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Comparison of iodine application methods in Rocket Plant

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Abstract. Iodine is an essential trace element for human health and is involved in the production of the thyroid hormone. Recently, a new idea has emerged: to meet people's need for daily iodine by increasing the iodine content of vegetables with high iodine bioavailability and assimilation. This study was carried out with iodine foliar application and root application methods to determine which is more appropriate when attempting to increase iodine content. An experiment was conducted in a greenhouse with 4 doses of iodine in the form of potassium iodide (0, 2, 4, 8 mM), two application methods (foliar and root application) and three replicates. At the end of the experiment, the plants' fresh and dry weight, relative water content, membrane stability, chlorophyll a, chlorophyll b and total chlorophyll were determined. The results were subjected to analysis of variance according to the randomized blocks experiment design using the MINITAB 17.0 statistical package program. The applications did not show a statistical difference in plant fresh weight and dry weight. There was no statistical difference in the relative water content between the applications, while a statistical difference ($P < 0.05$) in the membrane stability was observed for the interaction of application type and application dose. Chlorophyll a, chlorophyll b and the total chlorophyll amount were also statistically affected ($P < 0.05$) by the application doses. Foliar and root application methods have not caused significant loss of yield. Both methods are also considered to be appropriate to use in practice.

Key words: Iodine, Rocket, Vegetable, Thyroid.

INTRODUCTION

Iodine is an essential element for humans and animals, while not necessarily a nutrient for plants. Iodine, which is involved in thyroid hormone production, is an essential element for normal growth and development and for brain and body functions (Erbaş, 2008).

The amount of iodine in rocks is generally low. Iodine concentrations are between 0.2 and 0.8 ppm in basic extrusive. In acid eruptive, metamorphic rocks and some sedimentary rocks, the amount of iodine varies from 1 to 2 ppm. The average iodine content of the soil surface is stated as 5 ug g^{-1}. The iodine content of the soil is more than 20–30 times that of rocks (Halilova, 2004). According to Whitehead (1984), the basic source of iodine in the soil is atmospheric iodine and the main sources of atmospheric iodine are seas and oceans (Tsukada et al., 2008). Iodine can easily evaporate under the influence of daylight and temperature. Iodine, which is mixed with the atmosphere, reaches land with rain (Halilova, 2004). The losses which occur during the process of the iodine reaching the soil cause a small amount of iodine to be found in the soil and in the crops grown in these soils. According to the WHO, ICCIDD (International Council for the Control of Iodine Deficiency Disorders), Global Network and UNICEF, the daily amounts of iodine that people should take are as follows: 0–6 years – 90 micrograms (μg), 7–12 years – 120 μg, over 12 years – 150 μg and pregnant and lactating women – 250 μg. When the daily iodine requirement is not satisfied, a series of developmental and functional diseases known as Iodine Deficiency Disorders can occur. The total amount of iodine in the human body is 15–20 mg (Erdoğan & Erdoğan, 1999). Although iodine deficiency can be cured, it remains a health problem for about 35% of the world's population (Pearce et al., 2004; Winger, 2008; Landini, 2011). According to recent WHO surveys, around 2 billion people in the world are still confronted with insufficient iodine intake. Compared with the WHO regions, the European continent has the highest rate of iodine deficiency, at 45% of the population (Anderson et al., 2012; Daum et al., 2013). In Turkey, iodine deficiency remains an important public health problem.

The visible sign of iodine deficiency is the goiter which is seen in all age groups. The total prevalence of goiter in Turkey is as high as 30.5% and the visible goiter rate is around 6.7% (Yordam et al., 1999). According to a study conducted by Koloğlu in 1984, 29 provinces, excepting the Aegean and Marmara regions, are endemic goiter zones (Aydın, 1989).

Iodine deficiency causes growth retardation, deafness, dwarfism and hypothyroidism in infants, growth retardation, school failure, difficulties in understanding and learning in children and adolescents, hypothyroidism, inadequate mental functioning, weakness and inefficiency in adults (Pekcan, 2008). At the same time, iodine deficiency was found to reduce IQ by 13.5 points (Pekcan, 2008; Zimmermann, 2012). There is an increased risk of death in newborns with a lack of iodine. The prevention of iodine deficiency in China and Zaire has been shown to reduce the risk of newborn mortality (Erbaş, 2008).

In the human body and animals, 80% of the iodine is naturally supplied by edible vegetables (Welch & Graham, 2005; Weng et al., 2013) and the bioavailability of iodine in these vegetables is about 99%. The concentration of iodine in the soil in which vegetables are grown is usually too low to supply the needs of the human body (Weng et al., 2013). The iodine content of the plant that grows in soil with a high amount of iodine is high. The iodine deficiency in the soil in Turkey affects the goiter rate more than drinking water. As consumption of vegetables, fruits and grain low in iodine content is common in our country, the total amount of iodine entering the body remains low (Aydın, 1989).

Diet is the only way to take iodine into the body (Vitti et al., 2001; Pekcan, 2008). The use of iodized salt is the most common approach for iodine supplementation (Delange & Lecomte, 2000; Andersson et al., 2005). However, during activities such as cooking, storage and transportation, it is difficult to control the loss of iodine and iodine supplementation causes many problems during food processing (Winger et al., 2008; Landini et al., 2011). The level of iodized salt usage in our country is inadequate to control iodine deficiency (Özkan et al., 2004). Increasing iodine levels in food with vegetables which have a high rate of bioavailability and assimilation is a more effective way of controlling iodine deficiency in a cost effective manner (Dai et al., 2004; Weng et al., 2009; White & Broadley, 2009; Landini et al., 2011).

Some studies show that iodine application to the soil increases iodine accumulation in the edible parts of vegetables (Dai et al., 2004). Umaly & Poel (1971) reported that plants could use iodate more than iodide. Whitehead (1973) reported that iodine is useful for many plants, even at very low concentrations, regardless of the form (Landini et al., 2011).

In a study carried out in lettuce, iodine (0.25 and 0.50 kg ha^{-1}) applied to the leaves increased the content of iodine in the edible parts of the plant without reducing the yield and quality of the product (Daum et al., 2013).

Food consumption in Turkey is largely composed of foods which are of cereal origin. In addition, leafy vegetables play an important role in the nutrition program. The rocket plant (*Eruca vesicaria*) is a vegetable with leaves consumed as a salad and recently its consumption has increased. The rocket plant is rich in vitamin C and also has antioxidant properties.

Considering that rocket production and consumption is increasing in Turkey, that it is consumed raw in salads daily, and that iodine can be accumulated in the leaves, this study aimed to determine which method is more appropriate to increase the iodine content of rocket plants, namely, spraying iodine on the leaves or applying it to the roots.

MATERIALS AND METHODS

The experiment was developed under greenhouse conditions at Canakkale Onsekiz Mart University in Turkey. The rocket plant was chosen as study material because of its consumption as raw leaves. The experiment was conducted with a randomized block design with 4 doses of iodine in the form of potassium iodide (0, 2, 4, 8 mM), two application methods (foliar and root application) and three replicates. The plants were grown with a Hoagland nutrient solution. At the end of the experiment, the plant's fresh and dry weight, relative water content, membrane stability, chlorophyll a, chlorophyll b and total chlorophyll were determined. After harvesting, the fresh weight (FW) of leaves was directly determined. For dry weight (DW) determination, the leaves were dried at 70 °C for 48 h and weighed.

Leaf tissue was used for Relative Water Content (RWC) determination, as follows. A composite sample of leaf discs was taken and the fresh weight was determined, followed by flotation on water for up to 4 hr. The turgid weight was then recorded, and the leaf tissue was subsequently oven-dried to a constant weight at about 60 °C for 48 h. RWC was calculated as

$$RWC\% = (FW - DW) \div (TW - DW) \times 100 \text{ (Weatherley, 1950)} \qquad (1)$$

Membrane permeability was evaluated by the leaf relative electrolyte leakage (EL). Samples were washed three times with deionized water to remove surface-adhered electrolytes. Leaf disks were soaked in 10 ml distilled water at 40 °C for 30 minutes and then the initial electrical conductivity (ECi) was determined by measuring the electrical conductivity of the solution using a conductivity meter, whereas the final electrical conductivity (ECf) was obtained by boiling the same solution for 10 minutes. EL was calculated as a percent of the initial to the final conductivity.

$$\text{Electrolyte leakage}\% = (ECi \div ECf) \times 100 \qquad (2)$$

Accurately weighted 0.5 g of a fresh plant leaf sample was taken, and homogenized with 10 ml of 80% acetone. The homogenized sample mixture was centrifuged at

4,500 rpm for 5 minutes. The supernatants were separated and analyzed for Chlorophyll-a, Chlorophyll-b and total Chlorophyll content in a spectrophotometer.

The results were subjected to analysis of variance according to the randomized block experiment design using the MINITAB 17.0 statistical package program and the averages of the data with significant differences between them were compared with the LSD.

RESULTS AND DISCUSSION

The fresh and dry weight data obtained from the soil and foliar iodine application to the rocket plant are given in Table 1. When Table 1 is examined, it can be seen that the highest fresh weight is 67.66 g and the lowest is 60.74 g. Although the highest and lowest data showed a numerical difference, there was no statistical difference. When the plant dry weight is considered, the situation is seen to be the same. Although there are numerical differences between the application form and the application doses, this is not statistically significant. The plant dry weight ranged from 11.90 g to 9.88 g.

Table 1. Fresh weight and dry weight (g) averages and multiple comparison results

Method of	Iodine application doses (mM)			
Application	0	2	4	8
	Fresh weight(g)			
Soil	67.66	65.98	62.66	63.27
Foliar	60.74	63.29	65.56	62.40
	Dry weight (g)			
Soil	11.90	11.20	9.88	10.37
Foliar	10.48	11.21	11.35	10.42

The Relative Water Content and Membrane Stability data obtained from the iodine application to rocket plants by soil and foliar application are given in Table 2. When Table 2 is considered, it can be seen that there is no numerical or statistical difference between the relative water contents. When analyzed, the results of the membrane stability data indicated that interaction between the application form and the doses created a statistically significant difference ($P < 0.05$) in membrane stability. Membrane stability reached the highest value at the level of 8mM iodine application to the soil, while the lowest value was reached in the control group with soil application.

Table 2. Relative water content and membrane stability (%) averages and multiple comparison results

Method of	Iodine Application Doses (mM)			
application	0	2	4	8
	Relative water content (%)			
Soil	72.56	72.13	74.05	70.60
Foliar	71.10	70.73	72.75	71.00
	Membrane stability (%)			
Soil	27.96 B	41.80 AB	40.21 AB	57.63 A
Foliar	37.30 B	29.98 B	33.68 B	39.70 AB

Mean values marked with the same letter in columns do not differ significantly ($P < 0.05$) from each other.

The Chlorophyll-a, Chlorophyll-b and total Chlorophyll content data obtained from the iodine application to rocket plants with soil and foliar application are given in Table 3. When Table 3 is examined, it can be seen that the doses of iodine application statistically affect ($P < 0.05$) chlorophyll a, chlorophyll b and total chlorophyll content.When compared with the control group, the highest dose of iodine application (8 mM) increased chlorophyll content.

Table 3. Chlorophyll-a, chlorophyll-b and total chlorophyll content averages and multiple comparison results

Method of application	Iodine Application Doses (mM)			
	0	2	4	8
	Chlorophyll-a			
Soil	4.46	4.50	4.21	4.53
Foliar	4.18	4.02	3.70	5.41
Average	4.32 AB	4.26 AB	3.95 B	4.97 A
	Chlorophyll-b			
Soil	4.83	5.08	4.46	5.08
Foliar	4.63	4.49	4.26	5.95
Average	4.73 AB	4.78 AB	4.36 B	5.51 A
	Total Chlorophyll			
Soil	9.30	9.58	8.67	9.61
Foliar	8.82	8.51	7.97	11.37
Average	9.06 AB	9.05 AB	8.32 B	10.49 A

Mean values marked with the same letter in columns do not differ significantly ($P < 0.05$) from each other.

CONCLUSIONS

It is important that no loss of yield occurred in applications to increase the iodine content of the vegetables. According to the results obtained from the experiment, foliar and root application methods have not caused significant loss of yield. Both methods used in the experiment are also considered to be appropriate to use in practice. In addition, considering the differences in membrane stability and chlorophyll content, the application dose should be selected carefully, so that it gives no harm to the plant.

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Effect of pre-sowing and nitrogen application on forage quality of silage corn

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Abstract. In order to determine the best pre-sowing treatments and nitrogen rates on forage quality traits in silage corn (SC 704), a field experiment was conducted in a split plot based on a randomized complete block design (RCBD), with four replications during 2013–14 growing season in Karaj. Main plots consisted of four pre-sowing treatments (Black fallow, Farmyard manure and 2 green manure treatments including pre-sowing treatment of perko PVH and pre-sowing treatment of buko) and sub-plots included three rates of nitrogen (120, 240 and 360 kg ha^{-1}, utilized urea source). Results showed that the effect of pre-sowing treatments on DMD, NDF and forage yield was significant ($P \leq 0.01$), so that in all traits, perko PVH and buko treatments were the best ones to compare with black fallow and farmyard manure. Moreover, different nitrogen levels had significant ($P \leq 0.01$) effect on dry matter digestibility (DMD), neutrals detergent fiber (NDF) and forage yield, so that with the increasing rate of nitrogen, these traits increased. The interaction effect of pre-sowing treatments and nitrogen levels on water soluble carbohydrates (WSC), crude protein (CP), acid detergent fiber (ADF) and total ash was significant ($P \leq 0.01$). In general, results showed that the suitable component is perko PVH treatment by using 240 kg ha^{-1} nitrogen fertilizer.

Key words: Crude protein, Dry matter digestibility, Green manure, Water soluble carbohydrates.

Abbreviations: ADF: Acid Detergent Fiber; CP: Crude Protein; DMD: Dry Matter Digestibility; NDF: Neutrals Detergent Fiber; WSC: Water Soluble Carbohydrates.

INTRODUCTION

One of the most important factors limiting the development of animal husbandry and livestock production is to provide fodder to feed the country's livestock. Thus, the need for forage production is increasing every day (Armstrong & Albrecht, 2008). Forage crops have an undeniable role in providing nutrient requirements of the ruminants. In modern animal husbandry, the silo which is made from corn allocates an important part of the daily diet of ruminants (Kmicikewycz et al., 2015). Forage quality represents nutritional value and the amount of energy that is available for livestock. In other words, it is the amount of nutrients that animals obtain in the shortest possible time from the feed (Buxton, 1996). Using good quality forage in animal breeding, reproduction, meat, dairy, leather and wool is very useful and effective. So that nutrient in the diets of livestock, forage quality and the amount of that is very important (Suyama et al., 2007). An important factor in the production and management of forage plants is the quality of the forage and improving forage quality results in feed efficiency (Catanese et al., 2016).

Corn is widely cultivated due to many features including multiple use cases in many countries (Al-Kaisi & Yin, 2003). In addition to being very good forage for livestock; this plant is also suitable for livestock in terms of power supply. Because of having sugars, starch and high forage yield, corn is one of the most important crops for the production of green forage, silage and grain (Gholamhoseini et al., 2013). Silage corn yield in most of the arid and semi-arid areas of the country is low due to low organic matter of the soil and nitrogen deficiency (Ferreira et al., 2014). Although the use of chemical fertilizer significantly increases the performance of many products; however, some adverse environmental impacts and lack of response to the excessive use of fertilizers because of their indiscriminate use, food production in the coming decades will face difficulties (Villegas & Fortin, 2002). Black fallow as a traditional practice has been defined as farming perform wherein no crop is grown and all plant growth is controlled by cultivation or chemicals during a season when a crop might normally be grown (Haas et al., 1974). Unfortunately, soil loss under black fallow management due to water and wind erosion can be significant. A study involving the use of the revised universal soil loss equation (RUSLE; Renard et al., 1991) indicated the use of seeded fallow (green fallow) in central Spain would cut the area estimated to have greater than 6 t ha^{-1} soil loss to one-third the area under that risk when in unseeded or black fallow (Boellstorff & Benito, 2005). In central Croatia, Basic et al. (2004) measured a 5-yr average soil loss of 87 t ha^{-1} from standard black fallow USLE protocol plots (Wischmeier & Smith, 1978) on a 9% slope.

Recently, different cropping systems, including crop rotation, delayed planting and annual intercropping planting with legumes are introduced to increase production in agriculture (Carruthers et al., 2000). Today, for this purpose and instead of black fallow, pre-sowing (or green manure) can be cultivated for special purposes such as preserving and adding nitrogen and carbon in agricultural systems, improving C/N ratio and the soil erosion control. Three major groups of plants, including grasses, legumes and recently brassica family are cultivated as green manure. Brassica family are planted in many cases as a substitute for legumes and grasses which in addition to the properties of green manure, can significantly increase soil organic carbon and soil porosity (Collins et al., 2007). Nowadays, in Europe and parts of North America hybrid varieties of brassica species such as perko PVH and buko are planted as intercropping forage. Perko PVH plant is the hybrid between *Brassica napus* L. var. napus and *Brassica campestris* L. var. sensulato. Also, buko is the result of crosses between tetraploid rapeseed (*Brassica napus* L. var napus) and Chinese cabbage (*Brassica campestris* L. var. sensulato) and forage turnips (*Brassica campestris* L. var. rapa), which are in many ways superior to their parents. These hybrids also are used in livestock feed due to be palatable, according to grow and create quick cover on the soil surface and also high performance of aerial organs can be used as cover crop and green manure in organic and sustainable farms (Mihailovic et al., 2008).

Clark et al. (1997) reported that quantitative and quality of corn forage is very significant under the influence of cover crops. In another study, Holderbaum et al. (1990) have been reported increased corn yield with increasing nitrogen by studying lucky clover cover crop harvest management, which was 30–65% of increased performance compared to control corn yield using 90 kg N ha^{-1}. In the study of single and double cropping of sorghum, has been reported that by increasing the amount of nitrogen fertilizer, sorghum protein content has increased and also the protein content of sorghum was looking for rye cover crop cultivation was more than sorghum which was continuously growing (Buxton et al., 1999). Ranells & Wegger (1996) attributed the main reason for increasing in yield after planting cover crops to the release of nitrogen from crop residues. Gholamhoseini et al. (2013) reported that by increasing levels of nitrogen fertilizer protein percentage of corn forage is significantly increased. Increasing the amount of nitrogen will lead to the increased crude protein, total carbohydrates and total sorghum ash (Reiad et al., 1995). A significant increase in protein percentage and digestibility of corn by increasing the levels of nitrogen has been reported by other researchers (Lawrence et al., 2008; Gheysari et al., 2009). Most studies on corn silage were based on monoculture and single cultivation and report on the plants before cultivation is unavailable. Therefore, knowing the effect of organic fertilizers on forage quality and performance of silage corn requires lots of study and research. This study aimed to evaluate the effect of pre-sowing forage quality and nitrogen levels were implemented.

MATERIALS AND METHODS

Field experiment was conducted at the Faculty of Agriculture, Agricultural Research Station of Islamic Azad University, Karaj, Iran (35° 45' N and, 51° 56' E, altitude 1,313 m), during the 2013 and 2014 years. The region is characterized as semi-arid, with mean annual precipitation of 207 mm, which mostly falls during the autumn and winter months. The annual mean temperature is 16 °C. The average precipitation and temperature in 2013 and 2014 were similar to the long-term meteorological data trend. Prior to the beginning of the experiment, a composite soil sample was collected at depths of 0–30 cm, air-dried, crushed and tested for various physical and chemical properties. Physical and chemical analysis of the soil and farmyard manure is provided in Tables 1 and 2, respectively. In addition, meteorological data were obtained from a meteorology station, Meteorological Organization of Alborz, located 7 km away from the experimental field Province (Fig. 1). The experiment was conducted using a randomized complete-block design with a split plot arrangement of treatments through four replications. The first factor included four pre-sowing treatments (2 green manure treatments including pre-sowing treatment of perko PVH and pre-sowing treatment of buko, Farmyard manure and Black fallow) as main plot, and the second factor included three nitrogen rates (120 and 240 and 360 kg ha^{-1}, utilized urea source) as sub plot.

Table 1. Soil physiochemical properties site

Soil depth, (cm)	soil texture	pH_{CaCl2}	EC (dS m^{-1})	C_{org} %	N_t %	P (mg kg^{-1})	K
0–30	sandy-clay	7.8	2.83	0.81	0.08	11.8	342
30–60	sandy-clay	7.6	3.7	0.63	0.06	9.8	298

C_{org} – organic carbon; N_t – total N.

Table 2. Properties of the farmyard manure

K	P	DM (%)	C_{org}	N_t
1.25	1.07	91.74	21.12	1.11

C_{org} – organic carbon; N_t – total N; DM – dry matter.

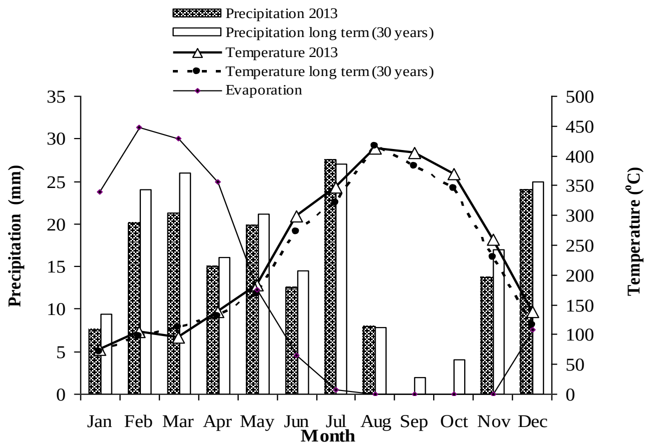

Figure 1. Monthly temperature, precipitation and Evaporation during the growing seasons in 2013 and long term.

Cultivating the pre-sowing plants was done in March 2013. For cultivating the pre-sowing plants, seeds of buko and perko PVH plants were considered linearly at the depth of 0.5 up to 1 cm and spacing of 15 cm. At the end of June and the end of the growth period of plants, pre-sowing plant returns to the soil in conducted. First, the plant floor action was done and then return operation was conducted by the crop rotator. Preparation of silage corn farm was done on 10 July. Plots were prepared after plowing and disk-harrowing. The plots were 5 m long and consisted of six rows, 65 cm apart. The distance between the plants in the rows was 13 cm; thus, the plant density was approximately 12 plants per m^2. There were 2.5 m gaps between the blocks, and a 1.5 m alley was established between the plots to prevent lateral water movement and other interferences. Based on the recommendations of soil tests, 36 kg ha^{-1} phosphorus of triple superphosphate and 70 kg ha^{-1} of potash of potassium sulfate resources were added to the soil before conducting the experiments. Also, nitrogen fertilizer is used in three steps by 10% in 5–6 leaves, 70% in the stem elongation and 20% in grain filling stage of urea resources. In addition, for applying the treatment of farmyard manure in plots, the amount of 7 ton ha^{-1} was given to the soil before the cultivation. Corn cultivation was done by pneumatic devices in July 10 in a mechanized way. During the growing season for weed control, weeding was done by hand. The irrigation was done by stacked barley and based on crop needs and environmental conditions every seven days in the early period of growth and every 10 days in the last period of growth. On 30 October 2014, when the moisture content of corn achieved to the 55–60%, the amount of 4 m^2 was taken from each port subject to the marginal effect.

Green forage which is immediately weighed and then a 2 kg sample of each plot (a total of 48 samples) after drying in 65 °C oven, first, the relevant samples are milled (at least 50 g) and then qualitative characteristics including DMD, WSC, CP, ADF, NDF and total ash are identified and used by near infrared spectroscopy which has the most accurate and at the same time the fastest technique for estimating the chemical composition of agricultural products. Data analyses are done using SAS statistic software Version 9.1.3 (SAS Institute, 2004). Mean comparison was done using the LSD test at the level of 5%.

RESULTS AND DISCUSSION

Water Soluble Carbohydrates (WSC)

The effects of pre-sowing treatments, N rates and the Pre-sowing × N interaction on the WSC were significant (Table 3). The maximum WSC (30.5% in dry matter) was observed for those plots that received 240 kg N ha^{-1} with perko PVH as pre-sowing treatment, and the minimum WSC (20.22% in dry matter) was obtained from application of the lowest levels of N fertilizer (120 kg N ha^{-1}) with farmyard manure as pre-sowing treatment (Fig. 2). Since the increase in WSC is known as a positive factor, thus, if the plant has enough reserves of soluble sugars can be further grown and don't lose due to the weakness and lack of food especially when photosynthesis is impaired or plants exposed to biotic and abiotic stresses (Buxton, 1996). Soluble carbohydrate that constitutes a significant portion of non-structural carbohydrates (Rostamza et al., 2011) are one of the most important components of determining forage quality which has the duty of supplying energy to the micro organisms of the rumen and maintain a healthy digestive system of livestock. Results showed that N application enhancement and perko PVH as green manure led to a significant increase in the WSC. The dominant reasons for this result are (i) N absorption by crop residues when N is more available (by applying N fertilizer) and (ii) the slow release of N by perko PVH residues during the corn growth period. Mirlohi et al. (2001) reported that by increasing the amount of nitrogen in the soil by plant debris and consumption of nitrogen fertilizer the percentage of forage WSC significantly increased.

Table 3. Analysis of variance (mean square) on different corn forage traits as affected by pre-sowing and N treatments

Sources of variation	d.f	#WSC	CP	ADF	Ash	DMD	NDF	FY
Replication	3	0.72	0.23	0.17	0.26	2.19	3.24	19.19
Pre-sowing (P)	3	86.15 **	21.27 **	124.39 **	10.48 **	158.37 **	300.25 **	487.82 **
Error A	9	1.05	0.10	1.08	0.21	1.69	3.04	52.87
Nitrogen (N)	2	33.94 **	5.84 **	75.88 **	11.06 **	253.85 **	15.39 **	845.63 **
P×N	6	5.71 **	0.79 **	5.28 **	1.33 **	5.72 n.s.	2.69 n.s.	118.90 n.s.
Error B	24	1.14	0.11	1.07	0.33	2.71	2.42	34.88
C.V. (%)		4.37	5.07	4.01	10.27	2.95	4.3	12.78

#WSC: Water Soluble Carbohydrates; CP: Crude Protein; ADF: Acid Detergent Fiber; Ash: Total Ash; DMD: Dry Matter Digestibility; NDF: Neutrals detergent fiber FY: Forage Yield; *, ** and ns: significant at 0.05, 0.01 probability level and no significant, respectively.

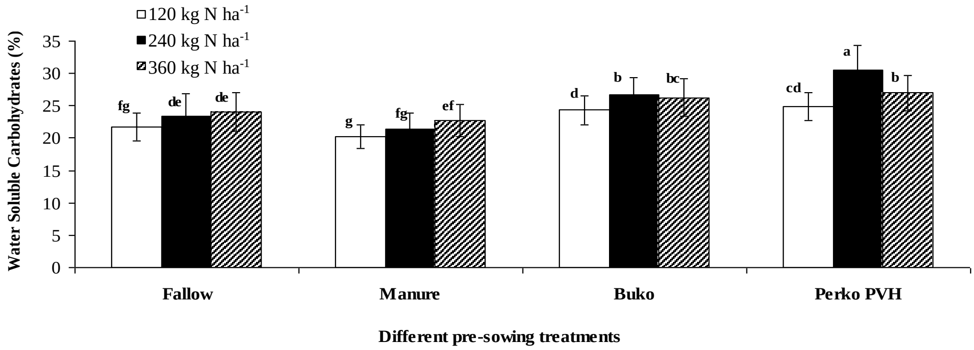

Figure 2. Interaction effect of Pre-sowing treatments × N rates on corn forage water soluble carbohydrates content. Means followed by the same letter are not significantly different ($P \leq 0.05$). Vertical bars indicate standard deviation (n = 4).

