -13.5 °C, duration of frost was from 11 to 16 hours per day. Similar period of frost occurred in October 28–31. Likewise, on the soil surface two periods of temperature below 0 °C occurred in October, and remained for nine (October 8–16) and three (October 29–31) days. For the first time in the growing season (October 8) soil temperature at the surface level dropped below 0 °C for 2 hours; five days later – for 7 hours (minimal hourly temperature -1 °C). Duration of negative soil surface temperature reached 15 hours per day (-2 °C) in October 30 and 31. However, during the observed period no negative soil temperature at 20 cm depth was recorded.

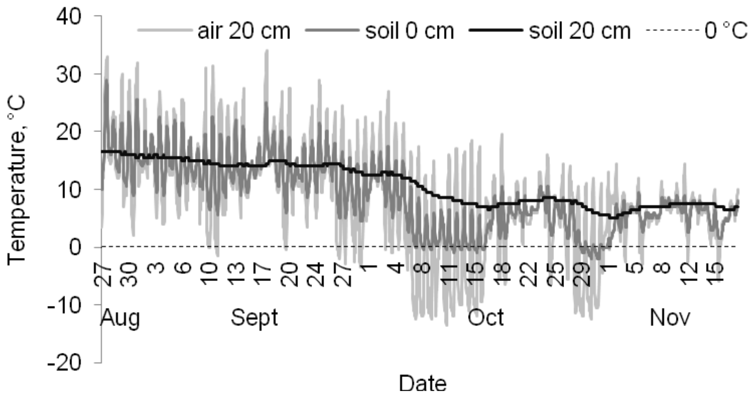

Figure 1. Temperature of air (20 cm above ground) and soil (at surface level – 0 cm – and in 20 cm depth) in Vecumnieki trial during 27/08/2015–18/11/2015.

Data of the long-term (30-year mean) measurements for the respective period were obtained from the nearest (distance ca. 30 km) weather station, located near Bauska (56º22`N, 24º13`E; Latvian Environment, Geology and Meteorology Centre). During the analysed period, mean diurnal air temperature did not decrease below 0 °C; but minimal temperature (30-year mean of the minimal temperature of the date) first dropped below 0 °C in November 11 (Fig. 2) – considerably later than in the study year. As can be seen the study year represent very specific meteorological conditions in the autumn thus authors did not had a chance to repeat the study with larger set of clones and sites during next growing season even so the experimental design was prepared.

In the beginning of June 2016, damage of stems (one-year-old shoots, emerged in the previous growing season) were observed. All trees had completely withered stems (no growth from the previous year shoots occurred), and part of them had new emerging shoots from the ground level. Tree height (± 1 cm) and diameter (± 1 mm; measured for 410 trees in 2 rows) at the stem base (root collar) of the highest one-year-old shoot (emerged in 2015) of trees were measured, number of stems (emerged in 2015) and number of branches (longer than 5 cm) per tree were counted, and length of branches (± 1 cm) was measured. Trees that had more than three stems and more than three branches were pooled into groups 'more than 3 stems' and 'more than 3 branches', respectively. For each tree occurrence (0/1) of browsing damage and occurrence of new emerging shoots (further called 'sprouting' (1) and 'dead' (0) trees) were recorded. In total, 3,025 trees were measured. In the middle of July, assessment of sprouting/dead trees was repeated in a part of the trial (5 rows, 1,018 trees).

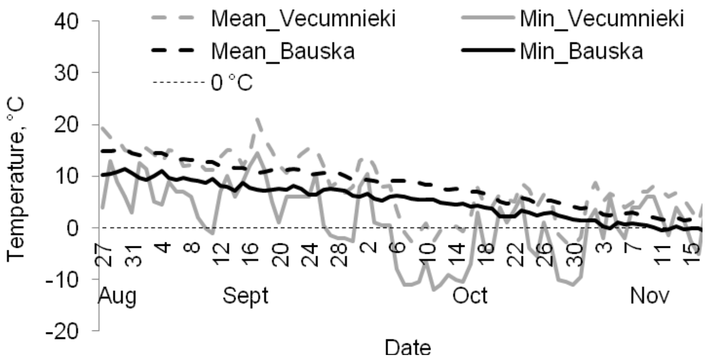

Figure 2. Air temperature in the study year in Vecumnieki trial and long-term measurements of the nearest weather station (Bauska) for the period of 27/08–18/11. 'Mean_Vecumnieki' – mean diurnal temperature, calculated from hourly measurements; 'Min_Vecumnieki' – the lowest recorded diurnal temperature; 'Mean_Bauska' – mean diurnal temperature, calculated from 30-year data of mean temperature of the respective date; 'Min_Bauska' – mean of the 30-year lowest diurnal temperature of the date.

To characterize the frost hardiness of poplars the data obtained in another poplar trial located in eastern part of Latvia (56°41´N, 25°58´E), near Kalsnava, were used additionally. Besides OP42, 17 other clones were included in this trial where autumn phenology observations were made. Frequently recurring freeze-thaw cycles at the same period as in Vecumnieki were observed in Kalsnava; the frost damages for leaves and stems of two-year-old trees were assessed visually (evaluated at five grade scale) in October 15th. The detailed description of methods, as well as the frost hardiness comparison at the clone level was performed by Lazdiņa et al. (2016). In our study, clones were pooled into five groups according to their origin (Sweden, Italy, Germany, Latvia) to estimate the relative frost hardiness of OP42 in comparison to other poplar clones.

The Shapiro-Wilk test was used to assess the normality of the data. Differences of mean height, diameter, and length of branches were assessed using one-way analysis of variance (i) between trees that had formed one or multiple stems, and (ii) between trees that had formed one, two, three or more stems. The Chi-squared test was used to assess (i) distribution of the number of branches, proportion of sprouting trees and proportion of browsed trees between trees that had formed one or multiple stems and among trees that had formed one, two, three or more stems, (ii) distribution of the proportion of sprouting trees between browsed and not browsed trees, (iii) distribution of proportion of sprouting trees among plantation rows. The statistical analysis of differences between proportions of trees among the leaf and stem damage grades was hampered by insufficient number of trees in several of the grades. Pearson's correlation was used to assess relationship between proportion of sprouting trees and mean tree height of the plantation rows. Spearman's correlation was used to assess the relationships between (i) number of branches and number of stems per tree, (ii) length of branches and number of branches, (iii) length of branches and number of stems per tree. Spatial autocorrelation of the sprouting trees was assessed using Moran's I. All tests were performed at $\alpha = 0.05$. Mean values and their confidence interval are shown both in text and figures.

RESULTS

In Vecumnieki at the end of the first growing season 89% of the planted cuttings had formed shoots. About half (49%) of trees had formed one stem, and about half had formed multiple stems – 31% of trees had formed two, 10% of trees three and 10% of trees four and more stems. Tree height varied from 10 to 251 cm (mean 118 ± 1.8 cm), and trees that had formed multiple stems were significantly ($P < 0.001$) lower (mean height of the highest stem 114 ± 2.6 cm) than trees that had formed one stem (mean height 121 ± 2.5 cm). No overall trend between number of stems and tree height was observed (Fig. 3).

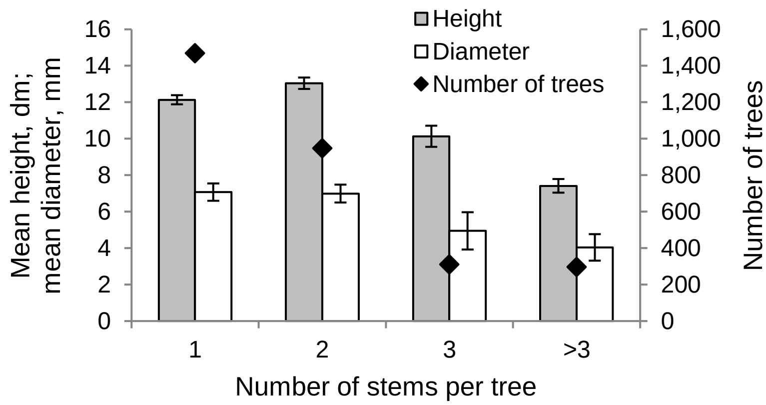

Figure 3. Mean tree height and diameter at root collar (both ± confidence interval) and number of trees according to a number of stems per tree for the clone OP42 in Vecumnieki.

Branches were observed for 30% of trees, mean number of branches was 3.3 (from 1 to 42). One branch was observed for 11% of trees, two branches for 6%, three branches for 4% and more than three branches – for 9% of trees. Proportion of number of branches between trees that had one and trees that had multiple stems was similar ($P = 0.86$). No relation between number of stems and number of branches was observed ($r_s = -0.060$, $P = 0.071$).

Mean length of branches was 30 ± 1.0 cm, and trees that had formed one stem had slightly, but significantly ($P < 0.001$) longer branches (32 ± 1.5 cm) than trees that had formed multiple stems (28 ± 1.4 cm). However, it was similar ($P > 0.05$) for trees that had formed one stem (32 ± 1.5 cm) and trees that had formed two stems (31 ± 2.1 cm), but trees that had formed three (26 ± 2.5 cm) and more (25 ± 2.4 cm) stems had significantly shorter branches. Mean branch length had weak but significant correlation with number of branches ($r_s = 0.089$, $P = 0.007$) and number of stems ($r_s = -0.154$, $P < 0.001$).

Mean diameter at stem base was 6.6 ± 0.32 mm. Similarly to tree height, trees that had formed smaller number of stems were thicker (Fig. 3). Mean diameter of trees that had formed one and two stems was similar ($P > 0.05$; 7.1 ± 0.48 mm and 7.0 ± 0.49 mm, respectively), and both significantly differed from diameter of trees that had formed three and more stems (4.9 ± 1.0 mm and 4.0 ± 0.73 mm, mutually similar ($P > 0.05$)). Browsing damages were observed for 7.6% of trees. Proportion of browsed trees significantly differed between trees that had formed one, two, three and more stems ($P < 0.001$), and it was 10.2, 6.6, 3.2 and 2.4%, respectively.

In the late June 2016, new emerging (sprouting) shoots from the ground level were found for 19.6% of trees. The sprouting trees had reached slightly, but significantly ($P < 0.001$) lower height in the previous growing season (110 ± 3.9 cm) than the dead trees (119 ± 2.0 cm), regardless of the number of stems (Fig. 4). Among trees that had formed one stem, height of the sprouting and dead trees was 114 ± 5.2 cm and 123 ± 2.8 cm, respectively; among trees that had formed multiple stems – 106 ± 5.7 cm and 116 ± 2.9 cm, respectively.

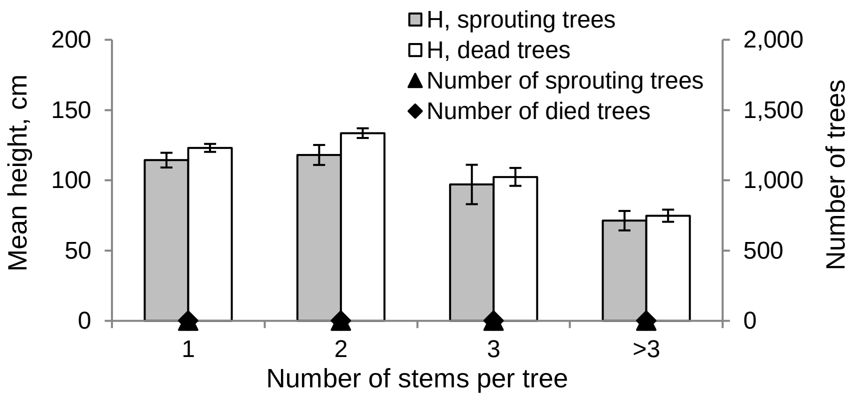

Figure 4. Mean height (H; ± confidence interval) and number (N) of sprouting and dead trees according to number of stems per tree for the clone OP42 in Vecumnieki.

Proportion of the sprouting trees that had formed one, two, three and more stems was similar ($P = 0.89$) – 19.4; 20.1; 20.0 and 18.2%, respectively. Significant ($P = 0.006$) difference of proportion of the sprouting trees was found between browsed (12.6%) and not browsed (20.1%) trees. Proportion of the sprouting trees differed significantly ($P < 0.001$) between the trial rows, but no relation ($P > 0.90$) between proportion of the sprouting trees and mean tree height in the row was found. Spatial distribution of the sprouting trees in the field was random ($P > 0.05$).

During the repeated assessment of sprouting/dead trees, proportion of sprouting trees was notably increased and reached 44%. However, the observed relation between measured traits remained – the sprouting trees were significantly ($P < 0.001$) lower than the dead trees (111 ± 4.6 cm and 123 ± 4.5 cm, respectively). Height of trees that had formed one and multiple stems was similar ($P = 0.058$) – 121 ± 4.5 cm and 115 ± 4.7 cm, respectively. Among trees that had formed one stem, height of the sprouting trees (116 ± 6.2 cm) was significantly ($P = 0.03$) lower than that of the dead trees (126 ± 6.4 cm). Similarly, among trees that had formed multiple stems, height of the sprouting trees (107 ± 6.8 cm) was significantly ($P = 0.002$) lower than that of the dead trees (121 ± 6.4 cm).

Frost damage of the same frost event was assessed in another progeny trial in Kalsnava. Analysis of two-year-old trees reveals differences between clone origins. All trees of clones that were collected across Latvia were dormant (Grade 0L) and had no frost damage of leaves. Clones of Italian origin had all trees still growing, and 66% of trees had moderately damaged (Grade 3L) leaves but no severe damage (Grade 4L) was observed. Clones from Germany had relatively large proportion (64%) of dormant trees; but among still growing trees 96% had severely damaged (Grade 4L) leaves. Similarly to clones of Italian origin, all trees of clone OP42 were still growing at the time of frost event, but in contrast, trees of OP42 were more intensely damaged – 96% of trees had severely (Grade 4L) and 4% of trees had moderately (Grade 3L) damaged leaves (Fig. 5).

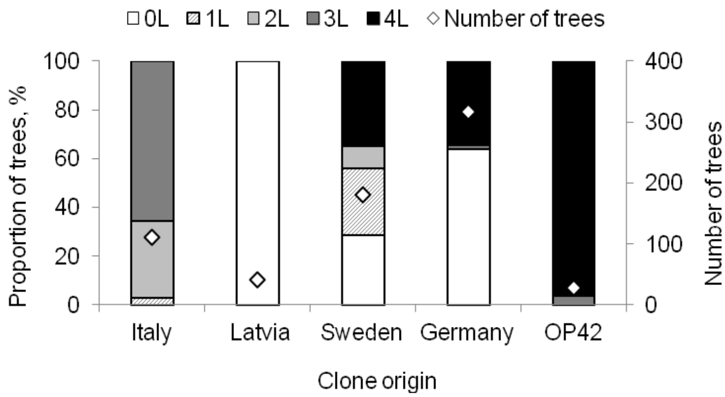

Figure 5. Proportion of two-year-old ramets with different leaf damage grades (0L, 1L, 2L, 3L and 4L) according to clone origin in Kalsnava. Damage grades: '0L' – leaves were already fallen before the freezing event and buds were set; '1L' – visually intact leaves; '2L' – several damaged leaves; '3L' – most of leaves in the current year leading shoot were damaged; '4L' – most of leaves on the whole tree were damaged.

Stem damage was less frequent – in total 8% of trees had stem damage of different intensity. No stem damage was observed for clones from Latvia, which also had no leaf damage, and for clones from Italy, which had moderate leaf damage. Similarly, clones from Sweden and Germany had 1% and 15% of trees with mild stem damage, respectively. Clone OP42 had severely damaged leaves but no stem damage was observed.

DISCUSSION

The results from rather rare combination of meteorological conditions in autumn were reported even so the study design was not initially intended for that. Considering the increasing use of the clone OP42 in Latvia and other Baltic states we did see the necessity to inform about the data so that land owners would have as objective information as possible while selecting the planting material. Although the result from the autumn phenology experiment in Kalsnava is not a direct replication of study in Vecumnieki, still it comes to similar conclusion on the risk of frost damages of the particular clone and provides a plausible reasoning for the observed problem.

Frost induced damage is not exclusively caused by extremely low temperatures; damage type and severity depends on frost event timing, duration and pattern, and the highest risk of frost damage occurs during the transition period between annual cycle of growth and dormancy (Charrier et al., 2015). We studied freeze-thaw damage in the early autumn. The freeze-thaw cycles are common to initiate embolism – bubbles are caused by dissolved air that freezes out of the sap (Tyree & Sperry 1989; Brodersen & McElrone, 2013), and sufficiently large bubbles may fill xylem conduits hampering water transport during the thawing (Pittermann & Sperry, 2006).

The first freeze-thaw cycles of the growing season occurred in the middle of September, and can be characterized by mild frost (ca. -2 to -3 °C). In the middle of October, more intense freeze-thaw occurred (Fig. 1) – temperature dropped below zero for 11–16 hours repeatedly for 10 successive nights, while the maximum temperature at day was from 6.5 to 18 °C. During the studied period the longest thaw period was 13 hours, indicating that trees experienced repeated embolism while the hydraulic conductivity had not recovered from the previous freeze-thaw cycle. Experiments of Just & Sauter (1991) showed that hydraulic conductivity of *P.* × *canadensis* stem segment decreased by ca. 60% after one freeze-thaw cycle, and almost no recovery was obtained during the first hour after thawing. It took 19 hours at the room temperature (21 °C) to recover 90% of the initial conductivity and about 2 days to recover completely.

Extremely high mortality in the studied site might be explained by timing of the particular freeze-thaw event. At the time of the frost all trees of clone OP42 were actively growing in the Kalsnava trial (Fig. 5), also a study of *P. tremula* × *P. tremuloides* in Latvia has revealed strong relation between height growth intensity and daily mean temperature (Zeps et al., 2015) and many other studies have showed delayed growth cessation for northward transferred species (Howe et al., 2000; Christersson, 2006; Friedman et al., 2008). Hence, most presumably clone OP42 was still growing also in Vecumnieki. During the growing season trees have higher water content than during the dormancy (Mäkinen et al., 2008; Pallardy, 2008), and more severe freeze-thaw damage is showed for trees that have high water content before the freezing occurs (Cox & Zhu, 2003). Also temperature regime before freezing significantly influences damage severity. *Betula* trees that were exposed to longer thaw period before winter freezing had increased percent length of shoot dieback and reduced length of new emerging shoots (Zhu et al., 2000). Longer thawing period was associated with increased dehardening of roots and root damage, thus, reducing root pressure, and resulted in poor recovery of embolism (Zhu et al., 2000).

Timing of the freeze-thaw cycle is also showed to affect tree recovery. *P. balsamifera* had different pattern of recovery after autumn and spring frost (Hacke & Sauter, 1996) – in the autumn when trees still had some leaves, recovery was slow and remained incomplete during the next growing season; in contrast, recovery after spring frost was complete in two months. Tree ability to recover largely depends on its vitality and damage severity. Zhu et al. (2000) have suggested that shoot damage might be directly dependent on ability of the unembolised vessels to maintain water supply for stem and branches. In turn, Cox & Zhu (2003) found that diffuse-porous trees that undergo permanent residual embolism in combination with root and shoot freezing injuries experienced higher bud mortality and shoot dieback than trees that can recover before next freeze-thaw cycle occurs.

Poplars are commonly propagated by cuttings, and trees develop shallow root system with no taproot and most lateral roots found near (within ca. 30 cm) soil surface (Puri et al., 1994; Crow & Houston, 2004; Johansson & Hjelm, 2012a). Fine roots are responsible for water uptake for trees (Block et al., 2006), and the highest biomass of fine roots was found down to 5 cm (Al Afas et al., 2008), 10 cm (Crow & Houston, 2004) depth for several poplar clones. During the studied period only slight temperature drop below zero on soil surface and no negative temperatures at the 20 cm depth was observed during the measurements (Fig. 1), therefore the particular frost event is not likely to cause substantial root damage. However, frequent recurrence of freeze-thaw cycles can cause accumulated embolism during autumn/winter (August–March) season (Sperry & Sullivan, 1992). Most deciduous trees refill frost embolized vessels in spring, and at this time nearly all conduits may be filled with air, causing as much as ca. 90% decrease of hydraulic conductivity (Sperry & Sullivan, 1992). Christensen-Dalsgaard & Tyree (2013) have observed ca. 90% loss of conductivity already after one light frost event, indicating high sensitivity of *P. deltoides* × (*P. laurifolia* × *P. nigra*) clone Walker. Later studies showed that for poplars most of the accumulated embolism occurred after the first few frost events (Christensen-Dalsgaard & Tyree, 2014). Autumn frost damage hamper winter hardening, which in turn increases vulnerability to damage (von Fircks, 1992), thus it could be assumed that root damage might occurred during the following winter when temperature dropped down to -25 °C (data from Latvian Environment, Geology and Meteorology Centre).

Root damage hamper the recovery of the hydraulic conductivity – Sperry (1993) has found that embolism of not-damaged *Betula* trees recovered from 81 to 88% during one month (April to May), while trees with root damage had 75% embolism after two months (in June). Similarly, no recovery of hydraulic conductivity was observed for *P.* × *canadensis* during more than three months if root pressure was absent (Hacke & Sauter, 1996). The reduced hydraulic conductivity, in turn, is showed to negatively affect timing of bud break – the lower the hydraulic conductivity at the end of the winter, the later the bud break (Wang et al., 1992). The observed emergence of new shoots of the otherwise vigorously sprouting clone OP42 (Johansson & Hjelm, 2012b) in this study was rather late and poorly performed, suggesting that trees were struggling to either rely on the remaining unembolized conduits or try to grow new conduits (Hacke & Sauter, 1996; Améglio et al., 2002).

We found that the sprouting trees were lower than the dead trees, regardless of the number of stems per tree (Fig. 4). Tree height has positive relation to vessel diameter (Martínez-Cabrera et al., 2011), which is the most important wood trait that determine vulnerability to embolism (Sperry & Sullivan, 1992). Plants most efficiently ensures conductivity by forming few, wide and long conduits (Sperry et al., 2008), but for a given tree height many small vessels are more resistant to embolism (Davis et al., 1999). Poplars are associated with high water consumption (Silim et al., 2009), and hence might have trade-off between hydraulic conductivity and resistance to embolism.

CONCLUSIONS

Interest of growing poplars in the Northern Europe is increasing. Yet, limited number of commercial clones is available, and their growth as well as suitability to climate should be thoroughly tested due to northward transfer. We observed reasonable growth of one-year-old poplar clone OP42. However, extremely high mortality occurred due to early autumn frost (freeze-thaw cycles) at the end of the first growing season, suggesting that frost prone sites are not suitable for establishment of plantations of OP42 clone. The results emphasize necessity to include frost risk assessment, especially for the first year of establishment, in site selection and economical calculations. Our experiment was carried out in a single trial and therefore information from other sites needs to be collected before any further generalizations. Resprouting of damaged trees was rather late and poorly performed, and further assessment of vitality and growth of the sprouts is needed.

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The effect of bedding amount on gas emissions from manure during storage

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Abstract. One of the major agricultural pollutants of environment is manure from livestock. We focused on dairy cows kept in the barns with straw bedding commonly used in the Czech Republic. We tested the hypothesis that the amount of bedding used daily relative to the number and size of animals kept has a significant effect on the emissions of gases from manure stored in a manure pile. In the experiment, a group of 10 dairy cows of Holstein and Czech Red Pied breed was housed in a stable bedded with various amounts of wheat straw (4–10 kg/livestock unit per day). The manure was removed from the stable after 48 h and mixed was stored in cubic containers with drain floor allowing measurement of manure leachate release. For 50 days we measured weight, volume, weight of manure leachate and manure temperature. Decreasing stored manure weight can be attributed to release of manure leachate and emissions of gases, primarily water vapor, as a result of microbial activity and increased temperature in the manure during storage. Using the calculated model, we found that daily production of emissions of water vapor and other gases was related to bedding amount in a statistically significant manner ($P < 0.001$). The cumulative amount of gas emissions grew rapidly in all treatments. Also total amount of emissions was related to bedding amount in a statistically significant manner ($P = 0.004$). We also found the relationship between internal manure temperature and the logarithm of the amount of emissions produced to be statistically significant ($P < 0.001$).

Key words: dairy cows, manure storage, bedding amount, gas emissions.

INTRODUCTION

Animal agriculture is a source of many substances having environmental impacts. It involves in particular the production of nitrogen, which is released into the environment from livestock housing as well as during storage and field application of manure from farm animals.

Nitrogen is released into the environment from livestock housing in the form of gas emissions (NH_3, N_2O, NO) and it pollutes water sources (as NH_4+, NO_3, organic N) through leakage and seepage during storage and field application of manure.

Animal excreta (urine and feces) contain environmentally reactive nitrogen, which, unless it is incorporated into a crop or converted into molecular nitrogen, begins moving into the air and water from the time it leaves the animal (Amon et al., 2001; Powell et al., 2013; Kohn, 2015).

Measures aiming at reducing environmental impacts occurring in connection with agriculture, and in particular animal farming, are being sought and introduced around the world. The European Union and many other developed countries have devoted a particularly great deal of attention to methods for storing and applying manure from livestock with the objective of minimizing their negative environmental impacts (Nitrates Directive, 1991; Ohio Livestock Manure Management Guide, 2006; Agricultural Waste Management, 2011).

This topic is also highly relevant in the Czech Republic, where annual manure production is estimated at 10.3 million metric tons. Many animals on Czech farms are kept in barns with bedding. In these systems, the space where the animals rest is covered with a certain amount of bedding in order to create a soft and dry bed. Sometimes bedding is also added to manure gutters. The mixture of feces, urine, bedding, and potentially feed residues and other substances is mixed and subsequently pushed out of the animals' pens by their movement, is mixed with other manure and unconsumed feedstuffs, is removed from the barn, and then is stored in a suitable manure pile.

In addition to the animals' species, feeding, and housing system, manure properties are also affected by the bedding that is used. The amount and properties of bedding affect in particular the content of nutrients, organic materials, and dry matter of manure produced in stables. Bedding also has a considerable effect on manure's physical and mechanical properties (e.g. dry matter, volume, density, porosity, and air content), which in turn affect the storage process, manure leachate production, nutrient loss, gas emissions, and other aspects. (Misselbrook et al., 2004; ASAE, 2005; Agricultural Waste Management, 2011).

Bedding and its properties and amount also affect the quantity of gas emissions from stored manure. Increasing proportions of bedding and correspondingly boosting the amounts of oxygen in the manure support microbial processes occurring in the manure during storage as well the overall amount of gas emissions (Jeppsson, 1999; Misselbrook & Powell, 2005; Aguerre et al., 2012).

The literature contains a large amount of data on the production of manure and manure leachate in connection with animal farming, much of which has a normative character (ASAE, 2005; Oeneme et al., 2007; Government of the Czech Republic, 2013). Nevertheless, there is a need for more-detailed understanding of changes in stored manure's properties through the storage process.

Aim of this work is deepen understanding of production of water vapor and other gases from stored manure in accordance with the amount and properties of bedding as well as storage duration. This understanding is important for defining principles for the proper storage and treatment of manure during storage and application.

To better understand the properties of stored manure and changes to them during the storage process depending upon bedding amount, we therefore established controlled experiments focused on understanding these variables within manure produced by dairy farming operations using straw bedding.

We adopted the hypothesis that the daily amount of bedding relative to the number and size of livestock expressed as livestock units (LU, with 1 LU = 500 kg live weight) would significantly affect the emissions of water vapor and other gases from manure during storage in a pile and these emissions are also significantly affected by the course of temperature within stored manure.

The objective of these experiments was to test the assumptions expressed in the hypothesis and contribute to a better understanding of emissions of water vapor and other gases from stored manure according to the amount of bedding and duration of that storage.

MATERIALS AND METHODS

The effect of bedding on manure properties was evaluated on a dairy farm in the Czech Republic (Central Bohemia region). A group of 10 dairy cows was housed in a pen bedded with varying amounts of wheat straw (Fig. 1). The pen had a flat and impermeable concrete floor. The determined amount of bedding was spread at regular intervals over the entire pen. Dairy cows were free to move about the entire pen and their movements mixed their excrement into the bedding. The experiment was carried out in the autumn months (September – November).

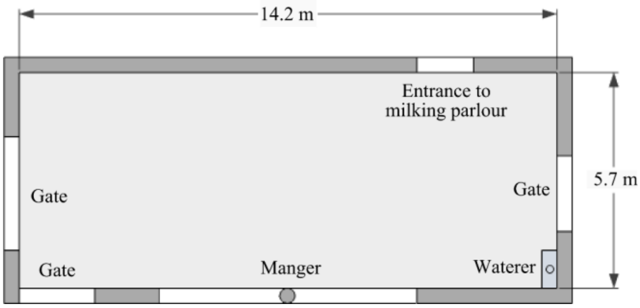

Figure 1. Floor plan of group pen for 10 dairy cows where in experiments utilizing various amounts of bedding were conducted.

Experimental Conditions

- ✓ Number of milk cows in the pen: 10;
- ✓ Pen area: 81 m^2;
- ✓ Cow breed: Holstein and Czech Red Pied;
- ✓ Mean cow weight: 650 kg (1.3 LU);
- ✓ Mean annual milk production: 8,500 kg;
- ✓ Bedding: wheat straw;
- ✓ Bedding dry matter: 84–86%;
- ✓ Bedding amount: 4–10 kg per LU per day (1 livestock unit = 500 kg live weight), i.e. 5.2–13 kg per cow per day;
- ✓ Feeding: TMR;
- ✓ Milking frequency: 2 times per day.

In order to create sufficient manure and ensure cow cleanliness and comfort, bedding corresponding to the amount to be provided over 2 d was put down in the pen at the start of the experiment. Straw was spread in the experimental pen by small wheel loader and manually.

Individual treatments were conducted under identical conditions other than to alter the amount of bedding added to the pen, the values for which are given in Table 1. Cows were weighted before the experiment to apply proper amount of the bedding.

Table 1. Bedding amounts added to the pen for individual treatments

Item	Treatment				
	4 kg per LU[2]	6 kg per LU	7 kg per LU	8.5 kg per LU	10 kg per LU
bedding amount, kg per LU per day**	4.0	6.0	7.0	8.5	10.0
bedding amount, kg per cow per day	5.2	7.8	9.1	11.1	13.0
total bedding amount in pen for 10 cows*, kg	104	156	182	222	260
specific amount of bedding in pen*, kg m^{-2}	1.3	1.9	2.2	2.7	3.2

* Bedding corresponding to twice the daily amount was put down in the pen at the start of the experiment.
** LU = livestock unit (500 kg live weight).

After each 48 h, the mixture of feces, urine, and bedding (collectively defined as 'manure' for the purposes of this article) was removed from the pen and mixed using a front-end loader and then unpressured placed in storage containers with watertight bottoms and walls and having volume 1,507 m^3 and the following inner dimensions: width 1,135 mm, length 1,135 mm, height 1,170 mm.

The containers' bottoms contained sealable drains enabling the daily drainage and subsequent weighing of manure leachate released from stored manure.

During the experiment, the containers were stored in shade on the northern side of the building and protected from sunlight. After being filled with manure, the containers were covered with corrugated roof panels to protect them from rain and enable continuous ventilation of gas formed during storage.

Throughout the experiment, containers with manure having the defined proportions of bedding were regularly weighed on a scale, the amount of released manure leachate was weighed, and the change in the volume of manure stored in the container was determined. The temperature of the manure at the center of the container was regularly measured using a rod thermometer.

Each treatment was repeated twice, always with the same amount of bedding, except that the treatment with 6 kg bedding per LU per day was repeated four times. This treatment was repeated more frequently due to the need to understand in more detail the behavior of stored manure with 6 kg bedding per LU per day. That amount is considered under Czech law to be the minimum necessary in order to store manure directly on the edge of land to be manured without the need for intermediary storage in a manure storage facility with a solid bottom.

Theory and modelling

In order to perform relevant comparisons of results from individual treatments and their variants, all measured values were converted for evaluation purposes to 1,000 kg manure weight at loading according to the equation:

$$m_p = m\frac{1{,}000}{M_1} \tag{1}$$

where m_p – the converted value relative to 1,000 kg manure weight, kg; m – the measured value, kg; M_1 – the manure weight at loading, kg.

Decreasing stored manure weight can be attributed to release of manure leachate and emissions of gases, primarily water vapor, as a result of microbial activity and increased temperature in the manure during storage. This was used to calculate gas emissions from stored manure. The daily amount of gas emissions was defined as:

$$E_n = m_n - m_{n-1} - h_n \quad (2)$$

where E_n – gas emissions for day N of storage, kg; m_n – manure weight at day N of storage, kg; m_{n-1} – manure weight at day, (N_{-1}) of storage, kg; h_n – daily manure leachate production for day N of storage, (kg.

The total amount of released water vapor and other gases was calculated according to the equation:

$$E_{total} = M_1 - M_2 - H_h \quad (3)$$

where E_{total} – the weight of water vapor and other gases released, kg; M_1 – manure weight at loading, kg; M_2 – manure weight at the end of storage, kg; H_h – the weight of manure leachate released over the entire storage period, kg.

The daily amount of gas emissions according to bedding amount treatment was calculated in a similar manner.

Daily changes in individual variables monitored over time according to the amount of bedding per LU per day were modeled using nonlinear mixed regression models. Time and bedding amount were taken as fixed effects. The effect of the container was considered a random effect as each container bore specific error in relation to the specific cows, time of year, weather, method of manure handling, and so forth. The studied relationships were displayed in graphs of both daily and cumulative changes.

In order to meet the conditions for those methods used, daily changes in manure temperature was square root transformed. The remaining variables were not transformed.

We studied the relationship between total gas emission over 50 d of storage and bedding amount. We modeled this relationship using quadratic regression.

Test results with $P < 0.05$ were considered as statistically significant. Statistical analyses were performed in R statistical package version 3.0.2 (R Core Team, 2014).

RESULTS AND DISCUSSION

Daily gas emissions from stored manure (Fig. 2) rapidly increased immediately after manure loading and peaked around day 6 to 8 of storage. The highest emission values were from manure with the most bedding. The emissions subsequently decreased and production of gas emissions from stored manure gradually ceased around day 35 of storage. The course of gas emission is clearly related to intensity of microbial activity and stored manure temperature, as can be concluded from the similar course of manure temperature (Fig. 3).

We modeled daily production of emissions according to the amount of bedding per LU using a linear mixed model as: (1/time + 1/exp(time)) × bedding amount. Using the calculated model, we demonstrated that daily production of emissions was related to bedding amount in a statistically significant manner ($P < 0.001$).

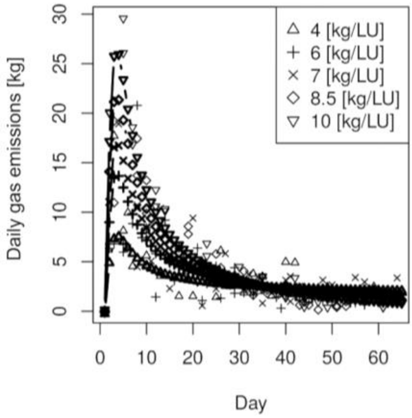

Figure 2. The course of daily emissions of water vapor and other gases from stored manure according to the amount of bedding per livestock unit (LU, with 1 LU = 500 kg live weight).

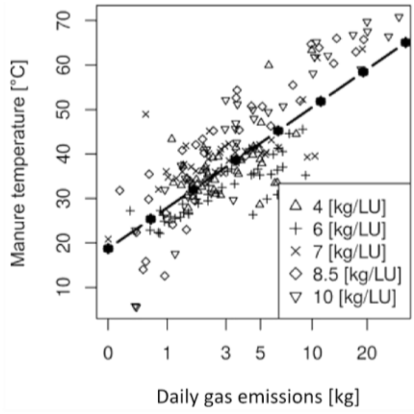

Figure 3. Correlation of internal manure temperature with various amounts of bedding per livestock unit (LU, with 1 LU = 500 kg live weight) and the daily amount of emissions produced.

Fig. 4 illustrates the cumulative course of gas emissions, clearly depicting that gas emissions depended on storage duration. The cumulative amount of gas emissions grew rapidly in all treatments. It is also possible to observe the effect of bedding on emission amounts.

Fig. 5 presents the total amount of gas emissions over 50 d of storage according to bedding amount. This figure also shows that the total amount of gas emissions grows with increased bedding amounts. This is clearly related to increased microbial activity and to the increased temperatures in manure with more bedding.

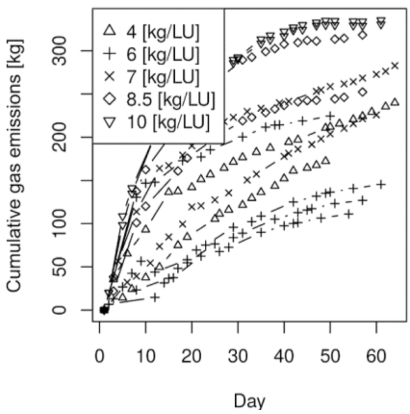

Figure 4. Cumulative emissions of water vapor and other gases from stored manure with various amounts of bedding per livestock unit (LU = 500 kg live weight) depending on storage duration.

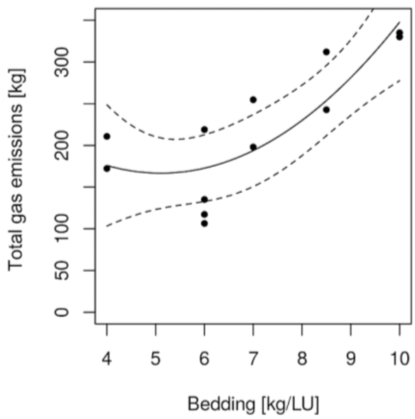

Figure 5. Total gas emissions over 50 d of manure storage according to the amount of bedding per livestock unit (LU, with 1 LU = 500 kg live weight). The solid line depicts the modeled relationship and the dashed line indicates the 95% confidence bands around the regression model.

We modeled the relationship between total gas emission and the amount of bedding per LU using quadratic regression. In the calculated model, the total amount of emissions was related to bedding amount in a statistically significant manner (P = 0.004). We have demonstrated that total gas emissions from stored manure for the entire duration of storage grew with increased bedding amounts.