Crude Protein

Crude protein is one of the major nutritious compounds in livestock feeding, and its deficiency in forage could reduce livestock production yield (Gholamhoseini et al., 2012). The effects of pre-sowing treatments, N rates, and their interaction on crude protein were significant (Table 3). The integration of the medium N level (240 kg N ha^{-1}) with the perko PVH pre-sowing treatment generated the highest crude protein (9.07% in dry matter). In contrast, 12 kg N ha^{-1} + farmyard manure yielded the lowest crude protein (4.60% in dry matter) (Fig. 3). It seems that increasing the percentage of corn crude protein after is related to the deep and wide perko PVH roots that can absorb nutrients from the soil depth and also the remains of its rapid decay which increase soil nitrogen. Moreover, the crude protein enhancement with increasing in fertilizer levels may be due to enhancement in amino acid formation affected by fertilization and N availability. By contrast, Muhammad et al. (2002) reported that crude protein index of fenugreek forage was impressed by organic fertilizers (farmyard manure) and nitrogen. However, the corn crude protein percentage significantly decreased with farmyard manure application in the different rates of N due to increase N leaching intensity (Yan-Wang et al., 2002; Basso & Ritchie, 2005) when N was available in soil after farmyard manure distribution.

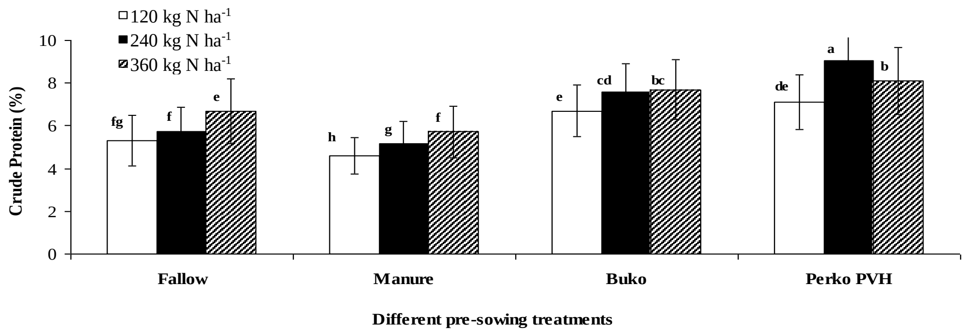

Figure 3. Interaction effect of Pre-sowing treatments × N rates on corn forage crude protein content. Means followed by the same letter are not significantly different ($P \leq 0.05$). Vertical bars indicate standard deviation (n = 4).

Acid Detergent Fiber (ADF)

Acid detergent fiber is an appropriate index to determine forage digestibility because it contains a high lignin ratio; thus, greater forage ADF decreased the digestibility of feed dry matter (NRC, 2001). In addition, Van Soest (1982) showed that ADF is the best index for representing of the nutritional value compared to the crude fiber and cellulose. Results revealed that the effects from pre-sowing treatments, N rates, and the pre-sowing × N interaction were significant for the ADF (Table 3). A comparison between the combined treatments indicated that under perko PVH pre-sowing treatment, application of 240 kg N ha^{-1} significantly increased the ADF when compared with other treatments (Fig. 4). The results demonstrated that both perko PVH and buko plants as pre-sowing treatments significantly enhanced the ADF under different N rates (Fig. 4). Also, results showed that the application of farmyard manure accompanied by N fertilizer consistently resulted in lower ADF than that found in other pre-sowing treatments. Valk et al. (2000) reported that increasing the nitrogen rate lead to the increasing of the ADF. Unfortunately, no one has reported the influence of pre-sowing treatments on the forage ADF percentage. However, it seems that increasing soil N by pre-sowing decomposing organic remains of plants is responsible for the ADF enhancement.

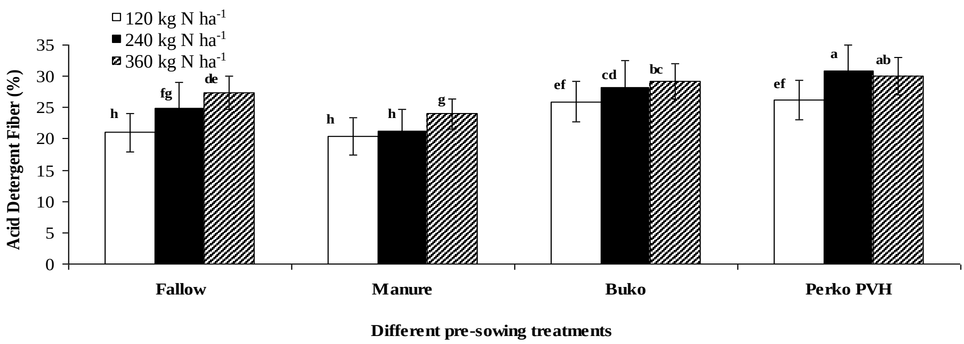

Figure 4. Interaction effect of Pre-sowing treatments × N rates on corn forage acid detergent fiber content. Means followed by the same letter are not significantly different ($P \leq 0.05$). Vertical bars indicate standard deviation (n = 4).

Total Ash

According to the data analysis, the effects from the pre-sowing treatments and N rates on the corn forage total ash were significant (Table 3); furthermore, the pre-sowing × N interaction was significant for this trait (Table 3). Comparison of means among treatments showed that the highest forage total ash was observed in plots fertilized with 240 kg N ha^{-1} and application of green manure (Perko PVH pre-sowing treatment) (Fig. 5). In contrast, the lowest ash content in forage was achieved in plots fertilized by minimum amount of chemical fertilizer (120 kg N ha^{-1}) accompanied by organic fertilizer (farmyard manure). The application of perko PVH and buko plants had a significant effect on forage quality traits, especially in those plots in which 240 kg N ha^{-1} was supplied by urea. The total ash content is used to determine the percentage of phosphorus, calcium, magnesium, potassium and other trace elements in the forage. It is quite obvious that mineral elements can be effective in forage quality (Sharma, 2002). Mineral elements in the forage are important as they involved in the animal metabolism and are necessary for body cell activity. In fact, ash forage represents the amount of minerals in plant tissues (Halil et al., 2009). So, in accordance with the direct relationship between total ash and forage quality, it is expected that increased total ash for corn forage in perko PVH and buko pre-sowing treatment accompanied with moderate N rate enhances forage quality and animal metabolism.

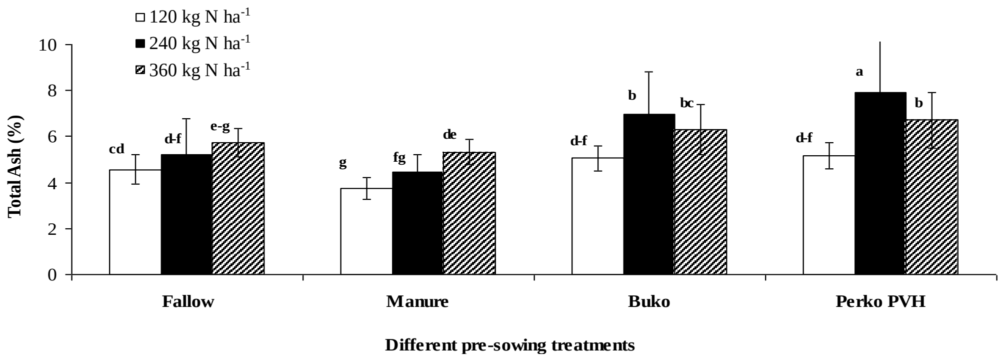

Figure 5. Interaction effect of Pre-sowing treatments × N rates on corn forage total ash content. Means followed by the same letter are not significantly different ($P \leq 0.05$). Vertical bars indicate standard deviation (n = 4).

Dry Matter Digestibility (DMD)

Digestibility usually calculated based on dry matter and is mentioned as a ratio or percentage. Also, digestion index is defined as preparing food for absorption by the digestive system of animal (McDonald et al., 1997). Results showed that corn forage DMD content was significantly affected by pre-sowing treatments and N rates (Table 3). An increase in the N fertilizer rates from 120 to 240 and 240 to 360 kg N ha^{-1} enhanced the forage DMD by 13 and 1%, respectively (Table 4). In fact, N efficiency was reduced by higher levels of this fertilizer. On the other hand, application of minimum amount of N fertilizer (120 kg N ha^{-1}) compared with other N treatments produced less DMD compared with the other N treatments. Almodares et al. (2009) reported that DMD will gradually increase with the increase of nitrogen, which is consistent with the results of the present study. Moreover, the maximum DMD (59.77% dry matter) was observed after the application of prko PVH plant as green manure, and the minimum DMD (51.26% dry matter) was observed after the application of farmyard manure in the soil. Since an increase in DMD is known as an advantage or positive factor, superior treatments of pre-sowing perko PVH and buko is important. In addition, it seems that, in those plots that received farmyard manure, leaching of N was the most important reason for DMD reduction.

Neutrals Detergent Fiber (NDF)

The amount of NDF in the feed is an indication of cell wall quantity, and the forage digestion coefficient can be predicted from the cell wall percentage (Gholamhoseini et al., 2012). The effects of pre-sowing and N treatments on the NDF were significant (Table 3). In N treatments the highest and lowest NDF values (37.27 and 35.44% dry matter, respectively) were generated using the 120 and 240 kg N ha^{-1}, respectively (Table 4). Suyama et al. (2007) reported that NDF value is significantly and negatively correlated with dry matter intake of ruminants, as NDF includes the structural cell wall components of plants (except pectins) and consists of the slowest digesting fractions (cellulose, hemicelluloses, lignin, and cutin). Therefore, it could be stated that high NDF restricts average daily body weight gains of cattle. In contrast, application of prko PVH and buko plants as pre-sowing treatments abrogated the increasing NDF such that the lowest NDF was observed from application of these plants as green manure compared with other pre-sowing treatments (Table 4). On the other hand, maximum forage NDF content was observed after the farmyard manure application (Table 4). Soluble fiber in neutral detergent includes sum of lignin, cellulose and hemicellulose and is a criteria for measuring the volume of the cell wall. By aging the plant, digestibility of dry matter and protein reduces and the amount of crude fiber and lignin increases (Halil et al., 2009). Since the reduction of soluble fiber is known as a positive factor in neutral detergent, so in this study, the treatment of 240 kg N ha^{-1} accompanied by pre-sowing of perko PVH and buko plants was the best treatment.

Forage yield

According to the data analysis, the effects from the pre-sowing treatments and N rates on the corn forage yield were significant (Table 3). The enhanced N application from 120 to 360 kg N ha^{-1} resulted in a 22% rise in forage yield (Table 4). Enhanced N fertilizer application enhanced the forage yield such that the highest yield (77,000 kg ha^{-1}) was from the 360 kg N ha^{-1} treatment, and the lowest yield (63,000 kg ha^{-1}) was from the 120 kg N ha^{-1} treatment (Table 4). It has been reported that N increases crop biomass through enhance of green area resulting in higher N assimilation (Gholamhoseini et al., 2013). Nitrogen assimilation enhancement is closely associated to an increase in net photosynthesis that finally results in enhanced plant dry weight. It should be stated that higher amounts of N above 240 kg N ha^{-1} did not significantly increase the corn forage yield (Table 4). On the other word, increase in N application from 240 to 360 kg N ha^{-1} increased forage yield only by 3%. These results suggested that increasing amounts of N application more than 240 kg N ha^{-1} does not increase yield production but does increase the environmental damaging side effects such as nitrate contaminated groundwater. The results demonstrate that application of green manure (especially perko PVH) increased the forage yield significantly (Table 4). Because the pre-sowing treatment of perko PVH increases the N availability and fertilizer efficiency, enhanced forage yield from this treatment is reasonable. Further, the ability of green manure to supply more nutritional elements gradually and during the plant growth period and improving soil physicochemical properties resulted in enhanced forage corn yield. Yield associated with various methods of fallow and pre-sowing treatments has been studied by many researchers (Biederbeck et al., 2005; Henry, et al., 2008). For example, Larsen et al. (2014) reported that, application of pre-sowing plants compared to the black fallow (unseeded fallow) leads to significantly higher corn yield quantities. They attributed the latter to the collapse of soil hard layer with plant roots, which makes better soil ventilation. It should be mentioned that C/N ratio in plant remains is critical for effectiveness of green manure. Therefore, low C/N ratio of buko and perko PVH plants (in the range of 15) is the reason for the increase of their effectiveness and rapid decay of buko and perko PVH remains. In comparison between black fallow and green manure, results showed that black fallow treatment had the lowest corn forage yield, being 12% lower than for perko PVH pre-sowing treatment (which had a maximum corn forage yield) (Table 4). This can be explained by the fact that corn forage yield is related to both nutrient and water availability and green manure (especially pre-sowing with perko PVH) unlike black fallow improved soil physical characteristics and resulted in greater corn root distribution and penetration. Only corn forage yield was enhanced at black fallow treatment compared with farmyard manure application treatment (Table 4) and this can be elucidated by greater nutrients leaching (especially N) due to manure activity in soil. Results showed that a significant and direct correlation between the total corn dry weight and leaf dry weight was existed. Ideally, those treatments that enhanced leaf dry weight (240 kg N ha^{-1} + pre-treatment of perko PVH or buko) because of their good digestibility can be categorized as optimum treatments.

Table 4. Main effect of Pre-sowing and N treatments on some corn forage traits

Traits Treatments	WSC* (%)	CP (%)	ADF (%)	Ash (%)	DMD (%)	NDF (%)	Forage Yield (ton ha^{-1})
Pre-sowing treatments							
Perko PVH	27.45 a	8.09 a	29.00 a	6.59 a	59.77 a	31.65 b	78.23 a
Buko	25.77 b	7.32 a	27.78 b	6.10 a	57.42 b	32.00 b	74.31 ab
Farmyard manure	21.48 d	5.16 b	21.92 d	4.50 c	51.26 d	40.95 a	63.71 c
Fallow	23.04 c	5.89 b	24.45 c	5.17 b	55.05 c	39.98 a	68.58 bc
Standard deviation (n = 4)	2.68	1.33	3.21	0.93	3.63	5.00	6.37
Nitrogen rates (kg ha^{-1})							
120	22.78 b	5.93 c	23.39 b	4.63 b	51.29 b	37.27 a	62.9 b
240	25.51 a	6.88 b	26.34 a	6.12 a	57.94 a	35.44 b	74.3 a
360	25.01 a	7.04 a	27.64 a	6.02 a	58.41 a	35.74 b	76.42 a
Standard deviation (n = 4)	1.45	0.60	2.17	0.83	3.98	0.98	7.27

WSC: Water Soluble Carbohydrates; CP: Crude Protein; ADF: Acid Detergent Fiber; Ash: Total Ash; DMD: Dry Matter Digestibility; NDF: Neutrals detergent fiber; Means with the same letter are not significantly different from each other (LSD test, $P > 0.05$).

CONCLUSIONS

According to the results it is clearly showed that pre-sowing of perko PVH and buko has a significant and positive effect on silage corn quality parameters and the reaction of the corn to the pre-sowing crops and N rates were different. Our results indicated that the best management treatments for the production of corn forage was an integrated treatment of N moderate rate in which N was combined with perko PVH and buko plants as pre-sowing treatments. These treatments improved corn yield and quality. In general, we can conclude that the cultivation of the pre-sowing plants and returning their remains to the soil, because of soil fertility and consequently improve the quantity and quality of corn forage, can be considered as one of the ways to achieve sustainable agriculture.

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Energy balance in production of chickpea in Turkey: A study performed in Adıyaman Province

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Abstract. In this study, it has been aimed to form the energy balance in the production of chickpea (*Cicer arietinum* L.) in Adıyaman province of Turkey. The material of the research consists of the chickpea enterprises in the center of Adıyaman province in the scope of the production season of 2015–2016. In this study, the number of enterprises for which is required to be made the study has been computed as 67 according to the simple random sampling method. Survey and observation studies have carried out in these designated enterprises. The energy equivalence of the chickpea samples taken from the enterprises has been determined by the calorimeter device. According to results of the study; the total energy input has been computed as 12,225.69 MJ ha^{-1} and the total energy output has been computed as 31,527.52 MJ ha^{-1}. The energy inputs in the production of chickpea have been 3,575.69 MJ ha^{-1} (29.25%), 3,523.08 MJ ha^{-1} (28.82%), 3,280.32 MJ ha^{-1} (26.83%), 1,230.39 MJ ha^{-1} (10.07%), 358.20 MJ ha^{-1} (2.93%), 131.52 MJ ha^{-1} (1.08%) and 126.50 MJ ha^{-1} (1.03%) as fuel energy, chemical fertilizers energy, seed energy, machinery energy, farmyard manure energy, human labour energy and chemicals energy inputs, respectively. In this study, indicators showing the energy ratio, specific energy, energy productivity and net energy were determined as 2.58, 7.07 MJ kg^{-1}, 0.14 kg MJ^{-1} and 19,301.83 MJ ha^{-1}, respectively. According to the results of the study, it is clear that chickpea production is an economical production for the 2015–2016 production seasons.

Key words: Adıyaman, chickpea, energy balance, specific energy, Turkey.

INTRODUCTION

Among the edible grain legumes in Turkey, chickpea makes up 52.5% of the total cultivation areas and 44% of the total edible grain legume production. At the same time, chickpea is ranked first among edible legumes, in terms of both cultivation area and production level in Turkey. With a total chickpea production of 535,000 tons, Turkey is ranked third in the world, following India and Australia (Anonymous, 2014; Küçükalbay & Akbolat, 2015). Chickpea appears as an important vegetable product to meet the protein need in nutrition, against the increasing population in Turkey and in the world. Because the dry grains of chickpea contain 18–31% protein, depending on the cultivar characteristics, environmental conditions of the cultivation area and the applied cultivation techniques. In addition, chickpea also has high biologic value. Digestible protein ratio is around 76–78% (Akçin, 1988; Erdin & Kulaz, 2014). Plant residues with low C/N coefficient rupture rapidly and increase soil fertility. By taking into crop alternation, it will lead to a great increase in the amount of product to be removed from the field (Azkan, 1999; Erdin & Kulaz, 2014).

Computing energy inputs of agricultural production is more difficult than the industry production due to the high number of factors affecting the production (Yaldız et al., 1993; Mohammadi & Omid, 2010). The main objective in agricultural production is to increase yield and decrease costs. Energy budget is important. Energy budget is the comparison of the relationship between energy input-output of a system in terms of energy units (Gezer et al., 2003; Mohammadi & Omid, 2010). In general, increases in the agricultural production on a sustainable basis and at a competitive cost are important to improve the enterprises' economic condition (De et al., 2001; Mohammadi & Omid, 2010).

Many researches have been done on energy balance analysis in several type of agricultural products, animal products etc. such as on energy balance activities of chick pea (Yaldız et al., 1993; Marakoglu et al., 2010), miscanthus x giganteus (Acaroğlu & Aksoy, 2005), vetch (Kökten et al., 2016), soybean (Mandal et al., 2002), wheat (Gökdoğan & Sevim, 2016), corn (Öztürk et al., 2006), corn silage (Barut et al., 2011; Pisghar-Komleh et al., 2011), cotton (Polat et al., 2006), sugar beet (Hacıseferoğulları et al., 2003), black carrot (Çelik et al., 2010), barley (Baran & Gökdoğan, 2014), maize (Konak et al., 2004), sweet cherry (Demircan et al., 2006), walnut (Baran et al., 2017a), dryland wheat (Ghorbani et al., 2011), rainfed wheat (Houshyar & Kiani, 2012), canola (Mousavi-Avval et al., 2011), orange (Nabavi-Pelesaraei et al., 2014), rice (Pishgar-Komleh et al., 2011), apple (Rafiee et al., 2010), orobanche (Semerci, 2013), pear (Tabatabaie et al., 2013), organic grape (Baran et al., 2017b), lamb (Köknaroğlu et al., 2007), beef cattle (Demircan & Köknaroğlu, 2007), broiler (Atılgan & Köknaroğlu, 2006), organic broiler (Inci et al., 2016) etc. In this study, the purpose is to determine the energy balance of chickpea production in Adıyaman province.

MATERIALS AND METHODS

The study has been done in Adıyaman province of Turkey. The province of Adiyaman is located at the Southeast Anatolia Region of Turkey (Anonymous, 2015). Surveys and observations have been done face to face with 67 chickpea enterprises, in production season during in 2015–2016 in Adıyaman province. Total energy input in unit area (ha) constitutes of each total of input's energy. Fuel energy, chemical fertilizers energy, seed energy, machinery energy, farmyard manure energy, human labour energy and chemicals have been computed as energy inputs. Chickpea grain was computed as output. The surveys done to the enterprises have been computed by using the Simple Random Sampling method proposed by Çiçek & Erkan (1996). The formula was provided as below. In the formula; n, is the required sample size; N, the number of total enterprises in the area; s, standard deviation; t, the reliability coefficient (1.96 which represents, 95% confidence); d, acceptable error (5% deviation). The acceptable error value has been defined to be 5%, and the sample size has been calculated as 67 (50 da ≤ enterprises), to achieve 95% reliability.

$$n = \frac{N \times s^2 \times t^2}{(N-1)d^2 + (s^2 \times t^2)} \qquad (1)$$

$n = \frac{73x(105.49)^2x(1.65)^2}{(72)x(6.08)^2+((105.49)^2\times(1.65)^2)}$ = 67 chickpea enterprises have been determined.

Energy balance computations have been made to determine the chickpea production productivity. The unit shown in Table 1 has been used to compute the values of the inputs in chickpea production. Input amounts have been computed and then these inputs data have been multiplied by the energy equivalent coefficient. By adding energy equivalents of all inputs in MJ unit, the total energy equivalent has been found. The energy ratio (energy use efficiency), energy productivity, specific energy and net energy have been computed using the following formulates (Mandal et al., 2002; Mohammadi et al., 2008; Mohammadi et al., 2010).

$$\text{Energy use efficiency} = \frac{\text{Energy output } (\frac{MJ}{ha})}{\text{Energy input } (\frac{MJ}{ha})} \qquad (2)$$

$$\text{Energy productivity} = \frac{\text{Yield output } (\frac{kg}{ha})}{\text{Energy input } (\frac{MJ}{ha})} \qquad (3)$$

$$\text{Specific energy} = \frac{\text{Energy input } (\frac{MJ}{ha})}{\text{Yield output } (\frac{kg}{ha})} \qquad (4)$$

$$\text{Net energy} = \text{Energy output } (MJ\ ha^{-1}) - \text{Energy input } (MJ\ ha^{-1}) \qquad (5)$$

Table 1. Energy equivalents in agriculture production

Inputs and outputs	Unit	Energy equivalent (MJ per unit)	References
Human labour	h	1.96	Mani et al., 2007; Karaağaç et al., 2011
Machinery			
Machinery	h	64.80	Singh, 2002; Kızılaslan, 2009
Combine harvester	h	87.63	Hetz, 1992; Çanakcı et al., 2005; Tipi et al., 2009
Chemical fertilizers			
Nitrogen	kg	60.60	Singh, 2002
Phosphorous	kg	11.10	Singh, 2002
Diesel fuel	l	56.31	Singh, 2002; Demircan et al., 2006
Farmyard manure	kg	0.30	Singh, 2002
Chemicals	kg	101.20	Yaldız et al., 1993
Seed	kg	18.224	Measured
Output	Unit	Energy equivalent (MJ per unit)	References
Chickpea grain	kg	18.224	Measured

The results have been tabulated after the analysis of data has been done using Microsoft Excel program considering the inputs. Examining the values of chickpea input-output and computations have been given in Table 2. The indirect energy consists of pesticide and fertilizer while the direct energy includes human and animal power, diesel and electricity energy used in the production process. On the other hand, non-renewable energy includes petrol, diesel, electricity, chemicals, fertilizers, machinery and renewable energy consists of human and animal (Mandal et al., 2002; Singh et al., 2003; Koçtürk & Engindeniz, 2009). Energy input-output and energy use efficiency computations in chickpea production have been given in Table 3. Direct, indirect, renewable and non-renewable energy forms have been given Table 4. For calorific values of chickpea IKA brand C200 model bomb calorimeter device has been used. For measuring purposes, the amount of fuel (~ 0.1 g) has been combusted inside the calorimeter bomb. The device has been measured a calorific value in MJ kg^{-1} unit. For samples, reading of the calorific value has been measured repetitively for 3 times and then the average value have been reported in chickpea study.

RESULTS AND DISCUSSION

In the enterprises, the amount of chickpea produced per hectare during the 2015–2016 production seasons have been computed as an average of 1,730 kg. In chickpea production, it is noteworthy that and diesel fuel energy, chemical fertilizers energy and seed energy have been used as the highest input. In this study, the energy input-output analysis of chickpea production in 2015–2016 has been given in Table 2. It can be seen that the first, second and third of the highest energy of inputs in chickpea production are 29.25% diesel fuel energy, 28.82% chemical fertilizers energy and 26.83% seed energy have been the inputs computed. In Table 2, The energy inputs in the production of chickpea have been 3,575.69 MJ ha^{-1} (29.25%), 3,523.08 MJ ha^{-1} (28.82%), 3,280.32 MJ ha^{-1} (26.83%), 1,230.39 MJ ha^{-1} (10.07%), 358.20 MJ ha^{-1} (2.93%), 131.52 MJ ha^{-1} (1.08%) and 126.50 MJ ha^{-1} (1.03%) as diesel fuel energy, chemical fertilizers energy, seed energy, machinery energy, farmyard manure energy, human labour energy and chemicals energy inputs, respectively.

Table 2. Energy balance in chickpea production

Inputs and outputs	Unit	Energy equivalent (MJ per unit)	Input used per hectare (unit ha^{-1})	Energy value (MJ ha^{-1})	Ratio (%)
Human labour	h	1.96	67.10	131.52	1.08
Machinery				1,230.39	10.07
Machinery	h	64.80	17.50	1,134	9.28
Combine harvester	h	87.63	1.10	96.39	0.79
Chemical fertilizers				3,523.08	28.82
Nitrogen	kg	60.60	39.60	2,399.76	19.63
Phosphorous	kg	11.10	101.20	1,123.32	9.19
Diesel fuel	l	56.31	63.50	3,575.69	29.25
Farmyard manure	kg	0.30	1194	358.20	2.93
Chemicals	kg	101.20	1.25	126.50	1.03
Seed	kg	18.224	180	3,280.32	26.83
Total				12,225.69	100.00
Output	Unit	Energy equivalent (MJ per unit)	Output per hectare (unit ha^{-1})	Energy value (MJ ha^{-1})	Ratio (%)
Output-Chickpea grain	MJ kg^{-1}	18.224	1,730	31,527.52	100.00

Energy input, energy output, energy use efficiency, energy productivity, specific energy and net energy in chickpea production have been computed as 12,225.69 MJ ha^{-1}, 31,527.52 MJ ha^{-1}, 2.58; 0.14 kg MJ^{-1}; 7.07 MJ kg^{-1} and 19,301.83 MJ ha^{-1}, respectively (Table 3). In previous studies, Yaldız et al. (1993), computed energy use efficiency in chickpea study as 3.33, Marakoğlu et al. (2010) computed energy use efficiency in chickpea study as 0.205–2, Baran & Gökdoğan (2016) computed energy use efficiency in sugar beet study as 8.35, Mobtaker et al. (2010) computed energy use efficiency in barley study as 2.86, Bayhan (2016) computed energy use efficiency in sunflower study 9.57–11.82, Yıldız (2016) computed energy use efficiency in wheat study 2.36.

Table 3. Energy balance computations in chickpea production

Computations	Unit	Values
Chickpea grain	kg ha^{-1}	1,730
Energy input	MJ ha^{-1}	12,225.69
Energy output	MJ ha^{-1}	31,527.52
Energy use efficiency		2.58
Energy productivity	kg MJ^{-1}	0.14
Specific energy	MJ kg^{-1}	7.07
Net energy	MJ ha^{-1}	19,301.83

The total energy input consumed could be classified as renewable 30.84%, non-renewable 69.16%, direct 30.32% and 69.68% indirect in chickpea production (Table 4). Renewable energy has smaller than non-renewable energy. Similarly, in previous studies, it has been determinated that the ratio of renewable energy has smaller than the ratio of non-renewable energy in sugar beet (Erdal et al., 2007), cucumber (Mohammadi & Omid, 2010), maize (Vural & Efecan, 2012), vetch (Kökten et al., 2016) and lentil (Asakereh et al., 2010).

Table 4. Energy input in the forms energy for chickpea production

Type of energy		Energy input (MJ ha^{-1})	Ratio (%)
Direct energy [a]		3,707.20	30.32
Indirect energy [b]		8,518.49	69.68
	Total	12,225.69	100.00
Renewable energy [c]		3,770.04	30.84
Non-renewable energy [d]		8,455.66	69.16
	Total	12,225.69	100.00

[a]Includes human labour and diesel; [b]Includes seed, farmyard manure, chemical fertilizers, chemicals and machinery; [c]Includes human labour, farmyard manure and seed; [d]Includes diesel, chemical fertilizers, chemicals and machinery.

CONCLUSIONS

Based on this study, following conclusions are explained:

1. Chickpea production consumed a total energy of 12,225.69 MJ ha^{-1}, which has the highest due to diesel fuel (29.25%). The energy input of chemical fertilizers (28.82%) and seed (26.83%) have the second and third share within the total energy inputs.
2. Energy use efficiency, energy productivity, specific energy and net energy have been determined as 2.58, 0.14 kg MJ^{-1}, 7.07 MJ kg^{-1} and 19,301.83 MJ ha^{-1}.

3. The renewable and non-renewable energy inputs were 30.84% and 69.16% of the total energy input, respectively.

4. Decreasing of diesel fuel and nitrogen consumption are important for energy management. Suitable combine machines may be used and farm fertilizer using may be increased.

5. In this study, the energy balance of chickpea production in the Adıyaman province has been determined. According to the evaluated results, chickpea production is an economic production in terms of energy efficiency (2.58).

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Impact of the seeding and nitrogen fertilizer rates of spring wheat that is used as a cover crop on the yielding ability of tetraploid red clover stand established at different seeding rates

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Abstract. In the years 2013–2014, a field trial was conducted at the Estonian Crop Research Institute in order to investigate a possibility of using spring wheat as a cover crop in the establishment of red clover seed field. In the trial the cover crop had four different seeding and fertilization rates. Two tetraploid red clover cultivars, 'Varte' (early) and 'Ilte' (late), were seeded at rates 2, 4, 6 and 8 kg PLS per hectare in four replications. In the year of sowing the height and density of generative tillers of spring wheat, the grain yield and its quality, the number of red clover plants per m², and the seed yield of red clover and its quality in the 1st year of harvest were determined. Economic feasibility was calculated based on the prices valid at the time of trial conduction. The trial confirmed that while establishing a red clover seed field, it is possible to replace the earlier recommended six-rowed early barley cultivars with early spring wheat cultivars. It is expedient to reduce the seeding rate and nitrogen fertilizer rate of cover crop by one third. The optimum seeding rate of tetraploid red clover cultivars was 4–6 kg PLS ha^{-1}.

Keywords: cover crop, seeding and fertilization rates of cover crop, tetraploid red clover, seeding rate, seed yield, economic feasibility.

INTRODUCTION

The seed yield of red clover, the main leguminous forage plant in Estonia, depends on the type of cultivar, the weather conditions during growth, abundance of pollinators as well as the used cultivation methods. As a rule, it is recommended to establish a red clover seed field by seeding under a cover crop, which enables the seed producer to get yield (income) in the year of sowing, the pressure of annual weeds on the young clover stand is smaller and the cover crop protects clover sprouts during the 1st half of summer against unfavourable weather conditions. But at the same time the cover crop has also a negative impact: higher competition for light, plant nutrients and humidity. To reduce the negative effects, it is recommended to use early six-rowed barley cultivars as a cover crop; after harvesting the cover crop there will be more time for clover sprouts to recover before the end of vegetation period. It is also recommended to reduce the seeding and nitrogen fertilizer rates of cover crop (Jaama, 1986; Undersander et al., 2014).

Due to global warming the period free from nocturnal frosts in Estonia has become longer by 17 days in the past 30 years (Sepp, 2015). At the same time early two-rowed barley and spring wheat cultivars with shorter straw and good standing ability have become available on the market. These facts leave us with the question whether six-rowed barley cultivars with a relatively poor standing ability are still needed for the establishment of clover seed field, or can they be replaced by other spring cereals.

Previously barley has been dominating among the cereals grown in Estonia. In recent years, due to economic considerations, the area under wheat has considerably grown (exceeding that of barley), which has also increased the interest among producers towards spring wheat as a possible cover crop for red clover.

The research carried out in Norway has indicated that thanks to the adoption of new cereal varieties with a better standing ability, it is not necessary to reduce the seeding and nitrogen fertilizer rates of cover crop any more (Aamlid & Havstad, 2011).

In Estonia it has been recommended to establish seed fields of red clover at the seeding rate of 6–16 kg ha^{-1} (Kotkas, 1969; Rand, 1992; Bender, 2006). Foreign sources also suggest very different seeding rates, but as a rule, these recommendations remain below ours. They usually are between 2–4.5 kg ha^{-1} (Bowely et al.,1985; Bouet & Sicard, 1998; Huebner, 2016). In case of a broader row space it is recommended to sow only 0.5–0.75 kg ha^{-1} (Clifford & Anderson, 1980; Rinker & Rampton, 1985). However, it is said that in practice the seeding rate for seed fields is still 6–13 kg ha^{-1} (Bowely et al., 1985; Rinker & Rampton, 1985; Marshall et al., 1998; Huebner, 2016). In Latvia, the seeding rate of red clover seed fields in case of narrow row space is 8–10 kg ha^{-1} (Jansone, 2008), in Norway 2–4 kg ha^{-1} (Aamlid & Havstad, 2011; Aamlid, 2016). As a rule, the literature does not indicate whether the sowing rate recommendations concern tetraploid or diploid cultivars. However, there is a significant difference in 1000 seed weight: for diploid cultivars it is 1.4–1.9 g and for tetraploid cultivars 2.5–3.2 g. With the same seeding rate we sow considerably less seeds of tetraploid cultivars.

The seed yield of tetraploid cultivars is considered to be approximately 40% lower than that of diploid cultivars (Sjödin & Ellerström, 1986). In Norway, according to the statistics bureau, the average seed yield of tetraploid cultivars was 164 kg ha^{-1}, that of diploid cultivars 247 kg ha^{-1}. The same indices for Sweden were respectively 225 and 300 kg ha^{-1} (Amdahl et al., 2016). Very little information is available in literature on how the method of seed field establishment and cultivation affect the seed yield of red clover cultivars.

As in Estonia the cultivation methods for red clover seed production have not been studied for several decades, a field trial was established in Jõgeva in 2013 in order to investigate and specify the following issues:

1) Is it possible to replace six-rowed early barley, recommended as cover crop for red clover, with early cultivars of spring wheat;

2) How the applied seeding and nitrogen fertilizer rates affect the yield of cover crop and its quality;

3) What is the impact of cover crop background on the formation of red clover stand and the seed yield of the 1st year of harvest;

4) What is the optimum seeding rate for the establishment of seed field of tetraploid red clover;

5) How the cover crop background and the seeding rate of red clover affect the monetary value of production in total of the sowing and harvest year, and what is the profitability expressed as a ratio.

MATERIALS AND METHODS

In the field trial that was established with the aim to get answers to the above-mentioned questions the early spring wheat cultivar 'Mooni' was used as the cover crop. The trial had the following background variants:

1) The seeding rate of cover crop was reduced (66% out of the normal rate), the nitrogen fertilizer rate was reduced (66% of the normal rate) – control variant;

2) The seeding rate of cover crop was not reduced (100%), the nitrogen fertilizer rate was reduced (66% of the normal rate);

3) The seeding rate of cover crop was not reduced (100%), the nitrogen fertilizer rate was not reduced (100%);

4) The seeding rate of cover crop was reduced (66% of the normal rate), the nitrogen fertilizer rate was not reduced (100%).

The variants were calculated on the basis of the seeding rate of spring wheat that was 600 live seeds per m^2 (100%), and the nitrogen fertilizer rate N 120 kg ha^{-1} (100%).

In all four cover crop variants the following seeding rates of red clover were studied: 2, 4, 6 and 8 kg ha^{-1}. The early tetraploid cultivar 'Varte' and the late tetraploid cultivar 'Ilte' were used in the trial. The trial plots had a completely randomized design and there were four replications.

The trial was established on a calcaric cambisol (K_0), which according to analysis data had the following content of plant nutrients: P 179, K 162, Ca 1,392 and Mg 56 mg per kg soil. The soil contained 2.0% of organic carbon, the soil reaction pH was $_{KCL}$5.4.

Phosphor and potassium fertilizers were applied to the trial plots manually (P 19, K 67 kg ha^{-1}), nitrogen fertilizer was applied as ammonium salpeter before the last soil tillage prior to sowing using a Saxonia drill. Later the trial was not fertilized. Cover crop was sown on the 30th of April, undersowing was done on the 2nd of May (planter Hege 80). To control short-lived dicotyledonous weeds, the trial area was sprayed with the herbicide MCPA 750 at the rate 1.0 l ha^{-1}.

In the year of establishment the number of generative tillers of cover crop per area unit (0.5 x 0.5 m, in 4 replications) was counted, and their height during flowering was measured (in 16 replications). The spring wheat 'Mooni' ripened and was harvested on the 10th of August. The yield was determined with the trial plot combine Hege 140. Cereal samples were dried, sorted and weighed. In the laboratory of the Estonian Crop Research Institute the following quality parameters of the yield were determined: volume weight, 1,000 seed weight, crude protein content, gluten content and gluten index.

In the year of sowing, prior to the end of vegetation period, the number of red clover plants per area unit (0.5 x 0.5 m, in 4 replications) was determined. In the harvest year of red clover seed (2014), the cultivar 'Varte' was harvested on the 20th of August, the cultivar 'Ilte' on the 2nd and 3rd of September. The combine Hege 125 C was used for harvesting. Seeds were dried, treated in the brush machine Westrup-400 and final cleaning was done with the Kamas-Westrup laboratory cleaner LALS. Seed germination and 1,000 seed weight were determined in the laboratory three months after combine harvesting.

The trials were located in the vicinity of an apiary (ca 300 m) with 8 beehives.

Statistical analysis of trial results was performed with the programme AGROBASE 20 TM.

Meteorological conditions of trial years

In the year of trial establishment (2013) air temperatures were above normal in May, June and July; in June, July and August precipitation was lower than normal (Table 1). These factors accelerated the ripening of spring wheat, but slowed down the growth and development of undersown clover plants. After the harvest of cover crop, the red clover plants had 79 days for recovering before the end of the vegetation period.

Table 1. Temperature and precipitation compared with meteorological average

Month	Average daily air temperature, °C			Precipitation, mm		
	2013	2014	Average*	2013	2014	Average*
May	14.3	11.5	10.3	84	64	50
June	17.7	13.1	14.5	38	158	67
July	17.6	19.2	16.8	35	48	80
August	16.7	16.6	15.3	70	114	89
September	10.9	11.5	10.5	32	28	66
October	6.6	5.2	5.4	58	49	65

* − average of the years 1922−2012.

Weather conditions in the year of red clover seed harvest (2014) were contradictory. May was warmer than normal (temperature was above 25 °C in 8 days). It was followed by an unusually cool June. On four occasions (on the June 24, 26, 27 and 28) frost was registered on the surface of plant stand. On the 17th of June snow and on the 23rd of June hail was falling. Early red clover started flowering on the 15th of June, but the work of pollinators was stopped until the end of month due to unfavourable weather conditions. The weather improved in July. Air temperature above the average of many years as well as a small amount of precipitation favoured the work of pollinators. From the viewpoint of red clover seed production the weather remained favourable until the 23rd of August after which frequent rains started. On the 23rd and 24th of August there was a heavy hail fall. It was possible to harvest the seed yield of early red clover in favourable conditions, the ripening of seeds of late red clover coincided with the rain period. Part of seeds were damaged due to germination in flower heads in the field.

RESULTS AND DISCUSSION

Spring wheat 'Mooni' as cover crop

The spring wheat 'Mooni' sown as cover crop grew 91–98 cm tall in the research area (Table 2). The reduction of seeding rate did not change the length of generative tillers. The reduction of nitrogen fertilizer rate by one third decreased the length of generative tillers by 3–4 cm. The difference was statistically reliable.

Table 2. Height of plants and density of generative tillers of the spring wheat 'Mooni'

	Cover crop background			
Seeding rate 400 live seeds m^2, N 80 kg ha^{-1}	Seeding rate 600 live seeds m^2, N 80 kg ha^{-1}	Seeding rate 600 live seeds m^2, N 120 kg ha^{-1}	Seeding rate 400 live seeds m^2, N 120 kg ha^{-1}	LSD 0.05
	Undersown early red clover 'Varte'			
	Height of plants, cm			
91	89	93	98	3
	Number of generative tillers, pcs m^2			
430	617	654	516	38
	Undersown late red clover 'Ilte'			
	Height of plants, cm			
93	93	96	96	2
	Number of generative tillers, pcs m^2			
448	627	644	474	42

The number of spring wheat's generative tillers per area unit was affected both by the seeding and nitrogen fertilizer rates. The nitrogen fertilizer rate had a bigger effect on the density of spring wheat. Spring wheat was sparsest in the trial variant, in which both the seeding rate and nitrogen fertilizer rate were reduced by one third compared to those used in production. In this variant 430–448 generative tillers were counted on square meter.

In our trial in 2013, we received contradictory yield data of the spring wheat 'Mooni' (Table 3). Increasing the seeding rate from 400 live seeds to 600 live seeds per m² reduced the grain yield by 143–147 kg ha^{-1}. The decrease in yield was not statistically reliable, but as the tendency was the same for the both undersown red clover cultivars, it must have been caused by weather conditions. This particular year with high temperatures and sufficient soil humidity had very favourable conditions for tillering, which probably eliminated the advantages of bigger seeding rate. At the nitrogen background N 80 kg ha^{-1} increasing of seeding rate somewhat increased the grain yield's volume weight, but reduced the 1,000 seed weight. In the part of experiment, where the early red clover 'Varte' was undersown, these changes werestatistically reliable, with the undersown cultivar 'Ilte' the changes remained within the limit of trial error. The reduction of the seeding rate of spring wheat 'Mooni' changed neither the contents of crude protein and gluten nor the gluten index. At the nitrogen background N 120 kg ha^{-1} the reduction of the seeding rate of spring wheat 'Mooni' did not change the grain yield.

As predicted, the increase of nitrogen fertilizer rate invoked bigger changes in trial data. The grain yield increased depending on the variant by 229–281 kg ha^{-1}. The crude protein and gluten contents of the yield were reliably higher than those of the yield grown at lower nitrogen background. In our trial the nitrogen rates did not affect the volume weight, 1,000 seed weight and gluten index.

The goal of spring wheat production should be the use of the yield for human consumption. Of the yield's quality parameters the relatively low protein content reduced the selling price. When 80 kg ha^{-1} of nitrogen was applied, the yield's protein content was slightly above 10%, which corresponds to the 5th category of food wheat's quality. In variants with full nitrogen rates the protein content was 12%. This raised the quality of yield to category III. The difference in selling price compared to category V was 14 euros per ton. At lower nitrogen backgrounds also the gluten content was problematic. Due to the gluten content the yield's quality class varied between categories IV–V. The full rate of nitrogen fertilizer raised the gluten content to a level (over 26%), which could have allowed to sell the yield as food wheat of categories I or II.

Table 3. Yield of spring wheat 'Mooni' and its quality in 2013

No	Seeding and N fertilizer rates of spring wheat	Yield, kg ha^{-1}	Volume weight, g l^{-1}	1,000 s. weight, g	Crude protein, %	Gluten content, %	Gluten index
		Undersown early red clover 'Varte'					
1	400 live seeds m^2, N 80 kg ha^{-1}	3,474	805	72.93	10.33	21.83	60.3
2	600 live seeds m^2, N 80 kg ha^{-1}	3,331	823	70,59	10.32	22.18	59.5
3	600 live seeds m^2, N 120 kg ha^{-1}	3,703	808	69.50	12.20	27.95	49.5
4	400 live seeds m^2, N 120 kg ha^{-1}	3,707	800	71.34	11.87	26.97	51.7
		Undersown late red clover 'Ilte'					
5	400 live seeds m^2, N 80 kg ha^{-1}	3,610	807	71.04	10.60	22.73	54.3
6	600 live seeds m^2, N 80 kg ha^{-1}	3,463	813	70.31	10.37	21.70	59.0
7	600 live seeds m^2, N 120 kg ha^{-1}	3,891	810	69.57	12.72	28.75	50.7
8	400 live seeds m^2, N 120 kg ha^{-1}	3,866	797	70.13	12.67	28.83	50.5
	LSD 0.05	211	9	2.03	0.72	2.12	5.0

Table 4 presents the densities of undersown red clover stands counted on the 30th of October 2013, i.e. at the end of the vegetation period of the sowing year. The data indicate that the number of plants per area unit depended more on the seeding rate of red clover and less on the differences in the seeding and fertilizer rates of the cover crop. The big value of LSD indicates that the density of red clover stands varied a lot even within the same seeding rate. There were both sparser and denser stands. It could depend on uneven sowing or heterogeneous moisture and light conditions under the cover crop.

According to literature, to obtain a good red clover seed yield it is sufficient to have at least 17 plants per square meter (Clifford & Anderson, 1980). In our research this level was achieved with all red clover seeding rates at all cover crop backgrounds except one – when cover crop was sown at full rate, nitrogen fertilizer was applied at the rate used in production and red clover was undersown at a smaller rate (2 kg ha^{-1}).

Table 4. Densities of red clover, sown under the spring wheat 'Mooni', stands in the autumn of the seeding year, plants pcs m^2

Clover seeding rate, kg ha^{-1}	Cover crop background				LSD 0.05
	400 live seeds m^2, N 80 kg ha^{-1}	600 live seeds m^2, N 80 kg ha^{-1}	600 live seeds m^2, N 120 kg ha^{-1}	400 live seeds m^2, N 120 kg ha^{-1}	
	Early red clover 'Varte'				
2	28	21	10	28	15
4	35	30	30	48	9
6	44	54	45	58	12
7	62	73	42	59	12
LSD 0.05	12	14	12	13	
	Late red clover 'Ilte'				
2	28	18	31	20	16
4	38	42	29	40	27
6	49	60	54	43	15
8	68	43	46	62	15
LSD 0.05	13	21	26	16	

Seed yield of red clover

The seeding and fertilizer rates of spring wheat had a relatively low impact on the seed yield of the undersown red clover 'Varte' (Table 5). The decrease in seed yield was statistically reliable only in the variant, in which the cover crop was sown at full seeding rate and fertilized at the nitrogen rate used in production, red clover was sown at the rate of 8 kg ha^{-1}.

Table 5. The effect of seeding and fertilizer rates of spring wheat 'Mooni' and that of seeding rate of the undersown red clover on the seed yield of the cultivar 'Varte' in 2014, kg ha^{-1}

Red clover seeding rate kg ha^{-1}	Cover crop background				LSD 0.05
	400 live seeds m^2 N 80 kg ha^{-1}	600 live seeds m^2, N 80 kg ha^{-1}	600 live seeds m^2, N 120 kg ha^{-1}	400 live seeds m^2, N 120 kg ha^{-1}	
2	316	310	290	311	56
4	325	306	312	319	50
6	340	329	319	325	82
8	366	302	283	305	73
LSD 0.05	53	45	54	62	
	Effect of cover crop background on the seed yield of undersown red clover 'Varte'				
2	100.0	98.1	91.9	98.5	
4	100.0	94.1	96.1	98.1	
6	100.0	96.8	93.6	95.6	
8	100.0	82.5	77.2	83.1	
	Effect of increase in the red clover 'Varte' seeding rate on the seed yield				
2	100.0	100	100.0	100.0	
4	102.9	98.8	107.7	102.4	
6	107.7	106.3	109.8	104.5	
8	116.0	97.5	97.4	97.9	

Increasing the seeding rate of the undersown red clover 'Varte' from 2 kg to 6 kg per ha increased the seed yield of the first harvest year, but the 4.5–9–8% increase was not statistically reliable.

The late red clover 'Ilte' sown under spring wheat gave a relatively good seed yield (Table 6). The seed yield varied between 332.8–406.3 kg ha^{-1} depending on the seeding and fertilizer rates of cover crop and on the seeding rate of red clover. When the seeding rate and nitrogen fertilizer rate of cover crop were not reduced, the seed yield of the late red clover 'Ilte' decreased in the first year of harvest by 8.9–10.1%. However, at the high value of LSD this decrease in seed yield was not statistically reliable. The seed yield of red clover 'Ilte' was highest in all variants of cover crop background, when the seeding rate of 4 kg ha^{-1} had been used for undersowing.

Table 6. The effect of seeding and fertilizer rates of spring wheat 'Mooni' and that of seeding rate of the undersown red clover on the seed yield of the cultivar 'Ilte' in 2014, kg ha^{-1}

Red clover seeding rate kg ha^{-1}	Cover crop background				PD 0.05
	400 live seeds m^2 N 80 kg ha^{-1}	600 live seeds m^2, N 80 kg ha^{-1}	600 live seeds m^2, N 120 kg ha^{-1}	400 live seeds m^2, N 120 kg ha^{-1}	
2	375	370	337	360	61
4	406	395	372	378	71
6	388	380	355	378	57
8	366	366	333	371	63
LSD 0.05	34	66	81	43	
	Effect of cover crop background on the seed yield of undersown red clover 'Ilte'				
2	100.0	98.7	89.9	96.1	
4	100.0	97.1	91.4	93.0	
6	100.0	98.0	91.5	97.5	
8	100.0	100.2	91.1	101.5	
	Effect of increase in the red clover 'Ilte' seeding rate on the seed yield				
2	100.0	100.0	100.0	100.0	
4	108.5	106.7	110.3	104.9	
6	103.5	102.8	105.3	105.0	
8	97.6	99.1	98.8	103.1	

In the year of sowing the seeding and fertilizer rates of cover crop and the seeding rate of red clover affected neither clover’s 1,000 seed weight nor germinability. For the cultivar 'Varte' these indices were between 2.857–2.989 g and 96–99%, for ‘Ilte’ respectively 2.813–2.949 g and 97–99%. The germinability of late red clover seed was somewhat surprising as the yield was affected in the field before harvest by continuous rain and seeds germinated partially in flower heads of heavily lodged stand.

Economic calculations

Of production inputs in the year of sowing major costs were related to phosphorous-potassium complex fertilizer (price 350 € t^{-1}), ammonium salpeter (300 € t^{-1}), spring wheat seed (C1 category 0.53 € kg^{-1}) and red clover seed (E category 10 € kg^{-1}). When the seeding rate of spring wheat was 600 live seeds per m² and the nitrogen fertilizer was applied at the rate of N 120 kg ha^{-1}, the inputs needed for the establishment of seed field cost 421.2 € ha^{-1} (Table 7).

Table 7. Economic result in 2013–2014 at the red clover seeding rate of 4 kg ha^{-1}

Cover crop background	Production input cost € ha^{-1}	Income from production sales € ha^{-1}	%	Ratio
	'Varte'			
Spring wheat 66%, N 66%	342.2	2,534	100	7.4
Spring wheat 100%, N 66%	385.2	2,396	94.5	6.2
Spring wheat 100%, N 100%	421.2	2,546	100.5	6.0
Spring wheat 66%, N 100%	378.2	2,563	101.1	6.8
	'Ilte'			
Spring wheat 66%, N 66%	342.2	3,042	100	8.9
Spring wheat 100%, N 66%	385.2	2,928	96.3	7.6
Spring wheat 100%, N 100%	421.2	2,934	96.4	7.0
Spring wheat 66%, N 100%	378.2	2,972	97.7	7.9

The reduction of spring wheat's seeding rate by one third reduced the establishment costs by 11.4%, the reduction of nitrogen fertilizer rate by one third by 9.3% and the reduction of both by 23.1%.

Based on the price level of 2015, the spring wheat yield could be sold for food consumption depending on the quality either as quality class III (182 € t^{-1}), IV (175 € t^{-1}) or V (168 € t^{-1}), the yield of red clover tetraploid cultivars as C_1 category seed at the price 6 € kg^{-1}. Spring wheat could not be classified into higher quality (and price) category due to low protein content, particularly in trial variants with the reduced nitrogen fertilizer rate.

Considering the cost of production inputs for the establishment of seed field, and the monetary value of production in the year of establishment and the first year of harvest, sowing of spring wheat as cover crop at reduced seeding and nitrogen fertilizer rates was economically more justified (Table 7).

CONCLUSIONS

Based on the trial results, the following can be concluded.

1. It is possible to establish a seed field of red clover by sowing it under early spring wheat varieties;

2. When weather conditions during tillering are favourable, the reduction of cover crop's seeding rate affects the density and yield of cover crop relatively little. This method enhances the development of red clover plants sown under cover crop and increases the seed yield in the first year of harvest by 3–6%;

3. The rate of nitrogen fertilizer has a significant effect on the density, grain yield and quality of yield of spring wheat. The nitrogen fertilizer rate applied to the cover crop had an impact on the development of red clover stand. The negative effect was evident in the density of clover stand, in the development of plants and strength of growth, and in the first year of harvest in the decrease of seed yield under sparser cover crop by 2–7%, under cover crop sown at full seeding rate even by 22%;

4. The seeding rate of tetraploid red clover between 2–8 kg ha^{-1} affected the seed yield relatively little. Better results were obtained with variants in which the seeding rate was 6 kg ha^{-1} for 'Varte' and 4 kg ha^{-1} for 'Ilte'. Based on the trial results we recommend

to use seeding rates 4–6 kg ha^{-1} for the establishment of seed fields of tetraploid red clover cultivars;

5. The seeding and fertilization rates of spring wheat and the seeding rate of undersown red clover did not affect the 1,000 seed weight and germinability of tetraploid cultivars 'Varte' and 'Ilte';

6. The red clover cultivars 'Varte' and 'Ilte' have a good seed yielding ability. 'Varte' yielded up to 366, 'Ilte' 406 kg ha^{-1};

7. Economically was justified to establish seed fields of both red clover cultivars under spring wheat by reducing the seeding and nitrogen fertilizer rates of cover crop by one third.

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Investigation of the influence of the parameters of the experimental spiral potato heap separator on the quality of work

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Abstract: The known designs of potato heap cleaners have a series of disadvantages, particularly on sticky soils. In the newly developed and patented potato heap cleaner of a spiral type there is used the vibration effect and other technical solutions which ensure efficient self-cleaning of the rollers. Laboratory-field equipment was worked out and made for the investigations which had a spiral potato heap cleaner of a new design mounted on it. Under the field conditions the real technological process of digging and cleaning of the potato tubers from the soil admixtures was simulated in one row of the potato plantation. By using the developed methodology of a multi-factor experiment dependencies were obtained characterising the impact of the design and kinematic parameters of the cleaner itself upon the quality indicators of its operation (the soil separation efficiency, the cleanness of the heap, the damage and losses of the tubers), allowing optimisation of the design.

Key words: potatoes, harvesting, separator, spiral tool, multi-factor experiment.

INTRODUCTION

Potatoes are an important food and fodder crop in the countries of Eastern Europe and the Baltic States, and improvement of the methods of their cultivation and harvesting is a pressing scientific and practical problem (Shpaar, 2004). One of the important problems of potato harvesting is cleaning of the extracted heap (the mass of potato tubers, soil and plant residues) from the soil and plant admixtures. After combine harvesting, fertile soil (up to 3–5% of the mass of potatoes) is carried away from the field together with the potatoes (Kanafojski, 1997; Barwicki et al., 2012). For instance, it is estimated that during only three years in Russia, which is one of the biggest producers of potatoes in the world, more than 11 million tons of fertile soil have been carried off the fields (Byshov, 2000). The basic reason for this is the fact that in mechanised potato harvesting a significant volume of soil is dug out and raised together with the tubers, in which the potato tubers themselves constitute only several percent.

Moreover, depending on the condition of the plantation at the moment of their harvesting, the humidity of the soil is from 7% to 20%. The systems of the separating tools used on the serial potato combine harvesters not always ensure a high separation degree from the admixtures (Petrov, 2004). Most often this happens as a result of intense sticking of the surfaces of the separating tools with humid soil (Pastuhov, 2014). The technical systems of more intense coercion upon the soil separation lead to increased undesired damage of the tubers. Separators of the potato heap should not only ensure reliable and qualitative execution of the technological process but also constantly carry out a self-cleaning operation during the working process. There is no doubt that the degree of damage of the potato tubers has to be as small as possible. There are many researchers and designers who have worked on the problem how to create efficient and reliable separators of the potato heap at the moment of its extraction, as well as on stationary potato cleaning sites (Byshov, 2000; Petrov, 2004; Wei et al., 2013; Norten Equipment, 2016). However, in spite of the great variety of the technological processes of cleaning the potato heap at the moment of its extraction, for the time present there are only relatively few investigations about the optimisation of spiral separators.