The acquired results demonstrated that bedding amount had a significant effect on the properties of stored manure and thus confirmed the results of studies by other authors (Amon et al., 2001; Misselbrook & Powell, 2005; Gilhespy et al., 2009; Powell et al., 2013).

The selected methodical approach enabled acquisition of new data about the effect of bedding on the properties of stored manure and the course of their changes during storage. Data on gas emissions enable specification of current recommendations for manure management and manure-storage facility design (Ohio Livestock Manure Management Guide, 2006; Agricultural Waste Management, 2011). They will also be used to specify Czech law (Government of the Czech Republic, 2013) and principles for nitrate management in Nitrate Vulnerable Zones pursuant to EU requirements (Nitrates Directive, 1991; Government of the Czech Republic, 2012).

CONCLUSIONS

The acquired results confirmed the formulated hypothesis that bedding amount affects the amount of daily gas emissions and the total amount of emissions over 50 d of storage. We demonstrated a correlation between the amount of gas emissions and the internal temperature of stored manure. The daily and total amount of gas emissions grows with increased bedding amounts. This is clearly related to increased microbial activity and to the increased temperatures in manure with more bedding. The results show a new perspective on manure storage in the relation to the amount of bedding. Data on gas emissions enable specification of current recommendations for manure management and manure-storage facility design.

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The advantage of Decision Support System for managing spring barley disease in Estonia

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Abstract. A Decision Support System (DSS) I-Taimekaitse focusing on use of timely applied and reduced fungicide rates in control of cereal diseases has been tested in field trials since 2003. We compared the conventional treatment and the DSS-based spray practices in 18 field trials in five agricultural locations over 7-year period. Efficacy of the control of net blotch caused by *Pyrenophora teres* (Drechsler, am *Drechlera teres* Sacc. Shoem), the main fungal disease in spring barley has been tested to determine the economic advantage of DSS use. Compared with the conventional spray practices, the advantage I-Taimekaitse resulted in reduction of application doses by 30 to 60% of the registered rate. According to I-Taimekaitse, the fungicides were applied mainly between heading and flowering growth stages (GS 55–65), whereas traditional routine spraying is commonly made at booting (GS 37–49). The experiment clarifies the cost-benefit of using DSS-based approach in barley disease management with average yield increase above the control in 12.8% and above the conventional treatment in 14.1%. I-Taimekaitse gave competitive disease control and average yield output reduction compared with conventional practice by 9%. In general the Treatment Frequency Index applied in conventional treatment was 0.65 and in DSS 0.41. Although the cost of treatment expense in DSS was 20% less compared with conventional practice, the performance of conventional used spray practices was outstanding in economic return.

Key words: Decision support system, economic return, fungicide, net blotch, spring barley.

INTRODUCTION

Disease control is an especially significant challenge for food security (Brown, 2010). The incidence and severity of plant diseases depend from combination of host plant and pest genotypes, climatic conditions and management practices. Environmentally sound and economically efficient disease control needs considering all these factors. To reduce the use of pesticides it is possible to improve the controls of disease with optimized fungicide application. Decision Support System (DSS) is based on using appropriate doses aimed at minimizing the overall pesticide input (Jørgensen et al., 2008; Shtienberg, 2013). DSS where specific field inspections are omitted and where regional disease data are relied upon may attract more farmers as they save the farmer's time (Jørgensen et al., 2008). For barley, major changes in the crop protection schemes are expected in the Baltic region due to northward spread of new pests and higher disease pressure from native pathogens due to higher precipitation (Olesen et al., 2011). The

DSS can help to inform users of plant disease risk (Gent et al., 2011) and develop a fungicide recommendation on the application time (Newe et al., 2003). The optimal input with fungicides depends on the disease pressure and the climate in the individual season, but the susceptibility of the cultivar in particular plays a major role for the optimal input (Jørgensen et al., 2008).

In Estonia cereals cover nearly 50% of the cultivated area and are the crops that required the largest volume of plant protection products (European Commission, 2007). In 2016, the spring barley growing area in Estonia was 132.6 thousand hectares and the average yield was 3,517 kg ha^{-1} (Statistical Database, 2016). A major foliar disease of spring barley (*Hordeum vulgare*) is net blotch, caused by *Pyrenophora teres* (Drechsler, am *Drechlera teres* Sacc. Shoem) have a significant negative impact on yield and quality (Sooväli & Koppel, 2010). The fungus overwinters in infected barley residue or as seed-borne mycelium. Infection of barley leaves is greatest during humid and cool weather conditions.

Estonian farmers were often use fungicides routinely at high doses. On average they use one spray between stem extension growth stage (GS 35) (Zadoks et al., 1974) and the flag leaf sheath swollen (GS 45) to control foliar diseases on barley (Koppel & Sooväli, 2012). Routine sprays with strobilurins and triazoles are applied to reduce disease pressure uncertainty associated with management of *P. teres* in barley (Heinonen et al., 2013). Strobilurin and Succinate dehydrogenase inhibitors (SDHI)-resistance is confirmed in *P. teres* in Denmark (Jørgensen et al., 2015).

Pest risk models aim at detecting the onset of pest infestations and timing pesticide treatments through economic thresholds integrated in the model algorithms, thereby helping to minimize and optimize pesticide use (Jørgensen et al., 2008; Sønderskov et al., 2014). Models of the Danish Decision Support Systems PC-P Diseases concerning diseases and weeds of cereals were tested and adapted to Estonian conditions. The DSS-based system (I-Taimekaitse at http://itk.etki.ee) focuses on dose-response of fungicide use in Estonia since 2003. Numerous field trials were conducted since this time to support the use of reduced rates. The DSS model of disease control in spring barley has been upgraded in collaboration of Estonian Crop Research Institute and Estonian University of Life Sciences in frames of the project 'Development and implementation of an Internet based decision support system in plant protection' supported by Estonian Ministry of Agriculture. The trials were focused on the testing and adapting the Danish computer-based DSS PC-Plant Protection for disease control under Estonian conditions. The decisions that are supported are recommendations for the timing of the fungicide application to control the disease. All recommendations are based on field and weather data. The decision support predictions include the optimisation of fungicide applications. A DSS pest forecasting model reduces the complexity of integrated pest management (IPM) that may otherwise deter farmers from adopting a full IPM portfolio (Been et al., 2009; Sharma et al., 2011).

The aim of the study was to assess economic return of fungicide use in spring barley. In addition, the DSS-based and conventionally used spray practices for disease control and yield were compared.

MATERIALS AND METHODS

Field trials were conducted during 2003–2009 at five different geographical sites (Saku, Northern Estonia, Jõgeva, Eastern Estonia, Väätsa and Jäneda, Central Estonia, Põlva, Southern Estonia). All trials were laid out in complete randomized block designs with three replicates containing fungicide treatments and untreated control. Treated seeds were sown in 10 m² plots at the rate of 500 germinating seeds per m². All trials were sown at optimal time in the first week of May. Fertilizer rates of 80 kg N ha^{-1} Kemira Power 18 (18 N, 9 P_2O_5, 9 K_2O) were applied at 500 kg ha^{-1}. Additional nitrogen AN43 80 kg ha^{-1} was applied at shooting stage (GS 21–23). *Pyrenophora teres* was allowed to develop naturally in each trial. The conventional treatment, a DSS-based reduced rate treatment and untreated control were compared. Experimental treatments consisted of the fungicides listed in Table 1.

Table 1. Fungicide active ingredients and prices used in field trials 2003–2009

Product and label dose L ha^{-1}	Active ingredient g L^{-1}	Price € L^{-1}
Artea 330 EC 0.4	Propiconazole 250, cyproconazole 80	58
Folicur 250 EW 1.0	Tebuconazole 250	35
Mirage 40 EC 1.0	Prochloraz 400	21
Tilt 250 EC 0.5	Propiconazole 250	43
Tango Super 1.5	Epoxiconazole 84, fenpropimorf 250	21

Fungicides were applied with a bicycle sprayer equipped with 6 Hardy nozzles 4110–12 on a 2.5-m boom using 200 L of water per ha^{-1}. The five varieties Anni, Barke, Baronesse, Inari and Mercada for the experiments were selected to maximize the severity of the *P. teres* for the purpose of the DSS. Untreated certified seed was used for all varieties.

Incidence and severity of net blotch was assessed at growth stages (GS 69–73) on the flag leaf on 3 main tillers at ten randomly selected places per each plot. The percentage of leaf area infected with net blotch was visually estimated. Plots were harvested each year using a plot combine harvester on mid of August and yield was corrected to 14% moisture content and measured in kg ha^{-1}. Treatment Frequency Index (TFI) is calculated by dividing the amount of used fungicide dose by the standard appropriate dose.

Gross margin results were calculated assuming a grain price of 120 € t^{-1} and an application cost of 7.7 € ha^{-1} per treatment. All prices were used without value added tax.

All data were analyzed using the factorial analysis of variance (ANOVA) using statistical software Agrobase (release 20; Winnipeg, Canada). Differences between treatments of disease severity data were analyzed using ANOVA with a factorial analysis. Standard analysis of variance was performed to determine the main factors and interactions. Mean separations were made for significant effects with LSD at probability $p < 0.05$.

RESULTS AND DISCUSSIONS

Timing of foliar fungicide application. Over the period 2003–2009 there were 18 field trials where DSS was compared with the standard conventional fungicide applications. Results revealed that standard routine spraying was used mainly in GS 37–49, whereas in majority of years the DSS-based often suggested application in GS 55–65 and recommended rates varied between 30 and 60% of label dose rate (Table 2). This shows that the DSS recommendation leads to use the fungicide generally later than standard routine. This shows also that farmers accustomed treatment practice was slightly earlier. Under high disease pressure in 2003, 2005 and 2009 early treatment was needed in barley net blotch control according to conventional or DSS.

Table 2. Varieties, fungicide doses and times of application in field trials 2003–2009

Year	Variety	Rate product L ha^{-1}		
		GS 32–35	GS 37–49	GS 55–65
2003	Anni	Artea 0.25	*Tilt 0.17	
	Barke		Artea 0.25	*Tilt 0.15
	Baronesse		Artea 0.25	*Tilt 0.23
2004	Anni		Artea 0.25	*Artea 0.12
	Baronesse		Artea 0.25	*Artea 0.13
	Barke		Artea 0.25	*Artea 0.15
2005	Anni		Tilt 0.25	*Artea 0.15
	Baronesse	Tilt 0.25	*Artea 0.13	
2006	Anni		Tilt 0.5	*Artea 0.15
	Baronesse		Tilt 0.5	*Artea 0.15
2007	Baronesse			Tilt 0.5, *Artea 0.18
	Inari		Tilt 0.5	Artea 0.2
2008	Baronesse			Tilt 0.25, *Tango Super 0.6
	Mercada			Tilt 0.25, *Artea 0.25
	Anni			Tilt 0.25, *Mirage 0.36
2009	Barke		Tilt 0.25	*Folicur 0.47
	Baronesse		Tilt 0.25	*Folicur 0.5
	Anni	Tilt 0.25, *Folicur 0.54		

GS – Growth stage (Zadoks et al., 1974), * – Decision Support System application.

The optimal fungicide input depends on the disease pressure and the climate in the individual season, as well as the susceptibility of the variety plays a major role for the optimal input. Increased incidence of cereal leaf spot diseases over the last 40 decades has been noted in Finland (Jalli et al., 2011). The choice of appropriate fungicide, dose, and spray time is difficult and cause farmers to use higher doses to minimize the risks (Day et al., 2008). The reason why growers use conventional treatment programs is the monitoring and identification of the pests is most difficult. Time management seems to be the main hindrance for integrating the use of monitoring and majority of growers currently use a conventional treatment (Epstein & Bassein, 2003). DSS takes into account field specific conditions, and determines when or if fungicides are needed and resulting spraying come in a later growth stages. Decision support system can help to inform growers of plant disease risk and thus assist in accurately targeting events critical for management (Gent et al., 2011). Our results from experiments using different fungicide timings showed that conventional practice is to apply a high dose of fungicides at flag emergence growth stage.

Effect of treatment program on disease control

The disease pressure varied yearly under the influence of different temperature and precipitation patterns. Diverse climatic conditions during the experiment period (2003, 2008 – exceptionally wet with reduced temperatures and sunlight in the summer; 2004, 2007 – normal, the sum of precipitation was similar as long term average; 2005, 2006, 2009 – drought, the seasons had very warm and dry July) enabled good assessment of treatment regimes in different conditions. This variability increases uncertainty for growers as to best management options. Conventional and DSS foliar application programs provided effective net blotch control. DSS treatment was significantly better than the conventional and untreated (Fig. 1). In 7-year mean the effect of high input treatment was weak compared to the untreated.

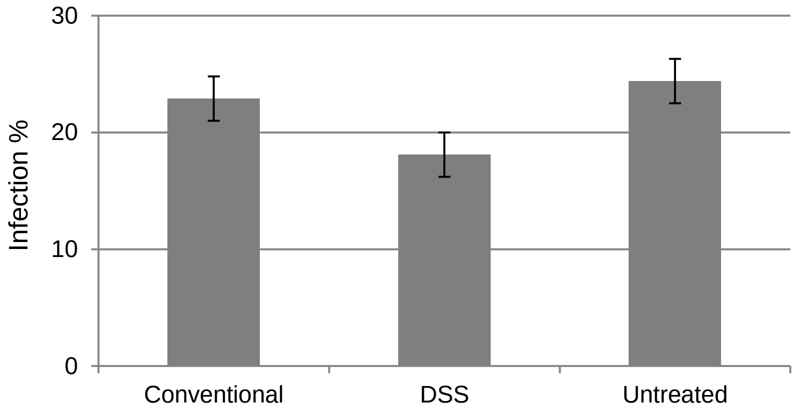

Figure 1. Percentage net blotch symptoms in spring barley leaf in average 2003–2009. $LSD_{0.05}$ = 13.03. DSS – Decision Support System.

Trials confirm control of net blotch is comparable weather conventional or DSS are used to make appropriately timed applications of fungicides. This is indication that the good disease control can be achieved from a relatively low fungicide dose, shown in Table 2. DSS uses weather data to predict infection periods and risk of epidemic progress (Audsley et al., 2005). Advice system DSS gave competitive disease control and reduced fungicide dose in line with or better control effect than the conventional treatments. Replacement of conventional treatment with 'environmentally driven' programs could reduce pesticide use in years with lower disease pressure (Epstein & Bassein, 2003). According to Cooper & Wale (1998) accurate timing of fungicide is crucial in the control of *P. teres* because of a short latent period and the potential for rapid disease development during optimum environmental conditions. The key time period for protection of barley is shortly before and after ear emergence (Young, 2012).

Effect of treatment time on yield

As experiment results differed between the years and there were different responses to fungicides but on average standard fungicide application worked better at GS 37–49 (Fig. 2). In average DSS-based fungicide application in later stage had good effect. The results illustrate that disease control effect for yield with DSS suggestions at earlier growth stages was low because used small doses did not protect the crop sufficiently for potential yield loss. To maximize the yield fungicides have to be applied between stem elongation (GS 32–35) and the heading to flowering (GS 55–65) depending on the susceptibility of the variety and the risk of disease. Furthermore the results verified that DSS-based late treatment gave very good effect with lower doses because DSS system took into account the prevailing weather conditions, the resistant level of the variety and pathogenic situation in the field.

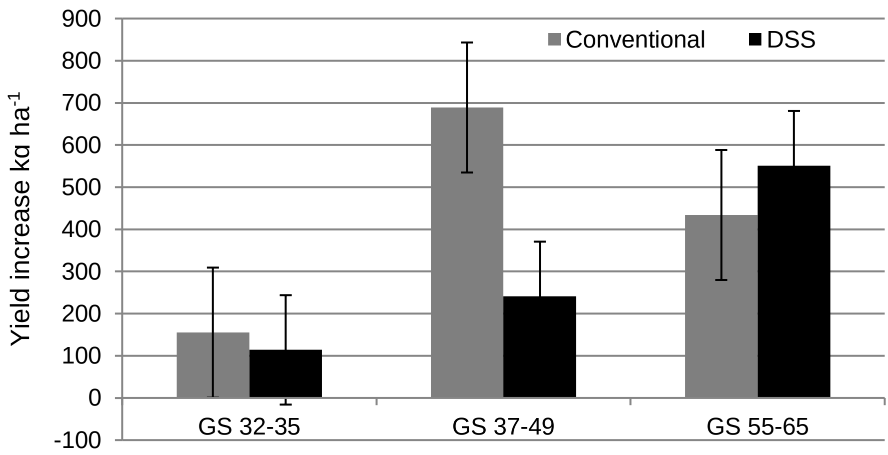

Figure 2. Average yield increase kg ha^{-1} in comparison with untreated crop at different treatment timings in spring barley in 2003–2009. $LSD_{0.05}$ = 572 (conventional), 436 (DSS). DSS – Decision Support System, GS – growth stages.

The results showed a higher yield response to applying a fungicide a conventionally compared to the DSS. As every year is different and there will be different responses to fungicide timings but on average traditional fungicide program seem to be economically justified. Only DSS fungicide program being applied later than conventional practice is likely to deliver bigger yield response in spring barley. Results are in accordance with these obtained by Day et al. (2008) that the optimization and the timing of fungicide treatment in relation to grain formation interacted with effect of disease development required a joined up approach to DSS.

Effect of different factors on disease severity and yield

Results of ANOVA verified that the year and location were major factors to influence the infection severity of net blotch (Table 3). Correlation (R^2 value) between growth region, year and variety contributed to the occurrence of net blotch about 88% (R^2 = 0.8797). The influence of the year correlated highly on the yield formation and had highest impact (R^2 = 0.8090).

Table 3. Sum of Squares of ANOVA of infection of net blotch and yield data

Effect	*d.f.*	Net blotch	*P*	Yield	*P*
Variety (1)	4	10.33	0.1140	6.94	0.0836
Year (2)	6	36.25	0.0000	52.03	0.0000
Location (3)	4	35.99	0.0000	11.45	0.0356
Treatment (4)	2	1.45	0.3019	7.22	0.0345
3 x 4	8	1.89	0.9001	0.76	0.9987
4 x 2	12	1.23	0.9981	1.94	0.9982
1 x 4	6	0.81	0.9590	0.56	0.9953
R^2		0.8797		0.8090	

d.f. – degrees of freedom, *P* – significance level, R^2 – correlation between trial factors.

Fungicide spray practices effects

The field trials clearly demonstrate that DSS recommended fungicide applications later than farmers conventionally used. In average of all trials the Treatment frequency index in common practice was 0.65 and in DSS programs 0.41 (Table 4). The cost of the different treatment expenses varied from 18.1 to 22.1 (€ ha^{-1}) and the reduction of expenses in DSS program was 20% less compared with farmers conventionally practices. Early season fungicide can be cost-effective depending on the variety and inoculum load.

Table 4. Treatment count and economic return on barley in different programs

	Conventional	DSS
Number of trials GS 32–35	3	1
Number of trials GS 37–49	11	1
Number of trials GS 55–65	4	16
Treatment frequency index	0.65	0.41
Treatment expense € ha^{-1}	22.1	18.1

DSS – Decision Support System, GS – Growth stage (Zadoks et al., 1974).

Selecting the appropriate dose is essential to optimize economic returns. Although, results suggest that, when fungicide dose was higher beyond crop needed, the increased dose would increase the economic loss. As defined by Paveley et al. (2001) and Jarroudi et al. (2015) the dose required to optimize positive return will vary substantially between years, sites and varieties. Profit is maximized when the dose of fungicide applied is just the amount that is needed.

Fungicide impacts on monetary terms

Comparing the benefit obtained by conventionally used practice and DSS-based applications, the average net revenue of conventionally used treatment strategy was higher (Table 5). In general, the long period results on yield and value for money show little bit better successful application time and dose were according to conventional practice. Hence the proposed DSS recommended use lower fungicide dose suggested that achieving cost effective means for maintaining sustainable barley production was less profitable. The average conventional treatment return was about 5 € more than the cost of DSS-based fungicide application in barley. Results implied that in average the minimal net revenue did provide no great an economic advantage.

Table 5. Spring barley average yield profit on a monetary basis

Treatment	Net revenue €	Benefit in terms of money € ha^{-1}
DSS	60.90	42.7
Conventional	65.44	43.3

DSS – Decision Support System.

Output from fungicide treatment and profit value

In average higher yield was achieved from convention treatment 4,670 kg ha^{-1} (Table 6). Over the trial period, the highest yield increase above the control due to conventional treatment was 55.1% and due to DSS was 53.6% in variety Baronesse in 2003. The results indicate that the average value of the yield output for Estonia, calculated at a price of 120 € t^{-1} varied between 42.75 (DSS) and 43.29 (conventional) € ha^{-1}. Results indicate that fungicides used in spring barley were more profitable when used as conventional treatments, but the trials data confirm comparable levels of control of net blotch can be achieved when DSS is used in place of conventional fungicide application.

Table 6. The highest output kg ha^{-1} and value € ha^{-1} for treated spring barley calculated by variety

Year	Variety	Yield kg ha^{-1}		Yield % of untreated		Benefit € ha^{-1}	
		Conventional	DSS	Conventional	DSS	Conventional	DSS
2003	Barke	4,507	4,455	1.1	-0.1	-16.62	-18.07
	Baronesse	4,666	4,622	55.1	53.6	176.47	178.55
	Anni	3,570	3,330	30.3	21.5	77.23	56.65
2004	Anni	4,261	4,337	4.1	6.0	-2.22	14.54
	Baronesse	5,630	5,620	30.6	30.4	136.03	141.87
	Barke	6,562	6,167	16.2	9.2	87.19	45.66
2005	Anni	4,633	4,516	2.8	0.2	-3.21	-15.31
	Baronesse	4,084	4,050	6.3	5.4	10.47	9.51
2006	Anni	4,019	4,125	3.9	6.6	-11.08	14.34
	Baronesse	4,578	3,827	19.6	0	60.8	-16.63
2007	Baronesse	4,497	4,656	4.7	8.4	-4.84	28.0
	Inari	4,552	4,483	20.0	18.2	61.88	63.36
2008	Baronesse	5,372	6,266	6.7	24.4	21.75	127.36
	Mercada	6,383	6,287	8.0	6.3	38.07	22.63
	Anni	5,429	5,340	15.5	13.6	68.79	61.41
2009	Barke	3,938	3,808	7.7	4.2	15.51	-8.24
	Baronesse	2,917	2,821	7.8	4.2	6.75	-10.47
	Anni	4,995	5,201	14.2	19.0	56.31	74.28
Average		4,670	4,662	14.1	12.8	43.29	42.75
LSD_{05}		458	491	6.8	6.7	26.33	28.73

DSS – Decision Support System, LSD – lowest significant difference.

The DSS has proved to give good control of pathogen and low product input. The information obtained from current study will use in further development of simplified DSS for the control of barley diseases. Such a system would improve control of net blotch while optimizing fungicide use. We agree with Day et al. (2008) that the decision system should give a ranked list of near optimal spray programs, not just the best and the potential of disease resistance of cultivars should be fully exploited and prophylactic spraying at present is unlikely to be profitable (Mercer & Ruddock, 2003).

CONCLUSIONS

This study confirms the importance of disease levels and fungicide used times and rates. The results allow growers to customize their fungicide treatments to minimise net blotch disease on susceptible varieties for cost-effective disease control.

1.DSS recommended the fungicide use in GS 55–65 that is generally later than conventional treatment in GS 37–49. DSS reduced fungicide application doses by 30 to 60% of the registered rate.

2.DSS-based treatment gave slightly better disease control effect compared with conventional treatment and untreated control.

3.A conventional treatment gave slightly higher yield response compared to the DSS.

4.DSS gave Treatment frequency index 0.41, reduction compared with conventional treatment was 1/3.

5.DSS reduced 20% the cost of treatment expense compared with conventional treatment.

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Analysis of *Arabidopsis* defensin-like genes and ovule development during fertilization and *Fusarium* infection

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Abstract. Defensins are small, highly stable antimicrobial peptides. Many defensin-like (DEFL) peptides found in flowering plant *Arabidopsis thaliana* are believed to have role in either natural immunity or cell-to-cell communication during fertilization. However, little is known about the DEFL peptides and their functions during these events. The goal of this work is to investigate the genes encoding selected DEFLs by observing their expression patterns during fertilization and *Fusarium graminearum* infection. According to the results 4 selected genes of interest (GOI) are downregulated after fertilization and infection and mock treatments are causing degradation and delay of development in treated ovules.

Key words: defensins, defensin-like peptides, development, double fertilization, *Fusarium graminearum* infection.

INTRODUCTION

Defensin-like genes form a large multigene family of more than 300 DEFL genes in the flowering plant *Arabidopsis thaliana*. These DEFL genes encode cysteine-rich peptides (CRPs) that are highly diverse and have conserved pattern of cysteine residues (Takeuchi & Higashiyama, 2012). Even though DEFLs are diverse, they mostly function in disruption of microbial membranes and have a ligand-like activity for cellular communication (Stotz et al., 2009). It is believed that DEFL genes have roles in male/female interactions such as intercellular communication during double fertilization and host/parasite interplays like defense reactions against pathogens (Ganz, 2003; Takeuchi & Higashiyama, 2012).

It is reported that *Arabidopsis* is applicable for studying *Fusarium* head blight (FHB), a devastating disease of cereal crops caused by *Fusarium* fungus. It is estimated that this pathogen causes huge economic losses each year worldwide (McMullen et al., 1997; De Wolf et al., 2003). Infection affects the amino acid composition, thus resulting in shriveled kernels (Beyer & Aumann, 2008). Grains harvested from infected plants are contaminated with dangerous mycotoxins, such as protein biosynthesis inhibiting deoxynivalenol and an estrogenic mycotoxin zearalenone (Häggblom & Nordkvist, 2015). In livestock, these toxins cause vomiting, reproductive defects, and liver damage and are considered harmful to humans as well (Gautam & Dill-Macky, 2011).

Mainly due to large size of genomes in cereal species and expensive resources required to research gene function in these plants, molecular basis of resistance to FHB is poorly understood. *Arabidopsis* has been noted for its potential for translational research in cereals (Brewer & Hammond-Kosack, 2015). Research done in *Arabidopsis* greatly reduces the time investment and expenses of the research due to short life cycle and small genome size of the model organism (Urban et al., 2002). The goal of this work is to investigate the *Arabidopsis thaliana* genes, encoding selected DEFLs, by observing dynamic change in expression of corresponding gene putative promoters during fertilization and *Fusarium graminearum* infection.

MATERIALS AND METHODS

In order to investigate expression and role of defensin-like proteins from *Arabidopsis* during fertilization and in the immune response to *Fusarium* various experiments were conducted. The whole research was divided into three parts (series of experiments). First part included the creation and analysis of marker lines to follow the activity of the corresponding putative promoters *in planta*. The second series of experiments were divided further into two sub-studies (pollination studies and pollination followed by infection (pollination-infection) studies). In this series marker lines were used to measure green fluorescence protein (GFP) signal intensity and quantify it in dissected ovules under the microscope. The third series were developmental studies where by using homogeneous marker lines the dynamic effect of various treatments on ovule development was observed. All experiments in detail are described in author's Master thesis available at University of Latvia Union Catalogue (Spalvins, 2016).

Marker lines (first series of experiments)

Paralogous genes identified by Takeuchi & Higashiyama (2012) were picked as a starting point. These 71 genes were then compared with the data of Huang et al. (2015) where expression profiles of 572 CRPs in ovule samples were analyzed. 53 of initial paralogous genes where also analyzed in these ovule samples, of which 18 genes followed the expression pattern of reaching peak expression at 6 hours after pollination. These genes were further investigated in publicly available expression database (Genevestigator) (Zimmermann et al., 2004). By analyzing mRNA sequencing data genes AT2G40995; AT5G38330; AT5G43285; AT1G60985 were picked because of the expression localization in developed flower and increased expression during treatment with various pathogens.

Putative promoters of genes AT2G40995; AT5G38330; AT5G43285; AT1G60985 were used for the creation of the marker lines.

Construct used for creation of the marker lines was pAT*G*****:NLS-(*x)eGFP line * (p – putative promoter, AT – *Arabidopsis thaliana*, AT* – chromosome number, G***** – gene number, NLS – nuclear localization signal (signal only expressed in nucleus), eGFP – enhanced green fluorescent protein, (*x) – number of tandem repeats of eGFP, line * – line number), p***** – * (p – putative promoter, ***** – gene number, * – line number) in short.

Consecutive scheme of making and analyzing marker lines is shown in Fig. 1.

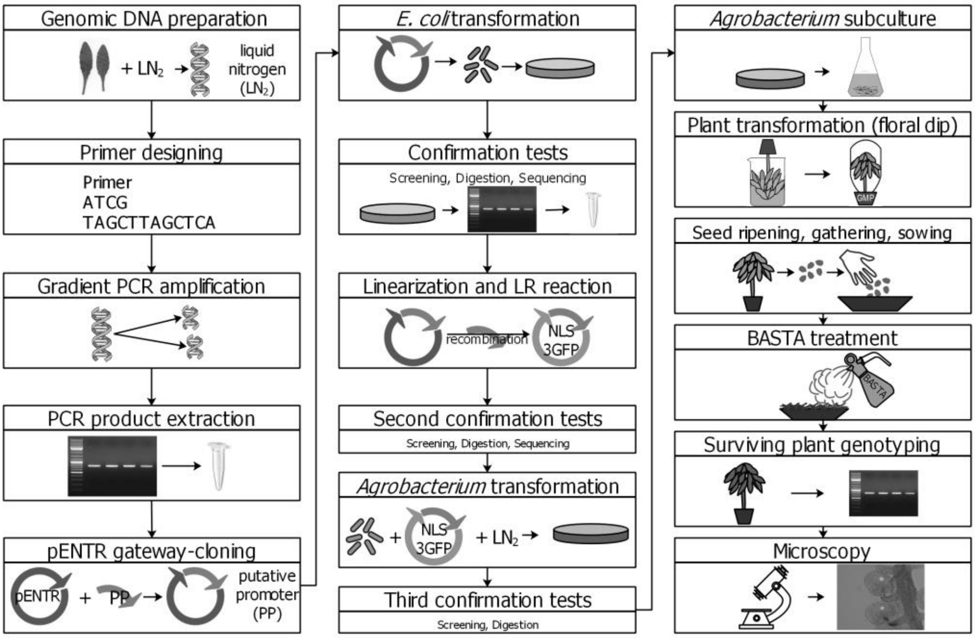


Figure 1. Overall scheme of creating and analyzing marker lines.

GFP signal quantification (second series of experiments)

In this series previously selected marker lines from the first series were used in order to investigate dynamic change of GFP signal intensity under such events as fertilization and infection. In order to have biological replicates for each gene two different lines were used.

In pollination studies flowers of the marker line plants were emasculated and pollinated. Pollinated pistils were collected and ovules dissected to measure changes in GFP signal intensity under the fluorescence microscope at 0 hours after pollination (HAP), 8 HAP, 24 HAP, 48 HAP. In Fig. 2, scheme of GFP signal quantification for pollination studies is shown.

In pollination-infection studies at specific time periods after emasculation and pollination, plants were infected with transgenic plant pathogen *Fusarium graminearum* DsRed(Fg8/1)and kept for 1, 2 or 3 days after infection (DAI)under moist conditions suitable for fungal growth.

Two controls were used in these studies – control and mock treatment. Control samples wereonly emasculated and pollinated with no other treatment applied. Mock treatment received exactly the same treatment as infection samples, only difference being that no pathogen was added in the mixture where plants were dipped in. After letting infection spread for 1, 2 and 3 days plants were removed from treatment chambers. In Fig. 3 scheme of GFP signal quantification for pollination-infection studies is shown.

Quantification process is described in detail in the next two sections: microscopy; signal quantification using ImageJ (Fiji) (Rasband, 2009).

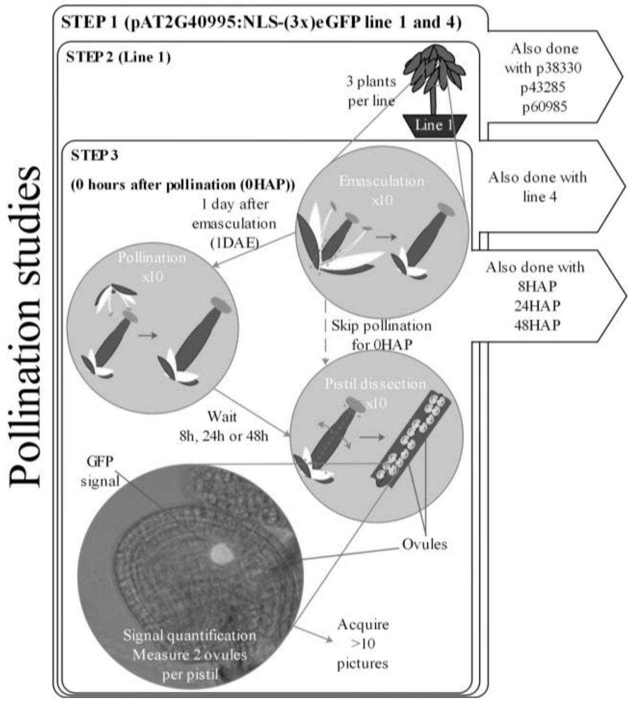


Figure 2. Overall scheme of GFP signal quantification for pollination studies.

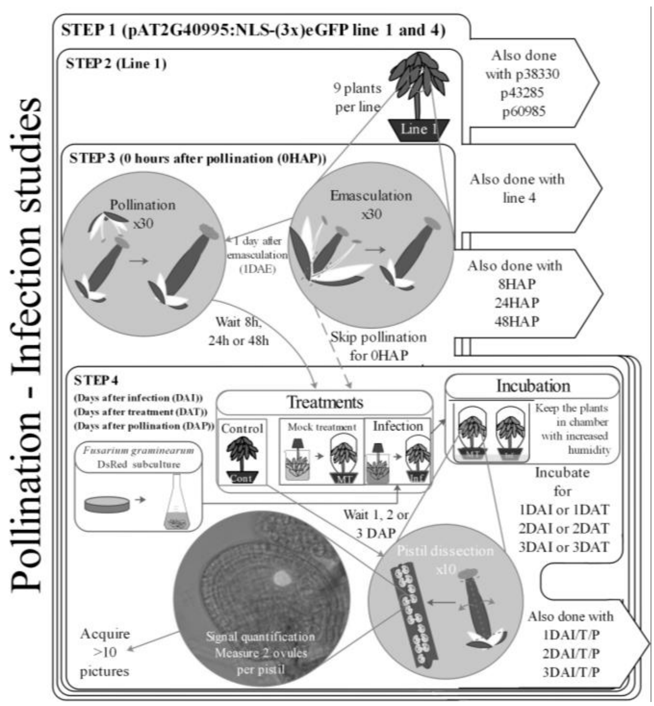


Figure 3. Overall scheme of GFP signal quantification for pollination-infection studies.

Fusarium graminearum DsRed (Fg8/1) Infection

Initially *Fusarium* was cultured in 5 g L^{-1} and 7.5 g L^{-1} of wheat media. Cultures were incubated in darkness at 22 °C for 1 week with moderate shaking. Before liquid *Fusarium* culture was set, additional infected potato dextrose agar(PDA) plates were prepared by spreading fungi with an inoculation loop on freshly made plates.

The concentration of *Fusarium* spores within the media was estimated with the following procedure: 50 mL of a liquid culture were filtered through autoclaved cotton gauze and centrifuged for 15 min at 22°C at 5,000 rpm. The supernatant was carefully removed, 30 mL of water were added to resuspend the pellet. The concentration of *Fusarium* spores was calculated by applying 20 µl of solution on the upper part of a counting chamber and then closing it with a coverslip. Spores within the gridded glass chamber were counted under the Nikon TS100 microscope. By knowing the volume in which the cells were initially re-suspended, the number of squares where spores were counted and the number spores within each square it was possible to calculate concentration. After that 1% Tween infection mixture was prepared in a beaker by diluting with water the *Fusarium* solution to a concentration of ~9*105 spores mL^{-1} in total volume of 300 mL.

Infection itself was done by floral dipping. After dipping, plants were covered with plastic bags and put into a treatment chamber (sealable 500 x 400 x 298 mm plastic box). Before closing the chamber, the bottom was thoroughly sprayed with water in order to keep humidity high. Other long day growth conditions remained as previously described. The treated plants were kept in the chamber for 1 DAI, 2 DAI, 3 DAI before collecting the treated pistils. The mock treatment proceeded as previously described, but without *Fusarium* on the dipping medium.

Uninfected control plants were emasculated and pollinated as previously described. While infected and mock treatment plants were kept in the treatment (incubation) chamber, control plants were kept in normal growth conditions for the same period of time before gathering the pistils.

Microscopy

Dissection under the stereo microscope was performed by fixing the pistil on double-sided adhesive tape and cutting with a syringe needle. Dissected ovules were put on new slide on 30 µl droplet of 10X PBS buffer and covered with a cover glass.

A method of quantification was developed to acquire data from dissected ovules. The exact exposure time was chosen based on properties of the marker lines. For example, if expression was not very intense the signal was weak, thus higher exposure time was necessary to document it. Because the aim of these experiments was to capture dynamic signal changes over time, the specific signal change after fertilization had to be taken in to consideration. If signal intensity decreased after successful fertilization, then it was crucial to choose such exposure time which would still be sufficient to capture presence of the signal even after expression decreased. It was also important to avoid picking high exposure time because that would result in oversaturation of the signal, thus calculated values of these oversaturated pictures could not be considered accurate.

Taking all these factors into consideration exposure times for each marker lines were chosen (Table 1).