The aim of the work was experimental investigation of the operation of the spiral potato heap separator of a new design in order to optimise its parameters and improve the quality indicators of cleaning the potato tubers from the soil admixtures.

MATERIALS AND METHODS

A new design scheme (Fig. 1) of a spiral potato heap separator (Bulgakov et al., 2002, Bulgakov et al., 2013) has been developed, consisting of three serially arranged spiral driving shafts 1, which are executed as cantilever (i.e. fixed only from one side) springs 2, fastened to hubs 3 and joined with the driving shafts 4 ensuring their rotary movement with a pre-set frequency. In order to avoid clogging of the sieving gaps with humid soil, spirals 2 are arranged overlapping each other but intensification of the heap separation and additional destruction of the lumps of soil is effected by means of eccentric fixation of the shafts 1 to the hubs 3.

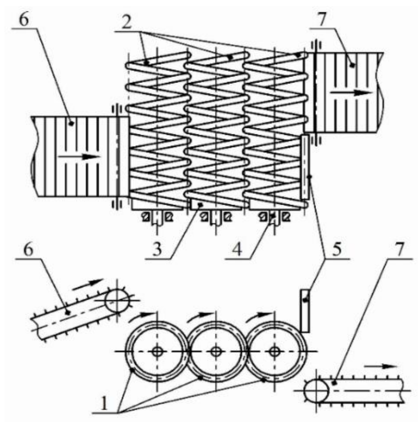


Figure 1. A design scheme of the spiral potato heap separator: 1 – spiral driving shafts; 2 – cantilever spiral springs; 3 – hubs; 4 – driving shafts; 5 – a flat screen; 6 – the feeding (input) conveyor; 7 – the discharge (output) conveyor.

The design of the spiral potato heap separator also provides for a feeding (input) rod chain conveyor 6 and a discharge (output) rod chain conveyor 7, the feeding (input) conveyor 6 being installed from the side of the hubs 3 of the spiral springs 2 and the discharge (output) conveyor 7 – from the side of the cantilever ends of the springs 2. Opposite the feeding (input) conveyor 6, with the outer side of the third roller there is installed a flat screen 5.

The technological process of operation of the improved spiral potato heap separator proceeds in the following manner. At first the potato heap is conveyed onto the first driving shaft 1 where an impact contact of the heap with the spiral spring 2 occurs. Yet this impact interaction does not induce any essential damage to the potato tuners because the surface of the spiral spring 2 which receives the impact is not an entirely solid surface (on the contrary, it is a surface with alternating projecting rods and empty gaps). Since the spiral springs 2 are to the hubs 3 only from one side, their ends are able to commit oscillatory movements in longitudinal vertical planes, which also mitigate the impact interaction. The tiny soil admixtures and the plant residues are immediately sifted down (outside the area of the separator) through the coils of the spring and the gaps between separate spiral springs. The potato tubers, left on top the active wavy surface formed by the spiral springs and inclined at a certain angle, are immediately involved in a complex movement both in a radial and an axial direction. As the spiral springs 2 are eccentrically mounted on hubs 3, the surface on which the potato tubers are situated commit compulsory wave-like movements over its entire surface with small amplitude, which promotes intense turning of the tubers and efficient sifting down of the soil residues outside the area of the separator. Here an important moment is that the spiral springs 2, due to their overlapping, clean themselves efficiently from the stuck soil. In the area of the third shaft further movement of the tubers is limited by a flat screen 5, and the tubers are directed towards the output conveyor 7. By means of the last spiral spring 2 the tiny soil admixtures and soil residues are conveyed outside the area of the cleaner into a gap between the last spiral spring 2 and the flat screen 5.

In order to conduct the research, laboratory experimental equipment was developed and made, on which a specimen of the new spiral potato heap separator was mounted. The amount of the soil and the soil residues sifted through the separator, the existence of soil stuck on the tubers of the potatoes and the degree of their damage were estimated according to a common methodology (Adamchuk et al., 2016). The design and kinematic parameters of the operation of the spiral separator were recorded in the process of investigation by means of sensors connected to the PC. The speed of the movement of the experimental equipment across the field was measured with the help of the track measuring wheel, also connected to the PC (Adamchuk et al., 2016). The design and kinematic parameters of the spiral separator of a new design (inclination of the separator in relation to the horizon, the peripheral speed of its spirals, the eccentricity dimensions of the adjacent spirals) were changed at the expense of different combinations of the dimensions of cogwheels, and controlled by sensors of revolutions. The amount of the potato heap to be fed into the spiral separator for cleaning was varied by changing the speed of the forward movement of the experimental equipment across the field. Under laboratory conditions, the input of the heap to be cleaned was estimated using prelimarily prepared and carefully weighed samples (there were prepared soil-tuber samples of standard dimensions). The condition of the potato plantation (the type of the soil, the crop capacity of the tubers, etc.) during the experimental research on the field was evaluated by the standard methodology (Standard UA7794).

To conduct experimental investigations of the spiral separator under laboratory conditions, one-row experimental equipment was made, equipped with a digging tool used on the standard potato digger L-651 and provided with two serially arranged rod chain conveyors having vibrators. Besides, on the rear part of the equipment (after the second rod chain conveyor) there was a spiral separator of a new designed installed. The laboratory equipment consists of a tracer roller 1 (with lateral cutting disks), a sectional digging share 2, the first 3 and the second 4 rod chain shaking conveyors, a spiral separator 5 of a new design, a blade 6 for receiving samples of the soil and plant admixtures at the output from the spiral separator 5, a blade 7 for receiving samples of soil and the plant residues sifted through the separator, a supporting wheel 8 (Fig. 2).

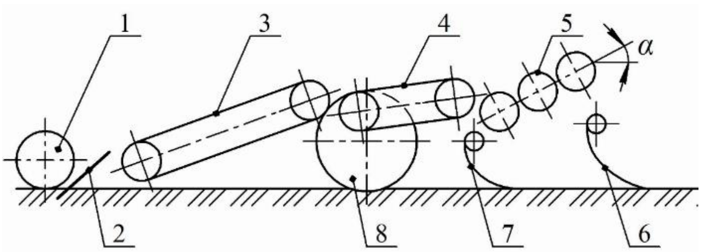


Figure 2. A design scheme of the experimental equipment: 1 – roller; 2 – a digging share; 3 – the first elevator; 4 – the second elevator; 5 – a spiral separator; 6 – a blade for receiving the mass of soil removed by the separator; 7 – a blade for receiving samples of soil passed through the separator; 8 – a supporting wheel.

When experimental investigations were carried out under stationary conditions, the experimental equipment was joined to the drive system by means of an electric motor and a transmission but the prepared samples of the heap of a corresponding fractional content were conveyed to the cleaning area. In addition, a task was posed to determine the impact of separate parameters of the cleaning technological process upon the operation quality of the separator. In accordance with the methodology, the limits of variation of the input parameters during the investigations were the following: the inclination angle α – 0…20°, the peripheral speed of the helicoidal surface of the shafts V – 1.81…2.37 m s^{-1}, eccentricity e – 0…10 mm, feeding of the potato heap Q – 15…25 kg s^{-1}. The other design parameters of the spiral separator were assumed during the experiments as constant. They included: the external diameter of the separator spirals – 133 mm, the inclination angle of the helical line of the spirals – 25°, the diameter of the metal coiling rod – 17 mm, the pitch of the coiling – 48 mm, the covering of the adjacent spirals – 6...8 mm, the minimal number of spirals – 3, the length of the spiral per one row of the harvested potatoes – 500 mm. The mode of the forward movement of the experimental equipment during the potato harvesting technological process was selected by means of different transmissions of the aggregated wheeled tractor; it was controlled using a track measuring wheel mounted on the experimental equipment and connected to the PC; it corresponded to such values: 0.53; 0.67; 0.83; 1.11 m s^{-1}. In each mode of speed of the experimental equipment the research was repeated five times. Using analytical calculations, on the basis of initial parameters of the row and conditions of digging (the digging depth – 27 cm; the width of the dug out layer – 55 mm; bulk density of the potato heap – 1,300 kg m^{-3}) the feeding rate of the heap into the middle of the experimental equipment per second were determined.

In the year 2016 the field experimental research was carried out on the experimental plot of the Ukrainian National Academy of Agricultural Sciences National Science Centre 'Institute of Mechanization and Electrification of Agriculture'. Harvesting proceeded from one row of the potato plantation, the widespread sort 'Lugovskoy'. The crop capacity of potatoes on the experimental plot was 40.35 t ha^{-1}; the potatoes were planted in ridges with 0.7 m row spacing. At the moment of the experimental research the characteristics of the potato field was: black soil, with a medium humus content, medium loamy; the average humidity of the soil 11% (in the top layer 6…8%, in the horizon 10 cm, and lower – 12…16%); hardness of the soil in the tuber-bearing layer – 0.3…0.5 MPa, weediness of the plot 1.8 t ha^{-1} (mainly haulm and stones).

According to the methodology of (Standard UA7794) under the field conditions, determination of the quality indicators was carried out while changing the forward speed, and a comparative evaluation of the heap purity in comparison with a serial machine without a separator was made.

The separation intensity was determined by the following formula:

$$q = \frac{\Delta m}{\tau \cdot S}, \text{ (kg s}^{-1}\text{ m}^{-2}\text{)} \tag{1}$$

where Δm – mass of the sifted soil (kg); τ – separation time (s); S – area of the separating surface (m^2).

The efficiency of separation is estimated as percentage of the sifted soil, and in the research it was determined according to the formula:

$$q = \frac{\Delta m}{m} \cdot 100\%, \tag{2}$$

where m – mass of the soil that enters the separator (kg).

RESULTS AND DISCUSSION

Preliminary investigations indicated that the greatest part of the soil in the experimental equipment was sifted onto the rod chain conveyors-separators but the operating area of the spiral separator was reached by approximately 30…32% of the total mass seized by the digging tools of the potato harvester. Besides, the fractional content of the potato heap was: the tubers – 43...62%, the soil admixtures – 25...40%, the plant admixtures – 11...25%.

At the first stage of the experimental research of the fractional factorial experiment an impact of separate factors was studied upon the operation quality indicators of the separator (Box et al., 2005). By using statistical processing of the results of experimental investigations for four factors mathematical models of the process were obtained in the form of linear regressions. At natural values they have the following appearance:

$$Y_1 = 118.396 + 0.25125\alpha - 12.2768V + 0.5325e - 0.3175Q \tag{3}$$

$$Y_2 = 126.3339 + 1.08\alpha - 43.107V + 0.+974e - 0.3081Q, \tag{4}$$

where Y_1– percentage of the sifted (separated) soil, (%); Y_2 – the separation intensity, (kg s^{-1} m^{-2}); α – the inclination angle to the horizon of the spiral separator, (deg); V – the peripheral speed of the rotary movement of the spiral shafts, (m s^{-1}); *e* – the installation eccentricity of the separator spirals, (mm); *Q* – feeding of the potato heap onto the spiral separator, (kg s^{-1}).

After completion of the regressive analysis (Binghman & John, 2010) of these equations one can draw a conclusion that the maximal effect upon the soil sifting and separation intensity is caused by the peripheral speed *V* of the separator spirals but the minimal effect – by feeding of the potato heap *Q*.

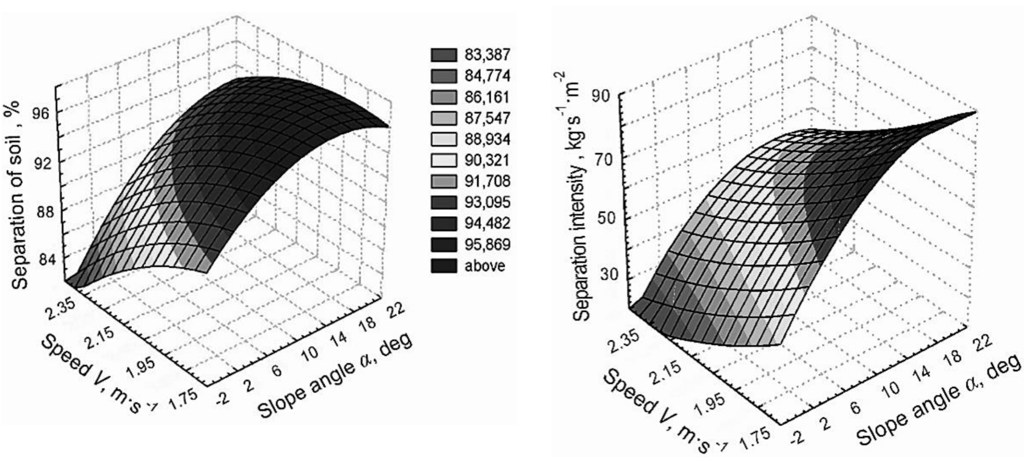
Increasing the inclination angle α of the separator and eccentricity e, sifting of the soil and the separation intensity increases. Consequently, in order to raise the percentage of the sifted soil and the separation intensity by the spiral separator, it is necessary to ensure reduction in speed *V* and feeding *Q*.

The following experimental studies had an aim to examine the soil sifting relationships as a function of the inclination angle α of the separator spirals to the horizon and the peripheral speed *V* of its spirals. After completion of the experimental investigations a multivariate regression analysis was carried out on the basis of the obtained results. By investigating the percentage of the sifted soil the separation intensity the following models were obtained in the form of a multivariate polynomial of the second degree (at the probability $P = 0.95$, and the value of the critical Student distribution point, equal to $t_a = 2.176$):

$$Y_1 = 66.9523 + 34.755V - 0.0227\alpha^2 + 0.3868\alpha V - 11.56669V^2 \quad (5)$$

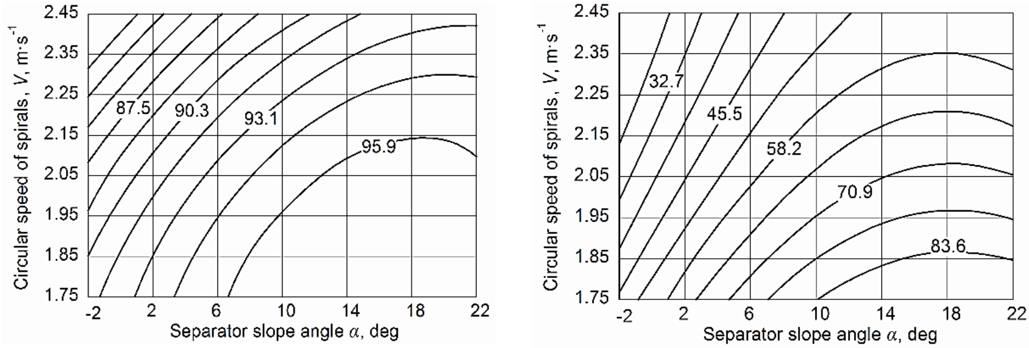
$$Y_2 = 141.6031 + 3.7093\alpha - 49.9049V - 0.0999\alpha^2 \quad (6)$$

On the basis of this mathematical simulation and data, obtained during the experimental research, graphs were built of the dependencies of the separated soil percentage and the separation intensity upon the inclination angle α of the spiral separator to the horizon and upon the peripheral speed *V* of its spirals (Figs. 3 and 4).



a) b)

Figure 3. A response surface of the inclination angle α of the spiral separator and the peripheral speed *V* of the rotary movement of the spirals: a) upon the percentage of the sifted soil; b) upon the separation intensity.



a) b)

Figure 4. A two-dimensional cross-section of the response surface of the inclination angle α of the separator and the peripheral speed *V* of the rotary movement of the spirals: a) upon the percentage of the sifted soil; b) upon the separation intensity.

At a constant value of the peripheral speed *V* (1.81 m s^{-1}) and change of the inclination angle α there were conducted investigations of the technological process of the separator operation for various soil humidities (Fig. 5).

The data obtained as a result of the research witness that, increasing humidity, the sifting process on the spiral separator takes place more slowly, which leads to increased soil contents in the cleaned heap in contrast to the heap of lesser humidity.

As it is evident from the dependency (Fig. 6, a), when the inclination angle is raised from 0 to 15°, an essential increase in the percentage of the sifted soil is observed. Yet after the following raising of the value of the inclination angle α of the spiral separator to the horizon, the variations of this indicator are not essential.

Increasing the peripheral speed of the spirals to 2 m s^{-1} (Fig. 6, b), the percentage of the sifted soil decreases slowly, but then rapid decrease is observed. This can be explained by the fact that, increasing the peripheral speed, the time of contact of the delivered mass with the operating tools of the separator diminishes, and the greatest part of the soil clods fly through the separator. The percentage of the sifted soil reaches its maximum value at the peripheral speed 1.81 m s^{-1} and the inclination angle of the separator to the horizon 15–19°.

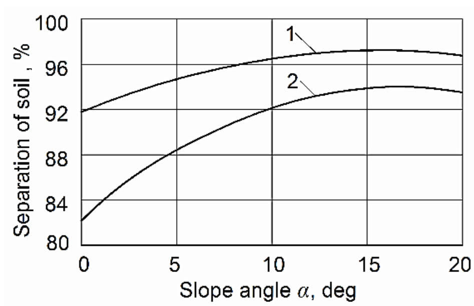
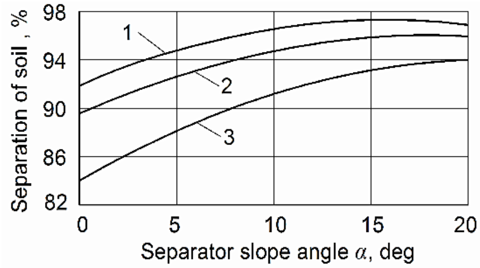
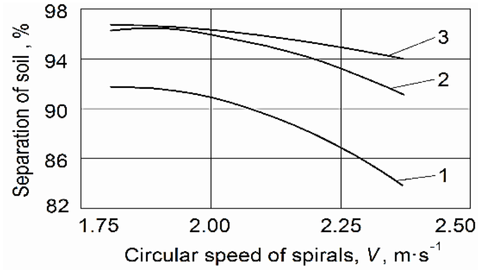


Figure 5. The impact of the inclination angle α of the spiral separator upon the percentage of the sifted soil at its humidity: 1) 7.0%; 2) 12.5%.



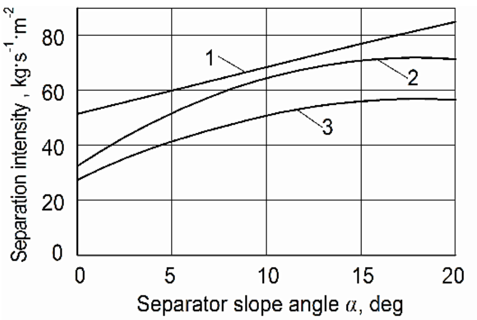
a)



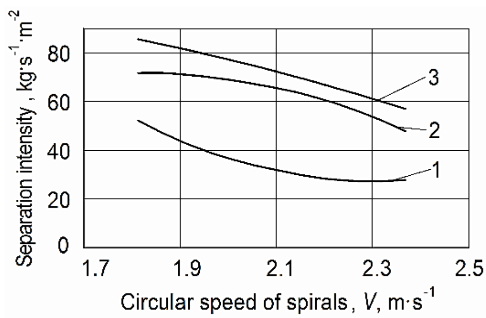
b)

Figure 6. Dependencies of the percentage of the sifted soil upon: a) inclination of the angle α to the horizon at the following values of the peripheral speed *V* of the spirals: 1) 1.82 m s^{-1}, 2) 2.09 m s^{-1}, 3) 2.37 m s^{-1}; b) the peripheral speed *V* of the spirals at the following values of the inclination angle α of the separator to the horizon: 1) 0°; 2) 10°; 3) 20°.

Dependencies of the separation intensity on the inclination angle α of the spiral separator to the horizon and on the peripheral speed *V* its spirals are presented in Fig. 7. As evident from the graphs, the dependencies mentioned above have an appearance close to the linear one.



a)



b)

Figure 7. The intensity of the separation depending on: a) angle *a* of the the separator inclination to the horizon: 1) 0°; 2) 10°; 3) 20°; b) circular speed V of spirals: 1) 1.82 m s^{-1}; 2) 2.,09 m s^{-1}; 3) 2.37 m s^{-1}.

By using the results of laboratory investigations, optimal values of the parameters were determined for the work under field conditions (for the speed of the movement of the experimental equipment 0.5–2.0 m s^{-1}): the inclination angle α of the separator to the horizon was accepted as equal to 15°, the peripheral speed *V* of the spirals was equal to 1.92 m s^{-1}, eccentricity e – 10 mm.

On the basis of the obtained structural parameters of the machine, a sample machine was made for the work on the field. Under the field conditions, determination of the quality indicators of operation was carried out while changing the forward speed, and a comparative evaluation was made with a potato digger which is popular among the potato breeders (Standard UA7794).

The dependency graphs of the separation efficiency of the potato heap, its frequency, losses and damage of potatoes are presented in Fig. 8–9. When the speed of the forward movement of the experimental equipment is increased, feeding of the potato heap (at constant values of the section of the dug up layer) increases. According to the results of the experimental investigations (Fig. 8), increase in the forward speed has an unequal effect on the agrotechnical indicators of the operation of the harvesting aggregate. Thus, the separation efficiency (Fig. 8, curve 2) in the region of the changing speed of the movement to 0.67 m s^{-1} increases gradually but, when the speed is changed from 0.67 m s^{-1} to 0.83 m s^{-1}, its slow falling is observed. Further increase in the forward speed of the experimental equipment leads to significant diminution of this quality indicator, and at the speed 1.11 m s^{-1} the separation efficiency is, on the average, 33.31%. In a similar way a change in the purity of the potato heap takes place (Fig. 8, Curve 1) since the purity of the cleaned potato heap also depends on the separation efficiency. In addition, when the speed of the movement is increased, damage of the potato tubers decreases (Fig. 9, curve 2). First of all this is due to the fact that the operating tools of the separator receive a greater amount of soil, which promotes reduction in the contact time of the root crops with the metal surface of the cleaner. The losses of the potato tubers (Fig. 9, curve 1) constitute 0.68% at the speed of the machine 0.67 m s^{-1}, but its further increase evidently leads to successive increase in their damage. When the speed of the movement of the experimental equipment is equal to 1.11 m s^{-1}, the losses constitute 2.23%.

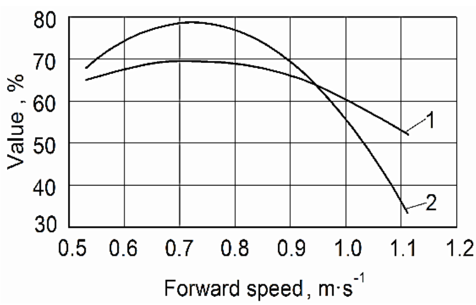


Figure 8. Influence of the forward speed of the experimental machine on the heap purity (1) and the percentage of the sifted soil (2).

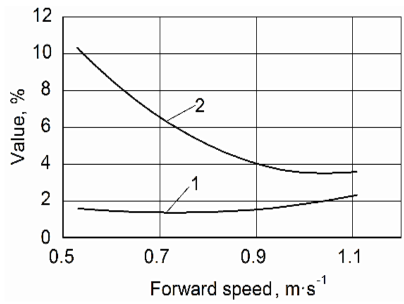


Figure 9. Influence of the forward speed of the experimental machine on the losses of tubers (1) and their damage (2).

As a result of the field experimental investigations on cleaning the potato heap, sort 'Lugovskoy', the crop capacity 40.35 t ha^{-1}, in the black soil, with a medium humus content, medium loamy; the average humidity of the soil 11% and hardness 0.3–0.5 MPa, weediness of the plot 1.8 t ha^{-1}, it was established that, using a potato digger with a spiral separator, there are losses of the potato tubers – 1.8%, and the total damage of the tubers up to 6.4%.

A statistical analysis of the research results of the damage caused to the potatoes by the spiral separator was also executed. Fig. 10 presents a distribution diagram of the types and degree of (%) damage of the potato tubers interacting with the spiral separator. As it is evident from the presented diagram, the greatest injuries (about 50%) are due to stripping the outer surface (skin) from ¼ of the tubers, the minimum (2.17%) – due to damage of the pulp of the tubers, which does not exceed the allowed norms. It is evident that it will be possible to lower the degree of damage of the potato tubers by rubberising the separator spirals envisaged by further plans.

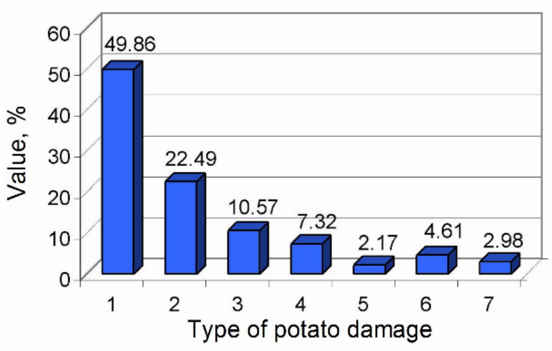


Figure 10. A distribution diagram of damage (%) of the potato tubers by their types: 1 – stripping of the outer (up to ¼) surface of the skin); 2 – stripping from ¼ to ½ of the outer surface of the skin; 3 – cracks 10…20 mm; 4 – damage of the pulp less than 2 mm; 5 – damage of the pulp to the depth 2…5 mm; 6 – traumas with the diameters of spots 5…10 mm; 7 – traumas with the diameters of spots 10…15 mm.

According to practitioners, in Ukraine the potato digger L-651 is considered one of the best machines of this purpose. Therefore, in 2016, evaluation of the the heap purity of the experimental machine was carried out also in comparison with the serial potato-digger L-651 (without a separator); which showed that the inclusion of the separator in the potato-digger construction ensures an increase in the purity of the potato heap by 27%. The conducted field experimental investigations confirm the high quality indicators of the potato heap separation using the new design of a spiral separator, and the possibility of its application in the designs of the contemporary potato harvesting machines. In the future, after completion of industrial testing (for the reliability of the design, etc.), it is envisaged to introduce the discussed design of the separator into the systems of the potato harvesting combines of areal application.

CONCLUSIONS

1) On the basis of the research results, using the method of a fractional factorial experiment and statistical processing of the results, mathematical models have been obtained which characterise the impact of the design, kinematic and technological parameters of the new spiral separator of the potato heap per cent of the sifted soil and the separation intensity of admixtures.

2) For the experimental spiral separator the optimal parameters are: the peripheral speed of rotation of the spirals 1.75–2.0 m s^{-1}, the inclination angle of the separator to the horizon 15–19°, the installation eccentricity of the spirals – 5–10 mm, the recommended speed of the movement 0.6–0.8 m s^{-1}.

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Assessment of the relationship between spectral indices from satellite remote sensing and winter oilseed rape yield

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Abstract. Winter oilseed rape (*Brassica napus* L.) belongs among the most common and strategic crops in the Czech Republic. Growth and vitality status, yield potential and yield prediction of oilseed rape on plots of different sizes can be effectively examined using remote sensing. That is why the main aim of this study was to discuss a possibility of deriving spectral indices for an assessment which spectral index is more adequate to forecast oilseed winter rape development and consequent yield in the Czech Republic. Information about the winter oilseed rape growth and yield was collected in three years – 2004, 2008, 2012. A relationship between grown crops and selected vegetation indices was evaluated. The Landsat 7 satellite images were selected as a source for deriving spectral indices. The relationship between each spectral index and yield was analysed in 2012 only. Five images on different dates during the whole life of winter oilseed rape were found during this year. The images from the years 2004 and 2008 were cloudier. The spectral indices showing the best relationship with yield from 2012 were then analysed in the images from 2004 and 2008. The results showed that Enhanced Moisture Stress Index is the most acceptable index from the selected indices used in this study. From an agronomical point of view no available index was found to be suitable for the winter rape growth evaluation due to dependence on precipitation conditions. For monitoring of the yield components in winter oilseed rape in conditions of the Czech Republic, it seems necessary to develop a new vegetation index which will reliably describe the winter oilseed rape growth stages during the whole vegetation season.

Key words: Remote sensing, spectral indices, winter oilseed rape, yield rating, Landsat 7 images.

INTRODUCTION

Rapeseed is among the three most important oilseed crops in the world (FAO, 2007). Its oil is used as a raw material to produce industrial and hydraulic oil, cleaners, soap, biodegradable plastics and for animal nutrition (Ghaffari et al., 2014). Besides, it is one of the cultivated medicinal food plants in Middle Asia, North Africa and Western Europe (Saeidnia & Reza, 2012). Rapeseed is also advantageous nutrition and fertilization plant in different soil tillage systems (Růžek et al., 2006). These advantages are the reasons why global cultivation has gradually been increasing over the last 10 years (Schoenenberger & D'Andrea, 2012). Since the 80's rapeseed is the most frequently grown oilseed crop in Europe, above all in the Czech Republic. In this country the area under rapeseed has increased to more than 400 thousand hectares (Krček et al., 2014) and rapeseed has become one of the strategic plants at economic and agricultural levels.

Satellite images are usually used for Earth observation, and many remote sensing applications are devoted to the agricultural sector, mainly in: (1) biomass and yield estimation, (2) vegetation vigour and drought stress monitoring, (3) assessment of crop phenological development, (4) crop acreage estimation and cropland mapping, and (5) mapping of disturbances and land use/land cover (LULC) changes (Atzberger, 2013). Technological advances in remote sensing have enabled the development of new applications in agriculture such as precision agriculture (Zarco-Tejada et al., 2014), irrigation management, time series (Tornos et al., 2015) and crop behaviour (Dominguez et al., 2015).

Only a few studies have been reported on the use of remote sensing methods for assessing winter oilseed rape biophysical parameters (e.g. Pan et al., 2013; Li et al., 2014). The area under winter oilseed rape in the countries of East-Central Europe such as Poland has increased in recent years. This is connected with the intensification of biofuel production. This should lead to the development of methods for the control of the condition of crops and forecasting yields (Piekarczyk et al., 2011). For example Piekarczyk et al. (2011) used hyperspectral radiometer measurements (a hand-held radiometer and multispectral images) for estimation of oilseed-rape yield. They found out that the strongest relationships ($R^2 = 0.87$) between the yield and spectral data recorded by both sensors occurred at early flowering stages.

Technological advances in the spectral data from World War Two to the mid-1960s encouraged scientists to use these data and to explore their applications (Cohen & Goward, 2004). The first spectral indices obtained from these data were developed as the ratio between reflection signals at 740 nm (near-infrared band, NIR) and 650 nm (red band, RED) (see Table 1) and they were used for different vegetation studies and called spectral vegetation index (SVI), simple ratio (SR), ratio vegetation index, formerly known as the environmental vegetation index (EVI) (Birth & McVey, 1968). SVIs are based on the relationship between the leaf structure and electromagnetic radiation reflectance by chlorophyll. A few years later, Landsat 1 was launched with a spectral resolution similar to that used in spectroscopy studies for the visible and NIR bands. SVI was the first spectral index used, however, for studying plant growth it is better to use the normalized difference vegetation index (NDVI), because pigments in plant leaves strongly absorb wavelengths of red light and the leaves themselves strongly reflect wavelengths of near-infrared light (Rouse et al., 1974). However, the NDVI exhibited no good correlation with the chlorophyll content. The best correlation was found in the ratio of the reflection signals at 800 nm (NIR) and 550 nm (green band, GREEN). The resulting spectral index was similar to NDVI, but replaced the red band by the green band. This index was called green normalized difference vegetation index (GNDVI) (Buschmann & Nagel, 1993). The optimized soil adjusted vegetation index (OSAVI) was developed as a modification of NDVI to correct for the influence of soil brightness when the vegetative cover is sparse. The OSAVI is structurally similar to the NDVI but with the addition of a 'soil brightness correction factor' (Rondeaux et al., 1996). Various spectral indices were used for nitrogen determination in maize, such as normalized green ratio (Norm G), normalized red ratio (Norm R), normalized infrared ratio (Norm NIR), and the green optimized soil adjusted vegetation index (GOSAVI) (Sripada et al., 2006). The close relationship between leaf nitrogen content and leaf chlorophyll content was analysed by means of chlorophyll vegetation index (CVI) (Hunt et al., 2011).

Table 1. Spectral range overview of Landsat 7 image

Landsat 7 Bands	Spectral Range	Wavelength (μm)
Band 1	Blue	0.45–0.51
Band 2	Green	0.53–0.59
Band 3	Red	0.64–0.67
Band 4	Near Infrared (NIR)	0.85–0.88
Band 5	Short-wave Infrared 1 (SWIR 1)	1.57–1.65
Band 7	Short-wave Infrared 2 (SWIR 2)	2.11–2.29

Soil and water content are other factors of great importance for plant growth and health. That is why short wavelength infrared bands (SWIR) were used in moisture stress index (MSI) and in enhanced moisture stress index (EMSI) (Rock et al., 1985; Dupigny-Giroux and Lewis, 1999). Water content in the leaves has been studied using the normalized difference water index (NDWI) (Gao, 1996). A modification of NDWI is to replace SWIR1 by SWIR2; this index was called enhanced normalized difference water index (ENDWI) (Chen et al., 2005). During a five-year (2001–2005) history of moderate resolution imaging spectroradiometer (MODIS), the NDVI and the NDWI were used to study drought and allowed to develop a new spectral index – normalized drought difference index (NDDI) and enhanced NDDI (ENDDI). The ENDDI ratio is calculated by dividing the difference of the NDVI and ENDWI between the sums of these spectral indexes (Gu et al., 2007).

It is clear from the above review of literature that remote sensing can be used for the assessment of plant biophysical parameters and a relatively high number of vegetation indexes was introduced. Satellite remote sensing is presented as an auxiliary tool in agriculture. However, it is necessary to analyse the various methodologies in order to obtain optimum performance of this tool and the relationship between satellite remote sensing and yield forecasting. Only a few studies examined biophysical properties of rape in the past despite the fact that rape is an increasingly popular crop under European conditions. Thus, the aim of this study is to fill this gap of knowledge and to assess which spectral index is the best for winter oilseed rape yield forecasting in the Czech Republic.

MATERIALS AND METHODS

Study area

The study area is an experimental field of 11.5 ha in size with Haplic Luvisol located in Prague-Ruzyně (50°05'N, 14°17'30"E), Czech Republic. A larger part of the field has a southern aspect and the elevation ranges from 338.5 to 357.5 m above average sea level (a.s.l). The average slope of the field is approximately 6%. The soil of this experimental plot can be classified as Haplic Luvisols partially covering fine calcareous sandstones with higher content of coarse silt and lower content of clay particles and clay. The value of cation exchange capacity in the top layer containing clay is 20–35%. The soil profile is neutral and the sorption capacity is from saturated to fully saturated.

Content of available minerals is from good to very good. In the slope positions and in loess loam profiles of Luvisols with remnants of alluvial horizon can be found. Some parts where the topsoil directly overlays the parent material of loess loam are strongly eroded. The average precipitation is 526 mm per year and the average temperature is 7.9 °C. Conventional arable soil tillage technology based on ploughing and fixed crop rotation was used in this field. Since 2001 the crop rotation has been as follows: sugar beet (2001), spring barley (2002), winter wheat (2003), winter oilseed rape (2004), winter wheat (2005), oat (2006), winter barley (2007), winter oilseed rape (2008), winter wheat (2009), oat (2010), winter wheat (2011), winter oilseed rape (2012), winter wheat (2013), oat (2014), winter barley (2015) and winter oilseed rape (2016) (Kumhálová & Moudrý, 2014). This crop rotation system is a common practice in the Central Bohemian Region (Czech Republic). Our experiment included the data from the years 2004, 2008 and 2012 only.

Field data

Yield was measured by a combine harvester equipped with an LH 500 yield monitor (LH Agro, Denmark) with a DGPS receiver with EGNOS correction. The horizontal and vertical accuracy of this system was ± 0.1 to 0.3 m and ± 0.2 to 0.6 m, respectively. Measured yield data were processed by an on-board computer on the combine harvester and saved together with the location data every 3 s. The grain moisture content was measured continuously and the yield was recalculated to 14% moisture content. The yield values were corrected using a common statistical procedure; all values that exceeded the range defined as mean ± 3 standard deviations were removed. Because of the large amount of data for every year studied (more than 8,000), the Method of Moments (MoM) was used to compute the experimental variograms. Experimental variograms of yield were computed and modelled by weighted least-squares approximation in GS+ software (Gamma Design Software, St. Painwell, MI, USA). A detailed description of this method can be found in Kumhálová et al. (2011a). Ordinary punctual kriging was done on a 6.5 m grid using the relevant data and exponential variogram model parameters for yield data visualisation (see Table 2). The data were processed in ArcGIS 10.3.1 software (ESRI, Redlands, CA, USA).

Total monthly precipitation and temperature data were provided by the agro-meteorological station at the Crop Research Institute in Prague-Ruzyne. Precipitation and temperatures for the observed years are also shown in Table 3.

Table 2. Summary statistics, variogram model parameters and the methods of interpolation used for yield in the experimental field

	Yield 04	Yield 08	Yield 12
Count	10,861.0	8,440.0	9,389
Mean	3.708	2.734	2.809
Median	3.739	2.527	2.942
Mode	3.073	0.677	2.626
Sample variance	0.878	7.283	6.623
Standard deviation	0.937	1.477	1.199
Minimum	0.304	0.059	0.100
Maximum	7.104	7.342	6.623
Skewness	-0.612	0.126	-0.252

Table 2 (continued)

Method of estimation	Method of moments (MoM)		
Variogram model	Exponential		
Distance parameter (*r*)	28.9	22.7	69.7
Approximate range = 3 *r*	86.7	68.1	209.1
Nugget variance	0.340	1.040	0.589
Sill variance	0.817	1.750	1.449
Method of interpolation	Kriging	Kriging	Kriging

Table 3. Precipitation and temperatures in different growth stages by BBCH scale recorded on the experimental field in the year 2004, 2008, 2012 for winter oilseed rape

	Precipitation (mm)			Temperature (°C)		
	Winter oilseed rape					
	2004	2008	2012	2004	2008	2012
BBCH 0-19	52.8	72.0	61.3	16.5	13.5	16.8
BBCH 20-29	103.4	105.3	167.8	5.4	5.3	4.3
BBCH 30-59	157.2	112.6	54.1	14.6	11.8	9.5
After BBCH 60	46.6	99.6	258.9	19.1	18.9	17.8
Sum	307.2	317.5	480.8	-	-	-
Mean	102.4	105.8	160.3	13.0	11.9	10.5

Remote sensing data processing

Landsat 7 Enhanced Thematic Mapper Plus (ETM^+) images were obtained from the US Geological Survey (USGS) (http://earthexplorer.usgs.gov/). All cloud-free images (see Table 4) available over the study area from the years 2004, 2008 and 2012 between March and June have been selected (path 191, row 25 and path 192, row 25). ENVI 5.3 (Excelis, Inc., McLean, USA) remote sensing software was used for processing all images.

Table 4. Available Landsat images for the selected years

Crop	Date	Sensor	Satellite
Winter Oilseed Rape	28-Apr-2004, 30-May-2004, 8-Jun-2004, 2-May-2008, 9-May-2008, 10-Jun-2008, 17-Mar-2012, 26-Mar-2012, 27-Apr-2012, 4-May-2012, 19-May-2012,	ETM+	Landsat 7

The images that could not be used because of cloud cover or because of striping with data gaps were also found. This is a problem of Landsat 7 images. It is caused by the scan line corrector anomaly in Landsat 7, which is inconvenient when using remote sensing in some study areas. In fact it was the reason why there were only a few images from Landsat 7 for this area. However, the images available in UGSS allowed analysing the relationship between the spectral index and the yield. Temporal distribution of the images along the observed three years included all the growth stages of winter oilseed rape for each year.

Selected spectral indices (see Table 5) were calculated by means of images converted into reflectance bands using the atmospheric correction model Line-of-sight Atmospheric Analysis of Hypercubes (FLAASH) (Li et al., 2014; Dominguez et al., 2015).

Table 5. Spectral indices evaluated in this study

Spectral Index	Algorithm	References
Ratio Vegetation Index (RVI)	(NIR / RED)	Birth & McVey (1968)
Normalized Difference Vegetation Index (NDVI)	(NIR – RED) / (NIR + RED)	Rouse et al. (1974)
Green Normalized Difference Vegetation Index (GNDVI)	(NIR_r – GREEN) / (NIR + GREEN)	Buschmann & Nagel (1993); Gitelson et al. (1996)
Normalized Green (NG)	GREEN / (NIR + RED + GREEN)	Sripada et al. (2006)
Normalized Red (NR)	RED / (NIR + RED + GREEN)	Sripada et al. (2006)
Normalized NearInfrared (NIR)	NIR / (NIR + RED + GREEN)	Sripada et al. (2006)
Chlorophyll Vegetation Index (CVI)	NIR × RED / GREEN)^2	Vincini et al. (2008)
Optimized Soil Adjusted Vegetation Index (OSAVI)	[(NIR – RED) / (NIR + RED +L)] × (1 + L)	Rondeaux et al. (1996)
Moisture Stress Index (MSI)	SWIR1 / NIR	Rock et al. (1985)
Enhanced Moisture Stress Index (EMSI)	SWIR2 / NIR	Rock et al. (1985)
Green Soil Adjusted Vegetation Index (GSAVI)	[(NIR – GREEN) / (NIR + GREEN + L)] × (1 + L)	Stripada et al. (2006)
Normalized Difference Water Index (NDWI)	(NIR – SWIR1) / (NIR + SWIR1)	Gao (1996)
Enhanced Normalized Difference Water Index (ENDWI)	(NIR – SWIR2) / (NIR + SWIR2)	Chen et al. (2005)
Normalized Drought Difference Index (NDDI)	(NDVI – NDWI) / (NDVI + NDWI)	Gu et al. (2007)
Enhanced Normalized Drought Difference Index (ENDDI)	(NDVI – ENDWI) / (NDVI + ENDWI)	Gu et al. (2007)

FLAASH correction consists of two parts. The first is conversion of digital numbers (DNs) to radiance values. This is calculated by the following formula:

$$L_\lambda = (Gain_\lambda \times DN7) + bias_\lambda \tag{1}$$

where L_λ is the calculated radiance [in W/(m^2 × sr ×μm)], DN7 is the Landsat 7 ETM$^+$ DN data or the equivalent calculated in step, and the gain and bias are band-specific numbers. The latest gain and bias numbers for the Landsat 7 ETM+ sensor are given in Chander et al. (2009).

The second part is to convert radiance data to reflectance data. Top of Atmosphere (TOA) Reflectance was calculated using the following expression:

$$R_\lambda = \frac{\pi L\lambda d2}{Esun\lambda \sin(\theta SE)} \tag{2}$$

where R_λ is the reflectance (unitless ratio), L_λ is the radiance calculated in the preceding step according to formula 1, d is the Earth-Sun distance (in astronomical units), $Esun_\lambda$ is the band-specific radiance emitted by the Sun, and ΘSE is the solar elevation angle.

The FLAASH module from ENVI software was used to correct Landsat data with the metadata file for each Landsat image (scene centre location, sensor altitude, pixel size, flight date and time), atmospheric model (Mid-Latitude Summer), aerosol model (rural), initial visibility (30 km) and aerosol retrieval [2-Band (Kaufman)].

The relationship between each spectral index and yield was analysed in 2012 only because in that year 5 images taken on different dates during the whole winter oilseed rape life were found. The spectral indices showing the best relationship with yield were then analysed in the images from 2004 and 2008. The statistical analysis of data was done by Statistica 8.0 software (StatSoft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

The correlation coefficients (R) between different spectral indices and yield in different years are shown in Table 6. The correlation coefficients were calculated for a 5% significance level. Winter oilseed rape yield in all the three growing seasons is represented in Fig. 2. All basic differences between yield data can be seen in Table 2. Fig. 3 shows the dependence between the EMSI spectral index and yield on three dates of crop monitoring in 2008 and Fig. 4 documents the same dependence on five dates of crop monitoring in 2012.

Table 6. Correlation coefficients (R) between different spectral indexes and yield in the years 2004 in terms:(1) 28–Apr, (2) 30–May, (3) 8–Jun; 2008: (1) 2–May, (2) 9–May,(3) 10–Jun; 2012: (1)17–March, (2) 26–March, (3) 27–Apr, (4) 4–May, (5) 19–May (5% significance level)

	NDVI	GNDVI	CVI	OSAVI	MSI	EMSI	NDWI	ENDWI	NDDI	ENDDI
2004										
(1)	0.07	0.007	0.005	0.0004	0.000	0.0001	0.0007	0.0006	0.007	0.009
(2)	0.0005	0.07	0.08	0.056	0.042	0.19	0.034	0.047	0.003	0.0004
(3)	0.013	0.09	0.09	0.07	0.077	0.08	0.085	0.05	0.002	0.047
2008										
(1)	0.009	0.004	0.033	0.01	0.005	0.02	0.0025	0.022	0.026	0.033
(2)	0.05	0.027	0.012	0.07	0.216	0.04	0.216	0.044	0.17	0.1
(3)	0.147	0.112	0.012	0.147	0.277	0.61	0.0314	0.192	0.2	0.013
2012										
(1)	0.32	0.18	0.11	0.31	0.22	0.46	0.22	0.24	0.23	0.06
(2)	0.25	0.30	0.17	0.22	0.33	0.52	0.28	0.32	0.20	0.06
(3)	0.24	0.20	0.08	0.20	0.28	0.56	0.27	0.22	0.21	0.15
(4)	0.002	0.006	0.01	0.002	0.006	0.036	0.01	0.0001	0.004	0.06
(5)	0.01	0.07	0.05	0.10	0.18	0.65	0.25	0.15	0.38	0.02

Total precipitation was average and mean temperature was higher in comparison with the other years during the 2004 winter oilseed rape growing season (Kumhálová et al., 2013). In that year, winter oilseed rape yield significantly differed from the other observed years (see Fig. 1). The yield was much more uniform overall field area and the mean of the yield was calculated to be 3.708 t ha^{-1}, which was about by 1 t ha^{-1} more than in the other two years (see Table 2). Relatively high yield and its uniformity were very probably caused by favourable water availability in the BBCH 30-59 development stages of winter oilseed rape. Sufficient available water caused the steady growth of plants on drier areas of the field. Less rainfall (see Table 3) was observed after the beginning of flowering. Nevertheless, this decrease of water availability hardly had any influence on the yield. The highest decrease in yield was observed at the headlands. It was probably caused by technogenic soil compaction at the headlands and other factors (pest attacks) (*Meligethes aeneus*, *Scierotinia scierotiorum*). It is quite clear from Table 6 that the correlation coefficients are generally very low. This fact may indicate that it is not possible to establish a relationship between selected indices in table 5 and rape yield. This is probably caused by specific development and leaf structure of rapeseed plants in comparison with grain plants. Another reason could be changes in the vegetation reflectance during the different rape phases, because rape changes rapidly the growth phases during the spring (Domínguez et al., 2015). That is why the spectral index values varied considerably. It is also clear from Table 6 that EMSI is the best index among the selected ones. EMSI is a spectral index that evaluates moisture stress and compares the relationships between band 4 (in Landsat 7 images), which contains information about the structure of the plant, and band 7, which contains data about water in the cells of the plant. EMSI showed low variability in band 4 and high variability in band 7 (Pan et al., 2013). Higher values of this index indicate greater water stress and lower water content. This fact could be seen in 2008 and 2012 in the later phase, but this dependence was very weak in 2004. Only three dates were evaluated in the 2004 season. The date 28.4.2004 was influenced by a change in the colour of vegetation during the BBCH 60 growth stage (beginning of flowering). Correlation coefficients (see Table 6) were very low for the other two dates in 2004. Both dates could be affected by the shooting stage, when due to the weather conditions water drops (dew in the morning during hot weather) were present on the leaves and reflectance values significantly changed. A significant relationship was found between the values of vegetation indices and yield in the 2004 season. Low correlation coefficients determined on 8. 6. 2004 could be caused by the weather conditions in the growth period after BBCH 60. This period was affected by dry and warm weather (see Table 3).

Average total precipitation and lower temperatures were observed in the 2008 growing season in comparison with the other observed years (Kumhálová et al., 2013). That year was good for plant mass production in the autumn season. Rape plants grew too large for winter. This consequently caused high infestation by fungal diseases (Kumhálová et al., 2011b), which significantly affected the yield on areas with standing water in the studied field. The field terrain probably had a negative effect on yield due to a positive effect on the plant disease development (Kumhálová et al., 2013). The average yield was 2.734 t ha^{-1}. Fig. 1 shows the influence of terrain topography and the influence of losses caused by fungal diseases on yield.

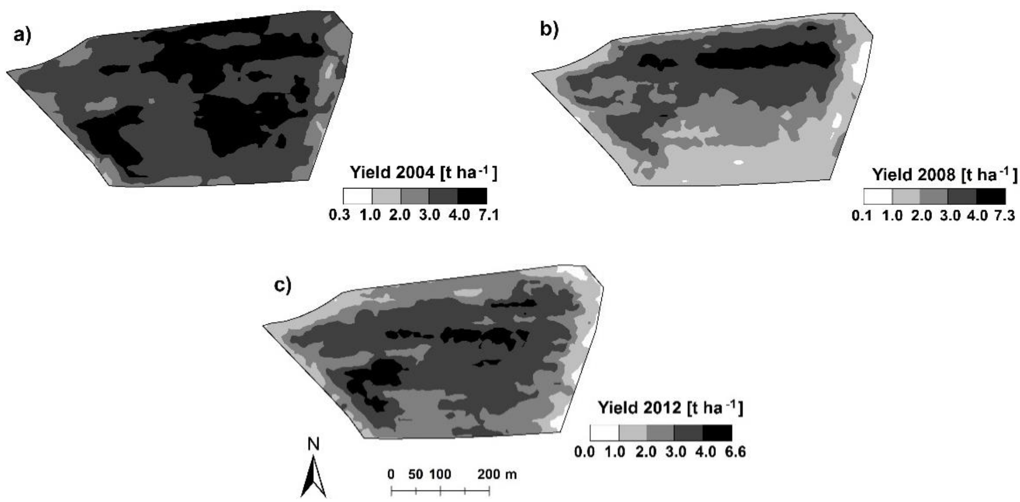

Figure 1. Winter oilseed rape yield.

Fungal diseases produce a coating on the leaves and physiological changes in the structure of plants. The influence of fungal diseases was much smaller in later growth stages (after BBCH 60). In the 2008 season there were two dates (May 2, May 9) of the flowering phenophase (BBCH 60-65 stage). A change in the colour of vegetation from green to yellow made it impossible to evaluate a relationship between yield and values of vegetation indices. The rape stand in terms of the growth phase was not quite uniform. Drier sites began to flower earlier than wetter ones. This corresponds to the variation in dependences between the yield and EMSI index (see Fig. 2). The value of correlation coefficient between the yield and EMSI index in the phase after BBCH 70 (June 10, 2008) increased to 0.61, which is acceptable for the evaluation of yield. This value is in agreement with other studies evaluating i.e. oat and wheat yield when the values of MSI index reached -0.68 or -0.60 for oat and -0.65 or -0.82 for winter wheat (Kumhálová et al., 2014). The rape stand at this time is light green with fully developed pods. Correlations between yield and other indices were generally very low on June 10, 2008.

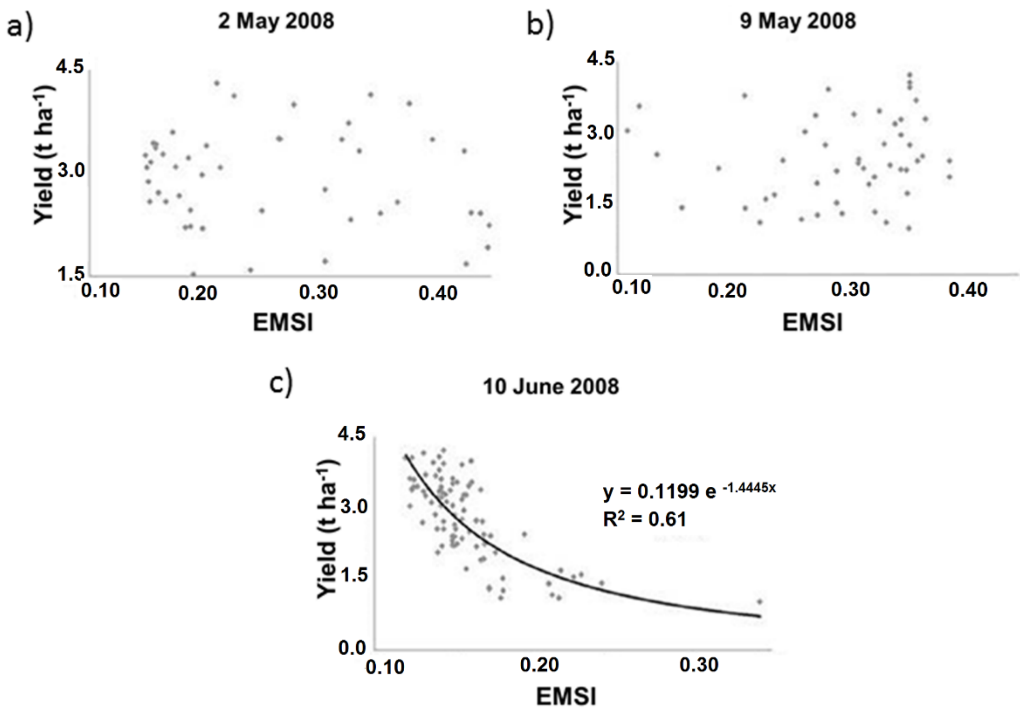

Figure 2. Relationship between EMSI on different dates and yield in 2008.

The year 2012 was the coldest and richest in precipitation. This weather pattern probably caused that the rape crop prospered well in the autumn season (especially in BBCH 10-19 stages). Nevertheless, the worst uptake of nitrogen fertilization at drier places of the field was probably caused by relatively low precipitation during flowering (BBCH 60-69). Kumhálová et al. (2013) described the influence of topography, which can be seen in the southwestern part of the field (see Fig. 1). The influence of soil compaction at the headlands can be seen.

Fig. 3 shows the dependence between the EMSI index and yield on several dates of taking images (in 2012). A good agreement was found between the values of EMSI and yield in the majority of the spring growth stages from the beginning of stem elongation (BBCH 30) to ripening. The correlation coefficients in 2012 between EMSI and rape yield gradually increased during the plant development from 0.46 (March 17) to 0.65 (May 19). It corresponds with the amount of precipitation in the BBCH 30-59 growth stages (54.1 mm) in 2012. As it was described in Kumhálová et al. (2011a), topography and weather conditions affected the yield in this field. On the contrary, the correlation coefficient dropped sharply on May 4, 2012. It was due to the beginning of the flowering stage (BBCH 60), when the colour of plants is changed in the individual storeys, which influences also reflectivity. Therefore in the flowering stage it is not possible to evaluate the rape stand by this index. The highest value of correlation can be seen in the evaluation of the images from May 19, 2012. At that time rape was at the BBCH 70 stage. The upper parts of plants were after the end of flowering. The development of pods occurred at this stage.