Table 1. Specific exposure times for signal quantification of the candidate maker lines

Marker line	Exposure time (Chanel 2*)
p38330 line 1 and 4	200 ms
p40995line 1 and 4	300 ms
p43285line 2 and 17	1,000 ms
p60985 line 1 and 18	200 ms

**for additional information see Table 2.*

Supporting information

Table 2. Settings used for Axio imager M2 microscope

Settings	
	10x/0.45 M27 20x/0.8 M27 40x/1.4 Oil DIC (UV) VIS-IR M27
Camera	Axiocam 503
Camera adapter	1x Camera adapter
Channel 1. (Default channel used in sample analysis and imaging)	
Reflector	Analy. DIC Trans.light
Contrast method	Brightfield
Light source intensity	2–6 Volt
Channel name	TL Brightfield
Channel color	White
Exposure time (depending on the sample)	5 ms –1 s
Binning mode	1.1
Channel 2. (If sample had GFP marker in it, then used in combination with channel 1)	
Reflector	38 HE Green Fluorescent Protein
Beam splitter	495
Filter ex. wavelength	450–490
Filter em. wavelength	500–550
Contrast method	Fluorescence
Channel name	EGFP
Channel color	Green
Excitation wavelength	488
Emission wavelength	509
Exposure time (depending on the sample)	20 ms – 1 s
Binning mode	1.1
Channel 3. (If GFP sample was infected with *Fusarium graminearum*DsRed, then used in combination with channel 1 and 2)	
Reflector	43 HE DsRed
Beam splitter	570
Filter ex. wavelength	538–562
Filter em. wavelength	570–640
Contrast method	Fluorescence
Channel name	DsRed
Channel color	Red
Excitation wavelength	545
Emission wavelength	572
Exposure time (depending on the sample)	100 ms – 1 s
Binning mode	1.1

Stacked pictures of the single ovule showing signal were taken at 40x magnification using an Axio Imager M2 microscope (see Table 2. for microscope settings). When the stacked pictures of the treated marker lines were taken, quantification could be done in ImageJ. Stacked pictures were extended to more dimensions to create hyperstack multidimensional images. Whole sets of images were projected in such a way that only the highest value pixels were shown, thus creating a single picture which projected only the maximum values of the stack. After creating these maximum projection images the values of the signal were measured and the overall value of each of the signals was calculated by subtracting its value from the mean value of the background.

Signal quantification using ImageJ

Following steps were performed in order to quantify the GFP signal from the marker lines: a stacked image of 30 pictures was acquired (Fig. 4).

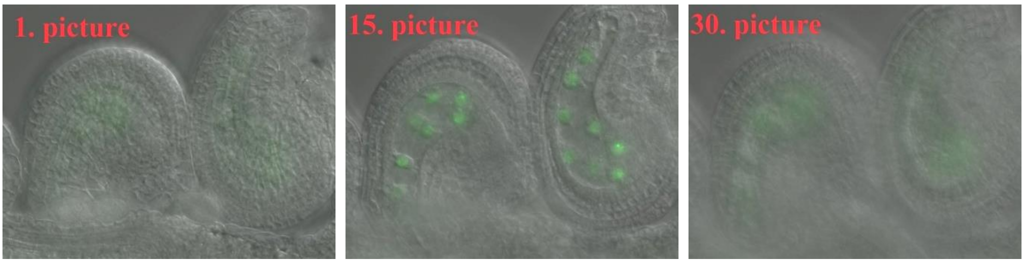


Figure 4. Example of pictures 1., 15. and 30 within single stacked image.

In ImageJ, the stacked image was opened and converted from image to hyperstack by going to Image/ Hyperstacks/Stack to Hyperstack. In Convert to HyperStack window following settings were inputted: Order: xyczt(default); Channels (c): 2; Slices (z): 30; Frames (t): 1; Display Mode: Color. After that image projecting the pixels of highest intensity was acquired by going to Image/Stacks/Z Project and in ZProjection window following settings were inputted: Start slice: 1; Stop slice: 30; Projection type: Max Intensity (see Fig. 5).

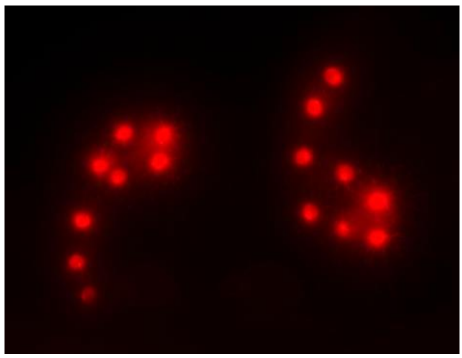
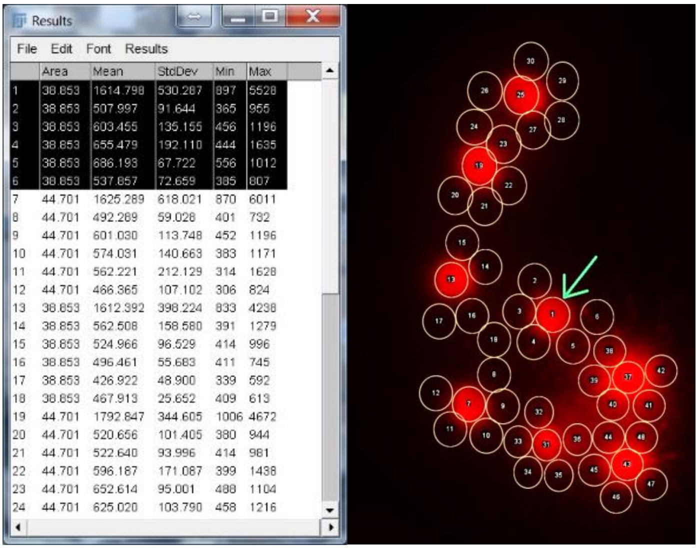


Figure 5. Example of stacked image projecting the pixels with highest intensity of all 30 pictures that the stacked image consisted of.

In the created image every nucleus that was showing GFP signal was circled around by using elliptical or polygon selection tool. After circling single nucleus M key was tapped in order to measure the intensity and T key was tapped in order to save the selection in ROI manager. For every nucleus measured, 5 same size circles around each nucleus were measured in order to acquire the background value (Fig. 6.).



Results

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	Area	Mean	StdDev	Min	Max
1	38.853	1614.798	530.287	897	5528
2	38.853	507.997	91.644	365	955
3	38.853	603.455	135.155	456	1196
4	38.853	655.479	192.110	444	1635
5	38.853	686.193	67.722	556	1012
6	38.853	537.857	72.659	385	807
7	44.701	1625.289	618.021	870	6011
8	44.701	492.289	59.028	401	732
9	44.701	601.030	113.748	452	1196
10	44.701	574.031	140.663	383	1171
11	44.701	562.221	212.129	314	1628
12	44.701	466.365	107.102	306	824
13	38.853	1612.392	398.224	833	4238
14	38.853	562.508	158.580	391	1279
15	38.853	524.966	96.529	414	996
16	38.853	496.461	55.683	411	745
17	38.853	426.922	48.900	339	592
18	38.853	467.913	25.652	409	613
19	44.701	1792.847	344.605	1006	4672
20	44.701	520.656	101.405	380	944
21	44.701	522.640	93.996	414	981
22	44.701	596.187	171.087	399	1438
23	44.701	652.614	95.001	488	1104
24	44.701	625.020	103.790	458	1216

Figure 6. Example of nucleus signal and background circling (left) and intensity measurement acquisition in ROI manager (right).

The average value of the five background measurements was subtracted (see example in Fig. 6 (right). Results, Mean column: rows 2–6 (average of 5 measurements – 598.196)) from the value of the nucleus (Results, Mean column: row 1 (1614.798)). Thus the signal intensity of single nucleus was acquired (See example in Fig. 6 (right). Calculated signal intensity of Nucleus 1 (green arrow) is 1016.602). Actions were repeated with every nucleus.

Mean value of all nuclei in single ovule represent single n (see Fig. 9).

Developmental studies (third series of experiments)

The third series of experiments were developmental studies where by using homogeneous marker lines the dynamic effect of pollination-infection treatments on ovule development was observed. In the beginning of these experiments, homogenous plants of the marker lines needed to be found. By using the data from pollination studies (second series of experiments) it was decided that pAT1G60985:NLS-(3x)eGFP line 1 was perfectly suited for the use as homogenous marker line with strong expression long after fertilization (see Fig. 9).

The plants were treated as described before in thesecond series of experiments for pollination-infection studies. The aim was to gather 10 pistils from each condition, dissect them and count, under the microscope, in what stages the ovules were. Fig. 7 represents how ovules looked like to be counted as being at one of the developmental stages. The following stages were recorded for each corresponding condition observed when looking at dissected ovules: degraded; 0 HAP/8 HAP; 10 HAP/12 HAP; 24 HAP; 48 HAP; 72 HAP; 96 HAP. Since pictures for this studies were taken at 10x magnification the ovules that looked like 0 or 8 HAP in this studies were counted in single data set because it was impossible to tell them apart at such a small magnification. Looking at the ovules and count them on larger magnification was not possible since capturing pictures of 10 pistils at every condition at 10x magnification already created data set of ca. 1,000 pictures. The ovules that looked like 10 and 12 HAP were also counted together because these ovules represented very small set of overall data (only 0.4–8.9% of all ovules counted; see Fig. 12).

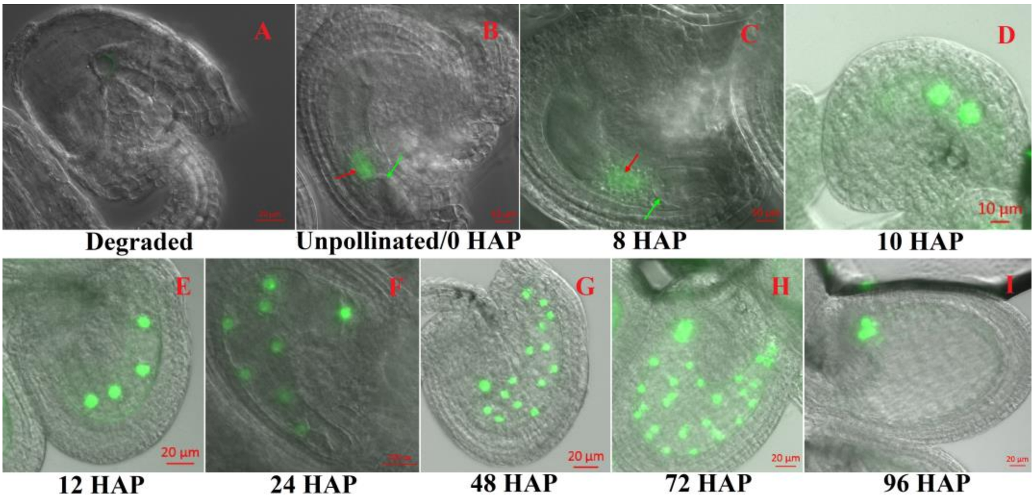

Figure 7. Developmental stages of the *Arabidopsis thaliana* ovules. HAP – hours after pollination. Green spots – GFP signal in central cell nucleus. A – degraded ovule, signs of degradation: ovule decrease in size (A compared to B); disintegration of outer and inner integuments; central cell is left covered with only a thin cell layer of endothelium. B – unpollinated/0 HAP, signs of unpollinated ovule: central cell nucleus (red arrow) still next to egg cell(green arrow). C – 8 HAP, signs of early pollination: central cell nucleus (red arrow) starts to move away from egg cell and synergids (green arrow). D – 10 HAP, first division of central cell nucleus (2 nuclei visible). For every following stage (E, F, G, H) number of GFP signals (number of visible central cell nuclei) doubles. I – 96 HAP, separate nuclei are not discernible anymore (picture taken 5 days after pollination was performed).

RESULTS AND DISCUSSION

Microscopy of the GFP marker lines

Individual T1 transgenic plants that survived BASTA treatment were marked and considered as individual line of the specific gene promoter construct. At the onset of flowering, pistils were dissected to check forthe presence of the GFP signal in the ovules. For each of the four DEFL gene (AT2G40995; AT5G38330; AT5G43285; AT1G60985)maker lines, two lines that showed the strongest signal were picked to grow the T2 generation for further studies (see Table 3). Most of the T2 generation marker lines showed GFP signal in the ovules, with majority of those lines showing signal in the nucleus of the central cell before (Fig. 8, A) and after (Fig. 8, B) fertilization suggesting that these genes might have some role in fertilization or development. Marker lines pAT5G43285:NLS-(3x)eGFP line 2 (p43285-2) and line 7 (p43285-7) showed signal in synergids (Fig. 8, C).

Signal smearing in the T2 generation of pAT2G40995:NLS-(3x)eGFP line 4 (p40995-4) can be seen in Fig. 8, E, where smeared signal (red arrow) is visible in the central cell, but it is not inside the nucleus (blue arrow) of the expressing cell even though

GFP had a signal for nuclear localization (NLS). T1 generation was showing proper signal, but analysis of the next generation suggested that marker line is not stable and thus unusable any further.

Table 3. Marker lines that were picked for further studies

Putative promoter DEFL gene marker lines	In short
pAT2G40995:NLS-(3x)eGFP line 1 and 4	p40995-1, p40995-4*
pAT5G38330:NLS-(3x)eGFP line 1 and 4	p38330-1, p38330-4
pAT5G43285:NLS-(3x)eGFP line 2 and 7	p43285-2, p43285-7
pAT1G60985:NLS-(3x)eGFP line 1 and 18	p60985-1, p60985- 8

* Unstable in T2 generation.

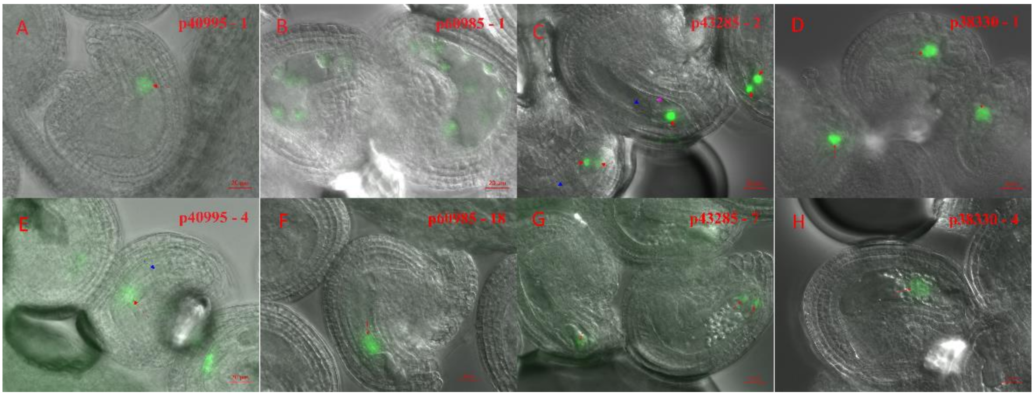

Figure 8. Example of signal expression in ovules of pATxGxxxxx:NLS-(3x)eGFP marker lines. A: red arrow – GFP signal in central cell nucleus; B: green regions – signal in central cell (endosperm) nuclei after fertilization; C: red arrow – GFP signal in synergids, blue arrow – central cell nucleus, violet – egg cell nucleus; D, F, G, H: red arrow GFP signal in central cell nucleus; E: red arrow – smeared GFP signal, blue arrow – central cell nucleus.

GFP signal quantification studies

In general, data acquired from pollination studies suggest that for all four genes promoter activity is being downregulated after fertilization (Fig. 9). Inthe two pAT5G38330 transgenic lines a noticeable upregulationwas observed at 8 HAP (Fig. 9, B), suggesting that this is characteristic of this specific gene during fertilization. Upregulation at 8 hours after pollination (HAP) suggests thatAT5G38330is involved in fertilization related process in the female gametes.

A trend of downregulation for the promotersof genes, pAT2G40995 and pAT5G43285 is observed in all of the respective lines (Fig. 9, A and C). Unfortunately, because there is only one line analyzed for AT2G40995, we cannot say for sure if this is a common property of this particular gene promoter. In relation to gene promoter pAT5G43285, both lines show the same trend of downregulation (Fig. 9, C). This data suggests that the genesare downregulated before fertilization occurs. For *Arabidopsis thaliana* it is known that fertilization takes place 6–8 HAP (Gooh et al., 2015). Therefore our data suggests that, unlike pAT5G38330 gene promoter, which has its peak expression during fertilization, pAT5G43285 has a role in the prefertilization events.

Because there is no data for earlier and intermediate time points like 2 HAP, 4 HAP, 6 HAP and 10 HAP. However, our observations correspond very well with other reports (Huang et al., 2015), thus proving that both our assumptions and quantification method are accurate.

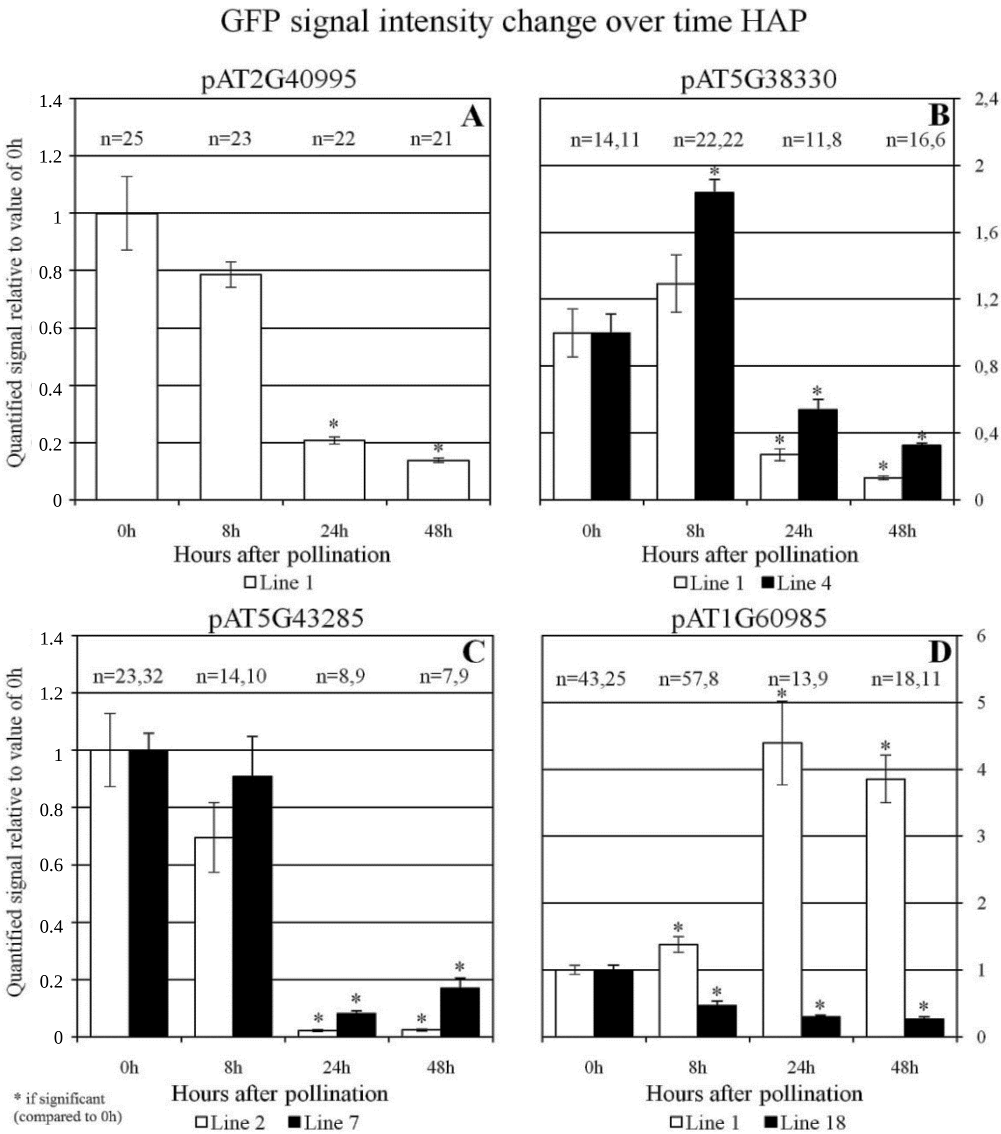

Figure 9. Pollination studies – GFP signal quantification of candidate marker lines at different time points after pollination. Columns represent the mean of the measured signal in multiple ovules (n – number of ovules), error bars represent the standard deviation of the data set. Significance test – One-Way ANOVA; unequal variance t-Test ($p < 0.05$). Note: 24 h and 48 h ovule samples had multiple nuclei with GFP signal in them, thus each n here represent mean of all nuclei signals within single ovule (for more detail see Fig. 7); also note the different scalings of y axes.

Gene promoter pAT1G60985 proved to be very problematic in these studies, because each of the lines show completely opposite trend of promoter activity (Fig. 9, D). With this data alone it is not possible to conclude which trend is representative of the actual properties of expression. If we compare our promoter activity data with the expression patterns of AT1G60985 during and after fertilization reported by Huang et al. (2015), it becomes clear that marker line p60985 – 18 accurately represent the nature of the gene. Concerning line 1 of pAT1G60985, we cannot use it to investigate the specific activity of the promoter for gene AT1G60985. Unlike line 18, it is active after fertilization takes place, showing increase in signal intensity as high as 4 fold at 24 HAP and 48 HAP when compared to the control value of 0 HAP (unpollinated) (Fig. 9, D). This peculiarity was perfect for using this line in developmental studies, where it was crucial to have strong signal after fertilization in order to make it easier to recognize the stage ovules are in (see Fig. 7).

In general, this study relies on the expression of gene promoter not the gene itself and thus might not be very representative of the characteristics of the actual genes in question, but because this method is relatively easy to perform, GFP signal intensity quantification is a good starting point for investigating the conditions in which the genes of interest might be transcribed.

In general, we propose ovule degradation takes place when ovules do not get fertilized for certain period of time. In our case first signs of degradation were usually documented 3 days after emasculation. If we take a look at ovules that have been emasculated and unpollinated in mock and infection treatments for 2 days, which means that since emasculations 3 days have passed, we can see morphological changes in ovules such as: ovule decrease in size (Fig. 10, A compared to C); disintegration of outer and inner integuments (Fig. 10, D, E, F); central cell is left covered with only a thin cell layer of endothelium (Fig. 10, E, F). Because all ovules in Fig. 10 were not pollinated, but degradation was present in both infected (Fig. 10, D, F) and mock treated samples (Fig. 10, C, E), it suggested that the cause for degradation might be the outcome of ovules not being fertilized.

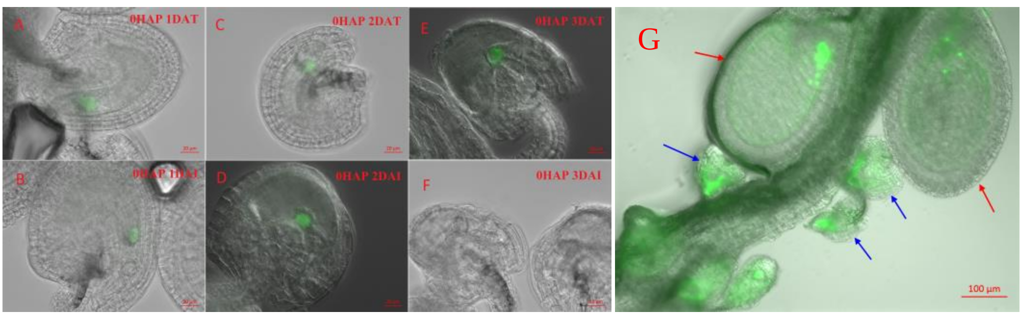

Figure 10. pAT2G40995:NLS-(3x)eGFP in pollination-infection studies. Ovule ageing and degradation. Signs of degradation: ovule decrease in size (A compared to C); disintegration of outer and inner integuments (D, E, F); central cell is left covered with only a thin cell layer of endothelium (E, F). HAP – hours after pollination; DAI – days after infection; DAT – days after mock treatment. Degradation of unfertilized ovules while fertilized ones continue developing (G). Red arrow – fertilized ovules (~120 hours after pollination); blue arrow – unfertilized, degraded ovules.

Unlike those ovules that were fertilized and resulted in normal seed development, unfertilized ovules probably degrade because they are not supplied with nutrients. To support this idea we can see in the Fig. 10, G how in the same ovary unfertilized ovules degrade while the fertilized ones continue development.

When observing the overall situation in the dissected ovules, degradation seemed more severe in samples that were infected when compared to the mock treatment samples (Fig. 10, C compared to D, E compared to F). This suggested the need for a detailed morphological characterization of ovule morphology before and after pollination and during infection.

Overall degradation would not have been a problem in these studies if it would not have affected the quality of the GFP signal. However in the 2, 3 DAI and DAT samples it was very difficult to find any ovules with signal in them. Most of the ovules in these samples looked like the ones seen in Fig. 10, E and F, substantially degraded and with no GFP signal.

Because initial 0 HAP pictures suggested that ovule degradation is caused by prolonged lack of pollination we also documented pistil samples 8 HAP, 24 HAP, 48 HAP which later on were subject to either 1, 2 and 3 days infection with *Fusarium* (1, 2, 3 DAI) or 1, 2, 3 days mock treatment (1, 2, 3 DAT). These samples got fertilized 1 day after emasculation and thus should not be affected by ovule degradation. By looking at these samples we found that, indeed, degradation was observed less frequently, but ovules were in very different developmental stages within a single pistil (Fig. 11, B), when compared to controls of the same age (measured in HAP) which were pollinated, but not infected or subject to a mock treatment (Fig. 11, A). By comparing the 48 HAP control (Fig. 11, A) with the picture of treated sample (Fig. 11, B), we can see that treated samples are in earlier stages of the development even though same time period has passed since pollination.

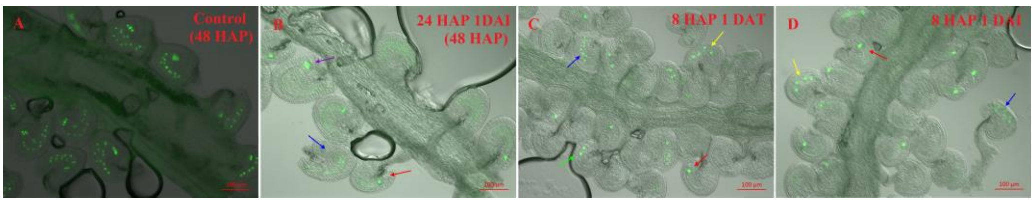

Figure 11. pAT2G40995:NLS-(3x)eGFP pollination-infection studies. Development delay of the ovules in treated samples when compared to control (B compared to A). A – all ovules are in stage of 48 HAP. Stages of the ovules (hours after pollination): red arrow – 0h/8h; green arrow – 10h; yellow arrow – 12h; blue arrow – 24h; violet – 48h. HAP – hours after pollination; DAI – days after infection; DAT – days after mock infection.

When comparing infection and mock treatments between each other (Fig. 11, C, D) we can see that both have ovules in very different developmental stages (from 0 HAP – 24 HAP) even though both pictures were taken 32 HAP (8 HAP combined with 1 DAT/I) and should have ovules mostly with eight nuclei (Fig. 11, C, D blue arrow; Fig. 7, F). This observation suggests that mock and infection treatments delay the development of the ovules.

In general, observations from pollination-infection studies further supported the need for a developmental characterization to investigate further the effect of pollination, infection and mock treatments.

Developmental studies

Developmental studies were perceived as necessary when initial pollination-infection studies were conducted and severe degradation and delay in development of the ovules were observed (see Fig. 10).

By looking at both 8 HAP and 24 HAP samples (Fig. 12; see deg. columns), percentage of degraded (deg.) ovules is increasing over time, with highest value being at 3rd day samples (Fig. 12, C, F; see deg. columns). This corresponds well with results gathered from pollination-infection pictures (Fig. 10). Thus confirming the previously proposed assumption that degradation increases over time.

Data also shows that degradation increases even if all samples were pollinated before the treatments, in contrast to previous results (Figs 10, 11). Here we see degradation increase (Fig. 12 from A to B to C and from D to E to F; see deg. columns) across all the samples. It is also important to point out that increase of the degradation is clearly visible in mock and infection treatment samples and to a lesser extent also in the control samples (Fig. 12, C, F; see deg. columns). This confirms that both treatments aggravate the degradation of the ovules.

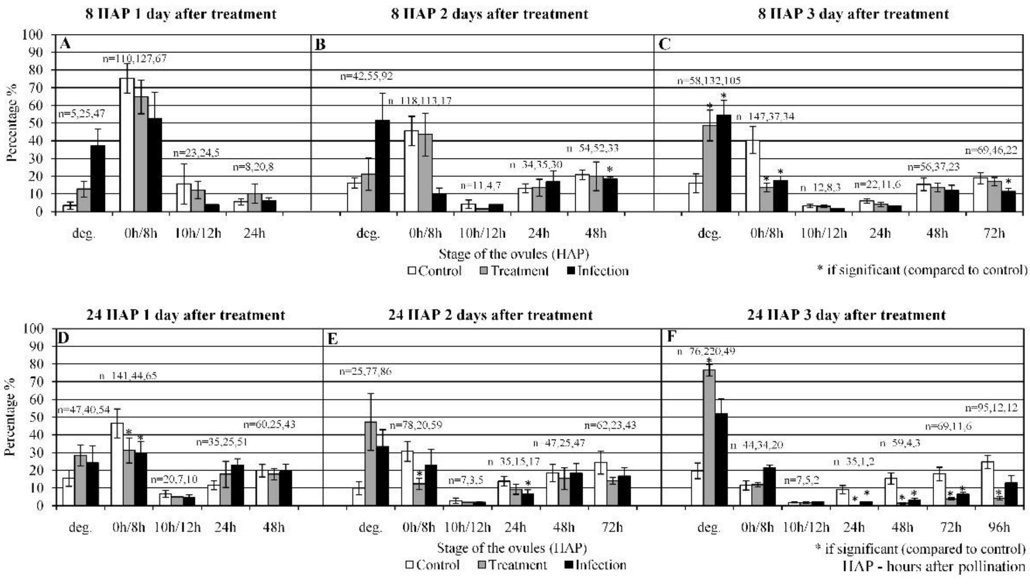

Figure 12. Results of developmental studies (A, B, C, D, E, F). HAP and control, mock or infection treatment effect on ovule development. Columns represent the percentage of stages ovules were in at given condition. Error bars represent the SD. Significance test – One-Way ANOVA; unequal variance t-Test: ($p < 0.05$). n – number of ovules at specific developmental stage counted within ca. 10 pistils (Example: figure A, from control (blue columns) samples in ca. 10 pistils 146 ovules were counted of which 5 were deg., 110 at 0h/8h, 23 at 10h/12h, 8 at 24h (for more detail see Fig. 7)).

Ovules at the stage 0h/8h (Fig. 12; see 0h/8h columns) show an opposite trend when compared to degrading ones (Fig. 12; see deg. columns), and decrease overtime (Fig. 12 from A to B to C and from D to E to F; see 0h/8h columns). The lowest percentage of 0h/8h stage ovules are in the 3rd day samples (Fig. 12, C, F; see 0h/8h columns). This makes sense since the 0h/8h ovules that generally look like and mostly are unpollinated ovules start to degrade over time. Interestingly in 8 HAP samples, infection treatment has highest degradation percentage (Fig. 12, C; see green deg. column), while for 24 HAP samples it was highest with mock treatment (Fig. 12, F; see red deg. column). It is not clear why time spent before treatment has such an effect, but it might be because at 8 HAP fertilization has not taken place in all ovules of a pistil (Gooh et al., 2015) making them perhaps more sensitive to infection.

When looking at samples, starting from 10h/12h to the oldest, we can see gradual increase of percentage by the stage in which the ovules were found (Fig. 12, B (see columns from 10h/12h to 48h), C (10h/12h to 72h), D (10h/12h to 48h), E (10h/12h to 72h), and F (10h/12h to 96h)). If we exclude degraded and 0h/8h data, we can see that the highest percentage ovules tend to be at the expected developmental stage corresponding to the treatment setting (Fig. 12, B, C, D, E, F; see from 10h/12h to oldest corresponding stage).

By performing statistical significance test (One-Way ANOVA; unequal variance t-Test) it becomes clear that mock and infection treatments delay the development of the ovules, at least in the 24 HAP 3 days after treatment samples (Fig. 12, F, see 24h, 48h, 72 h, 96 h columns). Overall, it is also clear that mock and infection treatments facilitate degradation of the ovules.

There is very little published information about ovule ageing, degradation and development delay in such specific conditions. Nonetheless, this study is giving a very interesting insight on how infection is affecting pollinated pistil and developing fruit. *Arabidopsis* is recognized as applicable model organism for translational research in cereals during *Fusarium* infection (Urban et al., 2002; Brewer & Hammond-Kosack, 2015). By improving the method so mock and infection treatments would not cause so much stress on samples and by looking for addition factors affecting the severity of degradation and development delay, as it has been done before (Carbonell-Bejerano et al., 2011), further research could prove to be very valuable in advancing the understanding of fungal infection effects on pollination, seed development and yield.

In this study three of GOI marker lines showed GFP signal in central cell and one in synergids. Many genes expressed in embryo sac are found to be essential for *Arabidopsis* reproduction and mutant analysis have identified multiple vital genes expressed in central cell, egg cell and synergids (Johnston et al., 2007). Central cells has vital role in pollen tube guidance (Chen et al., 2006; Li et al., 2015), while synergids trigger cell death after interacting with pollen tube (Sandaklie-Nikolova et al., 2007). Some CRPs have been recognized as essential in such reproduction events as pollen tube guidance (Li et al., 2015) in *Arabidopsis*. Even DEFLs are reported to have an important role in double fertilization (Okuda et al., 2009; Takeuchi & Higashiyama, 2012). Although our work lacks function studies, location of analyzed DEFLsis a good starting point for further investigation of these genes.

CONCLUSIONS

Marker lines of AT2G40995, AT5G38330 and AT1G60985 putative promoters are expressing GFP signal in the central cell of the embryo sac. Marker line of AT5G43285 putative promoter is expressing GFP signal in the synergids.

All four genes of interest (GOI) putative promoters – pAT2G40995, pAT5G38330, pAT5G43285 and pAT1G60985 – are downregulated after fertilization. Gene AT5G38330 promoter is upregulated 8 hours after pollination which is approximate time point of fertilization.

Mock and infection treatments are causing degradation and delay of development in treated ovules.

Method used for GFP signal quantification is reliable and can be used for investigating the conditions in which the GOIs are transcribed.

In future studies GFP signal quantification method can be used to advance our understanding of molecular basis of cereal resistance to FHB.

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Unified client service centres for rural development and smart governance in Latvia

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Abstract. Nowadays rapid increase of technological environment allows residents to be more mobile, choose working place, different from one's place of residence, start a new business or transfer company to rural areas, thus promoting polycentric development of a territory and increasing capacity of rural territories. It means that government should think of smart governance and service provision, providing different government services at one place. From June 2015 until December 2015, under the concept of improvement of public service system, the government of the Republic of Latvia established 75 unified state and local government customer service centres. Service centres operate on a local basis and in accordance with the uniform principles, provide customer with one place to access multiple public services. The objective of the present article is to evaluate the necessity and current activity of unified customer service centres and their contribution to smart governance of the country. The necessity for unified client service centres in the rural areas were therefore assessed with the analysis of attributable data, theory on establishment of such centres, and residents' survey, which showed that a big part of customers of the present centres are residents of neighbouring towns and cities where the regional governmental and local institutions are located, but they are attracted by the possibility to receive all the necessary services at one time.

Key words: unified service centres, smart governance, development, rural areas, etc.

INTRODUCTION

UNESCO (2010) states that globalisation is the ongoing process that is linking people, neighbourhoods, cities, regions and countries much more closely together than they have ever been before. This has resulted in our lives being intertwined with people in all parts of the world via the food we eat, the clothing we wear, the music we listen to, the information we get and the ideas we hold. While globalisation is not a new process, it has accelerated rapidly since World War II, and is having many effects on people, the environment, cultures, national governments, economic development and human well-being in countries around the world.

Though globalisation widens the possibilities of people, it also have a negative impact on population structure and density of certain areas – more and more countries face the fact that rural areas become uninhabited; people work and live in cities that can offer more necessary services than rural areas.

Globalization, itself, does not make any particular distinction between urban and rural areas, however the impact of their activities on rural areas is often much greater because rural areas are sparsely populated, spatially isolated, they lack the range and depth of social and financial resources, moreover, allocations for social services are seen as an exploitable resource by transnational corporations.

Such situation leads to monocentric development of countries, providing low competitiveness, insufficient urban development and weak links with surrounding territories. It should be taken into consideration that nowadays the majority of population, production and consumption occur in urban areas, thus, they are important units in the economic, social and environmental analyses as well as they are a base for the strategies of development policy. Evenly distributed urban network is beneficial for polycentric and balanced development of a country (Haite, 2013). In order to provide polycentric rural development of country, different measures sould be established to meet residents' requirements, which is so-called smart governance.

Since both 'smart government' and 'smart governance' terms are used in literature, a distinction must be given: 'Government occurs when those with legally and formally derived authority and policing power execute and implement activities' and 'Governance refers' to the creation, execution, and implementation of activities backed by the shared goals of citizens and organizations, who may or may not have formal authority or policing power. Therefore, it is concluded that smart governments implement smart governance initiatives (Maheshwari & Janssen, 2014).

Such problem is widely experienced also in Latvia, therefore the competent ministries are thinking how to provide smart governance in all territory of the country, thus stimulating residents stay or even move to rural areas.

In 2015, the Latvia's e-index was released to assess the necessary approaches and provide solutions for a more efficient development, as well as to identify the best examples implemented by other institutions and thereby enable exchange of experience and motivate further development of the digital transformation.

From June 2015 until December 2015, under the concept of improvement of public service system, 72 unified state and local government customer service centers of district significance and 3 centers of regional significance were established. Service centers operate on a local basis and in accordance with the uniform principles, provide customer with one place to access multiple public services. Such centers are significant for rural territories, since residents of rural areas can receive services of same quality and amount as residents of urban areas. The present approach can be considered as smart governance, increasing polycentric development of the territory and country itself, but the activity of the particular service centres should be assessed both by authorities and residents that have used their services.

MATERIALS AND METHODS

To carry out the present research, the authors used topic-related research papers, the analysis of attributable data, theory on establishment of such centres, and information available of the Central Statistical Bureau (CSB). The research methods employed the monographic and descriptive methods, analysis and synthesis, as well as logical and constructive methods. The main data was collected from survey that was provided from October, 2016 to January, 2017. The survey consisted of different questions concerning activity of unified client customer service centres, their identification, and provided services in order to determine, whether establishment of such centres develops the idea of smart governance in the country.