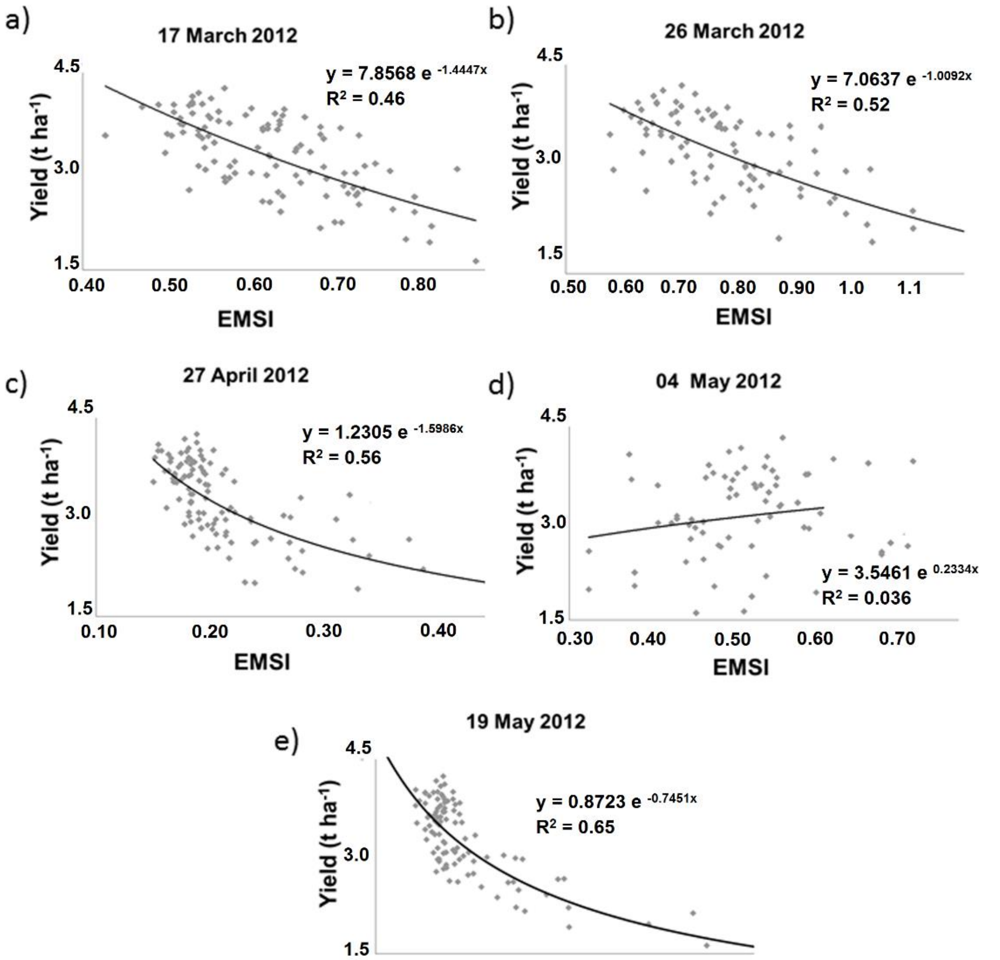

Figure 3. Relationship between EMSI on different dates and yield in 2012.

Some correlations between yield and the NDVI and MSI indices reached relatively high levels, but this cannot be generalized. The MSI index, due to the type of rape root system, does not have such an influence as e.g. in shallow rooted cereals. The behaviour of the CVI index is analogical. It is sensitive to the content of chlorophyll in plants. The highest values of all indices in 2012 were a general phenomenon.

In the literature, the studies about a relationship between spectral indices and crop yield are often mentioned. Many authors evaluated various crops grown on plots of different size and they used different sources of remote sensing data. In the last decades there has also been a rapid development of remote sensing systems, especially for targeted application. For agricultural purposes, it is possible to use remote sensing methods from hyperspectral to multispectral systems, from unmanned aerial vehicles or planes, spectroradiometers to satellite systems for monitoring the crop variability. For our study satellite remote sensing was chosen, Landsat 7 satellite data were used due to a good access to the database of Landsat images and a possibility of using several spectral bands, despite of their coarse spatial resolution. The spatial resolution of these images is 30 m. This resolution could limit the monitoring of spatial variability of crops. Nevertheless, Kumhálová et al. (2014) concluded that Landsat TM/ETM$^+$ images can be used for deriving spectral indices which can sufficiently explain plant variability in a field of 11.5 ha in size. Similar results were obtained for example by Chao Rodríguez et al. (2014) in the evaluation of a small water body (11.5 ha). They found out that the Landsat historical archives may still provide a wealth of environmental information. Wu et al. (2015) also used an experimental plot of 36.9 ha in size in their study to estimate the high-resolution Leaf Area Index from synthetic Landsat data (Landsat-7 ETM$^+$).

Many studies have indicated that remotely sensed vegetation indices can be used for crop variability monitoring (e.g. Vincini et al., 2008; Hunt et al., 2013) like in our study with winter oilseed rape. Vegetation indices can also be used to monitor the green vegetation component. At the leaf scale, leaf pigment concentration, leaf water content and leaf structure cause variations in leaf reflectance, transmittance and absorption (van Leeuwen & Huete, 1996). Reflectance and transmittance properties have been observed to be different between dicotyledonous and monocotyledonous leaves, because of differences in the mesophyll structure (Sinclair et al., 1971) and differences between adaxial (leaf face) and abaxial (leaf back) leaf scattering properties (e.g. Woolley, 1971). Van Leeuwen & Huete (1996) described that reflectance differences between vegetation and litter can be attributed to histological and optical properties. Senescence of plant components occurs during or after plant maturity or can be caused by stress factors like lack of water and nutrients or extreme temperatures. Senescence and decomposition of leaves will finally cause the breakdown of all pigments. These events may occur especially in winter oilseed rape and this corresponds with our research. Most vegetation indices tend to be species specific and therefore they are not robust enough when applied across different species, with different canopy architectures and leaf structures (Viña et al., 2011). For our research we chose traditional vegetation indices that were usually applied in other studies to evaluate plant and yield variability. Our results are then in good agreement with the findings of Piekarczyk et al. (2006). They found out that a very poor relationship between spectral data and all agronomic parameters of oilseed rape at the beginning of the spring growing season was caused by the presence of leaf litter on the ground. The spectral properties of plant litter affect vegetation indices and can cause errors in their response to the green vegetation cover. Optical remote sensing seems to be weak tool for oilseed rape evaluation. Huang et al. (2015) noted that a recently proposed alternative has used active microwave remote sensing, otherwise referred to as radar. Active microwave observations can be used to provide complementary information on vegetation properties, such as vegetation structures or level of vegetation growth. Radar vegetation index (RVI) can also be correlated well with the vegetation water content, Leaf Area Index and NDVI (Dinesh Kumar et al., 2013).

CONCLUSIONS

In this paper vegetation indices were evaluated in the stand of rape during three seasons. Very low levels of correlations were detected between vegetation indices and yield. The best results were obtained in the EMSI index even though the EMSI index fluctuated strongly in dependence on the growth phase and other conditions. In practice it appears problematic to use these indices for the rape stand evaluation. In further research it will be necessary to derive appropriate vegetation indices in optical part of spectrum or use RVI, along with their verification and presentation of model situations of a relationship to different stages of rape plant development. Indices in the SWIR 2 band seem promising. Despite the possible adjustment of indices it will not be possible in the future to evaluate oilseed crops during flowering with optical remote sensing methods. Despite the shortcomings mentioned in this article remote sensing used for the assessment of rape stands is a promising technique. European Sentinel mission (especially Sentinel 1) seems to be very promising way from this point of view.

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Biogas from wastes of pumpkin, marrow and apple

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Abstract. A lot of vegetables and fruits, which have been grown in Latvia or were imported from foreign countries, become waste, often due to unconformity to the marketing standards or biodegradation process fouling during storage. Waste biomass piles emissions during storage that contributes to global warming. It is appropriate to use such biomass as raw material for anaerobic digestion. This article shows the results of studies on evaluation of suitability of vegetable and fruit waste biomass for the production of biogas. Anaerobic digestion was investigated in 0.75 L digesters, operated in batch mode at a temperature of 38 ± 1.0 °C. The average biogas yield per mass unit of dry organic matter added (DOM) from digestion of pumpkin biomass was 1.095 L g^{-1}_{DOM} and the specific methane yield was 0.422 L g^{-1}_{DOM}. Average biogas yield from digestion of marrow biomass was 0.768 L g^{-1}_{ODM} and the methane yield was 0.274 L g^{-1}_{DOM}. Average biogas yield from digestion of apple biomass was 1.020 L g^{-1}_{DOM} and the methane yield was 0.451 L g^{-1}_{DOM}. All investigated wastes can be a very good source for biogas production. Anaerobic digestion may be a solution to treat waste biomass from food production facilities or supermarkets.

Key words: anaerobic digestion, pumpkins, marrows, apple, biogas, methane.

INTRODUCTION

The world is going through an era of rapidly diminishing fossil energy resources. It is therefore necessary to increase the usage of the renewable energy resources and, in first order, to recover the energy content of different organic wastes. Organic wastes, e.g. sewage sludge, vegetable residues, damaged products, whey, grain screenings and other residues from crop production, undergo biodegradation process, especially if stored in piles, so producing greenhouse gases and contributing to climate change.

The production of biogas is one of the most nature's friendly and prospective alternative energy technologies, and development of this technology will continue. Effectiveness of biogas production in Latvia will increase also for the benefits obtained from an environmental point of view, due to reduced GHG emissions and minimised losses of plant nutrients from manure. Fermented animal manure can serve better for substitution of commercial fertilisers, compared to unfermented manure, due to slower mineralization process and a better plant nutrients C: N ratio. Fermented manure can be applied directly for fertilization of crops in plants growing phase.

The use of cheaper raw materials will allow replacing the expensive corn silage. The effective operation of biogas plant will enable the use so far un-used organic wastes from cities and surroundings energy production and to reduce the usage of the fossil energy resources.

Governmental aid measures for biogas plant~~s~~ construction and electricity selling tariff facilitate~~s~~ the rapid development of biogas technologies in the last decade in Latvia resulting in 59 running biogas plants in year 2017. Any organic substances can be used for anaerobic fermentation process, and organic wastes are the most appropriate resources for anaerobic fermentation (Buffiere et al, 2006). In rural areas, such wastes can be manure, food residue, crop residue, municipal organic waste, sewage sludge, plant green biomass and other organic wastes sources from industry.

Raw material sources from large agricultural or food processing enterprises are suitable for biogas production (Hansen et al., 2004). Processing of biomass wastes in an environmentally friendly way is mandatory for every large enterprise, according to the Law On Pollution, Amendment 1, if production of such enterprises comply with the following conditions (The Saeima, 2001):

- pig farm with more than 2,000 places for pigs;
- pig farm with more than 750 places for sows;
- poultry farm with more than 40,000 places for poultry;
- fruit and vegetable processing plant with a capacity of more than 300 t d^{-1};
- animal raw materials (other than exclusively milk), with a finished product production capacity greater than 75 t d^{-1};
- milk production facilities with daily milk supply of more than 200 t d^{-1};
- slaughterhouses with a carcass production capacity greater than 50 tonnes per day

EC directives demand, that food processing plants, while producing biogas, must fulfil the necessary requirements for sanitation of some wastes. The technology for pre-treatment of slaughterhouses wastes and wastes originating from animal products for biogas production should include pasteurisation equipment requiring heating to 70 °C for 1 hour for 100% destruction of pathogenic micro-organisms.

All substrates or raw materials for biogas production are divided into the basic substrates and the additional substrates (co-substrates). Base substrates are manures produced in farm and additional substrates are usually different crops, plants by-products and food industry waste.

If the biogas plant is located nearby to the city, usage of manure is not desirable due to unpleasant odour. However, utilization of some other basic substrates produced in or nearby to the cities, e.g. sewage sludge, milk processing wastes, should be processed in biogas plant built at site of wastes origin to minimize the transport expenses and to improve environmental conditions, including odour.

Biogas volume obtained from a various raw materials is different (Baader et al., 1978; Angelidaki, I. et al, 2009). Researchers working with similar raw materials have obtained different results. Biogas volume depends on the substrate composition, on anaerobic conditions, organic matter (OM) and other factors (Chen et al., 1984). One of the most important factors that determine~~s~~ the potential of biogas production is the organic matter content and its composition, and especially the content of three main OM groups (Fernandez et al., 2001) carbohydrates, methane content of biogas can be determined using the Buswell-Symons formula (Symons, 1933, Liebetrau et al., 2016).

In practice, biomass is never fully degraded in fermentation processes; therefore, investigations should be carried out, in order to assess the methane potential for each specific biomass. Different methodologies are used for such the investigations. For investigation of the methane potential we have used a methodology similar to that described in a method guide used in Germany (Kaltschmitt & Hartmann, 2009; Kaltschmitt, 2010). In practice, many of the biogas plants digestate sample analyses reveal a still too high organic matter content.

The biogas and methane potential of abundant pumpkins, marrows and apples or of rotten fruits in Latvia has not been studied up till now. Such data were missing also in review information provided by German researchers (Becker, 2007).

The aim of this study is therefore the investigation of the potential of biogas production from vegetable processing plant residues and wastes: non-standard pumpkins, marrows and apples. The second aim is to investigate the effect of addition of a catalyst MF3. Positive results can give confidence to biogas plant owners on the feasibility of utilisation of mentioned biomass in biogas co-generation plants for electricity and heat production.

The results of the studies may be used throughout Latvia, where it is possible to work with the raw materials investigated during this study. The results of this study can be used also for calculation of the actual dose of biomass to be daily filled in biogas reactors.

MATERIALS AND METHODS

Materials, equipment and methods

Damaged pumpkin, marrow and apples were used in this study, Figs 1–3.

Figure 1. Pumpkin biomass.

Figure 2. Vegetable marrow biomass.

Figure 3. Chopped apple biomass.

Representative biomass samples were taken and their chemical composition was analysed using the standardized methodologies ISO 6496: 1999. Dry matter content, the organic matter content and the ashes content were investigated for the three raw materials and for the used inocula. Analyses were done using standard methods. The volume of biogas production was studied using laboratory equipment consisting of 16 bioreactors.

Each group of raw materials (pumpkin, marrow or apple biomass) and the inocula (fermented cow manure) were weighed and mixed together carefully. The inocula for digestion experiments of the three substrates and for control bioreactors was digester effluent of a continuously working bioreactor having inside almost completely biodegraded manure.

Study assays contained 20 g of the respective biowastes and 500 g inoculum in each of 14 bioreactors with a volume of 0.72 L. As references (controls) only 500 mL inoculum was placed in 2 bioreactors. An anaerobic fermentation temperature of 38 ± 0.1 °C was maintained inside the bioreactors during batch mode incubations.

1 mL of Metaferm MF3, a commercial catalyst for advancement of the anaerobic digestion process was added into R6-R9 bioreactors. Metaferm contains multiple ferments, micronutrients and B-group vitamins. The true composition of MF3 is not known due to proprietary rights of the producing company.

Dry matter, ashes and organic dry matter content was determined for every biomass mixture before filling of substrates into the bioreactors. All 16 bioreactors were placed into a large thermostat for anaerobic fermentation processing during 21 days. Accuracies of measurements were as following: ± 0.2 g for inocula and substrate weight (using scales Kern KFB HAS 16 KO2); ± 0.001 g for biomass dry matter, organic matter and ashes weight; ± 0.02 pH for pH (using instrument PP-50); ± 0.05 L for gas volume (using low flow gas meter) and ± 0.1 °C for temperature inside the thermostat (Memmert model).

Gases were collected in gas bags with a volume of 2 litres positioned outside of reactor and connected with reactors by plastic pipes. Gases volume measurements and gases content analyses were provided regularly during fermentation period. Biogas volume measurement was provided by Bioprocess control low flow gas meter, and normalized biogas volume (at standard pressure and temperature) was calculated. Biogas composition (CH_4, CO_2, O_2 and H_2S) was determined with a gas analyser GA 2000. Dry matter was determined by specialized unit Shimazy at temperature 105 °C, and ashing of samples was provided in oven Nabertherm at temperature 550 °C according to standard heating program.

All the substrates were weighed, dry matter, ashes and organic matter content measured after finishing of the anaerobic fermentation process. Standard error was calculated for each group of digesters using the standardized data processing tools.

RESULTS AND DISCUSSION

The results of the analyses of the raw materials before anaerobic fermentation are listed in Table 1.

The results of the analyses of the digestate in each bioreactor after anaerobic fermentation are shown in Table 2.

Raw biomass of pumpkins, marrows and apples have a low dry matter and organic dry matter content (Table 1), and are appropriate for biogas production, due to their high content of sugars and juice. The degradation rate of DOM was calculated using the data of the average organic dry matter content in raw biomass and of inocula before fermentation (Table 1) and data of the average DOM content in finished substrate (Table 2). Average dry organic matter (DOM) content in the finished substrate with pumpkins was 4.12 g and the average degradation rate was calculated as 54.92%. It is probable that some minor portion of DOM of the inocula still continues biodegradation in the anaerobic fermentation process. Average DOM in the finished substrate with marrow and inoculum was 5.01 g and the average degradation rate was calculated as 43.52%.

Table 1. The results of the analyses of raw materials

Bio reactor	Raw material	pH	TS, %	TS, g	Ashes, %	DOM, %	DOM, g	Weight, g
R1, R16	IN500	7.12	1.83	9.15	21.09	78.91	7.22	500
R2-R5	IN500 + P20		10.47	2.09	8.48	91.52	1.92	20
		7.11	2.16	11.24	18.68	81.32	9.14	520
R6-R9	IN500 + P20 + MF3		10.47	2.09	8.48	91.52	1.92	20
		7.13	2.16	11.24	18.68	81.32	9.14	521
R10-R12	IN500 + M20		9.0	1.8	8.48	91.52	1.65	20
			2.11	10.95	19.00	81.00	8.87	520
R13-R15	IN500 + A20		14.53	2.91	3.25	96.75	2.81	20
		7.12	2.32	12.06	16.84	83.16	10.03	520

Abbreviations: TS – total solids, DOM – dry organic matter (applied to organic dry matter of raw material to be investigated), IN – inoculum; P – pumpkin; M – marrow; A – apple; MF3 – commercial additive MF3 in volume of 1 mL.

Table 2. The results of the analyses of the digestate

Bio reactor	Raw material	pH	TS, %	TS, g	Ashes, %	DOM, %	DOM, g	Weight, g
R1	IN500	7.15	1.02	5.08	37.57	62.43	3.17	498.5
R16	IN500	7.15	1.03	5.13	37.62	62.38	3.20	499.0
R2	IN500 + P20	7.21	1.17	6.04	31.3	68.7	4.15	516.5
R3	IN500 + P20	7.23	1.16	5.99	31.25	68.75	4.12	516.4
R4	IN500 + P20	7.24	1.16	5.99	31.3	78.70	4.12	516.5
R5	IN500 + P20	7.20	1.15	5.94	31.20	68.80	4.09	516.6
R6	IN500 + P20 + MF3	7.21	1.10	5.68	24.8	75.20	4.27	516.8
R7	IN500 + P20 + MF3	7.30	1.08	5.59	23.68	76.32	4.27	518.0
R8	IN500 + P20 + MF3	7.37	0.92	4.77	19.64	80.36	3.83	518.6
R9	IN500 + P20 + MF3	7.35	1.12	5.80	23.49	76.51	4.43	517.5
R10	IN500 + M20	7.36	1.27	6.54	22.3	77.70	5.08	515.3
R11	IN500 + M20	7.15	1.25	6.44	22.27	77.73	5.01	515.5
R12	IN500 + M20	7.16	1.24	6.39	22.26	77.74	4.97	515.6
R13	IN500 + A20	7.19	1.47	7.59	22.15	77.85	5.91	516.6
R14	IN500 + A20	7.18	1.48	7.65	22.15	77.85	5.95	516.7
R15	IN500 + A20	7.18	1.49	7.70	22.20	77.80	5.99	516.8

Average DOM in the finished substrate with apple biomass was 5.95 g and the average degradation rate of organic dry matter was 40.68%.

Results confirm~~s~~ that pumpkin, marrow or apple biomass contains some substances that facilitates the bacteria growth resulting in better utilisation of other raw materials also, e.g. some residual organics of the inocula.

Methane and biogas yields from bioreactors with pumpkin, marrow and apple biomass are shown in Table 3.

Table 3. Produced biogas and methane

Bio reactor	Raw material	Biogas, L	Biogas, L g^{-1}_{DOM}	Methane, max %	Methane, L	Methane, L g^{-1}_{DOM}
R1	IN500	0.0			0.0	
R16	IN500	0.0			0.0	
R2	IN500 + P20	2.4	1.25	53.2	0.917	0.478
R3	IN500 + P20	1.9	0.99	58.8	0.751	0.391
R4	IN500 + P20	2.2	1.15	53.8	0.762	0.397
R5	IN500 + P20	1.9	0.99	61.8	0.809	0.421
Average R2-R5			1.095 ± 0.060			0.422 ± 0.040
R6	IN500 + P20 + MF3	2.1	1.093	52.9	0.738	0.384
R7	IN500 + P20 + MF3	2.0	1.042	64.1	0.869	0.452
R8	IN500 + P20 + MF3	2.1	1.093	62.0	0.944	0.492
R9	IN500 + P20 + MF3	1.9	0.99	51.8	0.604	0.315
Average R6-R9			1.055 ± 0.047			0.411 ± 0.078
R10	IN500 + M20	1.3	0.788	53.4	0.462	0.280
R11	IN500 + M20	1.2	0.727	54.6	0.434	0.263
R12	IN500 + M20	1.3	0.788	56.5	0.460	0.279
Average R10-R12			0.768 ± 0.026			0.274 ± 0.010
R13	IN500 + A20	3.0	1.068	67.9	1.342	0.478
R14	IN500 + A20	3.0	1.068	64.4	1.306	0.464
R15	IN500 + A20	2.6	0.925	70.0	1.158	0.412
Average R13-R15			1.020 ± 0.072			0.451 ± 0.033

Abbreviations: L g^{-1}_{DOM} – litres per 1 g organic dry matter (applied to organic dry matter of investigated raw material).

The obtained results are indicating a high efficiency of anaerobic degradation. Such good biomass degradation by micro-organisms can be explained by the beneficial chemical composition of substrates (high content of sugars and juice) for biogas production and by the buffering effect of the inocula during co-fermentation.

A high average specific methane volume of 0.422 L g^{-1}_{DOM} was obtained from pumpkins, calculated as methane volume released per 1 g initial organic dry matter. Less specific methane volume of 0.274 L g^{-1}_{DOM} was obtained from vegetable marrow. This may be explained by the fact that the vegetable marrow samples have relatively thick peels, which were difficult to degrade by bacteria. The highest specific methane volume of 0.451 L g^{-1}_{DOM} was obtained from apple biomass. The relatively high average methane content in the biogas from apples is explained by the fact that the raw material contains a lot of juice and sugars. From bioreactors containing pumpkins biomass and 1 mL of the additive MF3 the specific methane volume was 2.7% less compared to that obtained from bioreactors with pumpkins biomass but without the additive. This shows that the additive MF3 may contain some component(s) impairing the functioning of bacteria.

The volume of biogas and methane obtained from each bioreactor containing pumpkins biomass is shown in Fig. 4.

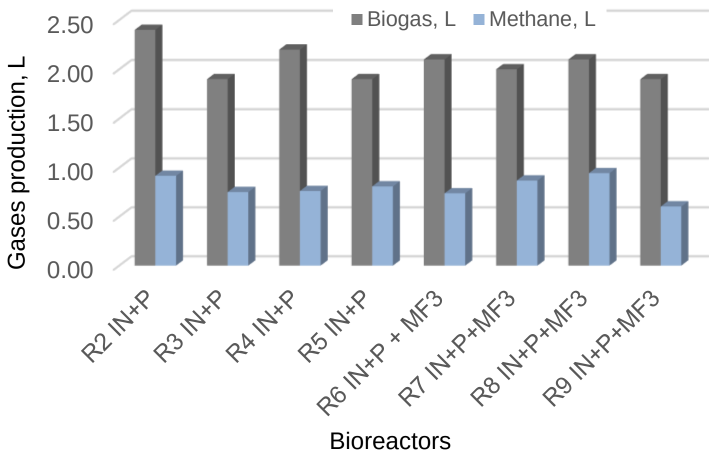

Figure 4. Volume of biogas and methane obtained from bioreactors with pumpkins biomass.

The specific volume of the biogas and methane per 1 g organic matter of raw biomass released from each bioreactor with pumpkins is shown in Fig. 5.

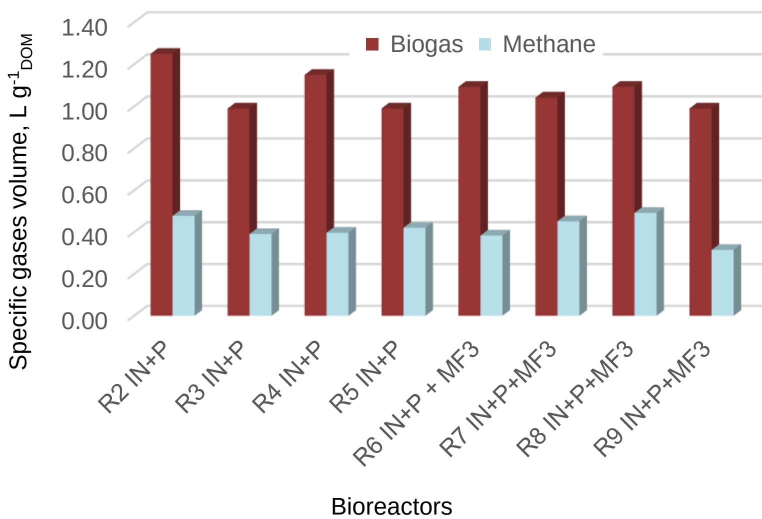

Figure 5. Specific volume of the biogas and methane released from each bioreactor with pumpkins biomass.

The maximal methane content in biogas from each bioreactor with pumpkins is shown in Fig. 6.

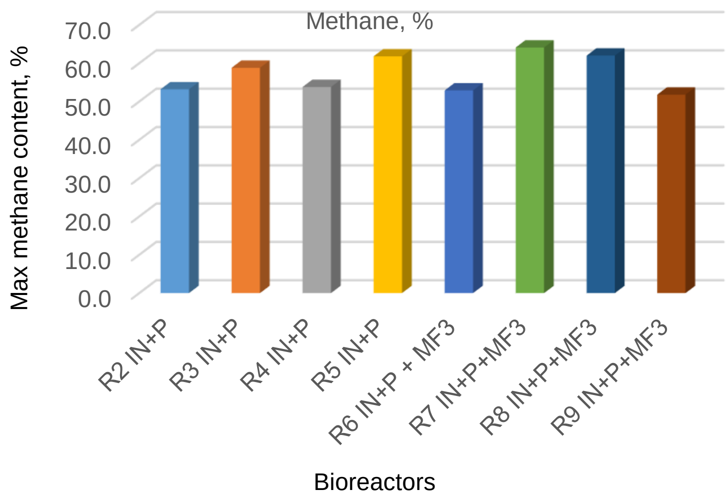

Figure 6. Maximal methane content in biogas from bioreactors with pumpkins biomass.

The biogas and methane volumes from each bioreactor containing marrow or apple biomass are shown in Fig. 7.

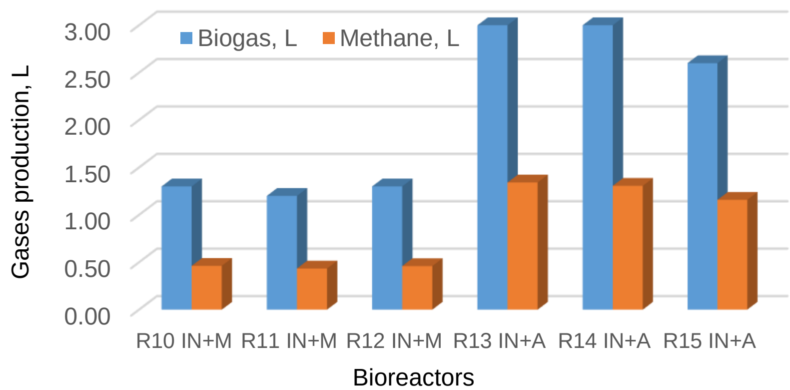

Figure 7. Volumes of biogas and methane obtained from bioreactors with marrow and apple biomass.

The specific volume of the biogas and methane released from each bioreactor containing marrow or apple biomass is shown in Fig. 8.

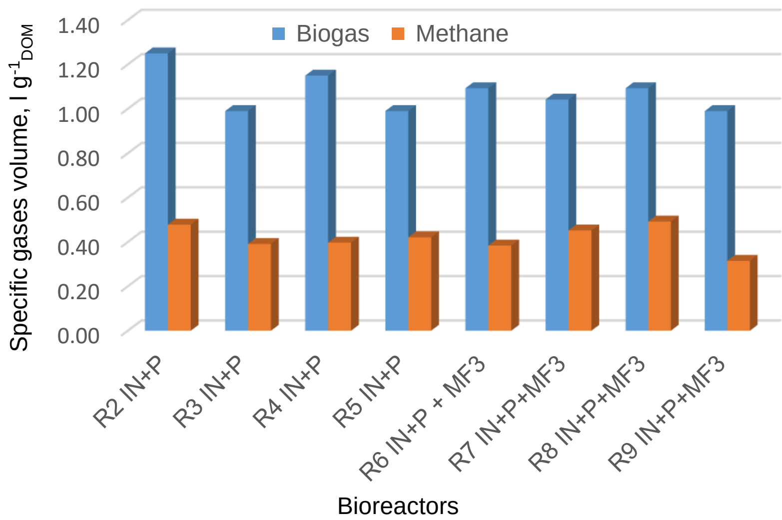

Figure 8. Specific volumes of the biogas and methane released from bioreactors with marrow or apple biomass.

The maximal content of methane in biogas from each bioreactors containing marrow or apple biomass is shown in Fig. 9.

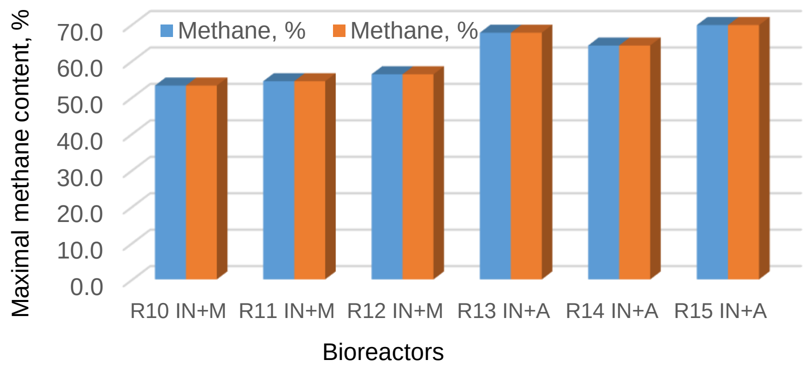

Figure 9. Maximal methane content in biogas from each bioreactor with marrow or apple biomass.

CONCLUSIONS

Pumpkin biomass is recommended for utilisation in biogas co-generation plants due to the high specific methane yield of 0.422 L g^{-1}_{DOM} on average.

The average specific methane yield from marrow biomass was 0.274 L g^{-1}_{DOM}.

The highest average specific methane yield was obtained from apple biomass and was 0.451 L g^{-1}_{DOM}. This was 6.4% or 39.2% higher as compared to the specific methane yield obtained from pumpkin or vegetable marrow biomass respectively.

Results of investigations show that pumpkin, marrows and apples biomass are good raw materials for biomethane production.

Addition of 1 mL MF3 results in lowering of the specific methane volume by 2.7% compared to that obtained from pumpkins biomass without this additive. It is proposed that the additive MF3 may contain some component(s) impairing the activity of bacteria.

Results of our investigations shows that co-substrates (raw materials) with a high content of sugars added to fermented cattle manure apparently "induce" additional biodegradation of finished digestate, therefore partial return of such the substrates into the cogeneration plant bioreactor can be regarded as useful.

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Yield components and quality parameters of winter wheat depending on tillering coefficient

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Abstract. Winter wheat (*Triticum aestivum* L.) is the main and most profitable cereal crop in Latvia, thus different aspects of its growth are widely researched. The aim of this three-year (2004–2005 and 2006–2007) long investigation arranged at Research and Study farm 'Peterlauki' of Latvia University of Agriculture (56° 30.658′ N and 23° 41.580′ E) was to evaluate importance of tillering for wheat yield, yield components and quality formation alongside the effect of cultivar, sowing date and rate, and research year. Three cultivars ('Cubus', 'Tarso', 'Zentos'), four sowing dates (starting with 30 August ± 2 days with 10-day intervals) and three sowing rates (300, 400 and 500 germinable seeds m^{-2}) were used. Soil and crop management was appropriate. Yield components were detected from sample sheets. Yield was affected substantially ($p < 0.05$) by all the investigated factor except sowing rate. Plants with tillering coefficient (TC) '1' to '6' formed yield, and the biggest proportion (20%) was given by plants with TC '3'. Grain number and weight per spike was substantially ($p < 0.01$) affected by TC, but changes in their values were irregular and further investigations are needed. Average values of crude protein, gluten and starch content, Zeleny index and 1,000 grain weight was not affected by TC substantially. Thus, tillering was found beneficial for winter wheat yield formation as part of yield compensation mechanism. Sowing rate was the least yield, its components and quality affecting factor, but environmental conditions (research year) – the most affecting factor. The effect of cultivar and sowing date was mostly substantial, but dependent on evaluated parameter.

Key words: yield; kernel number and weight per spike; 1,000 kernel weigh; protein, gluten, and starch content.

INTRODUCTION

Wheat (*Triticum* L.) together with maize (*Zea mays* L.) and rice *(Oryza sativa* L.) is between worlds' mega crops critically important for food. In 2014, according to FAOSTAT, 221 mill. ha were sown with wheat in the world, but harvested yield reached close to 730 mill. tonnes of grain. Worlds' average wheat yield per ha is still moderate – 3.3 t ha^{-1}. In Latvia, like in many other countries wheat (*Triticum aestivum* L.) has been the mainly grown cereal for many years. Sown area with wheat occupied 448.2 thous. ha in 2015 (38% from the total sown area), including winter wheat 290.6 thous. ha (43% from the total sown area with cereals). Sown area and harvested yield of winter wheat has increased dramatically in Latvia if compared with the time 10 years ago; in 2006, 152.3 thous. ha were sown with winter wheat and total yield was 461.7 thous. t (1,605.7 thous. t were harvested in 2015). An average yield per ha of winter wheat is also steadily increasing in Latvia, and 5.53 t ha^{-1} was obtained in 2015 (in comparison: 3.03 t ha^{-1} in 2006). The dominant position of wheat is the main reason for plenty of research on different aspects of its production and increase in yield, at the same time looking for economical and environmentally friendly methods.

Yield of any crop is mathematical function of separate yield components such as the number of plants per unit area and productivity of an individual plant. For cereals, mainly the number of spikes per 1 m^2, number and weight of kernels per spike, and 1,000 kernel weight (or one kernel weight) is measured (e.g. Slafer et al., 1996; Metho et al., 1998; Thiry et al., 2002). Some researchers analyzed the number of kernels per 1 m^2 and single kernel weight (e.g. Slafer et al., 1996; Frederick et al., 2001; Peltonen-Sainio et al., 2007). A lot of research is devoted to different factors affecting wheat yield and its components.

Yield potential of winter wheat is increased by breeding impressively (Feil, 1992) and increase is also continuing at present. Usually, researchers reported that the number of grain per 1 m^2 (formed by more spikes per 1 m^2 and more kernels per spike) gives the biggest contribution to higher grain yield if compared with single kernel weight in modern cultivars (Feil, 1992; Slafer et al., 2014). Peltonen-Sainio et al. (2007) researched 78 winter wheat genotypes during 30 years in Finland and found that winter wheat yield increase was promoted by both – an increase of kernel number per 1 m^2 and single kernel weight. As much as cultivar genotype also environmental conditions play an important role affecting formation of wheat yield and its components. Soil tillage system was found as a factor with little or irregular (Seibutis es al., 2009; Jug et al., 2011; Malecka et al., 2015) effect on wheat yield components' formation. Tillage effect in combination with crop rotation or production practices of previous crop (Frederick et al., 2001) was more expressed. Many researchers (Sieling et al., 2005; Feizabadi & Koocheki, 2012; Babulikova, 2014) found beneficial effect of crop rotation, especially when more crops were included in it. Appropriate N-fertilization not only increases the winter wheat yield and values of its forming elements, but can also reduce the effect of other unfavourable factors, e.g. crop rotation (Sieling et al., 2005). Fioreze et al. (2012) reported more tillers per plant and 1 m^2 when adequate level of phosphorus fertilizer was given. The use of suitable sowing rate at an optimal sowing date is very important for winter wheat. Researchers showed the compensation action of yield forming elements when different sowing rates were used at different sowing dates (e.g. Spink et al., 2000). Valerio et al. (2009) demonstrated that sowing rate can depend on cultivar tillering ability. Mostly all results of field experiments showed a strong effect of research year or location that also contributes to plasticity and compensation ability of yield components.

Nowadays, not only yield amount but also yield quality is important. For wheat, the main grain quality indicator is crude protein content and protein quality which could be measured by Zeleny index. Also, wet gluten content and starch content are measured frequently. Achievements of breeding ensure different kinds of wheat cultivars suitable for food, feed, ethanol production or other specific purposes. Genotype of cultivar is the first criterion determining specific grain quality parameters, as well as environmental and management conditions (Geleta et al., 2002; Zecevic et al., 2014), especially N-fertilizer rate and timing (Liniņa & Ruža, 2012) plays an important role.

For high winter wheat yield formation, the number of spikes per unit area is an important yield component (Metho et al., 1998), but it can be reached differently. Growers can use high sowing rates or sow little less seeds per 1 m^2 and allow plants to tiller more. Wheat tillering ability is high and plants depending on genotype and environment are able to produce from 1 to even more than 100 tillers (Šeļepov et al., 2013). Suitable number of tillers per plant or per 1 m^2 depends mostly on environmental conditions. Researchers concluded that wheat main stem is generally more productive if compared with the next level tillers (Metho et al., 1998; Elhani et al., 2007; Xu et al., 2015), and grain protein content varied depending on tiller's level and even on floret position in the spike (Metho et al., 1998). Data is hardly ever reported on average per plant values of wheat yield component and quality indicators depending on productive tillering coefficient.

The aim of this research was to evaluate contribution of plants with different tillering coefficient in winter wheat yield formation, and to evaluate yield components forming spike productivity, and grain quality, depending on tillering coefficient, cultivar, sowing date and rate, and conditions of research year.

MATERIALS AND METHODS

Field experiments were carried out at the Study and Research farm 'Peterlauki' of Latvia University of Agriculture (56° 30.658′ N and 23° 41.580′ E) during three seasons (2004–2005, 2005–2006 and 2006–2007). Field trials consisted of four target sowing dates with a 10-day interval from the end of August to the end of September (30 August ± 2 days (1 T); 10 September ± 2 days (2 T); 19 September ± 2 days (3 T) and 29 September ± 2 days (4 T)). Three bread winter wheat (*T. aestivum* L.) cultivars 'Cubus', 'Tarso' and 'Zentos' (originated from Germany) were sown using three sowing rates (300, 400 and 500 germinable seeds per 1 m^2). Field trial was arranged in a three-factorial split-plot design in four replications. Plot size was 25 m^2. Soil at the site was *Endocalcaric Abruptic Luvisol* (World Reference Base, 2014), silt loam; pH KCl = 6.9; available for plants content of P_2O_5 = 247 mg kg^{-1} soil; K_2O = 328 mg kg^{-1} soil, and organic matter content 1.4%.

Winter wheat was sown in bare fallow, but during previous year spring barley (*Hordeum vulgare* L.) was grown in the field. Conventional tillage system, which included mould board ploughing approximately one month before sowing and harrowing directly before sowing, was used. Plots were fertilized with 60 kg P_2O_5 ha^{-1} and 90 kg K_2O ha^{-1} before sowing. Split N-fertilizing (NH_4NO_3; 34% N) was used next spring: 150 kg ha^{-1} in total (N 90 kg ha^{-1} at the renewal of vegetation period and N 60 kg ha^{-1} at the GS 30–32). Certified treated seed was used and sowing depth was 3–4 cm. Plots were maintained free from weeds, pests and diseases using pesticides, and growth regulators were used in order to avoid lodging. Overall, crop management was performed according to the recommendations for the area.

Yield was accounted at the GS 90–92 harvesting and weighing each plot separately. Yield was recalculated in t ha^{-1} at 14% moisture and 100% purity. Winter wheat achieved GS 90–92 in all plots simultaneously (development differences depending on sowing time were observed in spring, but they equalized till milk ripeness stage), and harvesting of all plots was done on the same date (16 August 2005, 04 August 2006 and 02 August 2007).

Before harvesting, sample sheets were taken from 0.25 m^2 in each plot to detect productive tillering coefficient (later on: tillering coefficient – TC) for every plant. Plants were sorted according to the TC, and those with the same number of tillers per plant from the same treatment were joined together. The number of spikes was counted, and then grain was threshed by hand, cleaned, weighted and counted by grain counter Contador (Pfeuffer). Calculations were made as follows: the grain number per spike, grain weight per spike (g), proportion of stems from plants with the same TC per treatment (%), proportion of grain mass from plants with the same TC per treatment (%). Thousand grain weight was detected from the yield and 500 grains were counted twice (LVS EN ISO 520).

Grain quality was measured using Infratec™ 1241 Grain Analyzer (FOSS). It is a whole grain analyser for testing multiple parameters by use of near-infrared transmittance technology. Crude protein, wet gluten and starch content, and Zeleny index was detected. Measurements were done in cases when enough grain was obtained from plants with specific tillering coefficient per treatment.

Analysis of variance was used to evaluate impact of factors to investigation traits.

Pairwise comparisons among factors' levels have been done with Bonferroni test. The factor and differences between factors level were considered statistically significant when $p < 0.05$. Data processing was done using SPSS 15.

Meteorological conditions during three research years greatly differed. During the autumn vegetation period average air temperatures in September and October were slightly above the long-term average (11.7 °C and 6.8 °C respectively) in the region in 2004, but vegetation ended (average day-night temperature for the 5 succeeding days below 5 °C) in early November. Similar average air temperature was observed also in September and October 2005, but the beginning of November was very warm; despite this, vegetation ended in the middle of the 2nd ten-day period of November. Both winters were comparatively stable and did not cause serious wintering problems. In 2006, on the contrary, average air temperatures in September and October exceeded long-term average by more than 2 °C and together with enough precipitation promoted vigorous tillering and outgrowing of plants sown on earlier sowing dates. Vegetation period continued till the middle of December, and also later, till the middle of January 2007, average air temperature was above 0 °C. Temperatures below 0 °C were observed only in late January, but in February they dropped to -11.1 °C. It was a great stress for plants, especially those sown on earlier sowing dates and overgrown during the long and warm autumn vegetation period. This winter heavily affected yield formation, and enumerable yield for cultivar 'Cubus' sown on the first two sowing dates was not achieved. Vegetation started on 8–10 April 2004, 20–21 April 2006 and 25–26 March 2007. Vegetation periods of subsequent summers also differed. Temperature during the summer vegetation period is not a yield limiting factor for winter wheat in Latvia, but this can be grain quality affecting factor. Below the long-term average observations was average monthly air temperatures in May (-0.4 °C) and June (-1.0 °C) 2005, and in July 2007 (-0.5 °C). The long-term average air temperatures were exceeded in May (+0.8 °C) 2007, in June 2006 (+0.8 °C) and 2007 (+1.2 °C), and in July 2005 (+1.1 °C) and 2006 (+3.3 °C). Long-term average sum of precipitation in period May-July was exceeded in 2005 (121% if compared with the long-term average observations) and in 2007 (155%). Distribution of precipitation was comparatively even. In contrast, the summer 2006 was dry securing 77% from long-term average sum of precipitation in May, 50% – in June and 57% – in July. Overall, a severe winter affected winter wheat yield and its components formation in the season of 2006–2007, but a hot and dry summer – in the season of 2005–2006.

RESULTS AND DISCUSSION

Winter wheat yield and effect of plants with different TC in its formation

Average winter wheat yield was high (6.67–9.08 t ha^{-1}; Table 1) and was affected substantially ($p < 0.05$) by all the investigated factors except sowing rate (Table 1). Yield and effect of its determining factors were analysed in detail in previous paper (Ruza & Kreita, 2008).

Table 1. Grain yield of winter wheat depending on investigated factors, and contribution of plants with different tillering coefficient in its composition

Investigated factors	Yield, t ha^{-1}	Contribution of plants with different tillering coefficient in yield composition, %					
		1	2	3	4	5	6
	Cultivar (for yield: $p < 0.001$)			(for yield distribution: $p > 0.05$)			
Cubus	6.67^{a}	18^{A}	17^{AB}	19^{A}	14^{AB}	11^{B}	21^{A}
Tarso	8.96^{b}	9^{A}	16^{AB}	22^{BD}	18^{BDE}	23^{CD}	12^{AE}
Zentos	8.86^{b}	12^{A}	15^{AB}	20^{BC}	19^{BC}	22^{C}	13^{A}
Sowing time (for yield: $p < 0.001$)			(for yield distribution: $p > 0.05$)				
1 T	7.16^{a}	10^{A}	12^{A}	16^{AB}	16^{AB}	23^{B}	23^{B}
2 T	7.63^{a}	11^{A}	13^{AB}	19^{B}	18^{AB}	20^{B}	19^{B}
3 T	8.67^{b}	14^{ACD}	19^{BD}	23^{B}	17^{BC}	17^{BC}	10^{AC}
4 T	9.05^{b}	17^{A}	19^{A}	23^{A}	17^{A}	16^{A}	8^{B}
Sowing rate (for yield: $p = 0.557$)			(for yield distribution: $p > 0.05$)				
300	8.04	11^{A}	13^{AC}	20^{B}	18^{BC}	20^{B}	18^{BC}
400	8.15	13^{A}	16^{AC}	21^{BC}	18^{AC}	19^{AC}	14^{A}
500	8.29	15	18	20	16	17	13
Research year (for yield: $p < 0.001$)			(for yield distribution: $p > 0.05$)				
2005	9.08a	15^{A}	15^{A}	20^{A}	17^{A}	30^{B}	3^{C}
2006	7.94b	10^{A}	15^{AC}	21^{BC}	19^{BCD}	14^{AD}	21^{BC}
2007	7.37b	15^{AC}	17^{AC}	20^{A}	16^{AC}	12^{BC}	21^{A}
		average yield distribution depending on TC: $p < 0.001$					
On average per all factors	**8.16**	**13**	**16**	**20**	**17**	**19**	**15**

$^{AB...E}$ – data mentioned in the same row with different letters significantly differ ($p < 0.01$); ^{abc} – yield data mentioned with different letters significantly differ within the borders of investigated factor (in column).

Plants with TC ’1’ to ’6’ were detected in sample sheets every year. Such a good tillering was ensured by comparatively lower plant densities (field germination and wintering lowered the number of plants m^{-2}), good autumn weather conditions, especially for wheat sown at early sowing dates (1 T and 2 T and even 3 T) or suitable tillering conditions in spring (wheat sown on later sowing dates: 3 T and 4 T). Spink et al. (2000) in the UK reported the maximum number of tillers per plant at sowing density 320 seed m^{-2} 4.9, 5.6 and 7.1 depending on a research year. In our case, TC ’6’ was not achieved in 29% of all observations, but TC ’5’ in ~2% of observations. All these cases were mainly reported in 2005.

Average contribution of plants with different TC in yield formation was not affected substantially by any of researched factors (Table 1). The most average contribution was given by plants with TC ‘3’ (20%, Table 1), one percentage point less – by those with TC ‘5’ (19%). The least average contribution (13%) was given by plants which did not tiller at all (TC ‘1’) and had only the main stem. Plants with TC ‘2’, ‘4’ and ‘6’ contributed in the yield formation similarly (15–17%). Results correspond to the average proportion of productive tillers given by plants with different TC – the most number of tillers was given by plants with TC ‘3’ (~20%), but the least – by those which did not tiller at all (on average 13%).

Looking on average per investigated factors’ contribution of plants with different TC in the yield formation, numerical differences were found, e.g., plants with the TC ‘6’ contributed only 3% in average yield of 2005, and 8% on average for yield obtained when wheat was sown in late September (4 T). Comparatively high was contribution of plants with the TC ‘4’ and ‘5’ in the yield formation (on average 17 and 19% respectively, Table 1), and it gave evidence on high productive tillering ability of winter wheat, thus compensating the shortage of plants in wheat stand. This is illustrated also by lack of sowing rate’s impact on yield formation in this particular research (p = 0.557; Table 1). Researchers have expressed contrary views about the beneficial effect of tillering on wheat yield. E.g., Donald (1967) imagined the ideal wheat ideotype as a single stem plant with large ear. Elhani et al. (2007) investigating durum wheat (*T. turgidum* var. *durum*) in Spain, found no evidence for either a positive or negative effect of maximum tiller number on grain yield. The environment mainly affected suitable number of tillers. Protič et al. (2009) demonstrated positive effect of TC increase on wheat yield. Whaley et al. (2000) showed that grain yield was maintained with large reduction in plant density due to tillering. Summarizing different views, Šeļepov et al. (2013) wrote that the most yielding are cultivars characterizing with moderate tillering ability (4–5 productive tillers per plant).

Yield components characterising spike productivity

Average grain number and weight per spike were affected substantially by plants’ TC (Table 2). Still, these differences were small and irregular. Spike productivity (the number of grain as well as grain weight) of plants which did not tiller at all TC = 1 (Table 2) was the smallest. This is a case, when plants have only the main stem. Similar phenomenon, when plants with TC ’1’ were less productive, was mostly observed depending on researched factors – on the cultivar (Table 3), sowing rate (Table 4), sowing timing (except 4 T; Table 5), and year (Table 6). A lot of research is devoted to the differences in productivity of main stem and tillers showing main stems as more productive (in terms of grain number and weight per spike) if compared with tillers (Metho et al., 1998; Elhani et al., 2007; Xu et al., 2015). Our previous empirical observations make us think that tillers formed in autumn can be of the same productivity as the main stem (Ruža, 1995). This research showed that average spike productivity of plants with TC ’3’ to ’6’ can be at least of the same value or even higher if compared with plants with TC ’1’ and ’2’.

Table 2. Average values of wheat yield components and grain quality parameters for plants with different tillering coefficient

Parameters	Average per plants with different tillering coefficient						*p*-value
	1	2	3	4	5	6	
Grain number per spike	33.5	34.4	34.9	34.7	34.8	34.9	0.004
Grain weight per spike, g	1.45	1.50	1.52	1.51	1.52	1.49	0.006
1,000 grain weight, g	42.98	43.28	43.31	43.14	43.30	42.38	0.242
Grain crude protein content, %	13.5	13.4	13.3	13.3	13.4	13.4	0.870
Grain gluten content, %	25.4	25.2	24.6	24.8	25.0	25.8	0.881
Zeleny index	54	54	54	54	54	52	0.433
Grain starch content, %	65.5	65.7	65.8	65.8	65.7	65.8	0.280

Table 3. Cultivar means of yield components and grain quality parameters depending on wheat tillering coefficient

Cultivar	Average per plants with different tillering coefficient						Average per cultivar
	1	2	3	4	5	6	
	Grain number per spike						$p < 0.001$
Cubus	36.2^{a}	37.8^{a}	38.0^{a}	38.0^{a}	38.4^{a}	37.1^{a}	37.6
Tarso	31.9^{b}	32.3^{b}	32.7^{b}	32.9^{b}	33.0^{b}	33.5^{b}	32.7
Zentos	32.5^{b}	33.2^{b}	33.8^{b}	33.2^{b}	33.3^{b}	32.8^{b}	33.1
	Grain weight per spike, g						$p < 0.001$
Cubus	1.58^{a}	1.66^{a}	1.67^{a}	1.66^{a}	1.68^{a}	1.59^{a}	1.64
Tarso	1.26^{b}	1.29^{b}	1.31^{b}	1.31^{b}	1.32^{b}	1.29^{b}	1.30
Zentos	1.52^{a}	1.55^{c}	1.58^{c}	1.55^{c}	1.56^{c}	1.49^{c}	1.54
	1,000 grain weight, g						$p < 0.001$
Cubus	43.33^{a}	43.71^{a}	43.75^{a}	43.43^{a}	43.53^{a}	42.66^{a}	43.40
Tarso	39.20^{b}	39.67^{b}	39.80^{b}	39.66^{b}	39.79^{b}	38.20^{b}	39.39
Zentos	46.40^{c}	46.46^{c}	46.39^{c}	46.34^{c}	46.57^{c}	45.33^{c}	46.25
	Grain crude protein content, %						$p = 0.051$
Cubus	13.3	13.2	13.2	13.1	13.4	13.5	13.3
Tarso	13.4	13.4	13.4	13.4	13.4	13.2	13.4
Zentos	13.6	13.4	13.3	13.4	13.4	13.6	13.5
	Grain gluten content, %						$p < 0.001$
Cubus	24.4^{a}	24.3	23.9	24.1	25.1	25.5^{ac}	24.6
Tarso	25.4^{ac}	26.0	24.7	24.8	24.7	25.0^{a}	25.1
Zentos	26.4^{bc}	25.2	25.2	25.4	25.2	26.9^{bc}	25.7
	Zeleny index						$p < 0.001$
Cubus	54^{ac}	53	52^{a}	52^{a}	53	52^{ac}	53
Tarso	53^{a}	54	55^{b}	55^{ac}	55	50^{a}	54
Zentos	56^{bc}	55	55^{b}	56^{bc}	55	54^{bc}	55
	Grain starch content, %						$p < 0.001$
Cubus	66.1^{a}	66.2^{a}	66.1^{a}	66.2^{a}	65.7^{a}	66.8	66.2
Tarso	64.4^{b}	64.6^{b}	65.0^{b}	64.8^{b}	64.9^{b}	64.6	64.7
Zentos	65.7^{a}	66.1^{a}	66.3^{a}	66.3^{a}	66.3^{a}	65.7	66.0

Means of parameters in particular TC group mentioned with different letters in superscript are significantly different.

Table 4. Sowing date means of yield components and grain quality parameters depending on wheat tillering coefficient

Sowing date	Average per plants with different tillering coefficient						Average per sowing date
	1	2	3	4	5	6	
	Grain number per spike						$p < 0.001$
1 T	35.3^{a}	36.0^{a}	37.2^{a}	36.8^{a}	37.3^{a}	36.9^{a}	36.6
2 T	34.4^{ac}	35.7^{a}	35.7^{a}	35.6^{ac}	36.1^{a}	35.5^{a}	35.5
3 T	32.5^{bc}	33.3^{b}	33.6^{b}	33.9^{bc}	33.6^{b}	33.3^{b}	33.4
4 T	32.0^{b}	32.8^{b}	32.9^{b}	32.3^{c}	32.0b	31.8^{b}	32.3
	Grain weight per spike, g						$p < 0.001$
1 T	1.55^{a}	1.60^{a}	1.65^{a}	1.64^{a}	1.66^{a}	1.61^{a}	1.62
2 T	1.51^{a}	1.57^{a}	1.58^{a}	1.57^{a}	1.59^{a}	1.51^{a}	1.56
3 T	1.40^{b}	1.45^{b}	1.46^{b}	1.46^{b}	1.45^{b}	1.40^{b}	1.44
4 T	1.36^{b}	1.38^{b}	1.39^{b}	1.36^{b}	1.34^{c}	1.29^{c}	1.35
	1,000 grain weight, g						$p < 0.001$
1 T	43.29^{a}	44.08^{a}	43.83^{a}	43.93^{a}	44.20^{a}	43.24^{a}	43.76
2 T	43.60^{a}	43.60^{a}	43.94^{a}	43.69^{a}	43.79^{a}	42.19^{ac}	43.47
3 T	42.90^{ac}	43.25^{a}	43.21^{ac}	42.99^{ac}	43.19^{a}	42.04^{ac}	42.93
4 T	42.11^{bc}	42.19^{b}	42.27^{bc}	41.96^{bc}	41.89^{b}	40.68^{bc}	41.85
	Grain crude protein content, %						$p < 0.001$
1 T	13.9^{a}	13.7^{ab}	13.7^{a}	13.8^{a}	13.8^{a}	13.7	13.8
2 T	13.7^{a}	13.8^{a}	13.6^{ac}	13.6^{ac}	13.6^{a}	13.6	13.7
3 T	13.4^{ac}	13.2^{b}	13.1^{bc}	13.2^{bc}	13.3^{ac}	13.3	13.2
4 T	12.9^{bc}	12.8^{c}	12.7^{b}	12.7^{b}	12.7^{bc}	13.1	12.8
	Grain gluten content, %						$p < 0.001$
1 T	26.7^{a}	25.4	25.9^{a}	26.1^{a}	26.0^{a}	26.1	26.0
2 T	25.9^{a}	26.1	25.6^{ac}	25.8^{a}	26.2^{a}	26.3	26.0
3 T	25.1^{ac}	25.7	24.2^{bc}	24.4^{ac}	24.6^{ac}	25.4	24.9
4 T	23.8^{bc}	23.3	22.7^{b}	22.9^{bc}	23.1^{bc}	25.0	23.5
	Zeleny index						$p < 0.001$
1 T	56^{a}	56^{a}	56^{a}	57^{a}	58^{a}	54^{a}	56
2 T	56^{a}	57^{a}	57^{a}	56^{a}	57^{a}	53^{ac}	56
3 T	54^{a}	54^{a}	53^{ac}	55^{a}	54^{a}	50^{ad}	53
4 T	50^{b}	50^{b}	50^{bc}	49^{b}	48^{b}	49^{bcd}	49
	Grain starch content, %						$p < 0.001$
1 T	64.8^{a}	65.2^{a}	65.2^{a}	65.2^{a}	65.1^{a}	65.1	65.1
2 T	65.2^{a}	65.0^{a}	65.4^{ac}	65.4^{a}	65.3^{a}	65.0	65.2
3 T	65.6^{ac}	66.0^{b}	66.0^{bc}	66.0^{ac}	65.8^{ac}	67.2	66.1
4 T	66.2^{bc}	66.3^{b}	66.6^{b}	66.4^{bc}	66.5^{bc}	65.8	66.3

Means of parameters in particular TC group mentioned with different letters in superscript are significantly different.