RESULTS AND DISCUSSION

The meaning of terms ‘small government’ and ‘smart governance’

It is quite fashionable to speak about smart government and governance, provided by it nowadays, though there is no common consensus for the terms itself. Smart government is a term, which lacks a clear definition. Although the prior definition for ‘smart’ leads to a public administration that utilizes servant systems, such a definition comes to close to past electronic or digital or internet-based (or e-) or even open government definitions like: the utilization of the ICT by governments in order to become more effective, efficient, transparent and accountable (Anthopoulos, et al., 2015).

Different scholars provided alternative meanings of the term ’smart government’, providing diverse understanding of the present term. On the one hand, Mellouli et al. (2014) and Cellary (2013) name it as the extensive use of technology by governments to perform governmental tasks, while Taylor (2015) and Gil-Garcia et al. (2015) relate the terms ’smart city’ and ’government’ demonstrating innovation and intelligence for local or governments as the means to increase their efficiency and effectiveness. On the other hand, Harsh & Ichalkaranje (2015) present a claim that smart governments utilize the power of ’data’ in their attempt to improve public services; to enable an integrated, seamless service experience; to engage with citizens; to co- develop policies; and to implement solutions for well-being of the community.

Nevertheless, Anthopoulus & Reddick (2015) determined that smart government does not ‘ignore’ smart city. Instead, smart government leads smart city development, while it uses smart city as an area for its practice (collaboration and service co-production testing etc.). In this respect, there have to be complementary forces that interrelate these terms and have to be identified. It means that smart government develops smart city with help of different approaches that is called smart governance.

Direction of smart governance in Latvia towards rural development and policentricity

Smart governance is usually referred to technology in order to facilitate and support better planning and decision-making. It is about improving democratic processes and transforming the ways that public services are delivered. It includes e-government, the efficiency agenda and mobile working.

Nowadays rapid way of living request different approaches of delivering public services, streamlining electronic government and administrative modernization processes, etc. Countries adopt new laws concerning e-government, establishing new e-services to satisfy the needs of residents. For example, the German e-government (EGOV) law postulates simplified and reliable administrative processes, needs orientation, economic efficiency, ecological sustainability, modular and adequate ICT support, and a leading role in EGR; however, despite these high aspirations and its economic weight, Germany ranks only 15th in the most recent UN EGOV rankings (UN

E-Government, 2016). In 2015 Latvia started e-government development – the Latvia's e-index was released, being the first national-level initiative helping state and municipal institutions to evaluate their digital development, to assess the necessary approaches and provide solutions for a more efficient development, as well as to identify the best examples implemented by other institutions and thereby enable exchange of experience and motivate further development of the digital transformation (e-Government in Latvia, 2016). Nevertheless, Latvia ranks only the 45th in UN EGOV rankings, demonstrating the lowest rank among the Baltic States (comparing to 13th place for Estonia and 23th place for Lithuania).

Development of e-government in a country also provides development of rural areas, since e-services of different types can be provided in all territory of a country, thus decreasing monocentric model.

In monocentric model there is populated agglomerate with one leading centre, i.e., it is a settlement, where only one centre is dominant in all fields. Polycentric development in its turn is an alternative to monocentric development since it tries to equalize resources and ensure balanced growth of territory. The spatial structure of territory polycentrism has been created, resigning from hierarchical spatial organizations and developing horizontal network. The spatial structure of polycentrism is related to morphology of settlement system. Reviewing city systems from continental or state point of view, polycentrism appears if system is characterized by several cities in different levels instead of one city, which is dominant. Polycentrism exists in regional or local scale, if two or more cities have functions, which supplement each other and, moreover, if cities cooperate with each other in order to work together as one, greater city.

Nowadays Latvia represents relatively weak urban structure; habitation net regarding development and growth experiences high inequality. Initiating EU programs for promoting the development of Latvian territory the increase in population number has been set out as a significant effective indicator, which is the basic factor for promoting polycentric processes in country territory. The greatest part of Latvian residents ~ 61% live in cities, while only 39% of total population number live in rural areas (Fig. 1).

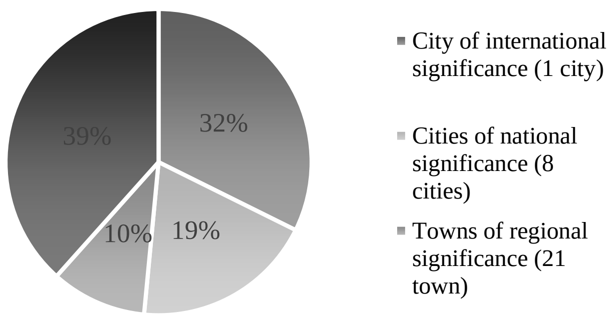

Figure 1. Distribution of Latvian residents according to their place of living in 2016, %.

The increase of Riga specific weight in total economic system has a direct relation with the increase in amount of population in Pieriga agglomerate. Concentrated resource mass (human resources, infrastructure of entrepreneurship and public services, finance capital etc.) in Riga exceeds the critical mass of other regions for several times. At the same time in Latvia there is a wide, comparatively equally located city network where the number of residents gradually decreases.

The government and local authorities of Latvia has provided different measures to attract specialists to rural areas, i.e., providing grants and scholarships to specialists of certain fields that are necessary for a particular territory (such as medicine, engineering etc.), encouraging employees for regional mobility by providing financial compensation of transportation and living costs for the first four months after the commencement of the employment (excluding Riga).

It should be mentioned that in 5 October, 2016 the Investment and Development Agency of Latvia and the Central Finance and Contracting Agency concluded an agreement No. 3.1.1.6/16/I/001 on the operational programme 'Growth and Employment' under priority 'Regional business incubators and creative industries incubator' that should be implemented until 31 December 2023. The project aims to support the establishment and development of new viable and competitive businesses in Latvian regions, providing entrepreneurial advice, training and measures of business issues, mentor support, the environment (premises) and co-financing grants of operating costs for individuals (authors of business ideas), which are or are going to carry out economic activities, small (micro), small and medium-sized enterprises. Under the present project 15 new business incubators in various Latvian towns, including specific creative industries business incubator in Riga were established and currently accept ideas of potential entrepreneurs.

Such measures in long-term could attract specialists and entrepreneurs to rural areas, but at the same time government should think of mechanism how to provide all the necessary services as close to entrepreneurs working place as possible, since it can be disturbing and difficult to run business if all necessary institutions are located in other municipalities or even regions.

The establishment of unified client customer centres as a part of e-government

All countries of the world understand that e-government is a significant part of smart governance, therefore tries to provide as many services as possible in electronic format. For example, Estonia, where 99% of public and municipal services are available in the electronic environment, spends on maintenance of its IT 40 times less than Finland and 400 times less than the UK. More than half of 300 different public services are currently available in Latvia in the electronic environment. This has contributed to the reduction in the administrative burden, increased efficiency of the work of public administration, also ensuring the availability of data for evidence-based decision-making. Thus, after its first year of operation, it was concluded that over 1.1 million euro per year or a man/day per businessperson might be saved from automation of the acquisition of necessary information on tax arrears, criminal records and insolvency for procurement purposes.

Digital tax administration and an electronic payroll tax book create truly 'tangible' time and money savings, while the availability of territorial development plans in the electronic environment has increased the transparency of this process. Overall, almost one third of service requests are made electronically and users are more satisfied with e-services than the use of services on site. Unified customer service centres are also important. Namely, an e-government allows offering services not physically available in the specific area in one place, while employees of these centres may help the population to use the electronic tools.

From June 2015 until December 2015, under the concept of improvement of public service system, 72 unified state and local government customer service centers of district significance and 3 centres of regional significance were established (Fig. 2). Service centres operate on a local basis and in accordance with the uniform principles, provide customers with one place to access multiple public services. Unified state and local government customer service centers are organized in a similar way: centers of district significance provide a standardized minimum service basket, for example, receive a service request and pass out a result of service; and offer consultancy on the content of the service and assistance in applying for e-service. Centers of regional significance of local government serve only those state service branches which are not located in these centers. It creates the availability of one place that offers state and local government services, on the basis of local government and in cooperation with the state. Centres offer consultancy on the extent of the state services and assistance in regard to the application for e-services. On a selective basis, state institutions come together ‘under one roof’ (e-Government in Latvia, 2016).

Figure 2. Distribution of Unified client service centres in Latvia, 2016.

One of Latvia’s digital environment success stories is ‘universal log-in’ or authentication for public services, which is used not only by the latvija.lv portal, but also by 30 other public administration portals and information systems. It is a solution envied by many EU countries and considerably easing us the introduction of trans-border electronic identification prescribed by the European Union in its eIDAS regulation, which will enter into force this July. According to this regulation, the population of European Union Member States with their national electronic identification tools will also have access to electronic services of other Member States (BiSMART technology platform, 2016).

Employees of unified client customer service centres not only teach their clients how to work with this universal log-in and therefore use all the services, provided by latvija.lv portal, but also provide certain services of such state institutions as:

1) State Employment Agency;
2) The Office of Citizenship and Migration Affairs;
3) State Labour Inspectorate;
4) State Revenue Service;
5) State Social Insurance Agency;
6) State Land Service;
7) Rural Support Service.

Unified client service centres are not new phenomena – such institutions have been established and fully works in different European countries, though some of them, such as Estonia, have gone further and provide electronical submission of documents (including online application for eID documents) in most of the abovementioned institutions. The equivalent in Latvia, Internet portal www.latvija.lv, currently provides mainly informative data, however section ‘e-Services’ are continuously developed, providing different new services.

Assessment of unified client service centres

In order to understand, whether project of unified client service centres are successful, useful for residents of Latvia and corresponds to idea of development of polycentrism in Latvia, a survey was provided. A survey was held in all Latvia from October, 2016 to January, 2016. The survey consisted of 10 different questions concerning the activity of unified client service centres. The survey was issued to randomly chosen people at unified service centres and it was available online that covered whole territory of Latvia. The total number of respondents was 733, covering all regions of Latvia.

After the data provided by the Central Statistical Bureau there were 1,968,957 residents of Latvia on September 2016. Taking into consideration the total number of residents and confidence level of 95% the sample size is 384 residents; therefore it can be determined the results are valuable; though the results of survey cannot be taken as nationally representative.

Public Relations companies, involved in the present project declare ‘there have been more than 230 articles in national, regional and local government newspapers, on internet portals and internet versions of newspapers, as well as on the websites of various institutions and organisations. Eleven television stories and several radio broadcasts were focused on the unified client service centres. Posters, brochures, internet banners, ads, direct e-mail and communication in the social media environment helped to deliver information about the centres to residents of cities and administrative districts that were involved in the project. This led to more than 13,000 clients visiting centres, and a survey of those people found that nearly all of them were satisfied with the services that were offered at the centres and the quality thereof’ (Comperio, 2015).

After the results of survey, it can be determined the information about such centres is unsatisfied, since only 48.6% of all respondents are informed about the existence of such centres. Taking into consideration the coverage of unified client service centres and length of their activity (in years) the government and local authorities should think of new marketing strategy to inform residents of possibility to receive the necessary services in one place.

However, 86.5% of those respondents who are informed about the centres have used the services at least once per year; moreover, they admit that quality of services provided is in high level and they definitely will use the services un unified client centre also in future, if it is necessary. The survey shows that residents in 2016 mainly used services of Rural Support Service (35% of residents have used such service for one time and 15% have used them two times and more) and State Revenue Service (25% and 8%, namely), but such services as State Labour Inspectorate and State Employment Agency are not used in 2016 (Fig. 3).

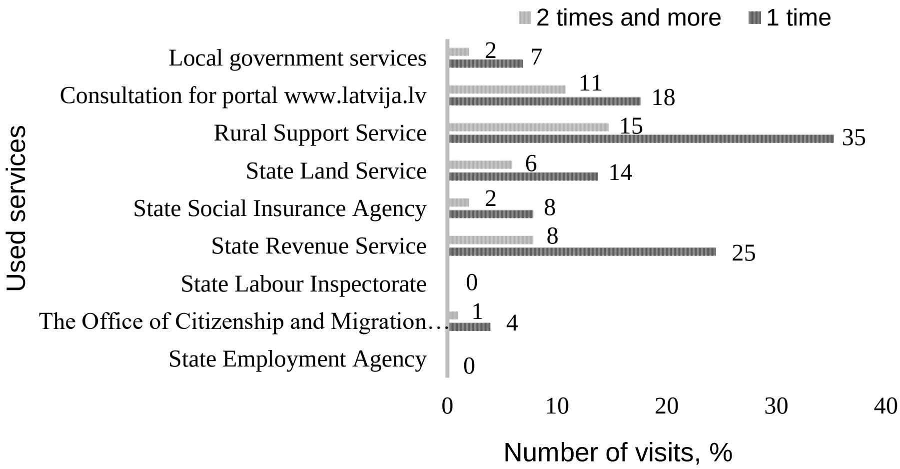

Figure 3. The purpose and frequency of respondents' visits to the unified client service centres in 2016, % (n = 356).

It needs to admit that quite frequently residents visited unified client centres to receive consultation for Internet portal latvija.lv, which means that further they will use Internet to receive different data and services. Such tendency show that people chooses electronic format to receive services instead of face-to-face visits, saving up their time and money.

Asking for the reasons why residents choose to go to the unified client centres, 53% stated that centres are located closely to their place of living; therefore, there is no necessity to spend time and money to visit regional centre or the town to receive services (Fig. 4). It is especially convenient in places like Roja (Kurzeme region) or Auce (Zemgale region), where the closest town for receiving services is within 40–50 kilometres.

It must be taken into consideration that Vidzeme region has the greatest number of unified service centres (13), they are not widely established in Kurzeme region – only 9 municipalities have implemented such service, moreover – none of them is located in centres of regional or national significance (Fig. 2). Northern part of Kurzeme is almost uncovered – there are only 2 unified service centres located, therefore in the future project stage should re-plan the location of unified client service centres, providing polycentric development.

38% admitted they use services of unified client centres, since it provides different services at one place, moreover 47% stated the employees were professional and responsive – if there were no possibility to provide some services at place, employees provided thorough consultation how to receive the necessary information and services, helped to make payments via i-bank and even helped to find information that was not related to provided services.

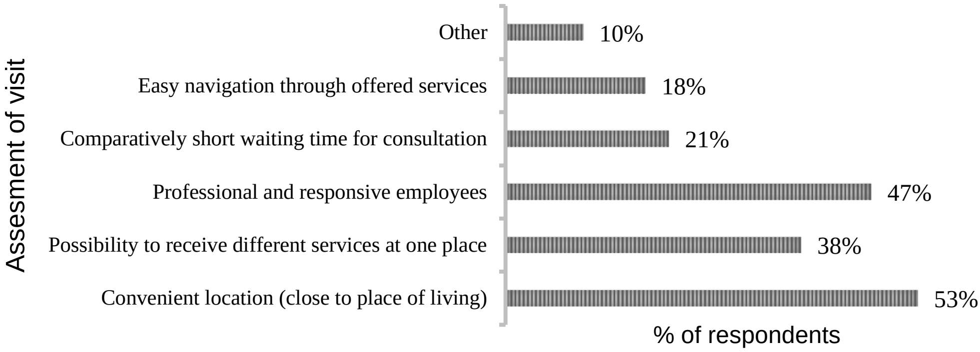

Figure 4. The assessment of unified client centres by respondents after visit in 2016, % (n = 356).

All respondents were asked to provide the overall assessment of unified client service centres in Latvia taking into consideration typical five-level Likert scale, where '1' stated 'there is no necessity of such centres' and '5', in its turn, stated 'the establishment of centres was successful; centres have future potential'.

The results were quite diverse, since the greatest part of respondents, as it was stated before, were not even informed about such centres (Fig. 5). Main part of respondents (37%) stated their attitude as neutral, but pointed out there is a lack of information about centres, their activity and offered services. Negative attitude about the establishment of centres showed 27% of respondents, but 34% of respondents, in their turn, considered the idea and establishment of unified client service centres as positive, though pointed out there is a necessity for more real function, not only advisory work.

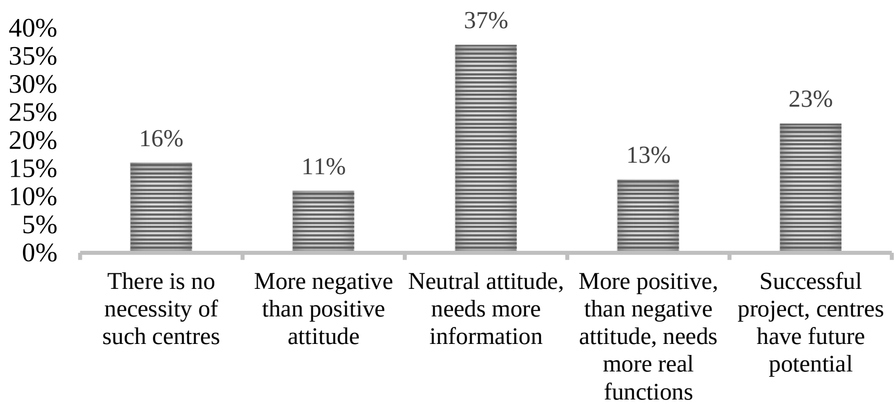

Figure 5. The overall opinion about the establishment of unified client service centres by the respondents (n = 356), %.

Taking into consideration the answers, provided by the respondents it can be stated that the project of unified client centres which has started already in 2014 has not justified itself and needs a massive future work in order to expand the project and make it functional. This project should implemented not only with more real functions, but also provide extensive marketing strategy directed towards different target audiences (middle-age, retired, non-residents, namely).

CONCLUSIONS

- Smart government involves the meaning of smart city, since smart government leads smart city development, while it uses smart city as an area for its practice. It means that smart government develops smart city with help of different approaches that is called smart governance.
- Concentrated resource mass in Riga exceeds the critical mass of other regions for several times. At the same time in Latvia there is a wide, comparatively equally located city network where the number of residents gradually decreases, providing monocentric situation in the country.
- Development of e-government in a country provides development of rural areas, since e-services of different types can be provided in all territory of a country, thus decreasing monocentric model.
- The government and local authorities of Latvia has provided different measures to attract specialists to rural areas that are necessary for a particular territory, encouraging employees for regional mobility by providing financial compensation of transportation and living costs for the first four months after the commencement of the employment.
- Unified client service centres are not new phenomena – such institutions have been established and fully works in different European countries. Latvia has established Internet portal www.latvija.lv that currently provides mainly informative data, however section 'e-Services' are continuously developed, providing different new services.
- After the results of survey, it can be determined that the information about such centres is unsatisfied, since only 48.6% of all respondents are informed about the existence of such centres. Taking into consideration the coverage of unified client service centres and length of their activity (in years) the government and local authorities should think of new marketing strategy to inform residents of possibility to receive the necessary services in one place.
- Asking for the reasons why residents choose to go to the unified client centres, 53% stated that centres are located closely to their place of living, therefore there is no necessity to spend time and money to visit regional centre or the town to receive services.

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Operator's behaviour measuring methodology inside off-road vehicle cabin, operator's focusing scheme

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Abstract. Operator's workplace design takes a priority to be developed in order to reach the highest possible level of Quality, Safety and productivity. Continual improvement of the workplace is yield from studies carried out on different approaches, each approach shall keep into consideration many aspects, in this research; the results will be used for feeding the productivity aspects with valuable and reliable input data using relatively simple engineering solutions. This research is made based on literature of the accumulated knowledge from diverse fields in which different studies and analysis are made to provide the necessary input for Human Centred Design process, adopting the-state-of-the-art technologies and methodologies used for data collection and analysis for Human behaviour inside the dedicated workplace. Better understanding of the operator's Gaze in addition to the change according to the mental and physical workloads inside the tractor cabin will lead to optimal designs for higher productivity operation.

Key words: off-road vehicle, operator's behaviour, eye tracking, focusing scheme.

INTRODUCTION

Human-centred-design approach is considered one of the most effective factors enhancing the productivity of vehicles used in the industrial and agricultural fields. The development of operator's workstation needs to be based on deterministic data which is validated, verified and dependable.

Due to the operational nature of multi-tasking off-road vehicles, operators need to spend long working hours; which increases the level of mental workload leading to human error. 'Li & Haslegrave (1999) introduced similar conclusion of which the vehicle design should be human oriented in order to maximize comfort and ability to perform the driving task perfectly and safely by reducing the human error possibilities'. Nowadays more and more agricultural machines are equipped with continuous measurement sensors e.g. measurement of soil resistance (Kroulik et al., 2015) to have more exact information on energy demand. This means that the driver attention is split by many signals.

Operating an off-road vehicle is a complex task, requiring a concurrent execution of various cognitive, physical, sensory and psychomotor skills (Young & Regan, 2007), additionally to control attached tools to perform in-field productive tasks such as agricultural and industrial operations. Ensuring the comfortable ride is considered essential for any vehicle, as well as executing happily and safely requested operational tasks, to that end, the driver ergonomics comes to play as considered as an important parameter that can't be neglected in the design phase of the vehicle (Hsiao et al., 2005).

Tractors are companions for many agriculture workers. Well-designed human – tractor interfaces, such as well-accommodated tractor operator enclosures can enhance operations productivity, comfort and safety Matthews (1977), Kaminaka et al. (1985), Liljedahl et al. (1996) and Hsiao et al. (2005).

Many studies have been carried on to find preferred locations of in certain types of tractor controls (Casey & Kiso, 1990), moreover; emphasizing how critical is the placement of controls in some tractors stating that, it actually creates an impediment to body movement (Hsiao et al., 2005).

When we are talking about automation, it is a general aim to improve comfort and safety (Sheridan, 1992; Endsley, 1996; Fukunaga et al., 1997; Scheding et al., 1999; Shen & Neyens, 2017), additionally, it is stated that, in the automated driving condition, driver responses to the safety critical events were slower, especially when engaged in a non-driving task. At the same time in their paper – dealing with driver visual attention 'Louw & Merat (2017) reached a conclusion shows that the drivers' understanding of the automated system increases as time progressed, and that scenarios which encourage driver gaze towards the road centre are more likely to increase situation awareness during high levels of automation'.

Generating dependable and deterministic data representing human behaviours inside the workplace using validated method will be beneficial for enhancing current cabin designs as well as the future cabin designs.

This research scope is the methodology part of studying operator's focusing scheme, which is one of the most beneficial behaviours to be recognized inside the tractor's cabin using simple engineering solutions to obtain useful results to be considered in the improvement of tractor's cabin design (i.e. upgrading notification system inside the tractor's cabin and allocating new equipment or components inside the tractor's cabin). In addition to enhancing the operation's work procedures design to increase the productivity of a specific agricultural operation (i.e. break time scheduling and feeding the risk analysis process with deterministic and/or probabilistic inputs).

Driving is not only a physical task but also visual and mental tasks. The eyes of a driver are indispensable in performing visual tasks such as scanning the road, and monitoring in-vehicle devices. Mental tasks are important during driving, and include such factors as understanding vehicle dynamics, making situation-dependent decisions, and judging time/space relationships (Kramer, 1990; De Waard, 1996; Brookhuis & De Waard, 2010; Marquart et al., 2015) were examined the eye-related measures of drivers' mental workload. The mental workload could be defined as the relation between demands resulted from various tasks to be performed on the operator and his ability to fulfil; with satisfactory; these demands Described those demands as multidimensional, as it involves tasks, operator and system demands together with other factors (Sporrong et al., 1998). Additionally; the studies showed that the need for well fitted architectural space to the operator's dimensions is considered crucial, additionally; the mental workload level is found to be increasing with the time passing.

For the purpose of this research, we focus on passive fatigue. This type of fatigue is characterized by being the indirect product of the human driver's exertion of a set of tasks whose demands are low, monotonous or repetitive (Saxby et al., 2013). These rules out any sort of physical fatigue or mental active fatigue.

A study conducted in 2015 by Gonçalves & Bengler claims that Highly Automated Driving (HAD) will be commercially available in a near feature, yet human factors issues like the influence of driver state can have a critical impact in the success of this driving paradigm and also in road and field safety. It is very likely that Driver State Monitoring Systems (DSMS) will play a bigger role than they have played so far.

However with this new driving paradigm shift is important to select highlight what is transferable from the previous systems. Due to lack of driving task engagement, driving performance metrics are no longer viable, creating opportunities for other approaches like detecting non-driving task engagement or fatigue countering behaviours. Eye based metrics will remain important.

MATERIALS AND METHODS

To the purpose of this research; the methodology part; the data extraction and analysis is limited to the laboratory test. However, the scope is subjected to be extended upon the accomplishment of the all research phases.

Selection of Operators

To the purpose of testing the methodology; one operator is selected to wear the eye-tracking equipment. The operator is mandated to spend several minutes inside the selected vehicle cabin to get familiar with the dashboard and equipment panels.

Selection of vehicle

CLAAS tractor (Model: ARES 567 ATZ) is selected to the purpose of accommodating the number of experimental trials (Fig. 1). This model has a covered workplace for the operator, which is helpful to control some of experimental conditions (i.e. temperature and humidity) keeping on the consistency of those parameters and conditions.

Figure 1. CLAAS tractor (Model: ARES 567 ATZ).

Selection of experimental field and operation

Experimental trials are conducted inside Szent István University Laboratories. In where the tractor is located. The operation part is limited to develop the operator's focusing scheme while exploring the cabin contents of the selected tractor. Spending several minutes as a familiarization process, the operator is introduced to the notification panel, main control panel and the side control panel components. However, and as previously stated, the scope of the full research is subjected to be extended to include several work fields for the same agricultural operation for 3 times for 5 operator with different work experience periods along 6 working hours.

Tobii Glasses 2 equipment

Tobii Glasses 2 (Fig. 2) is used to the purpose of obtaining the operator's focusing matrix from his/her real-time gaze analysis to predefined areas of interest. Which is feeding the research results with the main source of data regarding the target behaviour to be studied.

Building the mental-load topographical map on the areas of interest inside the workplace and accumulate it at the end of each working hour, will lead to measure the change of this topographical readings along working hours. Which is the behaviour to be studied 'Operator's focusing scheme and its change along working hours'.

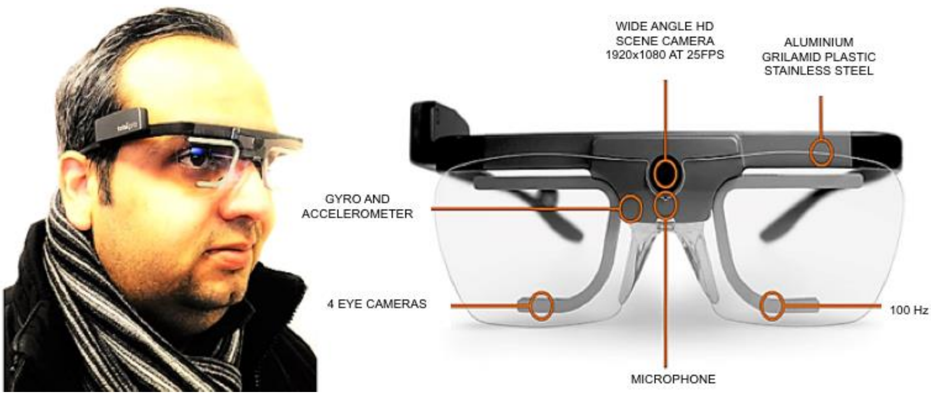

Figure 2. Tobii glasses 2.

Controller software

To record eye tracking data, the Tobii Glasses head unit must be fitted onto the test participant's head (similar to a standard pair of glasses). The system must then be calibrated separately for each participant. In the calibration process the test participant is asked to look at a Calibration Card held in-front of the participant for a few seconds. The researcher then starts the recording from Tobii Glasses Controller Software running on a Windows 8/8.1 Pro tablet or any Windows 8/8.1 or 7 computer. After the session, the researcher stops the recording and removes the head unit from the test participant. All interactions with the eye tracker (adding participants to test, initiating calibration, starting/stopping recordings etc.) are done through Tobii Glasses Controller Software.

The controller software also enables the researcher to view/hear the eye tracking session both in real-time (streamed through a wireless or wired connection) and after the recording. When viewing a recording, you can hear what was recorded on the integrated microphone of the Tobii Glasses 2 Head unit, the participant's gaze point also appears as a coloured dot on the scene camera video from the HD camera integrated in the Tobii Glasses 2 Head Unit.

Processing of raw data

The main processing tool of the operators' gazes is the Tobii Pro Lab (Fig. 3) which has a powerful post-analysis and visualization tools provide a full spectrum of qualitative and quantitative gaze data analysis and visualizations. Tobii Pro Lab logs events, defines areas of interest, calculates statistics, creates heat maps, and exports data for further analysis in other software.

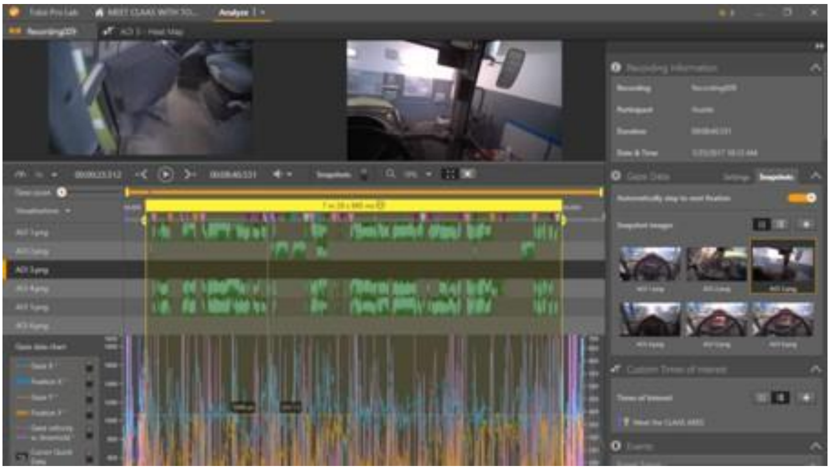

Figure 3. Tobii pro lab – analyser software.

Tobii Pro Studio has three different types of fixation filters to group the raw data into fixations and Tobii Pro Lab uses one type of fixation filter to process the data. These filters are composed of algorithms that calculate whether raw data points belong to the same fixation or not. The basic idea behind these algorithms is that if two gaze points are within a pre-defined minimum distance from each other (Tobii Fixation and ClearView Fixation Filter), or possess a speed below a certain threshold (Tobii I-VT Filter), then they should be allocated to the same fixation. In other words, the user has kept the eyes relatively still between the two sampling points.

Areas of interest (AOIs)

The area of interest is a concept and a Pro Lab tool that allows the eye tracking researcher or analyst to calculate quantitative eye movement measures. These include fixation counts and durations. Using this tool, the researcher simply draws a boundary around a feature or element of the eye tracking stimulus whether it's a button on a web page or actor walking across a scene in a video clip. Pro Lab then calculates the desired metrics within the boundary over the time interval of interest.

Real world mapping

Wearable eye tracking devices; such as Tobii Pro Glasses 2; produce eye gaze data mapped to a coordinate system relative to the wearable eye tracker and the recorded video, not to static objects of interest in the environment around the participant wearing the eye tracker. For most statistical/numerical analysis to be meaningful, the collected eye tracking data needs to be mapped on to objects of interest and into a new coordinate system with its origin fixed in the environment around the participant.

RESULTS AND DISCUSSION

Experimental procedure

An operator is selected to wear the Tobii Glasses 2 equipment which is connected to the central device running Tobii controller software by which the calibration process of operator focusing is conducted and recording process is controlled.

The used tractor (CLAAS tractor (Model: ARES 567 ATZ)) is located inside Szent István University Laboratories. Main areas of interested (AOIs) are defined inside the tractor cabin.

The operator is mandated to go through the calibration process, start the recording process and get in the tractor cabin for several minutes to get familiarized with the cabin components paying attention to the selected AIOs while receiving verbal illustration regarding each component.

Thereafter, the recording process is stopped, and the recorded video is processed by the Tobii Lab pro software using the automatic real world mapping tool, heat maps representing operator’s focusing scheme during the recording time are generated by the software, which leads to generate the statistic readings using MS Excel software.

Results

- In prior to start recording, the calibration process is done successfully and confirmed automatically by the Tobii controller software and the special calibration card (Fig. 4).
- 2 AOIs are selected inside the tractor cabin (Fig. 5) as follows:
    - AOI1: the notification panel in the tractor dashboard and the Air conditioning rotary switch.
    - AOI2: the side control panel in the tractor right side.
- The recorded video is processed using the Tobii Lab Pro software. The full recording time was about 520.53 seconds.

Figure 4. Calibration process using the special card and Tobii controller software.

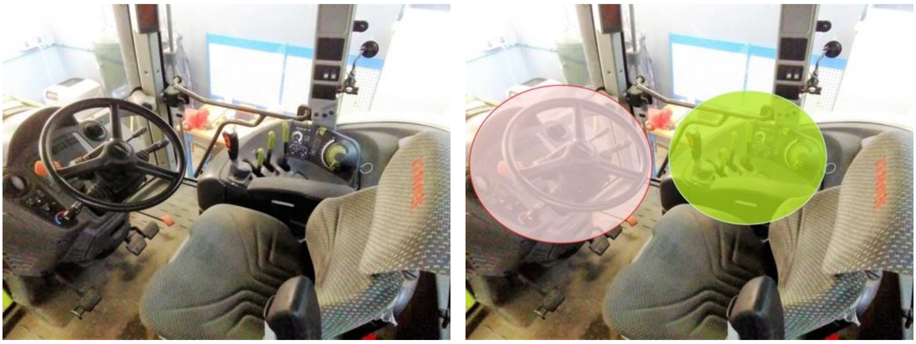

Figure 5. Tractor cabin and the selected AOIs locations.

- The AOI1 (Fig. 6) is represented into two components in the front dashboard. The Air conditioning rotary switch and the notification panel. From the variety of available data which the Tobii Lab Pro software is capable to provide, main collected data from AOI1 was limited to the accumulated gaze time spent on the selected components 'in seconds' and the counts representing the number of times in which each component is scanned by the operator (Table 1) and the heat map is generated (Fig. 7).

Figure 6. AOI1: the notification panel in the tractor dashboard and the Air conditioning rotary switch.

Table 1. AOI1: collected data

Total visit duration	AC rotary switch	Front dashboard	Sum	Total time o f interest duration	Total recording duration
Time (seconds)	0.67	100.11	100.78	290.13	520.53
Counts	4	239	243		

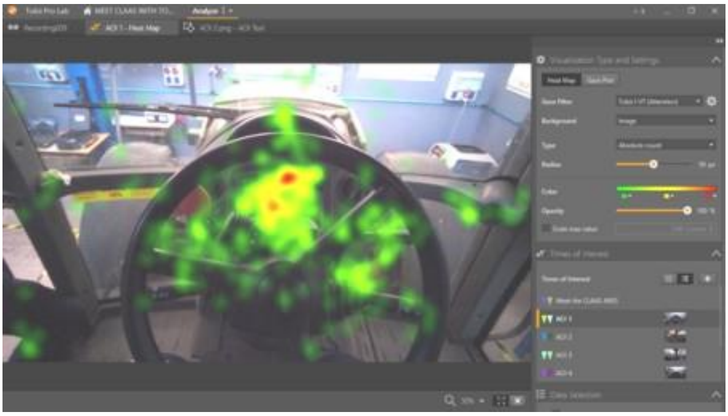

Figure 7. AOI1: Generated heat map.

- The AOI2 (Fig. 8) data is collected (Table 2) and the heat map is generated (Fig. 9).

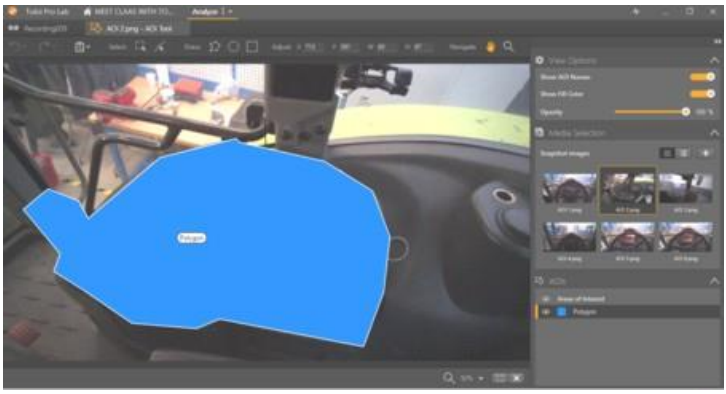

Figure 8. AOI2: the side control panel in the tractor right side.

Figure 9. AOI2: Generated heat map.

Table 2. AOI2: collected data

Total visit duration	Side control panel	Sum	Total time of interest duration	Total recording duration
Time (seconds)	33.88	33.88	54.94	520.53
Counts	77	77		

Discussion

The results showed very accurate and dependable data of the operators gaze on selected components inside previously defined AIO (i.e. the AC rotary switch in the AOI1). The used equipment and supporting software packages easily defined the time in which the operator paid his attention to the AC rotary switch (0.67 seconds) during the defined time of interest (520.53 seconds) during the familiarization with the tractor cabin. Such data is considered valuable to the designer of the front panel.

The operator's focusing scheme is represented in numbers and heat maps for the previously defined AIO (i.e. the side control panel in the AIO2). The resulted scheme is readable in term of number and sequence of hits representing the moments in which the operator paid attention to components inside the area of interest with ability to calibrate the sensitivity parameters and filters used to produce the data

CONCLUSIONS

The methodology used to generate deterministic results which are validated, verified and dependable to represent the operator's behaviour of the focusing scheme inside the workplace (tractor cabin).

The variety of filters and options available under the scope of the analyser software capability is found convenient to come over expected challenges during further research activities such as in-field experiments and outdoors activities.

Such generated results confirmed the feasibility of investing the followed methodology in studying more AOIs inside the tractor cabin to feed the design and/or development processes of the tractor cabin with valuable input data beside the conventional user experience feedback and continual research and development channels.

Additionally, by selecting a suitable time interval to generate operator's focusing scheme and providing comparison mechanism between generated schemes, the trend is found for the change of operator's focusing scheme along working hours. Which represents a dependable linkage between mental and physical workloads for the same operator inside his/her workplace for a specific productive operation along working hours. In other words, the fatigue indicator is became deterministic more than probabilistic value to be used in the design process of the operation (i.e. break times) to ensure the proper optimization for the productive operation.

Usability of research results

Increasing the efficiency and effectiveness of any agricultural or industrial operations that involves human operator – workplace interaction will be the main benefit of implementing this research methodology.

Current cabin designs are subjected to be enhanced with interactive guides and/or equipment for operators at the time in when and/or where it is expected to be needed because of the resulted numbers showing a decrement of focusing or response time.

Additionally, this research methodology is expected to be efficient comparison tool between prototypes of new cabin designs based on deterministic measures.

Future research

This research methodology is proposed to be developed for producing deterministic safety related measures to feed operator's workplace and operation design with the necessary inputs. Which might be dependable to produce the safety or risk assessment reports for certain vehicle or operation design.

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Effects of Irrigation water salinity on evapotranspiration and spinach (*Spinacia oleracea* L. Matador) plant parameters in Greenhouse Indoor and Outdoor Conditions

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Abstract. Response of spinach to irrigation water salinity under greenhouse indoor and outdoor conditions was investigated in this study to reveal different weather conditions on salinity tolerance of the plant. For this purpose, saline waters at six different salinities (0.65, 2.0, 3.0, 4.0, 5.0 and 7.0 dS m^{-1}) were applied to spinach (*Spinacia oleracea* L. Matador) grown in pots. Soil salinity increased linearly with increasing salinity of irrigation water. Threshold salinity is 2.35 dS m^{-1} and yield lost slope after this threshold is 3.51% for indoor and threshold salinity is 2.83 dS m^{-1} and yield lost slope is 3.3% for outdoor. Salinity harmful effect on spinach yield is higher for indoor conditions than for outdoor conditions because of higher indoor temperatures. These results apparently showed that spinach salinity response could change with changing weather conditions especially for temperature. Yield response factors (k_y), which is the ratio of relative evapotranspiration decrease to relative yield decrease, were close in the cases of irrigation water salinity in greenhouse outdoor and indoor (k_y = 2.4 and 2.1), respectively. Considerable water consumption decreases because of salinity were determined. Every 1 dS m^{-1} increment in soil salinity caused about 1.35% water consumption decrease for spinach. Therefore, depressing effect of salinity on water consumption should be considered in irrigation and salinity management to prevent excess saline water application and to protect environment.

Key words: Irrigation water salinity, Spinach, Plant water consumption, Plant growth parameters.

INTRODUCTION

Using rural and agricultural waste water and poor quality water in agriculture increase as world population increase to supply food need. Irrigation is one of the most reliable ways to increase productivity from unit area. Water demand of industrial, rural and agricultural sectors and global warming force to use poor quality saline and waste water resources (Rhoades et al., 1992; Shalhevet, 1994).

Sustainable production in agricultural lands is essential. Using saline water cause salinization of agricultural land and decrease productivity. To keep productivity and prevent salinization and water logging, proper irrigation and salinity management measurement are accomplished. Knowledge on plant water need and salinity tolerance of agricultural plants are vital for these purposes (Ayers & Westcot, 1989; Hoffman et al., 1992; Rhoades et al., 1992). If salt accumulation in root zone due irrigation water applied or high water table reduce crop yield lost, a salinity problem exists. Yield lost begins salinity level that the crop no longer able to uptake sufficient from salty soil solution and the crop experience water stress (Ayers & Westcot, 1989).

Edible flowering spinach plant (*Spinacia oleracea* L.) belongs to the family *Amaranthacea*. Spinach originated from southwestern and central Asia (Avşar, 2011). Turkey is the fourth largest spinach producer with 225 thousand tons after China, United States and Japan (FAOSTAT, 2017). Spinach includes high levels of vitamins and minerals. Vitamin A, vitamin B, vitamin K, vitamin C, vitamin B2 and B6, phosphorus, iron, potassium, folate, betaine, copper, protein, manganese, zinc, niacin, selenium and omega-3 are the ingredient included by spinach (Avşar, 2011).

Spinach was characterized as moderately sensitive plant to salinity. Threshold soil salinity was 2.0 dS m^{-1} and yield lost slope was 7.6% after threshold (Ayers & Westcot, 1989; Hoffman et al., 1992; Grieve et al., 2012). Ors & Suarez (2016) found different threshold values associated with Racoon spinach cultivar as 4.2 dS m^{-1} or higher for late fall and early spring period, as 3.3–4.2 dS m^{-1} for spring period and as 1.9–3.3 dS m^{-1} for late spring period in Riverside/California. These researchers stated that cool season spinach grown under cool period was more salt tolerant than that grown under warmer spring season. Spinach was also reported as a very sensitive to water stress (Yurtyeri et al., 2014).

In this study, some growth, yield and water consumption responses of spinach exposed to salinity under greenhouse indoor and outdoor conditions in the same period were investigated to reveal effects of different weather conditions, especially temperature on the plant salinity response.

MATERIALS AND METHODS

Two experiment were conducted under greenhouse indoor and outdoor conditions in 2007 to determine response of spinach (*Spinacia oleracea* L. Matador) to salinity in Gaziosmanpaşa University Agricultural Faculty in Tokat/Turkey. Six different saline water treatments as $S_0 = 0.65$ dS m^{-1} (control), $S_1 = 2.0$ dS m^{-1}, $S_2 = 3.0$ dS m^{-1}, $S_3 = 4.0$ dS m^{-1}, $S_4 = 5.0$ dS m^{-1} and $S_5 = 7.0$ dS m^{-1} were used to irrigate the plants grown under two different weather conditions in the same growth period. The experiment was designed in completely randomized plots with 5 replications. Therefore, totally 60 number pots were used for which 30 one greenhouse indoor and 30 one outdoor conditions. To prevent leaching due to rainfall, the outdoor experiment was conducted under a rain shelter. Tokat province of Turkey had a step climate with warmer dry summers and cooler rainy winters. Mean annual temperature was 12.6 °C and mean annual rainfall was 423.7 mm. Long term averaged temperatures of March, April and May, spinach growth period of these experiments, were reported 7.4 °C, 12.5 °C and 16.5 °C by Turkish State Meteorological Service (Anonymous, 2017).

The pot height and diameter were 27 cm and included 18 kg soil sieved from a 4 mm-sieve. Unit weight was 1.25 g cm^3. Sandy loam textured soil had 60.5% sand, 26.3%silt and 13.2% clay. Water contents (w/w) were determined as 33.5% at saturation point, as 23.6% at field capacity and as 9.8% at wilting point. Sowing date was 12 march 2007 and 16 spinach seed were sown to the pots. After seedling emergence, only 6 seedlings were left in each pot. Tap water (S_0) was applied to seedling until the seedling reached to 10 cm height. Harvesting was carried out on 14 and 17 may 2007 for indoor and outdoor experiment, respectively. Fertilizer needs were 120 kg ha^{-1} nitrogen, 100 kg ha^{-1} phosphorous (P_2O_5) and 50 kg ha^{-1} potassium (K_2O). Half of the nitrogen needs applied at sowing and another half applied 20 days later after seedling emergence.

To prepare saline waters at different salinity levels, three salts ($CaCl_2$, $MgSO_4$ and NaCl) were used. Amount of these salt types in saline waters were determined by using constant 1/1 Ca/Mg ratio (me l^{-1}) and sodium adsorption ratio (SAR = 5) (Ünlükara et al., 2008a; Unlukara et al., 2008b; Kurunc et al., 2011). Salinity levels of saline waters were checked by an electrical conductivity device and the salt's amounts in the saline waters were adjusted before applicating to the spinaches in the pots. Soil salinity were determined by soil saturation paste extract method from soil samples taken at harvest (Richards, 1954; Rhoades et al., 1992; Carter, 2000).

Water amounts applied to the treatments were determined by the following equation (Ayers & Westcot, 1986; Ünlükara et al., 2008a):

$$\mathrm{I} = \mathrm{ET}/(1 - \mathrm{LF}) \tag{1}$$

where I was amount of water applied (l), ET was plant water consumption (I) and LF was leaching fraction. Plant water consumption from pot field capacity was determined by weighing the pots just before irrigation. Pot field capacity was determined by weighing the pot drained after saturation (Öztürk et al., 2004; Yurtseven et al., 2005; Ünlükara et al., 2010). To prevent higher salt accumulation in root zone and obtain different soil salinity levels, leaching was carried out at each irrigation at constant leaching ratio (LF = 0.30) (Maas & Hoffman, 1977; Ayers & Westcot, 1989). Leaching water drained from the underneath orifices of the pots to the drainage cups and then measured. Plant water consumption for whole season were determined according to soil water budget method. Meteorological data was derived from Weatherlink meteorological station at Gaziosmanpaşa experimental area.

Depressing effect of soil salinity on plant water consumption was determined as stress coefficient (K_s) (Allen at al., 1998; Ünlükara et al., 2015):

$$\mathrm{K_s} = \frac{\mathrm{ET_{c\ adj}}}{\mathrm{ET_c}} \tag{2}$$

where $\mathrm{K_s}$was salinity stress coefficient; $\mathrm{ET_{c\ adj}}$ was water consumptions in liter from the saline treatments; ET_c was water consumption in liter from the control treatment. Relative decrease in water consumption due to salinity stress was related to relative yield lost by the following equation (Doorenbos & Kassam, 1986):

$$\mathrm{K_y} = \frac{(\Delta \mathrm{Y}/\mathrm{Y_m})}{(\Delta \mathrm{ET}/\mathrm{ET_m})} \tag{3}$$

where $\mathrm{K_y}$ was yield response factor caused by water stress created by salinity.

Salinity tolerance model of spinach were obtained by Maas & Hoffman (1977) model:

$$\frac{Y_a}{Y_m} = 1 - (EC_e - EC_{e\ threshold}) \cdot \frac{b}{100} \tag{4}$$

where Y_m was maximum spinach fresh yield (g) obtained from the control treatment; Y_a was actual fresh spinach yield (g) obtained from saline treatments; EC_e soil salinity or soil saturation paste extract electrical conductivity (dS m^{-1}); $EC_{e\ threshold}$ was soil salinity (dS m^{-1}) at which spinach begins to lost yield and b was yield lost slope (%) after the threshold.

Spinach plant height was measured weekly and apparent physiological changes were recorded. Plant biomass, stem diameters, root lengths were also determined at the harvest. Plant growth, development and yield values were evaluated by means over 5 replications. Plant leaf number were countered and leaf areas were measured by a planimeter. Plant roots were get from the pots by careful washing. Quantitative analysis was carried out to assess plant growth parameters (see Table 1) (Cemek, 2002).

Table 1. Plant Growth Parameters and Models Used for Quantitative Analysis (Cemek, 2002)

Growth parameters	Models
Leaf Area Ratio (LAR)	Total leaf area (cm^2) per Total Dry Biomass (g)
Specific Leaf Area (SLA)	Total leaf area (cm^2) per Total Dry Leaf Mass (g)
Leaf Mass Ratio (LMR)	Total Dry Leaf Mass (g) per Total Dry Biomass (g)
Root Mass Ratio (RMR)	Total Dry Root Mass (g) per Total Dry Biomass (g)

MS Excel 7.0 and SPSS 10 programs were used for statistical analysis of the results from spinach grown under indoor and outdoor greenhouse conditions to determine its salinity responses. Standard error bars were used to compare the results. Mean standard errors were obtained by Excel 7.0 and these standard error bars were installed mean values at $p < 0.05$ (Gomez & Gomez, 1984).

RESULTS AND DISCUSIONS

Mean monthly temperatures, minimum and maximum temperatures, relative humidity of indoor and outdoor greenhouse conditions were presented in Table 2 for plant growth season. Indoor mean temperatures varied between 13.3 and 24.2 °C while mean relative humidity varied between 40.7% and 66.1%. Outdoor mean temperatures ranged from 12.7 °C to 21.2 °C while mean relative humidity ranged from 40.8% to 54.8%. Therefore, the indoor spinach was exposed to 1.7 °C higher temperatures than the outdoor spinach.

Table 2. Temperature and relative humidity values for greenhouse indoor and outdoor conditions

	Indoor						Outdoor					
	Temperature (°C)			Relative Humidity (%)			Temperature (°C)			Relative Humidity (%)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
March	13.1	11.4	16.0	50.5	46.0	54.3	12.1	10.7	13.7	55.4	54.1	56.5
April	16.2	9.20	23.7	54.4	39.0	74.0	14.5	8.75	21.7	53.4	47.7	58.0
May	25.8	19.4	32.8	50.7	37.0	70.0	23.5	18.8	28.3	46.2	42.4	50.0
Mean	18.4	13.3	24.2	51.9	40.7	66.1	16.7	12.7	21.2	51.6	48.0	54.8

Effects of irrigation water salinity on spinach growth parameter under greenhouse indoor and outdoor conditions

Growth parameters of spinach grown under outdoor and indoor conditions were presented in Tables 3 and 4. Salinity effect did not found significant on leaf number and stem diameter for both conditions. Mean plant leaf numbers were 12 and 11.85 for indoor and outdoor conditions and the same stem diameter for both experiments (0.6 cm) were determined. Ors & Suarez (2016) concluded that salinity reduces spinach leaf area rather than leaf number.

Salinity affected plant root length significantly ($p < 0.05$) and higher root lengths were obtained in saline treatments for the both experiments (Tables 3 and 4). The smallest roots were observed in the control treatment (S_0) under two conditions but the longest roots were observed in all salinity treatments for outdoor and only in severe saline treatments (S_4 and S_5) for indoor condition. The root length increased from 9.13 cm for control treatment to 11.52 cm for S_5 under outdoor and from 11.57 cm to 14.07 cm under indoor. It could be concluded that salinity and higher temperature raised root length.

Table 3. Some growth response parameters of spinach to salinity for outdoor conditions

	Irrigation Water Salinity Treatments (dS m^{-1})							
	S_o 0.65)	S_1 (2.0)	S_2 (3.0)	S_3 (4.0)	S_4 (5.0)	S_5 (7.0)	Mean	P > F
Number of leaves	11.4$^{\#}$	12.2	12.5	11.9	12.0	11.8	12.0	ns
Root length (cm)	9.13 b$^{\Psi}$	10.6 a	10.5 a	10.8 a	10.6 a	11.5 a	10.51	**
Stem diameter (cm)	0.58	0.59	0.65	0.60	0.58	0.60	0.60	ns
Leaf area (cm^2)	501 a	350 b	268 c	243 cd	236 cd	216 d	302	**
Leaf area index	0.87 a	0.61 b	0.47 c	0.42 c	0.41 c	0.38 c	0.53	**
Leaf area ratio (LAR)	79.5 a	61.3 b	55.9 b	47.6 c	52.4 c	55.36 b	58.66	**
Specific leaf area (SLA)	95.0 a	71.9 b	66.7 c	54.0 d	60.6 d	65.0 c	68.9	**
Leaf mass ratio (LMR)	0.84 c	0.85 b	0.84 c	0.88 a	0.86 b	0.85 c	0.85	**
Root mass ratio (RMR)	0.16 a	0.15 a	0.16 a	0.12 c	0.14 b	0.15 a	0.15	**

Each value is mean of five replications; $^{\Psi}$ Within rows, means followed by the same letter are not significantly different according to Duncan's multiple range test at 0.05 significance level; *, ** Significant at the 0.01 and 0.05 probability levels, respectively; ns is non-significant.

Leaf area was significantly affected at 0.05 probability by salinity under both experimental conditions. The highest leaf areas (500.7 cm^2 and 484.2 cm^2) were observed in control treatment (S_0) while the lowest one (215.9 cm^2 and 211.3 cm^2) were observed in S_5 treatment outdoor and indoor conditions, respectively (Tables 3 and 4). Plant leaf area was decreased with increasing salinity. Changes in leaf area index (LAI) due to salinity was similar to leaf area changes. Generally, smaller leaves, shorter plant height and sometimes fewer leaves were resulted from salinity (Shannon & Grieve, 1999). Contrary to these results Ors & Suarez (2016) obtained increasing leaf area with salinity for late fall and early spring period. Decreasing leaf area with irrigation water salinity above 7 dS m^{-1} was reported by Ors & Suarez (2016) under warmer spring and late spring growth periods for Racoon spinach cultivar. Differences in spinach leaf area to salinity may result from different cultivars, environmental conditions and cultivating.

Leaf area ratio (LAR) was affected due to salinity for both conditions. The highest leaf area ratios (LAR) were 79.47 and 66.24 for controls of two experiments and the lowest LAR were 52.4 and 44.80 for S_4 under both conditions (Tables 3 and 4). LAR aligned in descendent order with increasing salinity except for S_5 under outdoor. It was concluded that matador spinach cultivar improved smaller leaf area per unit dry biomass due to salinity stress. Significant effect of salinity on specific leaf area (SLA) of spinach grown outdoor and indoor conditions, as similar as observed for LAR.

Table 4. Some growth response parameters of spinach to salinity for greenhouse indoor conditions

	Irrigation Water Salinity Treatments (dS m^{-1})							
	S_o (0.65)	S_1 (2.0)	S_2 (3.0)	S_3 (4.0)	S_4 (5.0)	S_5 (7.0)	Mean	P > F
Number of leaves	12.30$^{\#}$	12.10	11.23	11.20	11.60	12.67	11.85	ns
Root length (cm)	11.6 c$^{\Psi}$	12.2 bc	13.0 ab	12.4 bc	12.9 ab	14.1 a	12.7	**
Stem diameter (cm)	0.57	0.62	0.58	0.56	0.59	0.66	0.60	ns
Leaf area (cm^2)	484 a	376 b	253 c	241 c	233 c	211 d	300	**
Leaf area index	0.85 a	0.66 b	0.44 c	0.42 c	0.41 c	0.37 d	0.52	**
Leaf area ratio (LAR)	66.2 a	58.5 b	47.5 c	48.6 c	44.8 d	45.64 d	51.87	**
Specific leaf area (SLA)	78.1 a	67.2 b	52.6 d	54.8 c	50.6 d	55.6 c	59.8	**
Leaf mass ratio (LMR)	0.85 ab	0.87 a	0.90 a	0.89 a	0.88 a	0.82 b	0.87	**
Root mass ratio (RMR)	0.15 b	0.13 bc	0.10 c	0.11 c	0.12 c	0.18 a	0.13	**

Each value is mean of five replications; $^{\Psi}$ Within rows, means followed by the same letter are not significantly different according to Duncan's multiple range test at 0.05 significance level; *, ** Significant at the 0.01 and 0.05 probability levels, respectively; ns is non-significant.

Salinity caused significant differences in leaf mass ratio (LMR) and root mass ratio (RMR) under both of the conditions (Tables 3 and 4). The highest LMR for indoor grown spinach were observed in all of the treatments except in the severe saline treatment (S_5) while the highest LMR only observed in S_3 for outdoor grown spinach.

Root mass ratios (RMR) significantly varied between 0.12–0.16 for outdoor and between 0.11–0.18 for indoor conditions (Tables 3 and 4). The highest RMR in S_0, S_1, S_2 and S_5 but the lowest RMR in S_3 were observed under outdoor but the highest one only in S_5 under indoor conditions. Salinity effects on LMR and RMR occurred different manner for outdoor and indoor greenhouse conditions.

Robinson et al. (1983) applied irrigation water with 1,450 mg L^{-1} NaCl and nutrient to spinach every day. Soil salinity concentration reached to 11,600 mg L^{-1} and the spinach lost big than 50% biomass. Leaf thickness also increased by salinity but plant normally sustained development.

Effects of saline irrigation water on soil salinity and yield for greenhouse indoor and outdoor conditions

Soil salinity (EC_e), pH, spinach water consumption, spinach leaf fresh and dry weight caused by salinity were presented in Table 5 for outdoor conditions and in Table 6 for indoor conditions. Plant water consumption was significantly affected by salinity ($p < 0.01$) for both conditions. Plant water consumption decreases as irrigation water salinity increases. The highest water consumption (8.92 L per pot) occurred in S_0 while the lowest water consumption (7.90 L per pot) occurred in S_5 (Table 5) under outdoor. Therefore, the spinach exposed to salinity experienced water stress due decreased osmotic potential of the soil solution despite there was sufficient water in the root zone.

Similar water consumption trend was also determined for indoor conditions but higher water consumption of 36% was observed because of higher indoor temperature (Tables 2 and 6). Mean temperatures for the growth period were 18.39 °C and 16.71 °C for indoor and outdoor conditions, respectively. The highest water consumption was 12.23 L per pot for S_0 and the lowest water consumption was 10.7 L per pot for S_5. Although lower atmospheric evapotranspiration demand (ET_0) occurred during late fall and early spring growth period, higher spinach water consumption determined due to longer growth period than warmer spring growth season (Ors & Suarez, 2016).

Table 5. Irrigation water salinity effects on soil salinity and spinach yield for outdoor greenhouse conditions

	Treatments ($dS.m^{-1}$)							
	S_o (0.65)	S_1 (2.0)	S_2 (3.0)	S_3 (4.0)	S_4 (5.0)	S_5 (7.0)	Mean	P > F
ET_c (l)	8.92 a	8.82 a	8.29 b	8.26 b	8.19 b	7.90 c	8.40	**
EC_e ($dS.m^{-1}$)	2.36 f	4.29 e	6.96 d	8.25 c	8.78 b	11.73 a	7.06	**
pH	8.53 a	8.37 b	8.19 c	8.17 c	8.17 c	8.13 d	8.26	**
Leaf fresh weight (g pot^{-1})	129 a	126 a	111 b	109 b	101 b	91.2 c	110.9	**
Leaf dry weight (g pot^{-1})	5.3 a	4.9 ab	4.0 bc	4.5 b	3.9 c	3.3 d	4.31	**
Root dry weight	1.03 a	0.84 b	0.78 b	0.61 c	0.61 c	0.58 c	0.74	**
Total dry weight	6.3 a	5.7 a	4.8 b	5.1 ab	4.5 b	3.9 c	5.05	**

Each value is mean of five replications; $^{\Psi}$ Within rows, means followed by the same letter are not significantly different according to Duncan's multiple range test at 0.05 significance level; *, ** Significant at the 0.01 and 0.05 probability levels, respectively; ns is non-significant.

Soil salinity increased as irrigation water salinity increased in contrary to the water consumption trend for both outdoor and indoor conditions. Differences among soil salinity results were found significant at $p < 0.01$ for outdoor and at $p < 0.05$ for indoor conditions. The highest soil salinity resulted from application of the highest saline irrigation water for both of two conditions. Mean soil salinity of indoor experiment was found slightly higher than outdoor salinity (7.38 > 7.06 dS m^{-1}). Therefore, soil salinity over irrigation salinity ratios of S_0, S_1, S_2, S_3, S_4, S_5 treatments were 3.63, 2.15, 2.32, 2.06, 1.76 and 1.68 for outdoor and 2.95, 3.15, 2.34, 2.02, 1.84, and 1.68 for indoor conditions, respectively. Higher EC_e/EC_w ratios were obtained for lower EC_w application treatments due to depressing effects of salinity on water consumption. Plants extract nearly pure water from soil and left great amount of salt. More water consumption means relatively higher salt accumulation in soil and also in plant tissues.

Soil reaction (pH) varied between 8.53 and 8.13 for outdoor conditions and between 8.47 and 7.96 for indoor conditions. Soil pH decreased with increasing salinity (Tables 5 and 6).

Spinach fresh and dry leaf weight were significantly affected from salinity for indoor and outdoor conditions at $p < 0.01$. Mean fresh leaf weight were 110.9 g pot^{-1} and 95.7 g pot^{-1} for outdoor and indoor conditions, respectively (Tables 5 and 6). The highest fresh and dry leaf weight were observed from S_0 and S_1 treatments and the lowest one from S_5 for outdoor conditions. In indoor conditions, only S_0 treatment had the highest fresh leaf weight and S_4 and S_5 treatments had the lowest. Detrimental salinity effect on fresh leaf weight was more severe for indoor conditions than for outdoor conditions. Leaf dry weight decreased gradually with increasing salinity.

Detrimental effect of salinity on dry root weight was also observed for both indoor and outdoor conditions with one exception. Dry root weight gradually decreased with salinity until S_3 treatment and then stabilized for outdoor conditions (Table 5). Gradual decrease in root weight for indoor conditions was more steep until S_2 treatment and slight increase in root weight was occurred (Table 6).

Table 6. Irrigation water salinity effects on soil salinity and spinach yield for indoor greenhouse conditions

	Treatments (dS m^{-1})							
	S_o (0.65)	S_1 (2.0)	S_2 (3.0)	S_3 (4.0)	S_4 (5.0)	S_5 (7.0)	Mean	P > F
ET_c (l)	$12.2^{\#}$ a^{Ψ}	11.8 b	11.3 c	10.9 c	10.8 d	10.7 d	11.30	**
EC_e (dS m^{-1})	1.92 f	6.30 e	7.03 d	8.07 c	9.20 b	11.8 a	7.38	**
pH	8.47a	8.26 b	8.19 c	8.13 d	8.07 e	7.96 f	8.18	**
Leaf fresh weight (g pot^{-1})	114.6 a	104.5 b	94.3 c	90.8 c	85.4 d	84.9 d	95.7	**
Leaf dry weight (g.pot^{-1})	6.20 a	5.6 b	4.80 c	4.40 c	4.60 c	3.8 d	4.90	**
Root dry weight	1.11 a	0.83 b	0.52 c	0.57 c	0.60 c	0.83 b	0.74	**
Total dry weight	7.31 a	6.43 b	5.32 c	4.97 c	5.20 c	4.63 d	5.64	**

\# Each value is mean of five replications; $^{\Psi}$ Within rows, means followed by the same letter are not significantly different according to Duncan's multiple range test at 0.05 significance level; *, ** Significant at the 0.01 and 0.05 probability levels, respectively; ns is non-significant.

Total mean dry biomasses were 5.05 g and 5.64 g for outdoor and indoor, respectively. Dry mass differences among treatments were significant due to salinity for both conditions. Total dry biomass decreased from 6.3 g for S_0 to 3.9 g for S_5 under outdoor conditions. Similar dry biomass decreases were observed for indoor conditions but from 7.31 g for S_0 to 4.63 g for S_5. Shannon & Grieve (1999) stated that general effect of salinity in crops were depressed growth rate, shorter stature and sometimes fewer leaves.

Salinity Tolerance Model for Spinach

Salinity tolerance model according to Maas & Hoffman (1977) for spinach fresh yield were presented in Fig. 1. The salinity model for indoor conditions and outdoor conditions, respectively:

$$\mathrm{Y} = 100 - 3.51 \cdot (\mathrm{EC_e} - 2.35)$$

$$\mathrm{Y} = 100 - 3.30 \cdot (\mathrm{EC_e} - 2.83)$$

Threshold salinity of Matador spinach cultivar was 2.35 dS m^{-1} and yield lost slope after this threshold was 3.51% for indoor and threshold salinity was 2.83 dS m^{-1} and yield lost slope was 3.3% for outdoor. Indoor salinity threshold value was less than outdoor value. In contrary to the thresholds, indoor yield lost slope was steeper than outdoor yield lost slope. These means that indoor spinach yield lost would be higher than outdoor at the same soil salinity after the threshold. Higher air temperatures for indoor caused changes in spinach response to salinity.

Different threshold value results were obtained Ors and Suarez (2016) for spinach grown under three different growth stages. They obtained 4.2 dS m^{-1} or higher threshold value for late fall-early spring period while they determined lower threshold values between 3.3–4.2 dS m^{-1} for spring period and between 1.9–3.3 dS m^{-1} for late spring period due to warmer conditions.

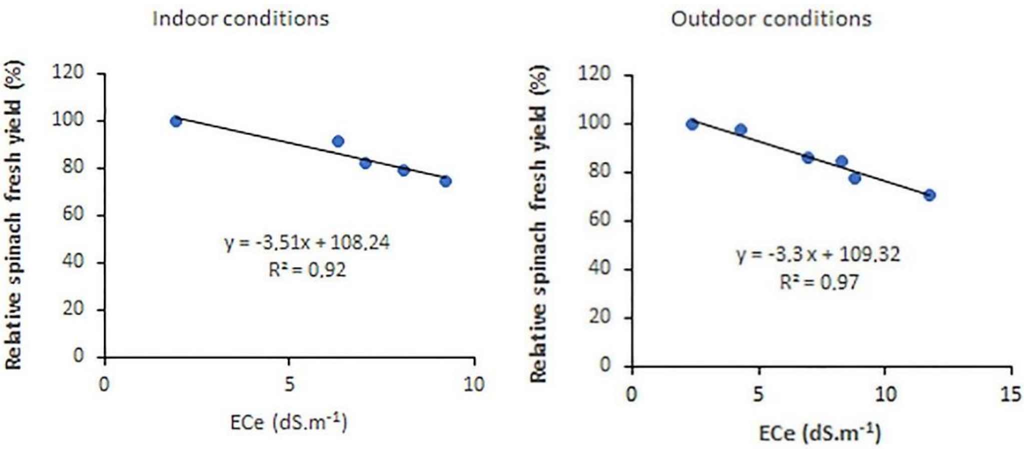

Figure 1. Spinach fresh yield salinity tolerance model for greenhouse indoor and outdoor conditions.

Spinach cultivar Matador was found moderately sensitive to salinity under both indoor and outdoor conditions. Long & Baker (1986) also reported that spinach had intermediate salt tolerance among herbaceous crops. Threshold soil salinity was 2.0 dS m^{-1} and yield lost slope was 7.6% after threshold (Ayers & Westcot 1989; Hoffman et al., 1992; Grieve et al., 2012). Spinach 50% yield lost occurred with watering 8.6 dS m^{-1} irrigation water (Maas & Hoffman, 1977; Shannon et al., 2000). Our both threshold values were higher than 2.0 dS m^{-1} but our outdoor and indoor yield lost slopes of 3.3 and 3.51% were not steeper than 7.6% (Fig. 1). In the past, NaCl salt often used to determine plant salt response to salinity. Besides of osmotic effect of Na and Cl ions were also reported toxic to cultivated plants and Na had detrimental effect to soil physical conditions (Hoffman et al., 1992; Rhoades et al., 1992). However, any water resource in nature is not consist of only NaCl. Perhaps, elimination of these more detrimental effect of NaCl by using three salts (NaCl, $MgSO_4$ and $CaCl_2$) in this experiment improved spinach salt response or using different cultivar under different conditions caused this result (Ünlükara et al., 2010; Kurunc et al., 2011).

Adjustment of Depressing Effect of Salinity on Plant Water Consumption and Yield Response Factor

Increasing salinity caused decreases in spinach water consumption under both indoor and outdoor conditions (Tables 5 and 6). This depressing effect of salinity on plant water consumption should be considered to manage irrigation and salinity, precisely. The relationship between soil salinity and relative plant water consumption is presented in Fig. 2. Very strong negative linear relationships were obtained between EC_e and ET_{adj}/ET for both conditions. Every 1 dS m^{-1} increment in soil salinity caused about 1.35% water consumption decrease for spinach.

Water deficiency caused plant yield lost. To evaluate plant tolerance to water stress, a yield response factor (k_y) often used (Katerji et al., 1998). K_y was used as a correlation coefficient between relative yield decreases and relative evapotranspiration decreases (Doorenbos & Kassam, 1986). Salinity also caused water stress because it decreased osmotic potential of soil water. The relationships between relative yield decreases and relative evapotranspiration decreases created by salinity were represented in Fig. 3 for Matador spinach cultivar. As seen in Fig. 3, $k_y = 2.1$ and $k_y = 2.42$ for indoor and outdoor conditions state that spinach was very sensitive to water stress created by salinity.

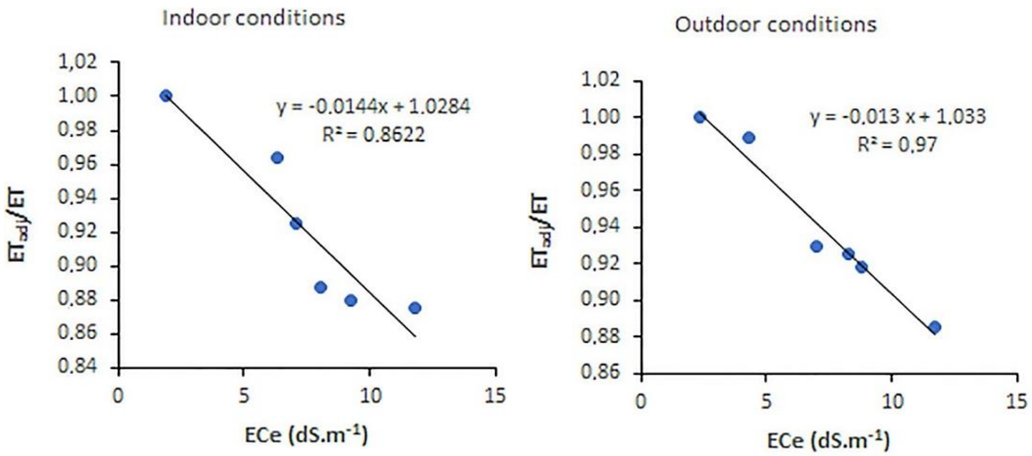

Figure 2. Soil salinity and spinach relative water consumption relationships for indoor and outdoor greenhouse conditions.

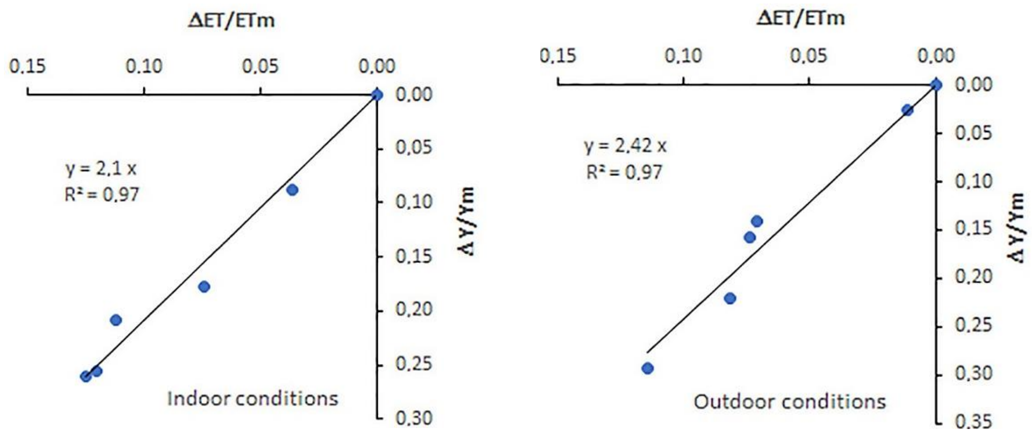

Figure 3. Relationships between relative yield decreases and relative evapotranspiration decreases created by salinity.

At the same time and same conditions, Yurtyeri et al. (2014) found Matador cultivar yield response factor created by water deficiency as 1.0 and 1.66 for the same indoor and outdoor greenhouse conditions, respectively. Spinach yield response factor was found higher under salinity conditions than reported ones under water deficiency conditions by Yuryeri et al. (2014).

CONCLUSION

Spinach responses to salinity investigated in this study under greenhouse indoor and outdoor conditions to reveal effect of different weather conditions on salinity tolerance. For this purpose, six different levels of saline waters (0.65, 2.0, 3.0, 4.0, 5.0 and 7.0 dS m^{-1}) were applied to spinach (*Spinacia oleracea* L. Matador). Spinach leaf number and stem diameter were not affected from salinity. Therefore, salinity promoted root length. Root mass ratio was found higher for non-saline and severe saline conditions. Medium salinity conditions negatively affected on root mass ratio. Severe saline conditions may cause spinach to develop more root to overcome salinity stress. Leaf area and leaf mass ratio decreased by increasing salinity.

Application of saline water increased soil salinity. Soil salinity to irrigation water salinity ratio was higher under relatively lower saline conditions due to more water consumption. Salinity had a depressing effect on spinach water consumption by causing osmotic potential decreases in soil water solution. This depressing effect should be considered to manage irrigation and salinity, precisely.

Spinach fresh yield was affected negatively from salinity. Matador spinach cultivar was found as a moderately sensitive plant to salinity. Warmer indoor greenhouse saline conditions caused higher yield decrease by a lower threshold value and steeper yield lost slope. Higher threshold value and smaller yield decrease slope were determined under cooler outdoor conditions. These results apparently showed that spinach salinity tolerance could change according to temperature.

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Prevention the impact of chemicals on the health of workers in fibreglass industry

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Abstract. Most of the chemicals make our everyday life easier and safer. However, there are a lot of new emerging risks connected with chemicals causing damage to people's health and environment. The results of the investigation: the chemical exposure index (EI) is between 0.16 to 25.98 (the last determined by the mould spray-up, outside of the protective masks). The air pollution index determined was between 16 to 760%. The ventilation rates for the remove of the volatiles from the workplace air are settled, the possibilities for substitution of hazardous chemicals to less hazardous are presented. When the concentration of a volatile is measured under the protective mask, which has a new filter, the tested substance concentration is lower that under the mask with an old filter, although the differences between these two were rather small. When the volatiles were measured under the protective mask, the concentrations of tested substances met the requirements.

Key words: chemicals' hazardousness, health risks, solvents, substitution.

INTRODUCTION

Increasing human consumption is accompanied by growing risk that the chemicals can cause to human health and the environment, so it is important that production and use of chemicals is managed with a proper responsibility (Latvian Free Trade Union, 2010).

To prevent the impact of chemicals on the health of workers, measures should be taken to reduce their concentration and exposure time in the work environment. On the other hand, the chemical can enter the lungs if it is in the dispersed state in the air. The source of the substance could be the equipment or device from where the chemical can be spread into the surrounding work environment. When chemicals are in the spread form in the work environment air, they may enter the employee's breathing area and from there get into his lungs (Samet & Spengler, 2003; Raaschou-Nielsen et al., 2013).

Factors of the working environment or working process, which adversely affect the body of workers and as a result of prolonged, intense exposure cause diseases, are called the harmful occupational factors (Latvian Association of Occupational Physicians, 2016).

Harmful occupational factors are commonly associated with:

- manufacturing process, technology and equipment (industrial dust, toxic chemical and radioactive substances, ionizing radiation, noise, vibration, high or low atmospheric pressure, increased or reduced temperature, electromagnetic radiation etc.);
- work process, its organization, intensity and duration (tension of nervous system, vision, hearing, voice, frequent repetitive movements, straining of certain group of muscles, prolonged forced body position etc. (Hakkola et al., 1997; Valavanidis et al., 2008).

Due to diversity and originality of occupational risk factors, their various combinations and effects on the body, a specific clinical structure of occupational diseases has formed. Polytrophic effect characteristics to the majority of harmful occupational factors result in the frequent formation of the visceral, neurological and other poly-syndrome abnormalities, which in specific combinations acquire a certain specificity (State of the Art Report, 2009). The aim of the study is to determine the influence of fiberglass particles on the workers' health in the Latvian companies and to compare the results with the other countries similar production areas air atmosphere.

The study objects in the company are the chemical risk factors, but the study subject is the reduction of chemical risk factors' effect on the workers' health at the fibreglass products manufacturing company.

THEORETICAL BASIS

Fiberglass production volumes in Europe continued a steady growth in 2015. Their output has reached 1,069 megatons, the highest level for eight years. This development is largely dependent on the economic trends in Europe in general. Fibreglass products are mainly used in transport and construction; therefore, the fiberglass products output volumes are dependent on the development trends in these industries. The last development in recent years in Europe, the output volumes of fibreglass products grow as well. The European fibreglass production is growing more slowly than generally in the world (Global, 2011).

Table 1 shows that the amount of fibreglass products annually manufactured in European countries is almost unchanged. The biggest changes have been observed in the Eastern Europe (where Latvia is also situated), which is associated with a higher number of countries here (Latvian Fiberglass, 2014; EUCIA, 2015).

The company is a fibreglass products manufacturing enterprise that started its activities in Latvia in 1996. Production is exported to Norway. The company annually produces up to 700 tons of fibreglass; in 2015 the output was 580 tons. The main raw materials are fibreglass fabric, resin, paint and hardener. The company has implemented the integrated quality management system – ISO 9001, ISO 14001 and OHSAS 18001. The company holds the B Category polluting activities permit. Fiberglass products are manufactured in three workshops of the company with use of three production technologies.

1) Manual technology: glass fibre and adhesives are applied on the product mould; the air is removed from the glass fibre by means of metal rollers. By means of manual lamination the company develops products where the product thickness and weight are

important, and which can be very well controlled when gluing the product with by manual lamination.

2) Vacuum technology: glass fibre is applied on the product mould at the required thickness, then the film is applied and the glue is sucked into the product by vacuum. This technology allows to obtain a very thin and lightweight, but durable product.

3) Spray-up plant by means of spraying technology produces roofs and engine covers of open-pit truck, ship safety equipment – accessory cabinets, life jackets storage boxes, battery boxes, fibreglass mini-golf courses, figures and leisure baths; water purification equipment. Spraying technology: glue together with glass fibre is applied by spraying with subsequent rolling and squeezing the air out of the glass fibre. This method speeds up the production laminating processes, but is not applicable to all types of products. In manufacture of certain products it is much more economical to use this spray-up method, since the manual gluing cannot provide such application rate as with the spray-up method.

Table 1. Fibreglass products in European countries (kilotons) (EUCIA, 2015)

	2012	2013	2014	2015
Great Britain, Ireland	134	140	146	150
Belgium, Netherlands, Luxembourg	43	42	43	44
Finland, Norway, Sweden, Denmark	44	44	42	39
Spain, Portugal	160	152	154	156
Italy	152	146	148	150
France	117	112	108	108
Germany	182	192	200	212
Austria, Switzerland	17	17	18	18
Eastern Europe (Poland, Czech Republic, Hungary, Macedonia, Latvia, Lithuania, Slovakia and Slovenia etc.)	161	175	184	192
Total, megatons:	1,010	1,020	1,043	1,069

Styrene emission at workplace changes depending on the production process (Stockton & Kuo, 1990). Manual technology and spray-up technologies are open type processes while vacuum technology is a process of closed moulds. Styrene emission into air in vacuum technology is only 2% compared to 94% in open mould technology. With vacuum technology, resin is introduced into the product under the film, therefore styrene does not evolve into the air (Kalkis, 2001).

Styrene emissions in Europe

When using unsaturated polyester resins, employees are potentially exposed to styrene effect. In all European countries the employer is responsible for the control of hazardous substances at the workplace and ensuring the observance of occupational exposure limits (OEL) established by competent national authorities. Table 2 provides an overview of styrene OEL across Europe. Styrene vapour concentration is shown in parts per million (ppm). Short term OEL is the maximum allowed value within a short period, usually 15 minutes. Some countries have high values, which may not be exceeded in any case.

Table 2. Overview of styrene occupational exposure limits in Europe (International Labour Organization, 2016)

State	OEL 8 h, part per million (ppm)	Short term OEL, part per million (ppm)
Belgium	50	100 (15 min)
Denmark	-	25*
Estonia	20	50
Finland	20	100 (15 min)
France	50	-
Germany	20	40 (30min)
Latvia	2,4	7
Lithuania	20	50
Netherlands	25	50 (15 min)
Norway	25	37.5 (15 min)
Poland	12	47
Sweden	20**	50 (15 min)
Switzerland	20	40 (4 x 10 min)
England	100***	250 (15 min)

As shown in Table 2, Latvian occupational exposure limit is the lowest in Europe, which is due to the fact that Latvian chemical industry is highly developed and this threshold is trying to limit the chemical industry. As shown, the highest occupational exposure limit value is in England, the reason being that chemical industry is not developed in this country. Value adopted in Europe when determining the styrene concentration in the air is parts per million – ppm. To convert mg m^{-3} into ppm, formula 1 is used:

$$\mathrm{PPM} = \frac{mg}{\mathrm{m}^3} \cdot \frac{\frac{g}{mol}}{L}, \quad (1)$$

where PPM – parts per million; g mol^{-1} – hazardous molar mass of substance; L – molar volume.

Air pollution index

Latvian University offers a simplified chemical risk assessment matrix based on the Air quality index (GPI) determination, taking into account the concentration of chemicals in working environment, *OEL* values, risk phrases, and determines the necessary preventive measures (Kalkis, 2001).

GPI is determined by formula:

$$\mathrm{GPI} = \frac{\left(\frac{c}{OEL}\right) \cdot t}{8} \cdot 100\% \quad (2)$$

where C – chemical concentration in working environment air (mg m^{-3} or ppm); *OEL* – occupational exposure limit for 8 workday hours (mg m^{-3} or ppm); t – time of workers' exposure to chemicals, h; GPI – value determining % concentration of chemicals in working environment air within time of workers' exposure to chemicals (Kalkis, 2001).

Chemical concentration measurements frequency is determined according to the chemical exposure index obtained by dividing the concentration of a chemical (occupational exposure concentration) in working environment air by the occupational exposure limit (*OEL*):

$$EI = \frac{C}{OEL}, \quad (3)$$

where EI – chemical exposure index; C – chemical concentration (occupational exposure concentration) in working environment air.

Substitution of chemicals for less hazardous substances

In order to reduce the workers' exposure of chemicals, it is necessary to consider whether the chemicals can be replaced with less harmful substances. One of the changes in the production process, which can provide the desired result, is substitution of a dangerous chemical for other, less hazardous (harmful). This particularly applies to consumables, such as solvents. Such possibilities are usually objected, referring that the potential substitutes usually do not exist, but if they do, then they are much more expensive. However, it should be tried to find any opportunity to replace hazardous chemicals with less hazardous ones (Hazardous, 2001; Oosterhuis, 2006).

Often these alternatives, less harmful chemicals, cannot be used throughout the process, but they can be used in some phases of the technological process (Bake et al., 2010; Tint et al., 2015; OSHA, 2016).

MATERIAL AND METHODS

The air quality assessing method was gas chromatography. As the measuring instrument for determination of styrene concentration in the air, gas chromatograph SHIMADZU 2010 was used. The air samples were taken with the syringe by the Pumped sampling method described in the ISO 16200-1:2001 'Workplace air quality – Sampling and analysis of volatile organic compounds by solvent desorption/gas chromatography – Part 1', method code 4.3 (ISO 16200-1:2001). The measurements errors range from 6.7 to 7.6%. The experiments were carried out in three (3) enterprises producing and manufacturing the fiberglass during different production procedures. The concentration of chemicals was measured under protective masks with filters; out of the protective masks, during gluing, colouring, spraying-up and packing. Measurements were made within the worker's breathing area outside and inside of the mask.

One of the methods for cleaning the workplace air is ventilation. Ventilation systems performance is characterized by the air exchange coefficient K, that is, the ratio between the discharged air amount and the ventilated space volume:

$$K = \frac{W}{V_t}, \quad (4)$$

where W – discharged air amount, $m^3\ h^{-1}$; V_t – premise volume, m^3.

RESULTS

In this part the results of measurements of chemicals in the work environment, the substitution possibilities of the hazardous chemicals to less hazardous, the effectiveness of using the personal protective equipment and the ventilation of the rooms as one of the main means for cleaning the workplace air.

Table 3 shows air pollution indexes in production shops both under and outside the protective masks. Outside the protective masks at the workplace in all production shops in accordance with the chemical risk assessment matrix intolerable risk V (GPI – 200%) was established.

Table 3. Air pollution index at the enterprise (GPI)

No.	Production shop (PS), works performed	t, h	C, mg m^{-3}	GPI, %
1.	PS 1, mould gluing, under protective masks with filters	3 h	4.4	17%
2.	PS 1, mould gluing, out of protective masks	3 h	129.9	487%
3.	PS 2, mould gluing, under protective masks with filters	4 h	6.4	32%
4.	PS 2, mould gluing, out of protective masks	4 h	151.6	760%
5.	PS 3, mould colouring, under protective masks with filters	3 h	8.8	33%
6.	PS 3, mould colouring, out of protective masks	3 h	216.5	812%
7.	PS 3, mould spry-up, under protective masks with filters	3 h	7.7	29%
8.	PS 3, mould spry-up, out of protective masks	3 h	259.8	259.8
9.	PS 3, packing, out of protective masks	2 h	45.0	113

Respiratory protection half-mask air purifying respirators with organic vapour cartridges and particulate filter P100 were used. Necessary measures: work with chemicals should be stopped immediately until the fulfilment of necessary measures ensuring the prevention of air pollution is guaranteed.

Calculating the exposure index (Table 4), it is evident that the company's employees must be sent to mandatory periodic health checks annually as well as that the company should carry out periodic measurements every 16 weeks.

Table 4. Chemical exposure index (EI)

No.	Production shop (PS), works performed	t, h	C, mg m^{-3}	EI
1.	PS 1, mould gluing, under protective masks with filters	3 h	4.4	0.17
2.	PS 1, mould gluing, under protective masks with filters	3 h	4.2	0.16
3.	PS 2, mould gluing, under protective masks with filters	4 h	6.4	0.32
4.	PS 2, mould gluing, out of protective masks	4 h	151.6	7.60
5.	PS 3, mould colouring, under protective masks with filters	3 h	8.8	0.33
6.	PS 3, mould colouring, out of protective masks	3 h	216.5	8.12
7.	PS 3, mould spry-up, under protective masks with filters	3 h	7.7	0.29
8.	PS 3, mould spry-up, out of protective masks	3 h	259.8	25.98
9.	PS 3, packing, out of protective masks	2 h	45.0	1.13

The air quality measuring method was a gas chromatography. The measuring instruments of styrene concentration in air were the chromatography SHIMADZU 2010. The measurements errors range – 6.7–7.6%.

The concentratilons of tested substances, measured under the protective mask, meet the requirements. If an employee uses the protective mask with a new filter, the tested substance concentration is even lower than OEL.

Checking the styrene and acetone concentrations, it was found that the styrene concentration exceeds the occupational exposure limit value (making measurements within the worker's breathing area outside at the mask). When measuring under the protective mask, the concentrations of tested substances meet the requirements. In general at the enterprise, the styrene occupational exposure concentrations in the working environment outside the mask significantly exceed the allowable norm. The highest occupational exposure concentration is with the spray-up operator and painter as at these workplaces chemicals are applied by spraying.

The results on the substitution possibilities of chemicals are presented in Table 5.

According to Table 5, almost all solvents which the company could use for cleaning the tools are volatile and harmful to the health of workers. As a substitute for acetone, the company can use the acetone substitute 'GRP Multi Cleaner', which is not classified as a hazardous substance and is a solvent, less harmful to the environment and workers.

Table 5. Acetone substitutes (Safety Data Sheets: 'Solvent Acetone', 'Safety Data Sheet RS-2', 'Solvent Vaitsol', 'Solvent Turpentine', 'Solvent Solveks spirit', 'Solvent 646', 'Solvent 2290', 'Solvent 647', 'GRP Multi Cleaner')

No.	Name	Ingredients	Ingredient %	Boiling t°, C	H-sentences
1	Acetone			56.3	H36, H66, H67
2	Solvent RS-2				H10, H43, H65, H66, H67, H51/53
		White spirit	65	150–200	
		Solvent	30	140–200	
		Turpentine	5	162–174	
3	*Vaitsol* solvent				H10, H43, H65, H66, H67, H51/53
		White spirit	90–100	150–200	
		Turpentine	< 10	162–174	
4	Turpentine			162–174	H38, H43, H65
5	*Solvent* spirit				H20/21, H38
		Xylene	90–95	137–143	
		Acetone	5–10	55	
6	Solvent 646				H20/21, H38, H41
		Xylene	45–55	121–127	
		2-butoxyethanol	5–10	171	
		n-Butanol	5–15	118	
		Acetone	5–10	55	
		n-Buthylacetate	5–15	137–143	
		Ethanol	5–15	78	
7	Solvent 647				H20/21, H36/38, H66
		n-Butyl acetate	25–35	121–127	
		Ethyl acetate	20–25	70–95	
		n-Butanol	< 10	118	
		Xylene	40–45	137–143	
8	Solvent 2290				H10, H66, H67
		n-Butyl acetate	> = 99	124–128	
9	GRP Multi Cleaner			200–230	

'GRP Multi Cleaner' boiling point is 4 times higher than the boiling point of acetone. It means that this product is not volatile and will not vaporize and be present in the air of the workroom. Disadvantage of this substance is that it is oily and inconvenient for workers because after cleaning of tools they are slippery and it is not possible to work properly with them. Taking into consideration that 'GRP Multi Cleaner' is not a hazardous substance and does not vaporize the company needs to replace the existing solvent – acetone with 'GRP Multi Cleaner'.

The Styrene concentration measurements are presented in Table 6.

Table 6. Styrene concentration measurements

No.	Air sample taking place	Characteristic of production conditions	Measured substance	Concentration of OEC mg m^{-3} tests results	Concentration of OEC admit table norm mg m^{-3}
	Shop 1				
1	gluing in masks				
1.1.	gluing out of masks	measurements	Styrene	4.4 ± 0.5	10/30
	Shop 2				
2.	gluing in masks	gluing process	Styrene	129.9 ± 13.0	10/30
2.1.	gluing in masks	gluing process	Styrene	6.4 ± 0.6	10/30
2.2.	gluing out of masks	gluing process	Styrene	151.6 ± 15.2	10/30
3.	Shop 3				
3.1.	Painting in masks	Painting process	Styrene	8.8 ± 0.9	10/30
3.2.	Painting out of masks	Painting process	Styrene	216.5 ± 21.7	10/30
3.3.	Spraying – up in masks	Spraying – up process	Styrene	7.7 ± 0.8	10/30
3.4.	Spraying – up out of masks	Spraying – up process	Styrene	259.8 ± 26.08	10/30
3.5.	packing	Measurements performed only of masks out	Styrene	45.0 ± 4.5	10/30

The results show (see Table 6) that styrene concentration exceeds OEL when measurements are performed out of masks. The highest concentration of styrene is marked both in spraying – up and painting process. In order to reduce harmful usage of styrene it is advisable to substitute it by less poisonous substance, such as resin 'Aropol M60HTB' (styrene contains 37%; standard resin contains – 50% styrene) or 'Apolo M105TB' contains styrene 41%. These resins have low emission; nowadays enterprises have already substituted 50% of high styrene emission resin.

Ventilation improvements in production shops

The ventilation system in improve. As the conclusion from the previous analysis in all the production shops the air exchange and the existing ventilation system are not sufficient and need to be improved in order to the air exchange coefficient $K = 8.96\ h^{-1}$.

The worst situation is in the Production Shop No. 2. There is also the highest yearly output of products and manual technology is used. The best indicator, in its turn, is in the Production Shop No. 2 spray-up room, where the styrene concentration is found highest by the experiments. Ventilation air exchange coefficient is presented in Table7.

Table 7. Ventilation air exchange coefficient

Production place	Room's volume m^3	Local ventilation system with capacity m^3 h^{-1}	Air exchange coefficient h^{-1}	Air exchange coefficient required by the enterprise h^{-1}
Shop 1	2,300	10,000	10,000/2,300 = 4.35	20,603/2,300 = 8.96
Shop 2	5,300	10,000	10,000/5,300 = 1.89	47,480/5,300 = 8.96
Shop 3	1,700	10,000	10,000/1,700 = 5.88	15,220/1,700 = 8.96

DISCUSSION

The following improvement measures can be proposed:

1)Improvement of work equipment.

To reduce the concentration of styrene in production shops of the company, it is necessary to wider the use of the vacuum technology. At present time, the vacuum technology is only used for certain products, approximately only 10% of all manufactured products. Increasing the production by vacuum technology, the company will reduce the occupational exposure levels in the working environment. The analogous statements are given also by Hablanian, 1997).

In the vacuum technology, resin is sucked by vacuum, placing special tubes into the article and into a bucket with resin. The bucket is open, which means that the styrene from resin evaporates into the air. In order to reduce the concentration of styrene in the working environment, it is recommended to use buckets with lids. The company should substitute the existing buckets for buckets with holed lid, thus it is possible to reduce the concentration of styrene in the air (Vacuum, 2010).

2) Storage of personal protective equipment.

Personal protective equipment shall be provided with a special, dedicated for this purpose storage place, which must be clean and in working order. The EU legislation demands the same in the whole European workplace area (Regulation (EU) 2016/425; Council Directive 89/686/EEC).

3) Isolation of the work operations.

In certain cases when it is not possible, both, to reduce the concentration of a chemical and to shorten the exposure time, it is possible to try the method where the work operation, during which chemicals are released, is isolated. One of most effective and most common methods is isolation of such operations, which includes a potential pollution occurrence in a room separated from other premises. A separate room allows more efficiently and economically organize the preventive measures compared to the operations, carried out in common production areas. At the same time, it allows to reduce and limit the number of people who are or work in a given premise and are exposed to risk (Traumann at.al, 2014; OSHA, 2016). For example, isolation of spray-up equipment depending on dimensions of the workplace is suitable. Spray-up equipment will be located at the confining site and the worker producing the products will put his arms into special holes, which in total will be three pairs and each pair will be located at different height because three people work at this equipment and the height will be adjusted for each of them (Isolation, 2017).

Isolating the work process, it is possible to reduce the occupational exposure concentration in production shop since the exhaust ventilation place will be located in the confining site. As well, it is possible to reduce the number of workers exposed to styrene emissions.

4) Mandatory health checks.

Company production workshop workers are sent to mandatory health checks once a year, while office employees – every three years. Taking into consideration the mandatory health check results, the company needs to reduce the impact of harmful working environment factors on employees' health. The effectiveness of health checks is analysed by different authors and they usually give positive results (Si et al., 2014).

To reduce the impact of harmful working environment factors on employees' health, the company needs to replace the existing chemical substances with less harmful ones as well as to improve the efficiency of ventilation.

CONCLUSIONS

1.When using unsaturated polyester resins, workers are potentially exposed to styrene impact.

2.In all production shops of the company according to the chemical risk assessment matrix there is intolerable risk V level (GPI – 200%).

3.Calculation of the exposure index shows that the company employees must be sent to mandatory periodic health checks annually. As well as the periodic measurements shall be carried out by the company every 16 weeks.

4.Concentration checks of styrene and acetone in the working environment air show that styrene concentration exceeds the occupational exposure limit value when measuring within the worker's breathing zone outside the protective mask.

5.According to the air exchange coefficient calculations, the existing ventilation system of the company is not efficient enough and needs to be improved so that the air exchange coefficient would be 8.96 h^{-1}.

6.Can be changed to 'GRP multi cleaner', which is not volatile and is not a hazardous substance.

7.In order to reduce styrene concentration in production shops of the enterprise, it is necessary to wider the use of the vacuum technology. At present time, the vacuum technology is only used for certain products, approximately 10% of all manufactured products. Increasing the production by vacuum technology, the company will reduce the occupational exposure levels in the working environment.

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Influence of tied ridging technology on the rate of surface runoff and erosion in potato cultivation

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Abstract. Water management and securing good condition of soil is becoming an important factor in agriculture one of the reasons being adaptation to the increasingly frequent extremes in weather. Tied ridging technology enables to reduce significantly the loss of water and soil from arable land. The effect of a tied ridger mounted on 2-row planter and effect of 6-row tied ridger on basin renewal was tested in potato cultivation on plots of land with length of 10 m. During entire season lasting 135 days the technology helped achieve 78% of efficiency in reduction of surface water runoff and 88% of efficiency in case of soil loss. In the case of furrows with only tied ridging the water retained represented additional 15% of total water from rainfall (37.5 mm of 250 mm) over untreated furrows. In the case of basins formed in furrows with wheel trails the water retained represented 15% of total water from rainfalls in the trail. This water retained on the land would have otherwise flown off and would not have been utilized without this technology. The renewal of dams after 1/3 of the season increased significantly the efficiency of this technology. The technology of tied ridging significantly contributes to sustainable agriculture management.

Key words: furrow damming, basin tillage, soil protection, water runoff, trail.

INTRODUCTION

At the present time, potato cultivation in the Czech Republic is an increasingly marginal issue for many companies and this is so even in regions with large potato cultivation areas such as the Vysočina region (No. of NUTS3 – CZ063, mean height 500 m a.m.s.l.). In order to reduce the very high costs arising in potato cultivation, it is important to maintain the best possible quality of soil.

Potatoes together with maize and sugar beet belong to wide row crops. Owing to their cultivation method, wide row crops are more prone to erosion compared to grains (Hůla et al., 2010).

By implementation of anti-erosion measures it is possible to reduce erosion to an acceptable extent which minimizes its harmful effects on principal environmental functions of soil and its production potential. Anti-erosion measures, which can be applied easily, are some agrotechnical measures (Javůrek et al., 2012; Hůla et al., 2016; Kovář et al., 2016).

One of suitable technologies for erosion reduction in wide row crops is tied ridging (Munodawafa, 2012). Tied ridging is a measure with lower cost and minimum impact on traditional cultivation procedure preserving the crop yield without increased input of plant protection agents (Billen & Aurbacher, 2007).

Agriculture equipment for tied ridging creates dams in furrows between ridges which form retention spaces for water. Commercially available devices for anti-erosion protection are relatively few and usually do not treat trail furrows left by the tractor wheels. These furrows add up to as much as 2/3 of area in potato cultivation. In the project of the Technology Agency of the Czech Republic (TACR), No. TA 02020123 anti-erosion devices were developed (Fig. 1). They were a tied ridger mounted on a two-row planter with the aim to merge the technology of planting and tied ridging into one working operation and an anti-erosion device for renewal of furrow dams (Vacek et al., 2015; Mayer et al., 2016). In 2016, trials were established with the aim to evaluate agrotechnical measures carried out in both types of furrows and their effect on erosion and surface runoff of water during potato cultivation.

MATERIALS AND METHODS

The field trials with potato cultivation took place on a field with sandy-loam soil (7% clay, 21.5% silt, 71.5% sand) with the slope of 5°. Stone windrowing was carried out before potato planting. The trial was founded with the aim to develop and deepen the knowledge about anti-erosion technology of tied ridging in connection with stone windrowing. Two-row planter with additional fertilization (RBM-2HP production year 1997, empty weight 610 kg, full weight 1,610 kg, Reekie, Algarkirk, UK) formed dams (0.1 m width) in the furrows creating basins between them. The basins had the following dimensions 0.4 m length, 0.25 m width and average volume 1.5–2 liters. Date of planting: 9.5. The renewal of dams by the 6-rows anti-erosion tied ridger (HEP-7 production year 2015, weight 800 kg, KOVO NOVAK, Citonice, Czech Republic, www.kovonovak.cz) took place on 23.6, which was 14 days after plant emergence (Fig. 4). This renewal can be carried out until the potato plants reach 0.3–0.4 m. Dimensions of reservoirs were the same as at the beginning. The tractor used was Zetor Forterra 125 with output of 95 kW.

Figure 1. Reekie 2-rows planter with tied ridging adapter and KOVO NOVAK 6-rows tied ridger.

On the trial field (N 49°38‘16“, E 15°29‘20“, 450 m a.m.s.l.) 4 variants of trial (F = Furrow, TF = trail furrow, RF = ridging furrow, TRF = trail ridging furrow) were established on 10 m long strips. Distance between ridges in F and RF was 0.75 m and in TF and TRF it was 1.05 m (Fig. 2).

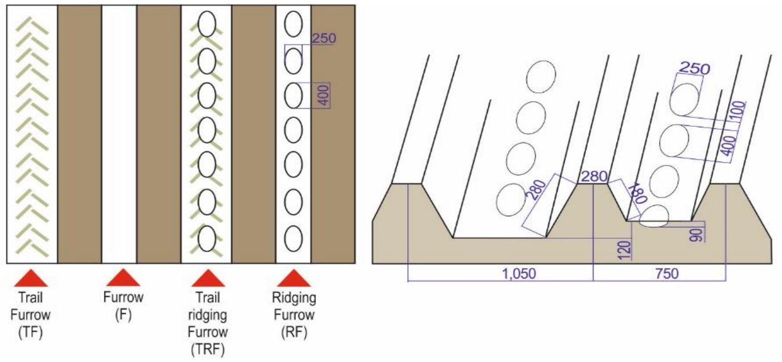

Figure 2. Layout and dimensions (mm) of variants and basins.

Each variant was established in 3 replicates. The rainfalls were recorded by a rain collector with the resolution of 0.3 mm. Volume of the basins was measured by vessel method in 10 replicates and on 8 dates. The water runoff from rainfalls was led to collection vessels separately for each variant by collectors (Fig. 3) and collection was carried out on 12 dates during the season (Fig. 4). By drying the samples of runoff liquid, the values of total soil loss from individual variants were obtained. The last term of runoff collection was carried out on 19.9., when the potato shoots were also removed. Harvest took place on 27.9.2016. The balance of runoff was calculated from the test areas. For the variant with tied ridging and for the variant without tied ridging the area was 54 m^2. Trail variants had the area of 31.5 m^2 (3 x 10.5 m^2) and non-trail variants 23.5 m^2 (3 x 7.5 m^2).

For statistical processing the software Statistica 12 was used employing one-way ANOVA Tukey HSD test.

Figure 3. Collectors for sampling and tied ridging in furrows.

RESULTS AND DISCUSSION

The volume of rainfalls for the whole period of potato cultivation (135 days from planting to shoot removal) was 250 mm m^{-2}. carried out in the first 45 days up to the date of dam renewal there was recorded 75 mm of rainfalls, which represented 30% of total rainfall for the season of potato cultivation. During those 45 days from planting there was recorded 13 out of 35 rainfall events. Eroded soil, owing to the greater rainfalls, especially with higher intensity (Fig. 4, Table 1), deposited in basins and reduced their retention volume (Fig. 5). Therefore, on 23.6. the renewal of dams was carried out by anti-erosion device in order to increase again the volume of basins. Reduction of reservoir volume was more significant in the case of variant with trail (TRF), 50% in comparison with 30% in case of RF.

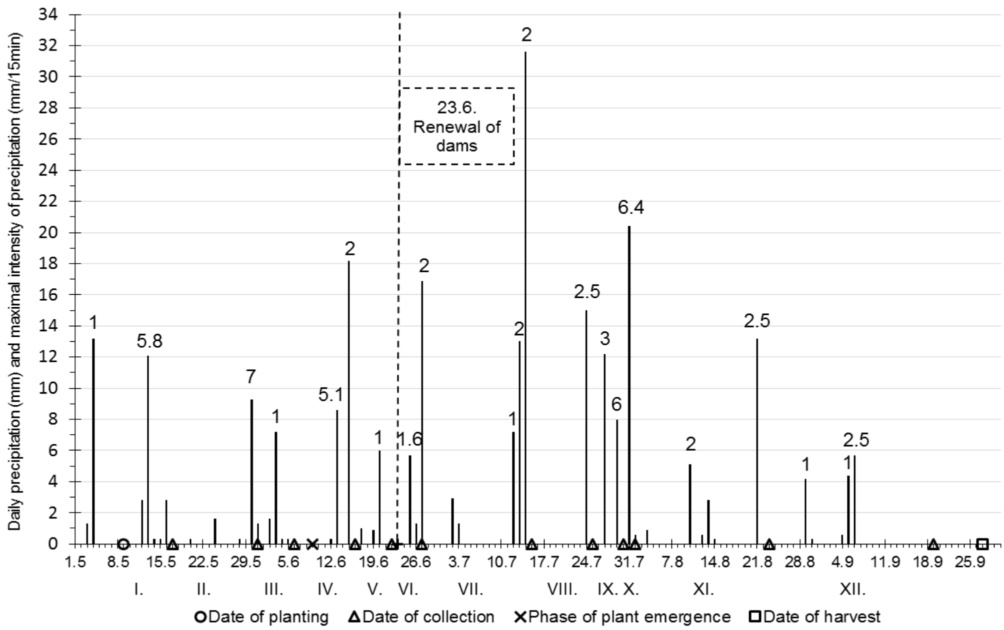

Figure 4. Time series of total precipitations, intensity of precipitation and date of collection.

Table 1. Precipitations in individual periods of collection

Periods of sampling	9.5.–16.5.	17.5.–30.5.	31.5.–5.6.	6.6.–15.6.	16.6.–21.6.	22.6.–27.6.	28.6.–14.7.	15.7.–24.7.	25.7.–29.7.	30.7.–31.7.	1.8.–22.8.	23.8.–19.9.
	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.	XII.
Total precipitation (mm m^{-2})	18.3	11.5	10.7	27.1	7.9	23.9	56.0	15.0	20.2	20.4	23.5	15.2
Max daily precipitation (mm)	12.1	9.3	7.2	18.2	6.0	16.9	31.6	15.0	12.2	20.4	13.2	5.7
Max intensity of precitipation (mm 15 min^{-1})	5.8	7.0	1.0	5.1	1.0	2.0	2.0	2.5	6.0	6.4	2.5	2.5

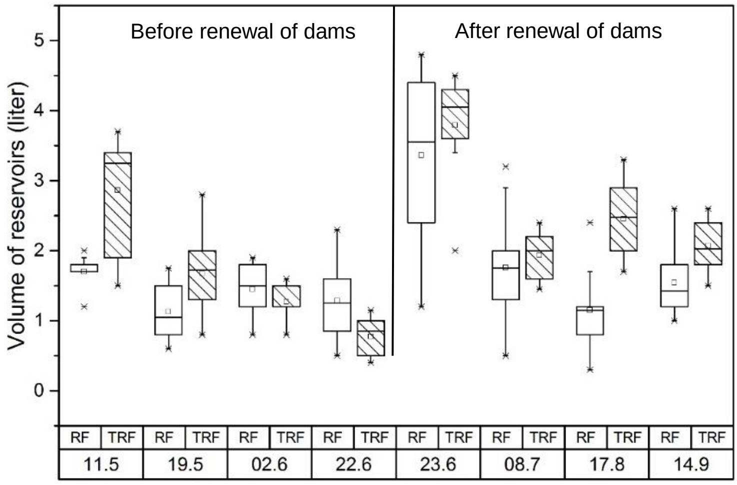

Figure 5. Reduction of volume of reservoirs of individual variants in time.

The volume of basins was higher in early days after the planting and even more notably after the dam renewal. In both cases the higher volume was caused by loosening of surface. The volume stabilized gradually and in 1–2 weeks it was 1.5–2 L. The filling of basins by eroded fine soil was less significant after their renewal on 23.6. This very likely relates to the coverage of sloping sides of ridges by potato shoots. The phase of shoot emergence took place 2 weeks before the renewal of dams. The shoots slowed the impact of rain drops and thanks to this erosion of ridge sides was reduced which led to better preservation of the reservoir volume in period after their renewal than before it. The greatest outflow in case of variants with tied ridging came in the IV period, when in short intervals there were recorded rainfalls with intensity of 5.1 mm in 15 min and quantity of 8 mm and daily rainfalls of 18 mm.

In case of variants without reservoirs the intensity of erosion was higher before 23.6. (1/3 of the whole season). This fact was probably caused by additional erosion from ridges as a consequence of 3 rainfall events with high intensity on 13.5., 30.5 and 13.6. In the remaining 2/3 of season the ridges were protected more by vegetation cover and erosion took place mainly in the furrows. Entrainment effect of running water on soil particles causing erosion is slowed with tied ridging by formed reservoirs. Daily quantity of rainfall of 32 mm and rainfalls with intensity of 6 mm in 15 min after dam renewal did not have any influence on runoff and the intensity of erosion in variants RF and TRF in comparison to the F and TF variants.

The renewal of reservoirs, carried out up to vegetation cover (23.6.) led to an increase of water retention from rainfalls, which considerably increased in further course of the season (Fig. 6). Tied ridging led to higher utilization of rainwater and lower losses of soil and after renewal of reservoirs these benefits increased further (Table 2 and Table 3). Comparing the trail variant (TF) and the non-trail variant without reservoirs (F) outflow and erosion were higher in TF (58% vs. 42%). Calculated to unit area m^2 it was 52% vs. 48%.

Retained water in case of TRF variant in the period up to the renewal of dams represented 7.7% of water precipitated on its area. After renewal of reservoirs, the retained quantity of water was higher – 15.7%. In total, for the whole season, reservoirs in the trail variant (TRF) retained 14.5% of total rainfall on its area which was 55% of total quantity of retained water in the technology of tied ridging (RF+TRF). Reduction in erosion intensity achieved in the case of TRF was 71% before renewal of reservoirs and 96% afterwards.

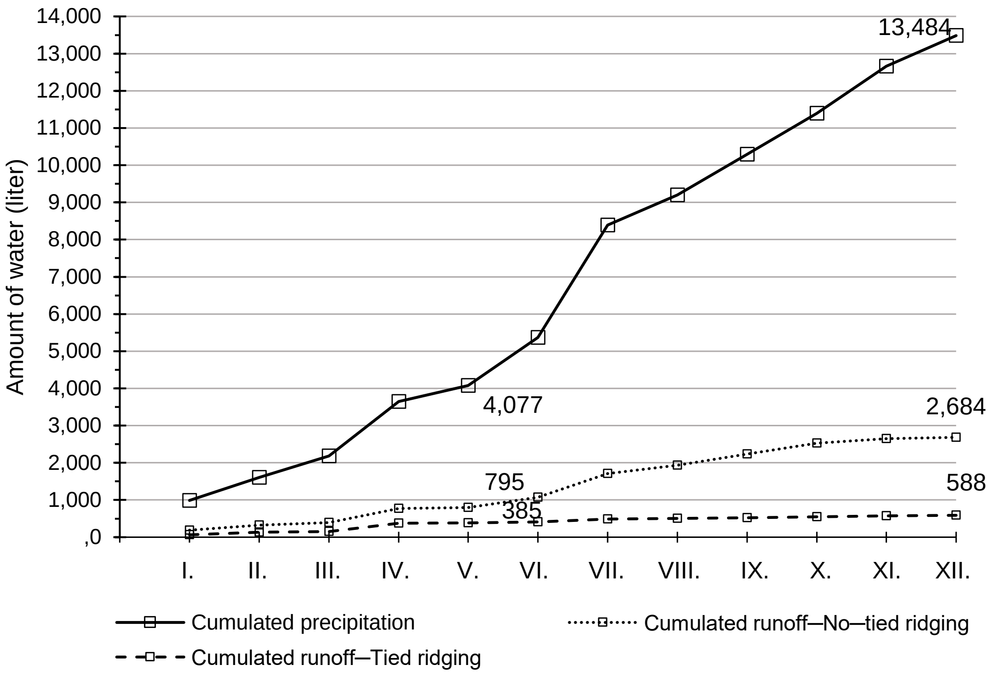

Figure 6. Balance of water on the research areas (54 m^2) in individual periods of sampling.

Technology of tied ridging retained 10% of rainwater before the renewal of reservoirs and 17% afterwards. In total, for the whole monitored period of 135 days following planting, the savings of water by using of this technology compared to regular technology were 15% from fallen rainfalls. This quantity of saved water, which represented 2,096 litres on an area of 54 m^2, would have drained away without the use of the tied ridging technology.

At the end of potato season, the variants of trial with the technology of tied ridging (RF+TRF) 78% lower surface runoff and 88% lower loss of soil (Tables 2 and 3) in comparison with variant without it (F+TF). On the F and TF variants the loss of soil was 107 kg of soil on an area of 54 m^2 across the monitored period.

Table 2. Runoff from individual variants and savings by tied ridging technology

Runoff in liter (l)	From planting until renewal of dams (9.5.–22.6.)	Save (9.5.–22.6.) of ridging technology in %	From renewal of dams until end of season (23.6.–27.9)	Save (23.6.–27.9.) of ridging technology in %	Whole season	Save of ridging technology in %
Tied ridging (RF+TRF)	385		204		588	
No-tied ridging (F+TF)	795		1,889		2.684	
Ridging furrow (RF)	92		60		152	
Furrow (F)	319		784		1,103	
Trail ridging furrow (TRF)	293		143		436	
Trail furrow (TF)	476		1,105		1,581	

Table 3. Erosion rate of individual variants and savings by tied ridging technology

Erosion in kilogram (kg)	From planting until renewal of dams (9.5.–22.6.)	Save (9.5.–22.6.) of ridging technology in %	From renewal of dams until end of season (23.6.–27.9)	Save (23.6.–27.9.) of ridging technology in %	Whole season	Save of ridging technology in %
Tied ridging (RF+TRF)	10.6		1.7		12.3	
No-tied ridging (F+TF)	54.8		52.1		106.9	
Ridging furrow (RF)	1.7		0.6		2.3	
Furrow (F)	23.4		21.1		44.5	
Trail ridging furrow (TRF)	8.9		1.1		10.0	
Trail furrow (TF)	31.4		31.0		62.4	

The significant differences of surface runoff between variants with tied ridging technology (RF, TRF) and without technology (F, TF) were proven by statistical analysis (Table 4). Significants differences could be also proven between variants in furrow (F) and in trail furrow (TF) without tied ridging technology. On the contrary, the differences between furrow (RF) and trail(TRF) in tied ridging technology were comparable and significant differences did not show.

Table 4. Tukey HSD test for surface runoff

Variants	Surface runoff (liter) – Mean	1	2	3
RF	4.219	a		
TRF	12.124	a		
F	30.761		b	
TF	45.161			c

Alfa = 0.05; RF – ridging furrow; TRF – trail ridging furrow; F – furrow, TF – trail furrow.

Tied ridging in interrows of potatoes, as an effective measure for reduction of surface runoff of water and loss of soil from land, is mentioned in case of trials in Canada on the Prince Edward Island by Gordon et al. (2011). By application of a single tied ridger without planter on fields with the length of 25 m with the slope of 3–5°, a reduction of surface runoff by 75% was achieved as well as 89% reduction of soil erosion. The basins had a length of 1 meter with a volume of 5.25 litres. Authors mention the reduction of retention space at the end of season by 1/3 in comparison with original volume at the beginning of season.

Positive effects of tied ridging technology during simulated 14 mm rainfall were observed in the trials of Müller et al. (2009) in Sachsen, Germany. Surface runoff on the 6 m plots on loess came after 400 seconds in the variant with tied ridging, however in the variant without this technology runoff started just after 50 seconds. During the time of runoff retention in reservoirs the water would carry away 270 kg of soil per hectar in the control variant.

Billen & Aurbacher (2007) measured the surface runoff in a trial with simulation of rain with intensity from 50 to 100 L m^{-2} per hour. The surface runoff was 1% from total rainfall in tied ridging technology against 14% to 52% in a variant without technology.

Olivier et al. (2011) carried out studies in the Flemish region in Belgium on 30 m plots with the length of basins 1.6 m. From May to September the efficiency of technology was monitored during various rainfall events. The authors mention a great effect on reduction of technology efficiency owing to an extreme rainfall. Up to the torrential rainfall in August of 40 mm, which was recorded on the 106th day from the beginning of measurement, the water runoff had been lower by 85% and after it only by 10–20%. In total, for the whole monitored season the authors achieved in their trials for 120 days runoff lower by 50% with the use of a single tied ridger in comparison with conventional technology.

Some disadvantages of this technology have been recorded from the experience of farmers. In the case that tied ridging is performed as a separate operation, the costs are rising and the soil is compacted repeatedly by machine passages. Another limiting factor of the system is the formation of bumps, which make the work of machinery on soil difficult (Applied Research Forum, 2007).

CONCLUSIONS

A high efficiency of agricultural anti-erosion technology of tied ridging to reduce surface runoff of water and soil erosion during cultivation of potatoes was confirmed. A substantial reduction in both surface runoff and erosion intensity are also mentioned in other studies worldwide. Extreme rainfalls can significantly reduce the efficiency of tied ridging technology. In our study, there were used greater number of smaller reservoirs of shorter length compared to the other studies and after 1/3 of season their renewal was carried out.

As a result of stronger rainfalls and lack of vegetation cover considerable erosion of soil can occur, which settles in reservoirs formed in soil in case of tied ridging technology. These reservoirs prevent erosion of soil from the field, but at the same time this material reduces the retention capacity of these reservoirs and it results in a reduction of efficiency of this technology. After 1/3 of season considerable filling of reservoirs has been recorded with reduction of initial volume by 30–50%. In order to increase the efficiency, it is suitable to carry out a renewal of reservoirs up to the time of closing of plant cover which will increase again their volume. As a consequence of developed vegetation cover formed by shoots there is not significant erosion and larger accumulation potential is restored for outflowing water which can be retained. Without renewal of reservoirs, the savings of water would range between 10–17% of the total rainfalls. By renewal of dams 1.7 x more water was retained in following 90 days from total rainfalls than in the previous 45 days.

By combination of tied ridging during the planting and renewal of reservoirs 15% of water from total rainfalls (37.5 mm of 250 mm) was additionally utilized in the whole period of 135 days. Otherwise this water would have drained away unused.

Soil protection technologies contribute significantly to sustainable agricultural production, reduction of harmful effects on the environment and reduction of consequences of weather extremes. Tied ridging technology improves considerably the water management on the fields and unification of tied ridging technology into one operation together with planting decreases costs and soil damage caused by agricultural machinery.

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AFLP-analysis of genetic diversity in soybean [*Glycine max (l.) Merr*.] cultivars Russian and foreign selection

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Abstract. Genetic diversity of 11 cultivars of soybean (Glycine max) from a VIR (N.I. Vavilov Institute of Plant Genetic Resources) collection was analyzed by the AFLP (amplified fragment length polymorphism) technique. From 18 tested primer combinations, both of them were selected for further analysis. From these two primer combinations (E32/M60 and E32/M59), 90 DNA fragments were obtained, 39 (43%) of them were polymorphic. Unique DNA fragments were found in accessions SibNIISHoz 6, Soer-4, Determinant. The Jaccard's similarity indices varied from 0.79 (between Sonata and Ugra) to 0.94 (between Ugra and Garmoniya) with an average of 0.775. Cluster analysis placed the cultivars into the several groups but separation in groups was not related to their origin or biological characteristics, even though cultivars Nordik and Soer-4 (cultivars recommended for Central Black Earth region of Russia) clustered together on the dendrogram. The obtained high values of Jaccard genetic similarity index and low level of polymorphism and differentiation illustrated a relatively low genetic diversity in our studied cultivars, which correlated with different other studies on soybean genetic diversity with AFLP-analysis.

Key words: Soybean, Amplified Fragment Length Polymorphism (AFLP), Genetic diversity, Molecular markers.

INTRODUCTION

Soybean (Glycine max (L.) Merr.) is the most important crop as the source of high-protein and oil for human consumption and animal feed. Soybean is known as the first crop among the oilseed crops in all over the world, soybean variety selection is the first step in producing a high-yielding soybean crop. Despite soybean plasticity, varieties have different level of adaptability to various environments, therefore, each country has its own cultivars genbanks. By increased demand on varieties (high yield, environmental factors), countries organize different breeding programs to create new improved cultivars, hence the requirement to introduce new genotypes to select breeding materials.

Knowledge of soybean genetic diversity is a fundamental importance for efficient breeding programs as it helps breeders and geneticists to understand the structure of germplasm, predict which combinations would produce the best offspring and facilitate to widen the genetic basis of breeding material for selection (Zargar et al., 2011; Bisen et al., 2015). Mentioned information is useful to organize a working collection, identify heterotic groups, and select parents for crosses. Genetic diversity between individuals can be estimated by using both morphological, biochemical and molecular markers, although the use of morphological and biochemical markers has its constraints, given that they are limited in number, stage specific and highly influenced by the environmental conditions (Cox et al., 1985). This problem has been overcome by using molecular markers, RFLP technique has also been used to study exotic soybean germplasm and it has allowed the identification of different gene pools (Kisha et al., 1998). Similar studies have been carried out using other types of molecular markers, such as RAPD markers (Abdelnoor et al., 1995; Brown-Guedira et al., 2000), simple sequence repeat (SSR) markers (microsatellites) (Diwan & Cregan, 1997) and amplified fragment length polymorphism (AFLP) markers (Zhu et al., 1999; Ude et al., 2003).

AFLP analysis is one of the most popular fast and highly reproducible methods that can detect very large number of DNA bands, thus enabling identification of many polymorphic markers. AFLP is employed for a variety of applications, such as: analysis of population polymorphism, phylogenetic relations, genetic diversity assessment within species or among closely related species, identification of loci linked to economically valuable traits, deduction of population-level phylogenies and biogeographic patterns, genetic maps design and determination of relatedness among cultivars (Renganayaki et al., 2001; Soleimani et al., 2002; Kim et al., 2010; Ovidiu & Schönswetter, 2012). This method also can be used by breeders to preliminary assess the initial genetic material to plan the strategy of crosses, to identify the best combinations of genotypes, and for general selection (Kim et al., 2010; Sensi et al., 2003; Portis et al., 2004; Zargar & Pakina, 2014).

Molecular markers have also been used in Russia to analyze genetic diversity in soybeans with RAPD (Seitova et al., 2004), SSR (Ramazanova et al., 2008) and ISSR (Kozyrenko et al., 2007) being the more prominent. However, studies of the genetic diversity on Russian soybean cultivars using AFLP markers are very few.

Keeping in view that fact, the objective of the present study was to assess the genetic diversity of 11 Glycine max cultivars from VIR-collection using the AFLP analysis. The 11 cultivars were chosen from a big collection of cultivars introduced in the central European part of Russia (Moscow region) from different regions of Russia and other countries. Some of mentioned cultivars were developed by different institutes for various regions of Russia, such as Mid-Volga, East-Siberian, Far East, Southeast Central Black Earth, lower Volga, mid-Volga, Volga-Vyatka, mid-Volga, Ural regions, and two cultivars were from other countries as Poland and Sweden. Some of these cultivars were developed from the same parental lines or, are from the same sub-species – spp. *Manshurica*. They were selected for additional studies, after showing good results in Moscow region, thus making them interesting for different breeding programs (Romanova et al., 2013; Shafigullin et al., 2016).

MATERIALS AND METHODS

Plant material: In this study, 11 soybean cultivars of Russian and foreign selection from a VIR (N.I. Vavilov Institute of Plant Genetic Resources) (Table 1) collection were analyzed using AFLP markers.

Table 1. A list of soybean cultivars used in AFLP-analysis

Accession name	Developers (Institute)	Region, Country
Sonata	All Russian Research Institute of Soya; Far East State Agrarian University	Russia
Determinant	All-Russia Research and Development Institute of Vegetable Crop Selection and Seed Growing	Russia
Altom	Altai scientific-research institute of agriculture	Russia
Soer-4	Ershov Experimental Station of Irrigated Agriculture, Research Institute of Agriculture of the Southeast	Russia
Nordik	Poland	Poland
SibNIIK 315	Siberian Federal Research Center of Agricultural Biotechnology of the Russian Academy of Science	Russia
SibNIISHoz 6	Siberian Research Institute of Agriculture of Russian Academy of Agricultural Sciences	Russia
Okskaya	Ryazan scientific research institute of agriculture of the Russian Academy of Agricultural Sciences	Russia
Garmoniya	All Russian Research Institute of Soya	Russia
Lidia	All Russian Research Institute of Soya	Russia
Ugra	Sweden Svalöf AB in Malmöhus	Sweden

(http://www.gossort.com)

DNA extraction: DNA was isolated from 5-day-old seedlings in accordance with the protocol of Doyle & Doyle (1987) by some modifications. The concentration of isolated DNA was measured in comparison to phage DNA of known concentration (Fermentas, Lithuania) with gel electrophoresis. DNA samples were stored at -20 °C.

AFLP-analysis: AFLP analysis was carried out in standard form (Vos et al., 1995).

For the *Restriction/Ligation,* 300 ng of genomic DNA of each accession was double-digested with 5 U *Eco*RI and 5 U *Mse*I restriction enzymes (NEB) in a final volume of 40 μl at 65 °C for 3 hours using Gene Amp PCR system 9700. The genomic DNA fragments were ligated using 1 unit of T4 DNA Ligase (Invitrogen) and 10 μl of Ligation solution (ligase buffer, 10 mM ATP, 10mM *Eco*RI, 10mM *Mse*I) at 36 °C for 6 hours. Then T4 DNA Ligase was inactivated at 65 °C for one hour. (Gene Amp PCR system 9700) ligated to 10 *Eco*RI and *Mse*I adapters overnight at 15 °C to generate template DNA for amplification.

For *Pre-amplification,* the ligation mixture was diluted to 4-fold with deionized water. The template DNA generated was first pre-amplified using the primer pair combination each having one selective nucleotide, 10 mM *Eco*RI+A and mM *Mse*I+C. The selective amplification PCR reaction was performed with a final volume of 15 μl containing 10 × PCR buffer, 25 mM $MgCl_2$, 2 mM dNTP, 10 mM each of *Eco*RI and *Mse*I primers, 0.5 U of BioTaq polymerase. The thermal profile: denaturation at 95 °C for 5 min, 12 cycles of 30 sec at 94 °C, 30 sec at 65 °C with 0.7 °C lowering for each cycle and 1 min at 72 °C, followed by 27 cycles of 30 sec at 94 °C, 30 sec at 56 °C and 1 min at 72 °C and final elongation at 72 °C for 10 min. PCR-reactions were carried out in the Gene Amp PCR system 9700. For quality control, the products of pre-amplification were visualized on a 1.2% agarose gel.

For *Selective amplification,* which's aim is to restrict the level of polymorphism and to label the DNA, we added three more nucleotides at the 3' end of the *Eco*RI and four nucleotides at the 3' end of the *Mse*I primers (Table 2). These additional nucleotides make the amplification more selective and will decrease the number of restriction fragments amplified (polymorphism). The selective amplification PCR reaction was performed with a PCR solution volume of 10 µl containing 10x PCR buffer, 25 mM MgCl2, 2 mM dNTP, 10 mM each of *Eco*RI and *Mse*I primers, 0.5 U of BioTaq polymerase. The thermal profile: denaturation at 95 °C for 5 min, 12 cycles of 30 sec at 94 °C, 30 sec at 65 °C with 0.7 °C lowering for each cycle and 1 min at 72 °C, followed by 32 cycles of 30 sec at 94 °C, 30 sec at 56 °C and 1 min at 72 °C and final elongation at 72 °C for 10 min. PCR-reactions were carried out in the Gene Amp PCR system 9700. The amplification products were separated in 6% polyacrylamide gel (PAAG) and stained with silver nitrate as described in (Benbouza et al., 2006). The length of the amplification fragments was assessed using the 100 bp DNA ladder (Invitrogen, United States) (0.05 g L^{-1}).

Table 2. Primers for selective amplification used in the study

Adapter/primer	Code	Nucleotide sequence
EcoRI-primer + A + AGC	E40	5′-GAC TGC GTA CCA ATT C + AGC-3'
EcoRI- primer + A + ACT	E38	5′-GAC TGC GTA CCA ATT C + ACT-3'
EcoRI- primer + A + AAC	E32	5′-GAC TGC GTA CCA ATT C + AAC-3'
EcoRI- primer + A + ATG	E45	5′-GAC TGC GTA CCA ATT C + ATG-3'
MseI- primer + C + CTC	M60	5′-GAT GAG TCC TGA GTA A + CTC-3'
MseI- primer + C + CAT	M50	5′-GAT GAG TCC TGA GTA A + CAT-3'
MseI- primer + C + CGA	M55	5′-GAT GAG TCC TGA GTA A + CGA-3'
MseI- primer + C + CTA	M59	5′-GAT GAG TCC TGA GTA A + CTA-3'
MseI- primer + C + CCC	M52	5′-GAT GAG TCC TGA GTA A + CCC-3'
MseI- primer + C + CTG	M61	5′-GAT GAG TCC TGA GTA A + CTG-3'
MseI- primer + C + CTC + C	M60C	5′-GAT GAG TCC TGA GTA A + CTC+C-3'
MseI- primer + C + CTC + T	M60T	5′-GAT GAG TCC TGA GTA A + CTC+T-3'
MseI- primer + C + CAT + C	M50C	5′-GAT GAG TCC TGA GTA A + CAT+C-3'
MseI- primer + C + CAT + G	M50G	5′-GAT GAG TCC TGA GTA A + CAT+G-3'
MseI- primer + C + CCC + A	M52A	5′-GAT GAG TCC TGA GTA A + CCC+A-3'
MseI- primer + C + CCC + C	M52C	5′-GAT GAG TCC TGA GTA A + CCC+C-3'

Both DNA isolation and AFLP analysis were carried out in the Laboratory of Plant Genetics of the Vavilov Institute of General Genetics, Russian Academy of Sciences (Moscow, Russia).

Data Analysis: The presence or absence of the amplification products was registered visually in the gel. Obtained data were loaded into a binary matrix, in which I corresponded to the presence of a fragment and 0 corresponded to its absence. For each combination of primers, the number of monomorphic and polymorphic fragments was estimated. The percentage of polymorphic fragments was assessed as the ratio of the number of polymorphic fragments to the total number of fragments. The Jaccard genetic similarity index was calculated using the PAST 3.09 program (Hammer et al., 2001). Dendrogram was also constructed with PAST 3.09 programs. Clusterization of the samples was performed using the UPGMA method.

RESULTS AND DISCUSSION

18 primer combinations were initially tested, but only two of them detected a high level of polymorphism, and were further used to analyse the genetic diversity in illustrated soybean cultivars (Table 3). The other 16 combinations showed a low level of polymorphism or were monomorphic, therefore were not used for further analysis.

The AFLP analysis of 11 soybean samples allowed us to identify 90 fragments, 39 (43.3%) of which appeared to be polymorphic (Table 3). The high numbers of fragments were obtained with the primer combination E32/M59. The number of polymorphic fragments varied from 18 to 21 per primer combination with the average of 19.5, and, the average level of polymorphism was 43.5%. In AFLP spectra of SibNIISKHoz 6, Soer-4, Determinant samples were found unique fragments, which can be used as molecular markers of these varieties.

Table 3. Characteristics of polymorphism in *Glycine max* cultivars using the selected AFLP primers

Primer combination	Amplifed fragments (a)	Polymorphic fragments (b)	Percentage polymorphism (b x 100/a)
E32/M60	40	18	45
E32M59	50	21	42
Total	90	39	43.3

Bonato et al. (2006) investigated the genetic similarity among 317 soybean cultivars released to Brazil by applying (AFLP) technique and achieved lower values of polymorphism level. The six primer combinations used to analyze generated 394 bands, but 78 (19.8%) of those were found to be polymorphic among genotypes. The average number of polymorphic markers per primer combination was 13 (Bonato et al., 2006).

Analysis of the 12 *G. max* and 11 *G. soja* accessions with 15 AFLP primers pair identified a total of 759 fragments, of which 274 (36%) were polymorphic, average number of polymorphic fragments for each primer pair was 18.3 (Maughan et al., 1996). Among the 274 polymorphic fragments detected, 37 were observed only in *G. max* and 147 were observed about *G. soja*. Satyavathi et al. (2006) used 12 AFLP primer pairs to assess genetic diversity in 72 soybean cultivars under Indian cropping systems, they produced 1,319 products, which 1,257 of them (95%) were polymorphic (Satyavathi et al., 2006).

Based on the AFLP spectra obtained in our study, the levels of Jaccard genetic similarity index in *G. max* samples were calculated. The maximum level of genetic similarity (0.94) was detected between the Ugra and Garmoniya samples, whereas t minimum level of similarity (0.79) was obtained between Sonata and Ugra samples.

Regarding to the mentioned AFLP analysis above, the values of the genetic similarity index achieved in our study show a significant level of similarity in our cultivars compared to other studies, although those studies analyzed a significant high number of cultivars. For example, the genetic similarity coefficients between Brazil soybean cultivars varied from 0.17 to 0.97 (Bonato et al., 2006). Jaccard's similarity coefficients among the Indian cultivars varied from 0.787 to 0.118 (Satyavathi et al., 2006). On the other hand, Maughan et al. (1996) found that in their study high similarity coefficients from 0.74 to 1.00 within the Glycine max varieties, and the examined cultivars were from different parts of the world (Maughan et al., 1996).

The clusterization of the samples was further perfomed (Fig. l).

On the dendrogram (Fig. 1) constructed according to AFLP-analysis the eleven accessions of *G. max* formed two clusters.

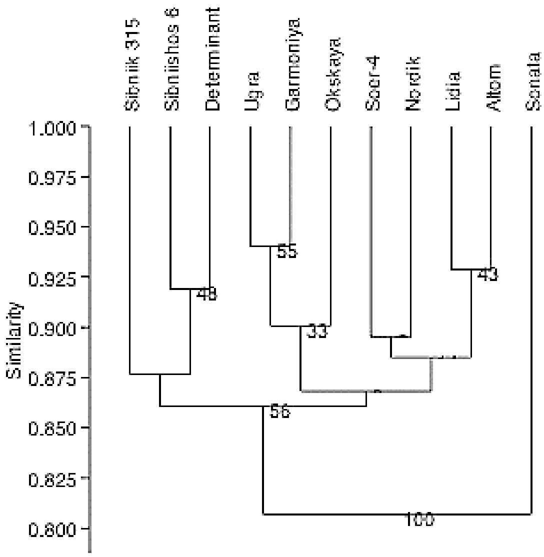

Figure 1. Dendrogram constructed on the basis of AFLP analysis of 11 soybeans samples using UPGMA method.

The first cluster includes one variety Sonata and the second cluster involved the rest of the samples. Within the second cluster, examined samples were formed two sub-clusters with a similarity index of 0.86. The first subcluster was formed by the varieties SibNIIK 315, SibNlISHoz 6, Determinant. Cultivars Ugra, Garmoniya, Okskaya, Lidia, Altom, Nordik, Soer-4 formed the second subcluster.

Generally, separation of samples in groups is not related to their geographical origin although Nordik and Soer-4 (varieties recommended for cultivation in the Central Black Earth region of Russia) clustered together also on the dendrogram.

The high values of Jaccard genetic similarity index, the low level of polymorphism and differentiation illustrated the relatively low genetic diversity present in the studied soybean varieties. The obtained results of low genetic diversity was similar to that obtained by Nimnual et al. (2014), which also showed that cultivars didn’t cluster according to their origin (Nimnual et al., 2014). The explanation given to the high genetic diversity obtained by Yan et al. (2014) compared to our results, was the bulking and mixing of cultivated gene pool from geographically distant populations by crossbreeding under condition of artificial domestication (Yan et al., 2014), but in our case studied cultivars were developed by collaborating organizations, from probably the same parental forms. The other explanation of the low genetic diversity can be the fact that some cultivars are from the same subspecies *manshurica*, some varieties were created by the same organization or from the same parental form and were developed for the same ecological zone.

CONCLUSIONS

AFLP markers used in present study to assess the genetic diversity in 11 cultivars revealed a relatively low level of genetic diversity. From the 18 primer combinations initially chosen for the study only two primer combinations (E32/M60 and E32/M59) allowed to detect polymorphic fragments. However, even with them we obtained a low level of polymorphism – of the 90 DNA fragments obtained, 39 (43%) were polymorphic. Unique DNA fragments were found for varieties SibNIISKHoz 6, Soer-4, Determinant. Jaccard genetic similarity index between varieties was high. Cluster analysis separated varieties into several groups but that separation was not related to their origin or biological characteristics. Such low genetic diversity level can probably be explained by the fact that all examined cultivars belong to subspecies *manshurica*, some varieties were created by the same organization or from the same parental form and can grow in the same ecological zone.

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Dimethyl ether as a renewable fuel for diesel engines

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Abstract: The area of automotive fuel, or fuel components, which can be produced from biomass also includes dimethyl ether, otherwise known as DME. The issue of the use of DME as a fuel is one which has been monitored until recently. Biomass can also be used as the raw material for the production of DME. DME has therefore replaced the previously-used CFCs (chlorofluorocarbons), which are now banned for their role in dangerous levels of ozone depletion. With regard to its physical properties and combustion characteristics, it is currently expected that DME will soon apply significantly as a fuel in the municipal sector and in households, and as an alternative fuel for motor vehicles with diesel engines. DME is a suitable fuel for diesel engines and can be considered as one of the most promising diesel fuel replacements. DME is a suitable fuel for diesel engines mainly due to its low self-ignition temperature and good cetane figures. It is well miscible with most organic solvents and because the polar solvent is water-immiscible. The advantage is its high levels of purity, and its being free of sulphur, nitrogen, or metals. The physical properties of DME are very similar to the physical properties of LPG. DME requires relatively complex and costly fuel accessories, but the original compression ratio of the diesel engine is maintained. A diagram of the fuel system is illustrated in the paper. The paper analyses the dependence of vapour pressure on temperature, the dependence of the density on temperature, kinematic viscosity, the flash point, the boiling point, and the solubility of water. The objective is to evaluate this interesting energy source for applications in diesel engines.

Key words: biofuel, biomass, liquefied petroleum gas, vapour pressure, density.

INTRODUCTION

There are many reasons why we should deal with alternative fuels. The main one is probably the fact that sooner or later oil reserves will exhausted and mankind will lose its ability to produce gasoline and diesel – fossil fuels without which today's traffic cannot get by. Further, using these classic carbon fuels delivers negative consequences in terms of ecology. Although there is significant emphasis on fuel quality in order to reduce emissions and fuel-efficient vehicles are being designed, these measures are not effective enough. All of this is thanks to increasing energy consumption levels in terms

of oil production (which should culminate in 2020) and the related increase in emissions. Road transport continues to produce still larger quantities of substances which have a harmful effect not only on the human body, but also on the world's overall climate on a global scale (De Wit & Faai, 2010; Thamsiriroj et al., 2011; Hönig et al., 2015).
Among the most-discussed alternative fuels are:

- **Biodiesel** – a methyl ester made of vegetable or animal oil or, to put it more precisely, fat. The quality corresponds to conventional diesel which is used as biofuel.
- **Bioethanol** – an ethanol made of biomass or by using the biodegradable fraction of waste.
- **Biomethanol** – methanol made of biomass. This methanol is used as biofuel.
- **LPG** (Liquefied Petroleum Gas) – a blend of liquefied propane and butane.
- **Biogas** – a name for a fuel gas made of biomass or made by using the biodegradable fraction of waste that can be purified to natural gas quality and used as biofuel.
- **Natural gas** – a natural mixture of gaseous hydrocarbons with a majority of methane.
- **Biohydrogen** – a hydrogen made of biomass and/or from the biodegradable fraction of waste, which is used as a biofuel (Schlaub & Vetter, 2008; Küüt et al., 2011; Pointner et al., 2014).

Apart from suitable physico-chemical properties, an alternative fuel should meet a number of requirements. Material from which the fuel is obtained must be accessible, renewable if possible, and not too expensive. Actual fuel production must not be too high-tech and energy-intensive. Extracted fuel must be economically competitive and environmentally friendly. In terms of thermal cycles, the fuel should have adequate calorific value. Another criterion is the need for this fuel to be applicable for use in commonly-used cars with petrol or diesel engines, with a minimum of necessary design adaptations being required. If the fuel requires a special redesign of the entire propellant system, it would be desirable that such an equipped vehicle is affordable and is available with a large selection. In order to be able to implement a totally new fuel also requires the construction of a distribution infrastructure – the fuel must be sufficiently available. Fuel should be easy to store, should be spoilage-resistant, and handling it should be as safe as possible (Ju et al., 2014; Wang et al., 2016).

Amongst automotive fuels or fuel components which can be produced using biomass, ethers also belong to this group, whether dimethyl ether (DME) or ethyl tert-Butyl ether. DME is a colourless gas with a chloroform odour. It burns with a slightly luminescence flame and is not toxic. It mildly irritates the respiratory system and has possible narcotic effects. When mixed with air, oxygen, chlorine, and hydrogen chloride it forms an explosive mixture (Nazari et al., 2015).

It is estimated that today's worldwide annual consumption of dimethyl ether (DME) is around 150,000 tons (Šebor et al., 2006). The vast majority of dimethyl ether (90%) is used as a propellant in the manufacture of aerosols. DME has therefore replaced the previously-used freons (chlorofluorocarbons, or CFCs), which are now banned thanks to their being dangerous to the ozone layer (Laurin, 2007). DME is used in the production of methyl acetate and acetic anhydride. It is a perspective material for the production of light olefins, especially ethylene and propylene. With regard to its physical properties and combustion characteristics it is currently expected that DME will soon apply significantly as a fuel in the municipal sector and in households, and as an alternative fuel for motor vehicles with diesel engines. The issue of the use of DME as a fuel was raised pretty recently (Nazari et al., 2015).

DME can be made by using various materials, such as natural gas and biomass (biodimethyl ether). For the production of one ton of DME we need three tons of wood, which corresponds to a yield of 500 litres of DME from one ton of wood (Laurin, 2007). DME is currently produced via the catalytic dehydration of methanol (a fixed catalyst base) according to this Eq. (1):

$$2CH_3OH \rightarrow CH_3OCH_3 + H_2O \qquad (1)$$

As a catalyst for the dehydration of methanol to DME, γ-alumina alone or γ-alumina saturated with phosphoric acid is being recommended (Laurin, 2007; Liu & Chen, 2015). Methanol preheated to 300 °C is dosed into a reactor which is filled with shape-alumina or, as the case may be, diluted with inert beads (such as glass, which offers better heat conduction). The reaction mixture is conducted from the bottom of the reactor at a maximum temperature of 400 °C. It operates at a pressure of up to 1.7 MPa. Under these conditions it is possible to produce a product which contains an average of weight of 58% DME, 20% methanol, and 22% water (Šebor et al., 2006; Ju et al., 2014).

For the large-scale production of DME from natural gas it is preferred that the production of methanol and DME be integrated into a single process; this integration is also advantageous in terms of thermodynamics. The direct synthesis of DME includes, aside from the above reaction (1), both basic methanol-producing reactions (2) and (3).

$$CO + 2H_2 \rightarrow CH_3OH \qquad (2)$$

$$CO_2 + 3H_2 \rightarrow CH_3OH + H_2O \qquad (3)$$

All three reactions must proceed simultaneously, which is something that is achieved by fitting a common catalyst for methanol synthesis and a catalyst for its dehydration (Šebor et al., 2006). In methanol factories, where methanol is produced at low pressure, DME as a by-product does not originate. There are developed processes in which methanol production is modified so that the synthesis product is a mixture of DME and methanol. In order to be able to yield the maximum levels of DME, there must be an available catalyst which is active both in the synthesis of methanol and in the subsequent dehydration to DME. Many producers work hard on the development of this technology. The production equipment is similar to that required in the manufacture of methanol. The recommended capacity of DME production is 7,000 tons per day. Expected investment costs are in advance of five hundred million USD. The investment costs were quantified as needing to be lower by 4–8% when it comes to the production of DME in comparison to the similar production of methanol. This is because energy consumption in the production of DME is about 5% lower when compared to the production of methanol (Liu & Chen, 2015).

If DME is made using biomass, then the finished product is marked as being bioDME. The USA produces DME under the trademark 'Dymel'. For its use in vehicles, DME is compressed to 0.5 MPa, which means that it is liquefied and that the liquid is refuelled into the tanks. It is processed similar to the method used for LPG, and therefore a similar infrastructure for its transportation and for providing refuelling may be used. Similar modifications may also be made in the fuel system (Šebor et al., 2006).

DME has similar physical properties to LPG. At a temperature of 20 °C it is a gas, but at a pressure of 0.5 MPa it liquefies (Kumar et al., 2011). In terms of installation, vehicle modifications consist of the addition of pressurised tanks. In order to achieve the same full-tank range, the lower specific energy is provided with compensation by installing larger tanks. Engines have a special fuel pump and injectors, an adapted control program, and common rail injection. For DME and LPG-propelled buses we need to solve the problem regarding the placement of tanks. For low-floor buses the installation of composite tanks on the roof can be considered, while in the case of medium and high-floor buses tanks can be placed under the floor (Laurin, 2007; Lee et al., 2016).

DME is injected into a cylinder in the same way as diesel fuel. Injecting DME into the combustion chamber is sufficient when compared with the injection of diesel under relatively low injection pressures, approximately 30 MPa, which ensures the required fuel dispersion. For instance, the accumulator injection system with electrically controlled injection valves, known as 'Common rail', is suitable. One of the typical arrangements for the fuel system is shown in diagram form in Fig. 1 (Laurin, 2007).

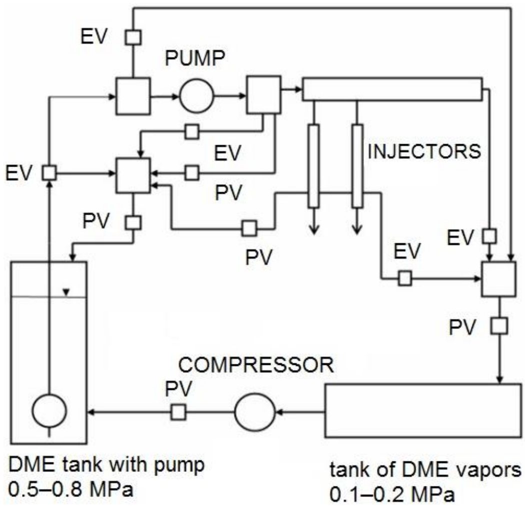

Figure 1. A diagram of the DME fuel system: EV electromagnetic valve; PCV pressure control valve; PV pressure relief valve.

Liquefied DME is propelled around the system by means of a pump which is located in the fuel tank, using a pressure of around 0.8 MPa in the high pressure pump where it is compressed to an injection pressure of 30 MPa, controlled by a regulation valve (PCV). After that, it is brought into the tank and from there to the individual injectors. Fuel injection quantity and injection timings are controlled by means of electromagnetic valves, which are controlled by the engine control unit (Laurin, 2007; Wang et al., 2016).

In order to prevent the penetration of the fuel through jets into one of the engine's cylinders after stopping the engine, the fuel must be moved from the high pressure section of the fuel system back into the tank. This option is provided up to certain pressure-relevant electromagnetic (EV) and pressurised (PV – pressure relief valve) valves. After the further reduction of the pressure levels, the DME is delivered from the fuel system into a closed tank, where it evaporates and the gaseous DME is transported via a compressor into the fuel tank. This measure will increase the safety of the entire fuel system and it also allows standard components to be used in hydraulic systems instead of perfectly gas-resistant components (Laurin, 2007; Wang et al., 2016).

In 2010 the Volvo Trucks company produced fourteen Volvo FH trucks for testing, all of which were equipped with a 13-litre engine with an output of 440 hp, which is adapted to run on DME.

This paper aims to determine the DME parameters in terms of it being a suitable energy source. Besides the parameters for applications in diesel engines, also being evaluated is the dependency of vapour pressure on temperature. The determination of this parameter is also very important when it comes to using the gaseous fuel if DME is to become an alternative for LPG fuel.

MATERIALS AND METHODS

For utilisation in diesel engines as a substitute for diesel fuel, it is necessary to determine density depending upon temperature. This is due to DME being injected into an internal combustion engine as fuel. There is a relationship between density and the calorific value of the fuel which is being analysed in this paper. For the safe storage and handling of DME it is necessary to know the flashpoint. A determination of the kinematic viscosity of DME in its liquid form is important for the prediction when it comes to mixing fuel with air in a combustion engine and the possibly of utilising a stress pump. An analysis of the solubility of water in DME is also analysed in the paper, as water is considered to be a corrosive environment.

Also, the dependence of vapour pressure on temperature was checked and compared to that of propane and butane in advance of the possibility of DME becoming an alternative to LPG (Liquefied Petroleum Gas as a blend of propane and butane in different proportions).

A sample of DME in p.a. quality was used for experimental analysis. The purity of the sample was at > 99.6% wt, with a methanol content of < 0.05% vol, and dissolved water at < 0.01% wt. Propane and butane gases were purchased from Linde Gas Company.

A diesel fuel sample was used which was compliant with the EN 590 standard without the presence of fatty acid methyl esters for laboratory tests.

Due both to the properties of the fuel and its boiling point, it was necessary to cool the fuel in the test tank by means of a cooling coil (Fig. 2). As the cooling fluid which would help to determine the boiling point, flash point, and kinematic viscosity, propyl ethylene glycol and ethanol were used.

Figure 2. Cooling coil.

The following parameters were measured:

- Volumetric and calorific value on isoperiobolic-incinerated calorimeter IKA C200 (IKA, Germany). The measurements corresponded to ISO 1928 (Fig. 4).
- The dependence of vapour pressure on temperature according to ASTM D 323 (Fig. 3).

Figure 3. An apparatus for measuring vapour pressure.

Figure 4. Isoperiobolic incinerated calorimeter IKA C200.

- The dependence of density on temperature according to the EN ISO 3675 standard.
- Kinematic viscosity according to the EN ISO 3104 standard.
- The flashpoint according to EN ISO 2719.
- The boiling point according to EN ISO 3405.
- The solubility of water in DME on Coulometer WTD (Fig. 5, Table 1).

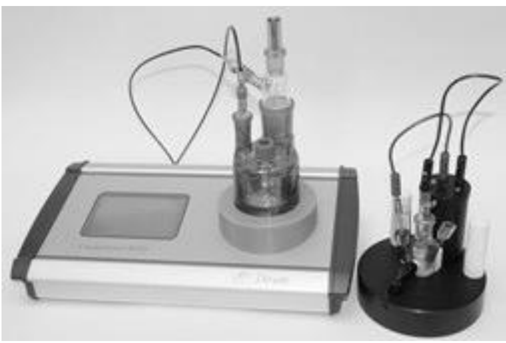

Figure 5. Coulometer WTD.

Table 1. Technical characteristics of coulometer WTD

Measuring range	1 ppm – 5% H_2O
Measurement error	0.5%
Titration flow	max. 330 mA
Indicating current	1–25 μA
Sample weight	0.002–2 g
Result display	μg, ppm, %
Power	230 V AC, 35 W
Titration container	200 mL with or without diaphragm

RESULTS AND DISCUSSION

Based on the rating of the vapour pressure by DME (Fig. 6) the practical use of DME as a substitute for commercially used LPG has been shown. For comparison, the vapour pressure of LPG at 20 °C is between 215 kPa and 770 kPa depending upon its composition (represented by propane and butane as shown in Fig. 6).

The horizontal axis *x* (Fig. 6) represents the temperature in degrees Celsius, while the vertical axis *y* represents the vapour pressure according to the Reid method (RVP) in kPa. For each sample three measurements were carried out. All of them always reached the same value on the manometer. The expected uncertainty of the result is ± 1% of the value of the result.

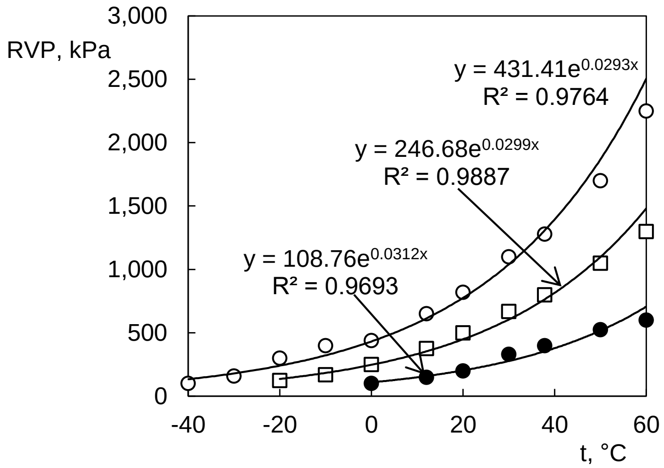

Figure 6. The dependence of vapour pressure upon the DME, plus propane and butane temperature.

An analysis of the DME vapour pressure dependant upon temperature was necessary also from the aspect of transportation, storage, distribution, and safety demands.

The horizontal axis *x* (Fig. 7) represents the temperature in degrees Celsius, while the vertical axis *y* represents the density of the fuel. For each sample three measurements were carried out. The results did not vary according to the evaluation methods, while the expanded uncertainty is ± 0.5kg m^{-3}.

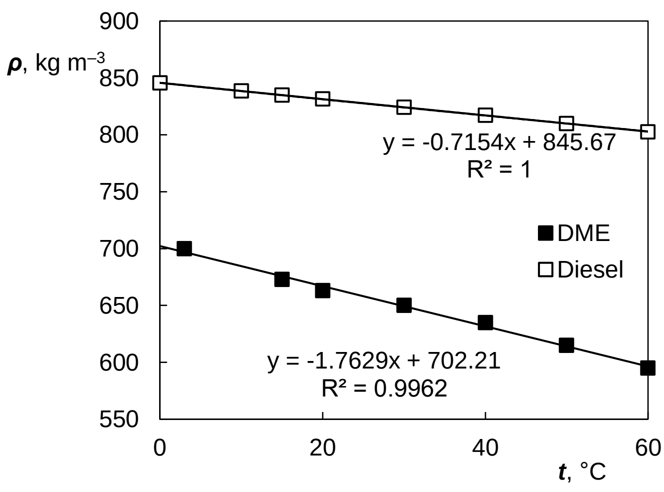

Figure 7. The dependence of the DME and diesel fuel density on temperature.

The lower density of DME when compared to diesel fuel had already signalled a lower calorific value, unlike diesel fuel. Compared with diesel fuel, the volumetric calorific value of dimethyl ether is almost half (Table 2). Engines running on DME, however, achieve a comparable performance and levels of efficiency, as in the case of the combustion of diesel (Šebor et al., 2006).

Table 2. Other measured parameters compared to the diesel fuel

	DME example	Diesel example
Kinematic viscosity at 20 °C ($mm^2\ s^{-1}$)	0.75 as a liquid (0.8 MPa)	4.09
Boiling point (°C)	-24.5	180–360
Flash point (°C)	-42	67
Water solubility (% wt.)	< 6	-
Density at 15 °C ($kg\ m^{-3}$)	673	834.37
Calorific value ($MJ\ kg^{-1}$)	28.4	36.1
Calorific value ($MJ\ l^{-1}$)	18.9	42.6
Vapour pressure at 37.8 °C (kPa)	803	-

DME has a high cetane figure, between 55 and 60 (Šebor et al., 2006; Ju et al., 2014), and is therefore suitable as a fuel for diesel engines. The compression ratio for the diesel engine also meets the DME. With the stoichiometric air-to-fuel ratio (wt) set at 9.0, the heat in vaporisation lies between 460 to 470 $kJkg^{-1}$ (Šebor et al., 2006; Wang et al., 2016).

In comparison with diesel fuel, DME contains no sulphur and has significantly different properties:

- high levels of compression;
- a low boiling point, and DME must be stored in tanks under pressure (Table 2);
- a lower density and a low energy content per unit volume of the liquid, and DME has a low calorific value (Table 2);
- a large dependence upon density levels is influenced by temperature (Fig. 7);
- a high oxygen content provides a positive influence upon the combustion process,
- low viscosity levels place a high demand on the tightness of the fuel system (Table 2);
- low water solubility levels are important in terms of limiting the corrosive environment and the efficiency of the internal combustion engine (Table 2);
- an aggressive reaction towards rubbers and plastics;
- a very low flash point (Table 2).

The boiling point of dimethyl ether is -24.5 °C (Table 2); therefore it is necessary to store it in pressurised tanks which can be filled only to 80% of their total volume due to the great dependence of the specific volume upon the temperature. Low DME viscosity places a demand on the quality of the fuel system in term of leaks. In order to avoid any damage being caused to moving parts in the fuel system, it is necessary to increase the levels of lubrication by means of suitable lubricating additives.

Compared to diesel fuel, the lower ignition temperature of dimethyl ether is an advantage. Dimethyl ether's own combustion rates also has a very positive effect on a large amount of the contained oxygen. DME has a high cetane figure between 55–60, which means that in terms of its use in diesel engines clearly provides it with an advantage (Šebor et al., 2006).

Dimethyl ether produces substantially less solid particles and nitrogen oxides (up to 90%) during the combustion process when compared to diesel fuel. On the contrary, diesel fuel combustion produces less carbon monoxide than dimethyl ether combustion. In terms of the treatment of exhaust gases only a simpler system is needed. The advantage over diesel fuel is also a lower engine noise level (Kim & Park, 2016; Lee et al., 2016).

The DME synthesis based on biomass is also currently still a subject of research. The Swedish National Energy Agency stated that influences on the price of DME which was produced from biomass included, primarily, the price of input raw materials and investment costs. The investment costs which are required for a unit with an annual capacity of 200,000 tons are estimated at around € 390 million, ie. € 2,000 per ton, € 0.27 per litre or € 14 per GJ respectively. Since DME has almost half of the calorific value of diesel fuel, the price of an equivalent amount of DME energy is around € 0.50 per litre (Laurin, 2007; Kim & Park, 2016; Lee et al., 2016).

CONCLUSIONS

Conceptual studies in this field are counting on the fact that the production and consumption of DME will increase significantly. It is expected that DME will be used as a substitute for diesel fuel and as a substitute for LPG with regard to its similar properties according to the EN 589 standard, and also as a petrochemical raw material for further synthesis.

DME is a suitable fuel for diesel engines and can be considered as being one of the prospective energy sources of the future. For use in diesel engines its liquefied form comes into consideration. In the process of switching from diesel fuel to DME no change is required in the compression ratio of the diesel engine. Switching to DME fuel however requires relatively complex and expensive fuel accessories. The DME engine output parameters engine may be the same as in the case of the diesel engine. Claims for transportation, storage, distribution, and safety are similar for DME as they are for LPG in petrol engines.

Application of the DME as a fuel for diesel engines is still in the process of development, undergoing experimental verification and the implementation of demonstration projects.

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Substantial factors influencing drivers' comfort in transportation

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Abstract. Research shows that driver stress is associated with workload and fatigue, and an inappropriate microclimate in the driving cabin can have an impact on overall driver's safety. The aim of this scientific study is to examine whether driver stress, across various urban and field drive conditions, can affect performance in a confined environment and whether the natural breathing process can also compound these effects and aggravate health hazards. This paper will address the influencing parameters associated with driver comfort of everyday job occupations in the urban communication network of Prague city public transport. In this research paper the authors will characterize cardinal components directly accountable to the safe operation elements; the concentration of carbon dioxide (CO_2) and the relative humidity (Rh_i) in the driving cabin, affecting the contentment of the drivers comfort while performing their duties. Similar inquiries were carried out on ventilation emphasis and air intake impact in drivers' cabin, recommending a design to minimize safety problems associated with comfort. Data on the concentration of carbon dioxide and internal relative humidity in the respective cabins have been collected carefully for detailed analysis. This research paper is the outcome of these findings.

Key words: driver, bus, tram, metro, microclimate, ventilation.

INTRODUCTION

Drivers in all transport categories, in the course of their daily operations, are affected by microclimate, which is determined by air temperature, air velocity, relative humidity, carbon dioxide and thermal radiation. The driver's exposure to a variety of temperatures whilst driving needs to be addressed for the safety and well-being of drivers in different climatic conditions, particularly in the driver's space, which has, so far, been given insufficient attention. The objective of this research is to examine the microclimate value measured in the driver's cabins in the professional city transport system. Microclimate in the driver's cabin significantly affects human thermal comfort; the cabin environment has an emphasis on thermal comfort not only for reasons of convenience, but also safety. It is necessary to ensure a suitable microclimate in the car cabin even in extreme operating conditions. The recommended values of microclimate in the cabin of the car, are according to researcher Vlk, (2003) as follows: Air temperature 18–22 °C and relative humidity 40–60%; air velocity 0.1 m s^{-1} at 18 °C and 0.4 m s^{-1} at 24 °C; air exchange per person (clean air): 25–50 m^3 h^{-1} of fresh air; maximal concentration of pollutants: 0.17% CO_2, 0.01% CO and 1 mg m^{-3} of dust. Scientific studies, Anderson, (1998) in the past have shown the effects of inappropriate working conditions on fatigue, which significantly applies to prolonged driver's working hours. A suitable microclimate is necessary and the systems must ensure it, as it is one of the most important safety features of vehicles.

The driver's cabin features a large flat windscreen. A small volume of air inside and relatively low heat insulation, result in a greater degree of influence on the operating conditions. If the temperature in a driver's cabin is below 17 °C, the body starts to cool down, resulting in a reduction of efficiency and a risk of muscle fatigue. In addition, inaccuracy and constraints of movements are observed. If the temperature is above 25 °C, reactions slow down and the rate of physical tiredness accelerates. At a temperature above 30 °C, mental activity will worsen. Generally, drivers are sensitive to humid air because the human body uses evaporative cooling as the primary mechanism to regulate temperature. Under humid conditions, the rate at which perspiration evaporates on the skin is lower than it would be under arid conditions. Because human beings perceive the rate of heat transfer from the body rather than temperature itself, we feel warmer when the relative humidity is high than when it is low (Zewdie & Kic, 2016b).

Some drivers experience difficulty breathing in humid environments. Some cases may possibly be related to respiratory conditions, while others may be the product of performance anxiety disorder. In times of extreme stress, a driver may shake uncontrollably, hyperventilate (breathe faster and deeper than normal) or even vomit in response, causing sensations of numbness, faintness, and loss of concentration, among others (Gladyszewska, 2011). Air conditioning reduces discomfort in the summer not only by reducing temperature, but also by reducing humidity. In winter, heating cold outdoor air can decrease relative humidity levels indoor to below 30% leading to discomfort such as dry skin, cracked lips and excessive thirst (Zewdie & Kic, 2015).

Passenger transportation safety is a priority task and so maximum attention should be given to the comfort of drivers and their working conditions. Thermal comfort and preferable local microclimate conditions include the combination of local air velocity and temperature. Moreover, the system should be related to attaining and keeping local skin temperature within comfort range, which will give the sensation of thermal comfort, penetrate natural airflow around the body avoid draught or eye irritation, and supply the breathing zone with fresh clean air. In other words, avoiding discomfort is not a guarantee that thermal comfort will be obtained, and *vice versa* (Zewdie & Kic, 2015). The oversized windshield parameters unfortunately have become the reason for and contribute to the problems associated with increased solar radiation in summer and the formation of moisture related to humidity inside in winter due to dew point temperature.

Generally, transport technology improvement is correlated to safety. In this research paper, the authors place a strong emphasis on an area that has not been well examined. Microclimate composition rate is an important index factor affecting the contentment of drivers in the cabin. Numerous researchers used different measurements to assess the driver workload under diverse driving conditions. The conclusions reached by monitoring measures support an objective and continuous analysis in a dynamically changing microclimate situation (Zewdie & Kic, 2016a).

A suitable microclimate is necessary and the systems must ensure a suitable microclimate as it is one of the most important safety features of vehicles. In a confined environment, our natural breathing process too can compound these effects and aggravate health hazards. In the course of breathing, we exhale CO_2, which can displace O_2 in an indoor environment such as a vehicle cabin, leaving the environment O_2 deficient. Such high CO_2 and low O_2 concentrations can cause adverse human health effects. Various independent studies, Galatsis et al. (2001) have also shown that through this process, the concentrations of O_2 and CO_2 may come to exceed safety limits. Interestingly, a study on fatal single vehicle crashes highlights that the vehicle is more likely to have closed windows and a heater on than to have fresh air and air conditioning fitted, Maroni et al. (1995). An O_2 deficient environment has been termed 'hazardous' when the O_2 concentration is less than 19.5%, Galatsis et al. (2000). Low O_2 levels can impair judgment, increase heart rate and impair muscular coordination. In the conclusion in their respective findings that thermal state of internal microclimate inside the drivers' cabins has a strong correlation with drivers' comfort which has influence on safety of drivers. The authors of this paper have confirmed the influences CO_2 and Rh_i based on collected data and statistical analysis, the airflow inside the cabin is crucial in removal of health hazardous pollutants generated by the driver himself.

MATERIALS AND METHODS

The authors carried out research on three public city transport modes; buses, trams and the subway cars (metros) accordingly. Detail category was denoted by the type of vehicles. For research implementation, the authors applied four buses Karosa KbN SOR, four trams Tatra T3 of surface transportation, which are Czech brands and four subway cars series 81-71M of Russian make for underground transportation; all modes of transportation were not air-conditioned. Data on the microclimate conditions in all drivers cabin were collected from measurement devices which were installed on the dashboard of the respective vehicles. The thermal comfort in the space was continuously measured by globe temperature (measured by globe thermometer FPA 805 GTS with operative range from –50 to +200 °C with accuracy ± 0.01 K and diameter of 0.15 m) together with temperature and humidity of surrounding air measured by sensor FH A646-21. The temperature sensor NTC type N with operative range from –30 to +100 °C with accuracy ± 0.01 K. and air humidity by capacitive sensors with operative range from 5 to 98% with accuracy ± 2% was installed. The concentration of CO_2 was measured by the sensor FY A600 with operative range 0–0.5% and accuracy ± 0.01%. All data was measured continuously and stored at intervals of one minute to the measurement instrument ALMEMO 2690–8 throughout the measurement process.

The data collections were carried out roughly in hot climate conditions in the summer of 2014 in the month 28th and 29th of August at an average external temperature t_e = 25 °C on buses 1 and 2. Buses 3 and 4 were measured at an average external temperature t_e = 29 °C. In July 7 and 8, 2015 at an average external temperature of t_e = 28 °C trams measurement was held. Similarly, on subway cars in June 2016 at noon with average subway platform temperature of t_e = 20 °C was measured. While data collecting process, the large side windows of buses and trams were open for ventilation whilst driving. Only subway car side windows were closed due to dust appearances in the tube. The drivers' cabin in all transport modes is bounded; drivers are limited and are not in contact with passengers. The duration of the measurement limited on the availability of the vehicles and capacity of the measuring devices. For buses measurement we had about 40 min, for trams about 50 min. and for subway car nearly 50 minute depending on the metro line, where the data collection was performed.

Assuming steady conditions, with a uniform distribution of pollutants in space the required volume air flow for ventilation V_c is calculated according to the Eq. (1).

$$V_c = \frac{M_p}{c_i - c_e} \quad (1)$$

where V_c – required volume air flow for ventilation, m^3 h^{-1}; M_p – mass flow of produced pollutant, uniformly leaking into the space, kg h^{-1}; c_e – concentration of pollutant in inlet air, kg m^{-3}, (usually is $c_e = 0$); c_i – concentration of pollutant in outlet air, kg m^{-3}, (usually considered OEL – Occupational Exposure Limits or MEL – Maximum Exposure Limits).

The data on CO_2 concentration, the thermal index composed of internal globe temperature t_g, and internal temperature t_i as well as internal relative humidity Rh_i are carefully collected for further analysis. The obtained results of CO_2 including relative humidity Rh_i were processed by Excel software and verified by statistical software (*Tukey HSD Test*). Different superscript letters (a, b, c, d) mean values in common are significantly different from each other in the rows of the (*ANOVA; Tukey HSD Test;* $p \leq 0.05$), e.g. if there are the same superscript letters in all the rows it means the differences between the values are not statistically significant at the significance level of 0.05.

RESULTS AND DISCUSSION

The mean values including standard deviation were calculated from the results of measurements for each internal and microclimatic parameter: internal temperature t_i (°C), internal relative humidity Rh_i (%), internal globe temperature t_g (°C), concentration of CO_2 (%) and calculated volume of ventilation air flow V_c (m^3 h^{-1}) in the driving cabin. The results of the measurement in the buses Karosa KbN SOR cabin are presented on Tables 1, 2., in trams Tatra T3 cabin on Tables 3, 4 and in subway cars 81–71M cabin on Tables 5, 6 respectively.

Table 1. The results of measurements indoor parameters in driving cabins of buses 1 & 2

Model			Bus Karosa (1)					Bus Karosa (2)		
Driver			1					2		
Parameter Units	CO_2 %	Rh_i %	t_g °C	t_i °C	V_c m^3 h^{-1}	CO_2 %	Rh_i %	t_g °C	t_i °C	V_c m^3 h^{-1}
Mean	0.040	33.5	30.4	26.5	224.2	0.043	46.5	35.1	31.5	158.2
± SD	0.006	2.0	3.8	1.0	95.6	0.010	0.6	1.9	1.6	133.7
Minimum	0.033	26.2	25.5	24.9	44.2	0.035	45.3	31.9	29.2	41.6
Maximum	0.065	39.5	38.7	30.1	471.4	0.069	48.1	37.4	34.9	707.5
Median	0.033	33.7	29.3	26.4	235.7	0.043	46.5	35.5	31.1	128.6

The results of the measurement in the bus Karosa (1) cabin, Table 1, shows that the mean value of CO_2 is a normal recommended. The value of the intake fresh air V_c

approaches to 224.2 $m^3 h^{-1}$, which is more than fourfold compared to the recommended range V_c = 25–50 $m^3 h^{-1}$. At the maximum value of CO_2, the fresh air intake reaches more than nine fold compared to the recommended range, i.e. V_c = 471.4 $m^3 h^{-1}$.

The result of the measurement in the Karosa (2) cabin, Table 1, indicates that the mean value of CO_2 is at the recommended value despite the high globe temperature t_g and internal temperature t_i. The internal relative humidity Rh_i records the acceptable value with the ventilation air flow V_c = 158.2 $m^3 h^{-1}$ is adequate. The driver apparently felt comfortable even though the temperature t_i = 34.9 °C and t_g = 35.1 °C. The amount of CO_2 concentration was within the normal recommended range. The authors believe that, due to the maximum fresh air flow which reached fourteen fold V_c = 707.5 $m^3 h^{-1}$ played a vital role for moderate internal relative humidity Rh_i = 40.1%.

Similar data were collected from the bus Karosa (3) cabin, Table 2. It demonstrates acceptable concentration of CO_2.The large amount of side window ventilation air flow V_c = 243.5 $m^3 h^{-1}$, which was twelve fold exhibits the contribution to low amount of internal relative humidity Rh_i and CO_2. The mean values of internal temperature t_i = 22.2 °C and the globe temperature t_g = 24.2 °C exposes that the measurement was held at a slight warm suitable summer days.

Table 2. The results of measurements indoor parameters in driving cabins of buses 3 & 4

Model			Bus Karosa (3)					Bus Karosa (4)		
Driver			3					4		
Parameter	CO_2	Rh_i	t_g	t_i	V_c	CO_2	Rh_i	t_g	t_i	V_c
Units	%	%	°C	°C	$m^3 h^{-1}$	%	%	°C	°C	$m^3 h^{-1}$
Mean	0.034	22.1	24.2	22.2	243.5	0.048	29.9	35.1	31.5	121.5
± SD	0.003	3.4	1.5	0.7	67.8	0.008	5.1	2.8	1.7	5.4
Minimum	0.033	14.0	20.7	20.7	83.2	0.033	19.9	29.7	27.7	44.2
Maximum	0.050	27.3	26.6	23.9	471.4	0.083	40.1	40.9	34.7	353.6
Median	0.033	22.5	24.0	22.1	235.7	0.050	27.8	35.3	31.5	94.3

The result of the measurement on bus Karosa (4) Table 2, demonstrates the mean values CO_2 and Rh_i are within the accepted range. The ventilation air flow V_c = 121.5 $m^3 h^{-1}$; all values at internal temperature t_i = 31.5 °C and extremely high internal globe temperature t_g = 35.1 °C. In this measurement, it was observed that the maximum concentration of CO_2 which is higher than the mean value and the ventilation air flow V_c = 353.6 $m^3 h^{-1}$ has reached four fold.

The results obtained from buses through measurement and mathematical assessments, the authors denote that the extreme high fresh air intake through ventilation V_c has immense temperature impact in the driver's cabin. The large side windows ventilation may play significant role for the substantial part of the ventilation performance.

Fig. 1. Demonstrates the values of CO_2 concentration in buses 2 and 3 driver cabins. From the Figure, it is clearly seen that all mean values are within the recommended margin. Interestingly, the courses of the graphs demonstrate the driver's preference of fresh air intake V_c for ventilation. Bus 3 in Fig. 1, indicates that the driver had a small break at 7th, 24th and 32th minutes and the value of CO_2 corresponds to collected data.

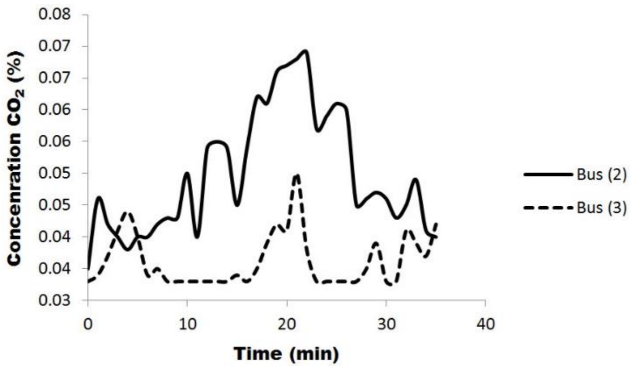

Figure 1. The dynamics of CO_2 concentrations in driving cabins of the buses 2 and 3

Obtained data through measurement of Tram T3 (1), from Table 3, shows that the mean value of CO_2 is at a normal recommended range due to a slightly more air flow which is $V_c = 189$ m^3 h^{-1} where as Rh_i which indicates insufficient internal humidity. Throughout the course of measurement it happens to be a very slight increase of CO_2, at $V_c = 0.083$ m^3 h^{-1}.The internal temperature t_i and the globe temperature t_g values indicate that the ventilation was sevenfold, $V_c = 353.6$ m^3 h^{-1}. Both values indicate that the measurement was held at a mild summer and increased to hot climate.

Table 3. The results of measurements indoor parameters in driving cabins of the trams 1 & 2

Model			T3 (1)					T3 (2)		
Driver			5					6		
Parameter Units	CO_2 %	Rh_i %	t_g °C	t_i °C	V_c m^3 h^{-1}	CO_2 %	Rhi %	t_g °C	t_i °C	V_c m^3 h^{-1}
Mean	0.048	20.3	22.0	20.9	189.0	0.052	28.7	17.9	17.5	88.5
± SD	0.013	4.2	2.5	2.9	90.7	0.009	3.1	0.1	0.2	49.8
Minimum	0.033	13.1	19.4	19.4	48.8	0.033	24.6	17.7	17.1	29.5
Maximum	0.083	27.7	30.5	30.2	353.6	0.081	34.2	18.1	17.9	353.6
Median	0.046	20.2	21.2	19.9	202.0	0.051	27.8	18.0	17.6	78.6

The result of the measurement of Tram T3 (2), on Table 3, shows that the data collection was held on a mild summer time. All mean values are in a recommended range except the internal relative humidity which was a slightly lower than normal value. At maximum value of CO_2 registered, $Rh_i = 34.2\%$ has reached the acceptable value and fresh ventilation intake air value $V_c = 353.6$ m^3 h^{-1} extended seven fold.

The results obtained from Tram T3 and third participant of the research indicated on Table 4, clearly shows that all mean values are in ideal ranges of the recommended values. Even though, the maximum value of CO_2 scored a slightly more the mean value, it still satisfy the acceptable range.

The case for Tram T3, fourth participant, Table 4, demonstrates an increased mean values of $CO_2 = 0.113\%$, $t_g = 33.2$ °C and $t_i = 31.6$ °C. The maximum value of $CO_2 = 0.174\%$ was slightly higher than the recommended 0.170%. The internal temperature $t_i = 40$ °C and globe temperature $t_g = 39.9$ °C have identical maximum values, which indicates as an extreme hot summer period. The maximum fresh intake air $V_c = 94.3$ m^3 h^{-1} was insufficient to moderate the acceptable internal temperature. From the values obtained the authors assume the cause for higher measured values to improper ventilation and small sized side ventilation windows of the tram.

Table 4. The results of indoor parameters' measurements in driving cabins of the trams 3 & 4

Model			T3 (3)					T3 (4)		
Driver			7					8		
Parametr Units	CO_2 %	Rh_i %	t_g °C	t_i °C	V_c $m^3\ h^{-1}$	CO_2 %	Rh_i %	t_g °C	t_i °C	V_c $m^3\ h^{-1}$
Mean	0.072	70.4	19.4	19.2	46.4	0.113	59.4	33.2	31.6	28.0
± SD	0.022	1.6	1.0	1.0	32.7	0.045	4.4	3.3	3.6	20.1
Minimum	0.033	67.7	18.3	18.2	19.1	0.048	47.0	28.9	27.6	10.0
Maximum	0.107	75.1	21.2	21.3	157.1	0.174	65.7	39.9	40.0	94.3
Median	0.073	70.2	18.9	18.8	33.3	0.114	59.9	33.4	29.9	17.5

Figs 2 and 3, demonstrate the impact of fresh air intake for ventilation V_c and concentration of CO_2 pollutant in driving cabins for two different trams; Tram 3 and 4. From the values of both graphs, it is clearly indicated as fresh air intake for ventilation has a significant impact on the pollutant concentration in the driving cabins.

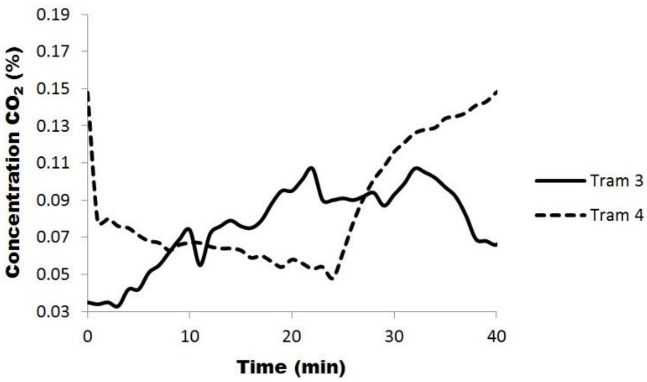

Figure 2. The dynamics of measured CO_2 concentration in drivers' cabins of the trams 3 and 4.

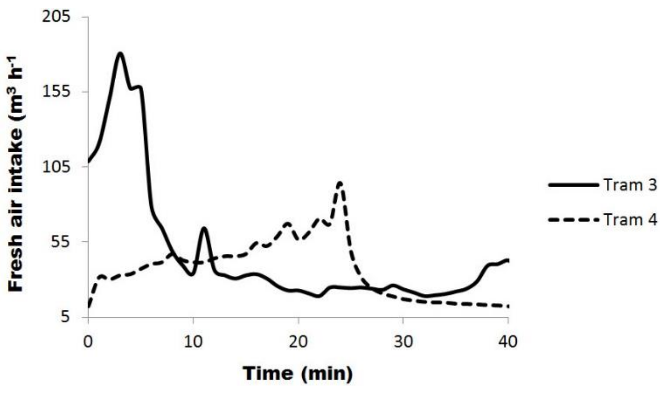

Figure 3. The dynamics of fresh air intake for ventilation in driving cabins of the tram 3 and 4.

Generally, most of subway cars transport operates underground. The data collection was held from the subway cars operating underground subway routes, where the influence of direct radiation temperature t_g (°C) and external temperature t_e (°C) do not play significant role. The data collected on subway cars M1 (1), Table 5, it is clearly shown that the mean values CO_2 and Internal relative humidity Rh_i and fresh air intake ventilation value V_c = 41 m^3 h^{-1} is within the recommended values all at suitable internal climate condition. The maximum value of CO_2 = 0.153% was a slight higher and has appeared on the margin of a tolerable value.

Table 5. The results of indoor parameters' measurements in driving cabins of the subway cars 1 & 2

Model			M1 (1)					M1 (2)		
Driver			9					10		
Parameter Units	CO_2 %	Rh_i %	t_g °C	t_i °C	V_c m^3 h^{-1}	CO_2 %	Rh_i %	t_g °C	t_i °C	V_c m^3 h^{-1}
Mean	0.076	56.8	22.2	21.7	41.0	0.082	63.7	23.9	23.7	82,5
± SD	0.025	2.1	0.4	0.4	18.1	0.029	1.8	0.8	0.2	52,8
Minimum	0.043	53.5	21.5	20.5	11.8	0.035	60.7	27.0	23.3	26,4
Maximum	0.153	60.5	22.8	22.1	88.4	0.140	68.5	26.6	24.1	176,8
Median	0.069	57.1	22.0	21.7	39.3	0.081	64.1	23.8	23.5	59,0

The attributes obtained for subway car M1 (2) on Table 5, shows the identical trends of mean values as subway car M1 (1). All mean values correspond to the recommended ranges. A slight increase of maximum value of CO_2 was observed. The relative internal humidity Rh_i, globe internal temperature t_g, internal temperature t_i and the fresh air intake ventilation V_c = 82.5 m^3 h^{-1} are all at the recommended values.

The case of the research on subway car M1 participant number three, on Table 6, clearly shows that the mean value is CO_2 and all other mean values corresponds to the acceptable ranges. On the maximum value of CO_2, the authors witnessed an increase, whereas the rest of other maximum values remain in accepted ranges.

The fourth participant of the subway car driving cabin M1 (4), Table 6, the mean value of CO_2 = 0.101% which means the mean values remained enjoyable. For the maximum values of all parameters, more or less stays at the recommended level.

Table 6. The results of indoor parameters' measurements in driving cabins of the subway cars 3 & 4

Model			M1 (3)					M1 (4)		
Driver			11					12		
Parametr Units	CO_2 %	Rh_i %	t_g °C	t_i °C	V_c m^3 h^{-1}	CO_2 %	Rh_i %	t_g °C	t_i °C	V_c m^3 h^{-1}
Mean	0.087	60.4	25.6	23.6	37.1	0.101	58.4	22.7	22.2	32.0
± SD	0.022	1.3	0.3	0.1	32.4	0.042	4.0	0.4	0.4	20.5
Minimum	0.042	57.8	23.2	23.5	17.0	0.048	47.0	22.5	21.5	10.3
Maximum	0.116	62.0	24.0	23.8	157.1	0.170	64.7	23.1	22.8	94.3
Median	0.094	60.8	23.6	23.6	23.2	0.079	58.0	21.8	22.0	30.8

The collection of internal cabin data indicates that the CO_2 and RH_i to be similar. The same applies to the internal globe temperature t_g and internal temperature t_i.

Interestly, there happened to be higher concentration of CO_2 in driving cabins of all four subway cars. The authors only assume that the high concentration of CO_2 was caused by insufficient ventilation and probably due to the prevention of excess dust concentration in the subways tube.

From the behaviour of functions plotted on Fig. 5, the concentration of CO_2 in subway cars 3 and 4 cabins differs. At the initial stage, at 3rd and 4th minute the graph; it is clearly shown that both cabins are insufficiently ventilated. Cabin for subway car 4 slightly exceeded the recommended value CO_2 = 0.170%. In general, subway cars cabin ventilation system is improper and inadequate. The authors of this research have a close talk with the drivers and the management of the institution concerning the ventilation system.

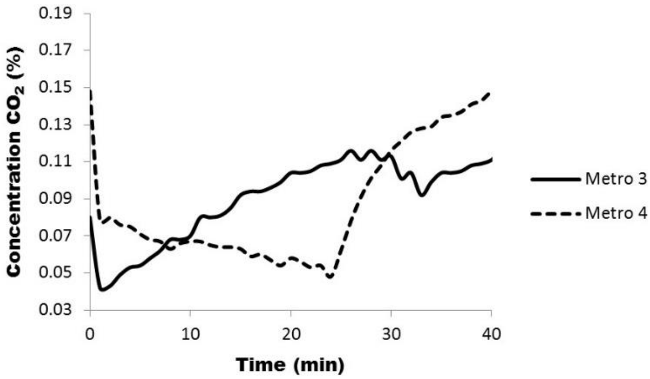

Figure 5. The dynamics of CO_2 concentration in the drivers' cabins of the subway car engines 3 and 4.

Table 7 shows the statistically significant data obtained for comparison in all twelve transport driving cabins. The data are mean value ±SD. Different letters (a, b, c, d) in the subscript are the sign of high significant differences *(Tukey HSD Test; p ≤ 0.05)*.

Table 7. Statistical parameters of CO_2 and Rh_i in driving cabins of the buses, trams and subway cars

Vehicle	pollutant unit	CO_2 % ± SD	Rh*i* % ± SD
	1	0.040 ± 0.060^a	33.5 ± 2.0^a
	2	0.043 ± 0.010^b	46.5 ± 0.6^b
	3	0.034 ± 0.003^c	22.1 ± 3.4^c
	4	0.048 ± 0.008^b	29.9 ± 5.1^d
	1	0.048 ± 0.013^a	20.3 ± 4.2^a
	2	0.052 ± 0.009^a	28.7 ± 3.1^b
	3	0.072 ± 0.022^b	70.4 ± 1.6^c
	4	0.113 ± 0.045^c	59.4 ± 4.4^d
	1	0.076 ± 0.025^a	56.8 ± 2.1^a
	2	0.082 ± 0.029^a	63.7 ± 1.8^b
	3	$0.087 \pm 0.022^{a,b}$	60.4 ± 1.3^c
	4	0.101 ± 0.042^b	58.4 ± 4.0^d

± SD – Standard deviation; Different superscript letters (a, b, c, d) mean values in common are significantly different from each other in the rows of the (*ANOVA; Tukey HSD Test; p ≤ 0.05*).

Based on the result of measurement and statistical evaluation of the lowest concentration of CO_2 level measured was in the bus 3; $CO_2 = 0.034\%$. For the comparison, the CO_2 level in the cabin of the tram 1 was $CO_2 = 0.048\%$ and for the subway car 1 was $CO_2 = 0.076\%$. The lowest relative humidity Rh_i was measured again in cabin bus 3, $CO_2 = 22.1\%$.

The highest concentration was measured in the drivers cabin of tram 4, $CO_2 = 0.113\%$, compared to bus 4, $CO_2 = 0.048\%$ and underground car (metro) where $CO_2 = 0.101\%$. Similarly, the highest level of Rh_i was measured in cabin tram 3 where $Rh_i = 70.4\%$; followed by subway car 2, $Rh_i = 63.7\%$ and subway car 3, where $Rh_i = 60.4\%$. The CO_2 concentration difference between buses 2 and 4 is not statistically significant. The relative humidity Rh_i between buses the differences are statistically significant.

The difference between CO_2 concentrations in tram 1 and 2 is not statistically significant. The CO_2 concentration in trams 3 and 4 are significantly higher. Relative humidity Rh_i in all trams are significantly different.

The differences of CO_2 concentration in subway cars 1, 2 and 3 are not statistically significant; the CO_2 differences between subway cars 3 and 4 are statistically significant. Differences of relative humidity Rh_i in all four subways cars are statistically significant.

In this research paper, the findings demonstrated that the concentration of CO_2 was the lowest in all buses and approaches the external CO_2 level. The level of the relative humidity Rh_i was at the minimum and hazardous to health problem. Both parameters were influenced by the large side windows where a considerable amount of air flow while driving had strong impact on thermal state. The higher concentration of CO_2 was registered in Subway car cabins which were caused by insufficient ventilation. This research paper verifies the early investigations carried out by Vlk (2003) and Zewdie & Kic (2015; 2016a and 2016b).The measurement method extends the principle of early warning and the presence of harmful substances in the driver's cab and safety adjustments at the intervals in the work process. The measurements would be extended in different seasons and different types of vehicles. The authors were obliged to stick by only public transport research.

CONCLUSION

The internal conditions in the cabin of the surface transport mode i.e. the buses and the trams are strongly influenced by solar radiation, particularly with a larger proportion of cabin glazing. Based on the result of the measurements in the bus maximum internal temperatures have occurred in Buses Karosa (2) t_i and Karosa (4) t_i. The internal relative humidity was in the normal recommended values Rh_i and very low CO_2 concentrations. The internal global temperature t_g in case Karosa (1) increased to t_g in Karosa (2). This indicates, the influence of solar radiation has increased rapidly compared to the internal temperature in all four Karosa buses. Despite the increase in internal temperatures, interestingly, the contents of CO_2 and the relative humidity Rh_i, correspond to the recommended values.

Data from the Trams Tatra T3 measurements also reveals the major influence of radiation on Tram (4). The maximum concentration of CO_2 which is slightly higher than the recommended 0.170% and the Rh_i shows that the drivers cabin was insufficiently ventilated. Surprisingly, all other tram values are within the accepted ranges. Though out the course of data collection, Tram and Bus drivers had open the large side windows. The low concentration of CO_2 and relative humidity Rh_i could be explained by the huge amount of fresh air intakes for ventilation, V_c.

The nature and specific characteristics of subway car transportation differ from the surface means of transportation. Due to the large amount of dust concentration in the subways, which was a result of the crumbling concrete surface, the drivers were not willing to open the side window for ventilation. The results indicate that the high concentration of CO_2 in the drivers' cabin has appeared on the margin of a tolerable value. Divers should ventilate sufficiently even in colder outdoor conditions to let in fresh air (O_2) and exhaust the polluted air (CO_2 and odours).

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