Grain number per spike for plants with the same TC depending on cultivar was different in all the cases ($p < 0.05$). The most number of grain per spike was noted for cultivar 'Cubus' (36.2 for plants with TC '1' to 38.4 for plants with TC '5') (Table 3). Differences between max. and min. number of grains per spike depending on tillering coefficient were 1.3, 1.6 and 2.3 grains for cultivars 'Zentos', 'Tarso' and 'Cubus' respectively. Gradual and even increase depending on TC increase from '1' to '6' was established only for cultivar 'Tarso'. Also, the biggest average grain weight per spike ($p < 0.001$) was noted for cultivar 'Cubus' (1.64 g) and significant cultivar impact on this parameter was observed for plants in groups of all evaluated TC. The biggest grain weight per spike was established for plants with TC ’2’ to ’5’ for ’Cubus’, with TC ’3’ to ’5’ for ’Tarso’ and with TC ’3’ – for ’Zentos’ (Table 3). Sowing time effect was substantial on average grain number per spike ($p < 0.001$; Table 4). Later sowing caused regular reduction of grain per spike and grain weight per spike on average and in all cases in groups with different TC.

Sowing rate increase caused small decrease of grain number and weight per spike, but changes were not linear: sometimes the smallest values were established when sowing rate 400 germinable seeds m^{-2} were sown (the number of grain per spike when TC was ’2’and ’6’; grain weight per spike when TC was ’1’, ’2’ and ’6’; Table 5).

Table 5. Sowing rate means of yield components and grain quality parameters depending on wheat tillering coefficient

Sowing rate	Average per plants with different tillering coefficient						Average per sowing rate
	1	2	3	4	5	6	
	Grain number per spike						p = 0.001
300	34.3	35.3	35.3	35.0	35.4	34.7	35.0
400	33.2	33.9	34.9	34.9	34.8	34.4	34.3
500	33.1	34.2	34.3	34.1	34.2	34.6	34.1
	Grain weight per spike, g						p = 0.01
300	1.50	1.54	1.54	1.52	1.54	1.48	1.52
400	1.43	1.47	1.52	1.51	1.52	1.45	1.48
500	1.44	1.49	1.50	1.49	1.48	1.47	1.48
	1,000 grain weight, g						p = 0.736
300	43.21	43.27	43.38	42.92	43.46	42.49	43.12
400	42.89	43.20	43.24	43.34	43.18	42.02	42.98
500	42.83	43.36	43.32	43.18	43.23	41.90	42.97
	Grain crude protein content, %						p = 772
300	13.5	13.3	13.4	13.3	13.4	13.4	13.4
400	13.4	13.4	13.3	13.3	13.3	13.5	13.4
500	13.4	13.3	13.2	13.3	13.3	13.5	13.3
	Grain gluten content, %						p = 0.685
300	25.7	25.1	24.8	24.7	25.2	25.8	25.2
400	25.3	25.6	24.7	24.8	24.9	25.8	25.2
500	25.1	24.8	24.3	24.8	25.0	25.6	24.9
	Zeleny index						p = 0.013
300	54	53	55	55	56	52	54
400	55	54	55	54	54	52	54
500	54	54	53	54	53	52	53
	Grain starch content, %						p = 0.402
300	65.3	65.5	65.7	65.7	65.6	65.4	65.1
400	65.5	65.6	65.8	65.8	65.7	65.3	65.6
500	65.6	65.7	65.8	65.7	65.7	66.4	65.8

Spink et al. (2000) and Whaley et al. (2000) noted significant reduction of number of grain per spike with plant density increase, but in their trials wide range of seed rates or plant densities were studied (20 to 640 seeds m^{-2} and 19 to 338 plants m^{-2} respectively).

The effect of year conditions on grain number and weight per spike was substantial ($p \leq 0.01$; Table 6) and similar differences were observed in all the groups depending on TC. The biggest values were established in 2007 when wintering conditions reduced the plant number per 1 m^2 considerably, but the following spring and summer favoured an increase in spike productivity. The smallest spike productivity was noted in 2006 when lack of precipitation was observed together with overly warm temperatures if compared with long-term average data. McMaster et al. (1994) showed that irrigation increased grain number per spike and grain weight per plant in the US Great Plains where water is commonly wheat yield limiting factor.

Table 6. Year means of yield components and grain quality parameters depending on wheat tillering coefficient

Research year	Average per plants with different tillering coefficient						Average per year
	1	2	3	4	5	6	
	Grain number per spike						$p < 0.001$
2005	33.8^a	34.0^a	34.4^a	34.1^a	34.8^a	40.7^a	35.3
2006	30.1^b	31.7^b	31.9^b	31.9^b	32.1^b	31.7^b	31.6
2007	36.1^c	37.7^c	38.3^c	38.0^c	37.6^c	36.4^c	37.4
	Grain weight per spike, g						$p < 0.001$
2005	1.54^a	1.56^a	1.58^a	1.55^a	1.59^a	1.88^a	1.62
2006	1.16^b	1.21^b	1.22^b	1.22^b	1.24^b	1.20^b	1.21
2007	1.67^c	1.73^c	1.76^c	1.75^c	1.73^c	1.66^c	1.72
	1,000 grain weight, g						$p < 0.001$
2005	45.42^a	45.66^a	45.55^a	45.37^a	45.48^a	46.22^a	45.61
2006	37.62^b	38.30^b	38.39^b	38.09^b	38.56^b	38.12^b	38.18
2007	45.89^a	45.88^a	46.00^a	45.97^a	45.97^a	45.55^a	45.88
	Grain crude protein content, %						$p < 0.001$
2005	12.9^a	12.9^a	12.9^a	13.0^a	13.2^a	13.0	13.0
2006	13.9^b	13.8^b	13.6^{bc}	13.6^{bc}	13.7^b	13.6	13.7
2007	13.3^a	13.3^a	13.3^{ac}	13.3^{ac}	13.2^a	13.3	13.3
	Grain gluten content, %						$p < 0.001$
2005	21.5^a	22.9^a	21.9^a	22.1^a	22.7^a	21.6^a	22.1
2006	27.6^b	26.7^{bc}	26.7^b	26.7^b	26.7^b	26.7^b	26.9
2007	25.2^c	25.2^{ac}	25.1^c	25.3^c	25.3^c	25.2^c	25.2
	Zeleny index						$p < 0.001$
2005	60^a	60^a	60^a	60^a	63^a	61^a	61
2006	56^b	55^b	54^b	54^b	53^b	54^b	54
2007	49^c	49^c	49^c	49^c	48^c	49^c	49
	Grain starch content, %						$p < 0.001$
2005	66.9^a	66.8^a	66.8^a	66.7^a	66.6^a	66.8	66.8
2006	64.9^b	65.1^b	65.1^b	65.4^b	65.2^b	66.1	65.3
2007	65.2^b	65.4^b	65.4^b	65.3^b	65.3^b	65.2	65.3

Means of parameters in particular TC group mentioned with different letters in superscript are significantly different.

Changes of spike productivity depending on TC within specific year were not so regular. The largest grain number and weight per spike in 2005 were observed when TC was '5' and '6', in 2006 – the grain number per spike when TC was '3' and '4', but weight per spike – when TC was ’5’, but in 2007 the most values of detected parameters were established when TC was ’3’.

It was shown by many authors (e.g. Zwer et al., 1995; Thiry et al., 2002; Abdoli & Saeidi, 2012; Li et al., 2016) that 1,000 grain weight (or one grain weight) is a strongly cultivar-dependent and comparatively stable characteristic or yield component.

TC of plant did not affect significantly average values of 1,000 grain weight (TGW) ($p = 0.242$; Table 2). Used cultivar affected TGW on average and when plant groups with different TC were evaluated ($p < 0.001$; Table 3). The difference observed between the lowest and highest TGW values depending on TC within the same cultivar was 1.09 g for ’Cubus’, 1.60 g for ’Tarso’ and 1.24 g for ’Zentos’. The lowest TGW for all the cultivars was observed for plants with TC ’6’, but the highest – for plants with TC ’3’ for cultivars ’Cubus’ and ’Tarso’, and with TC ’5’ – for cultivar ’Zentos’. It has to be remarked that difference between 1,000 grain weight (except the smallest and biggest values) of plants with two different TC was small.

Sowing rate effect was not signifficant on average TGW ($p = 736$; Table 5). Li et al. (2016) investigating four very contrastive plant densities (75 to 525 plants m^{-2}) found significant TGW differences within the investigated plant densities which depended on cultivar (i.e., significant cultivar × plant density interaction effect on TGW was observed). On the contrary, Zecevic et al. (2014) investigating five cultivars sown with two sowing rates (500 and 600 seed m^{-2}) three years found TGW increase with sowing rate increase in all genotypes and investigated years.

Later sowing date caused gradual decrease of average values of TGW ($p < 0.001$; Table 4). Gradual decrease of average TGW was observed also for plants with the same TC sown in different sowing times (only two exceptions were observed: if TC was ‘1’ and ‘3’ the highest TGW was observed if wheat was sown on 2 T). This agrees with results of Spink et al. (2000) who found well expressed TGW reduction by later sowing date (three dates were used) in one of trial years, in other one – decrease was not so clear between the 2nd and 3rd sowing dates, and in the third research year later sowing promoted even higher TGW.

Conditions of research year caused substantial ($p < 0.001$; Table 6) TGW differences. Similar average TGW values were observed for 2005 and 2007 (Table 6), but insufficient amount of precipitation, especially during the grain fill and TGW formation reduced TGW in 2006 by more than 7 g if compared with two other research years. Our results agree with findings of Abdoli & Saeidi (2012) who wrote that post-anthesis water stress decreased TGW. The smallest difference between the TGW max. and min. value depending on TC within the same year was observed in 2007 (0.45 g between TGW of plants with TC ’3’ and TC ’6’), but the biggest – in 2006 (0.94 g between TGW of plants with TC ’5’ and TC ’1’) (Table 6).

Although TGW is genetically stable yield component, almost all researchers showed substantial environmental or year effect on TGW in field trials (e.g., Spink et al., 2000; Frederick et al., 2001; Elhani et al., 2007; Jug et al., 2011; Zecevic et al., 2014).

Grain quality

Wheat TC did not affect average values of any of measured quality indicators ($p > 0.05$; Table 2): crude protein (CP), wet gluten (WG) and starch content, and Zeleny index.

As all three cultivars used in trial are bred for bread making purposes, differences in their average crude protein (CP) content were not substantial ($p = 0.051$; Table 3); difference between max. and min. values was 0.2%. CP content in grain obtained from plants in groups with different TC also was not substantially different ($p > 0.05$) depending on cultivar. Similarly, sowing rate did not affect substantially average CP content ($p = 0.772$) and CP content in different TC groups (Table 5). Metho et al. (1998) reported small, but not significant differences in CP content between the main stem and tillers whereas Geleta et al. (2002) established CP increase with the sowing rate decrease. At the same time researchers concluded that the quality traits are greatly influenced by the environment and in less extent by the sowing rates.

Sowing date and trial year showed mathematically substantial effect ($p < 0.001$) on average CP content. CP content differed essentially also in every TC group depending on the sowing date (Table 4) and trial year (Table 6). The sowing date delay to 3 T caused CP decrease for 0.5–0.6 percentage points, but till 4 T – even for 0.9–1.1 percentage points in most cases. The highest average CP content was observed in 2006, which was dry and warm during the grain fill and protein synthesis.

The cultivar influence on average WG content was substantial ($p < 0.001$) that agrees with many other results (e.g. Linina & Ruza, 2012; Zecevic et al., 2014); difference between max. and min. values was 1.1 percentage point. Analysing average WG values of cultivars within the specific TC groups, influence of cultivar on WG content was not consistent: it was substantial only in groups with TC '1' ($p = 0.007$) and TC '6 ' ($p = 0.02$). Sowing rate did not influence WG content essentially ($p > 0.05$). Zecevic et al. (2014) investigating two comparatively high sowing rates (500 and 650 seed m^{-2}) in Serbia found that higher sowing rate is beneficial for increase of WG content. Substantial sowing date influence on average WG content was established. Similar were values of WG content if wheat was sown at 1 T and 2 T, but obviously lower – when it was sown at 3 T and 4 T. This regularity was observed in all groups with the specific TC. Similarly to CP also average WG content was affected by trial year substantially confirming the verity that the environment affects grain quality greatly.

Although all values of Zeleny index correspond to demands for grain of good baking quality still the cultivar, sowing date and research year affected them substantially on average and within the groups with specific TC. Sowing rate affected Zeleny index on average ($p = 0.013$; Table 5), but substantial effect of sowing rate in any of groups with specific TC was not observed ($p > 0.05$). If sowing rate 500 seeds per m^{-2} was used, the least average Zeleny index (53; Table 5) was observed and it is contrary to Zecevic et al. (2014) who concluded that with a sowing rate increase also Zeleny index increases. Similarly to CP and WG, also Zeleny index decreased substantially if wheat was sown at 3 T and 4 T. The most expressed was influence of research year similarly to many other research results: the max. value was established in 2005 (61), but the min. value – in 2007 (49) (Table 6).

Starch is a grain quality indicator that is not so important for bread baking, but is more important if wheat is used as feed for livestock or for ethanol production. In our trial, an average starch content (65.7%) was not extremely high, but it was characteristic for bread wheat. This indicator was substantially affected by cultivar, sowing date and research year on average and in every group with different TC, and it was not affected by sowing rate ($p = 0.402$; Table 5). Starch content values were mostly inversely related to CP values. It is a well-known fact that CP and starch content in most cases correlates negatively.

CONCLUSIONS

Plants with tillering coefficient (TC) ‘1’ to ‘6’ were detected during all three research years, but the biggest average contribution in yield formation gave plants with TC ‘3’. Depending on investigated factor and environment, also any other TC group can give similar contribution.

TC showed substantial effect on two yield-forming components: the number and weight of grain per spike. Though differences were inconsistent, and further investigations are needed. Clear regularity was found that both spike productivity indicators were higher in groups with TC ≥ 2 if compared with plants that did not tiller at all. Thousand grain weight and grain quality indicators (crude protein, wet gluten and starch content, and Zeleny index) were not affected by TC. Thus, tillering is beneficial for winter wheat yield formation and is important mechanism in compensation of yield components.

Sowing rate mostly did not affect investigated yield forming elements and quality indicators. Contrariwise, winter wheat cultivar used and sowing time mostly affected investigated parameters substantially. If winter wheat was sown on 20 September and later (3 T and 4 T) the yield increased, but the quality of grain mostly decreased (except starch content) insignificantly. Conditions of research year substantially affected all investigated yield components and quality indicators, thus showing that the environmental effect is more critical if compared with the effect of sowing rate and in some cases even if compared with the effect of cultivar.

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Soil tillage and crop rotation differently affect biodiversity and species assemblage of ground beetles inhabiting winter wheat fields

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Abstract. This paper continues studies on ground beetles (Carabidae) in differently managed winter wheat (*Triticum aestivum*) fields in Latvia. The main task of those studies was to assess how different soil tillage regimes (ploughing and non-inverse tillage) and different pre-crops (winter wheat and spring rapeseed (*Brassica napus*) affect assemblage and biodiversity of ground beetles in winter wheat fields. The research was carried out in the Latvia University of Agriculture Research and Study Farm 'Pēterlauki' (56°30'39.38''N; 23°41'30.15''E) during vegetation season of 2013. The results were compared with the results of similar research carried out at the same place during 2012. Totally 57 ground beetle species were observed in studied fields in 2013. Total species assemblage varied between both consecutive vegetation seasons of the research, however these were minor differences not connected with studied agro-ecological factors. Dominance structure of ground beetle species was significantly different between both vegetation seasons – species which were dominant and subdominant in 2012 became subdominant and dominant one year later, accordingly. Annual effects of soil tillage regime and pre-crop on ground beetle dominance structure also were observed, however some differences were recognized between both vegetation seasons. In case, if weed control was successful, higher ground beetle biodiversity might be observed in ploughed fields pre-cropped with spring rapeseed. Otherwise, significantly higher ground beetle biodiversity may be observed in harrowed soil independently from the pre-crop.

Key words: Carabidae, *Triticum aestivum*, ploughing, non-inverse tillage, pre-crop, Latvia.

INTRODUCTION

Ground beetles (Carabidae) are well known inhabitants of almost all terrestrial habitats including different agrocenoses. From the economic point of view, those are beneficial insects consuming various pests and seeds of weeds in all field and horticultural crops (Holopainen & Helenius, 1992; Holland & Thomas, 1997; Cromar et al., 1999; Bohan et al., 2000; Arus et al., 2012; Renkema et al., 2012). There have been even attempts of mass culturing of some ground beetles to use them later as biological control agents in greenhouses and tunnels (Symondson, 1994). Overall, presence of diverse ground beetle species assemblage is a good indicator of successful integrated plant breeding in any agrocenosis. Since the integrated pest management (IPM) must be implemented in farms within EU, issues on more efficient use of natural enemies of pests became scientifically topical. As other invertebrates, also ground beetles are exposed to agricultural activities applied in crops. These insects are especially affected by soil tillage and crop rotations – factors which are mentioned as major components of the IPM. In the field crops, soil ploughing and crop rotation are mentioned as effective methods to reduce amount of pests, weeds and causal agents of plant diseases (Salt & Hollick, 1949; Brust & King, 1994; Dosdall et al., 1998; Bankina et al., 2013; Ruisi et al., 2015). On the contrary, non-inverse soil tillage or direct sowing and crop monocultures can dot the opposite effect. It is still unclear how significantly soil tillage and crop rotation affect ground beetles. Several authors had found out that more intensive soil tillage negatively affects ground beetle density, species richness and other parameters (Cárcamo, 1995; Thorbek & Bilde, 2004; Aviron et al., 2005; Cole et al., 2005; Hatten et al., 2007). However, other studies have opposite conclusions reporting that there is lesser activity density of ground beetles in the minimally tilled soil than in ploughed one (Hole et al., 2005). The third point of view is also available – different soil tillage regimes have not significantly different effect on ground beetles due to their migration abilities and breeding cycles adjusted to the tillage regime (Purvis & Fadl, 2002; Belaoussoff et al., 2003; Mason et al., 2006). Connections between ground beetles and crop rotation have been studied comparatively lesser than between beetles and the soil tillage, but mostly there is evidence that crop rotation positively affects ground beetles promoting higher activity density and diversity in the arable land (Brust & King, 1994; O'Rourke et al., 2008; Bourassa et al., 2010).

In Latvia, studies on ground beetles inhabiting winter wheat (*Triticum aestivum*) fields with different soil tillage intensities (ploughed and non-inverse) combined with different pre-crops (winter wheat, spring wheat, spring rapeseed) started in 2012. During this year, more than 60 ground beetle species were observed in the studied fields. Results of data analysis showed that ploughed soil promotes significantly higher activity density of small sized beetles (< 5 mm). Eight species – *Amara plebeja*, *Bembidion guttula*, *B. obtusum*, *Harpalus rufipes*, *Loricera pilicornis*, *Poecilus cupreus*, *Pterostichus melanarius* and *P. niger* – were the most frequent ones in the ground beetles' assemblage, but their proportion was not equal in the fields with all management types. For example, *B. guttula* and *L. pilicornis* over-dominated all other species in the ploughed and non-inverse tilled fields, respectively, but *A. plebeja* was 4–20 times more frequent in the non-inverse tilled fields pre-cropped with spring wheat than in other sample plots. Soil tillage combined with crop rotation also significantly affected biodiversity of ground beetles. Two management types of fields – ploughed soil and spring rapeseed (*Brassica napus*) as pre-crop (1) and non-inverse tilled soil and spring wheat as pre-crop (2) – promoted significantly higher biodiversity than other management types. Contrary, the lowest biodiversity of ground beetles was observed in the non-inverse tilled fields pre-cropped with spring rapeseed (Gailis & Turka, 2014a; Gailis & Turka, 2014b).

In the growing season of 2013, the research on the ground beetles inhabiting differently tilled and pre-cropped winter wheat fields had been continued in the same study place. Objectives of this research were to find out how soil tillage intensity and different pre-crops affected the species assemblage and biodiversity of ground beetles (1); to study whether similar management regime of fields causes equal effect to the species assemblage and biodiversity of ground beetles in two consecutive years (2); to discuss possible reasons of potential unequal between-years effects caused by both agro-ecological factors (3).

MATERIALS AND METHODS

Field studies were carried out during 2013 in stationary trial place created in 2009 for researches on good agricultural practice for the most popular field crops. This trial place belongs to the Latvia University of Agriculture Research and Study Farm 'Pēterlauki'. It is located near Poķi village 14 km south from Jelgava town (56°30'39.38''N; 23°41'30.15''E). Since the establishment, in this place, all agricultural activities, e.g., soil tillage, sowing of crops, usage of fertilizers and pesticides and crop harvesting were performed in accordance to conventional agricultural practice as in any usual field.

The trial place consists of a grid of 24 rectangular sample plots (0.25 ha; 30 x 85 m). The grid was surrounded by conventionally farmed arable land. A narrow strip (35 x 510 m) of circa 60 years old deciduous forest was located 30 m south, but the closest rural settlement – 120 m west from the study site. Stripes of land (2.5 m wide) separated sample plots from each other and from near crop fields. Vegetation of wild herbaceous plants covered those land stripes. The soil at this place is an Endogleyic Calcisol (GLu) with pH KCl 6.8 and low humus content – 20 g kg^{-1} (Dubova et al., 2013). Since 2009, the main soil treatments were conventional ploughing (0.22–0.23 m) with mouldboard plough and non-inverse tillage (0.10–0.11 m) with disc harrow for each 12 sample plots. For the growing season of 2013, these activities were executed in 10 August 2012. Other soil tillage activities were performed in accordance with the conventional agronomic practice as in any commercial field.

Six ploughed and six harrowed sample plots were sown with winter wheat (variety 'Zentos') for the growing season of 2013, and these sample plots were used for this study. Other sample plots were sown with other field crops. Winter wheat was pre-crop in two ploughed and two harrowed sample plots, but spring rapeseed was pre-crop in four ploughed and four harrowed sample plots. Thus there were four combinations of both agro-ecological factors – soil tillage and pre-crop – represented in the studied sample plots (Fig. 1).

After crop harvesting, straws and other plant remnants were left on the ground as fertilizer, but sample plots were fertilized with mineral fertilizers each year, as well. After monitoring, authorized fungicides, herbicides and retardants, but not insecticides were applied in the sample plots.

Red dead-nettle (*Lamium purpureum*), wall speedwell (*Veronica arvensis*), cleavers (*Galium aparine*) and knotgrass (*Polygonum aviculare*) were the most common weeds in all studied sample plots, but loose silky-bent (*Apera spica-venti*) was very common in plots, especially in harrowed ones, were wheat was sown after wheat each year. Total weed density was evaluated twice in the season – in May 10 (seven days before the application of herbicides) and in July 8. Before the application of herbicides, weed density varied between nine and 60 plants m^{-2} in different sample plots, but harrowed plots were comparably weedier especially ones pre-cropped with winter wheat. Weed control was comparatively ineffective – weed density either decreased insignificantly or increased between both accountings. Especially significant increase of weed density occurred in harrowed sample plots pre-cropped with winter wheat. In these fields, 80–137 weeds m^{-2} were observed during the second accounting (Fig. 1).

H	P	P	H
No. 1 Winter wheat* (Winter wheat)** W.D.: 38/137	**No. 2** Winter wheat (Winter wheat) W.D.: 60/66	**No. 3** Winter rapeseed	**No. 4** Spring rapeseed
No. 5 Spring rapeseed	**No. 6** Winter rapeseed	**No. 7** Winter wheat (Spring rapeseed) W.D.: 8/8	**No. 8** Winter wheat (Spring rapeseed) W.D.: 33/32
No. 9 Winter wheat (Spring rapeseed) W.D.: 3/19	**No. 10** Winter wheat (Spring rapeseed) W.D.: 7/8	**No. 11** Winter rapeseed	**No. 12** Spring rapeseed
No. 13 Winter wheat (Winter wheat) W.D.: 51/80	**No. 14** Winter wheat (Winter wheat) W.D.: 22/12	**No. 15** Winter rapeseed	**No. 16** Winter rapeseed
No. 17 Winter rapeseed	**No. 18** Winter rapeseed	**No. 19** Winter wheat (Spring rapeseed) W.D.: 15/14	**No. 20** Winter wheat (Spring rapeseed) W.D.: 40/39
No. 21 Winter wheat (Spring rapeseed) W.D.: 9/6	**No. 22** Winter wheat (Spring rapeseed) W.D.: 19/14	**No. 23** Winter rapeseed	**No. 24** Winter rapeseed

Figure 1. Scheme of sample plots in stationary trial place in Study and Research Farm 'Pēterlauki'. (H – harrowed with disc harrow; P – ploughed; * – crop in 2013; ** – pre-crop; W.D. – weed density in 10 May 2013 / 8 July 2013; pre-crops and weed density values are showed only for winter wheat sample plots used for this research).

Transparent plastic glasses (vol. 200 ml, 65 mm opening diameter) were used as pitfall traps for collecting of ground beetles. The traps were half filled with 4–5% acetic acid solution with few drops of detergent. Ten traps were placed in cornerwise transect in each winter wheat sample plot, distance between traps were three meters. Exposition of traps started when first active ground beetles were observed in spring (23 April 2013), but ended few days before harvesting of winter wheat (30 July 2013). The traps were emptied and filled with fresh acid every seven days. During the same periods of time, precipitation and average air temperature were registered using Davis Vantage Pro2 weather station, which was located 100 m away from sample plots. The meteorological situation is showed in Fig. 2.

Species of ground beetles were identified after Freude et al. (2004), but Check-List of Latvian beetles (Telnov, 2004) was used for nomenclature. Species assemblages of ground beetles were expressed as the dominance structure calculated according to Engelmann (1978). This scale anticipates to classify species into five groups according to their proportion in the species assemblage: eudominants (40.0– 100.0%), dominants

(12.5– 39.9%), subdominants (4.0– 12.4%), recedents (1.3– 3.9%) and subrecedents (<1.3%). The dominance structure was calculated for each of four studied winter wheat management types (the main soil tillage method in combination with the pre-crop). Proportion of each particular species in each particular management type was calculated using total number of individuals of particular species and total number of all ground beetle individuals caught in all traps throughout vegetation season. Biodiversity of ground beetles was assessed by calculating Simpson's index (D_s) for winter wheat fields with each management type:

$$D_s = \sum \frac{n_i\,(n_i - 1)}{N(N-1)} \tag{1}$$

where: n_i – the number of individuals of the i^{th} species per trap; N – the total number of individuals per trap (Magurran, 2004).

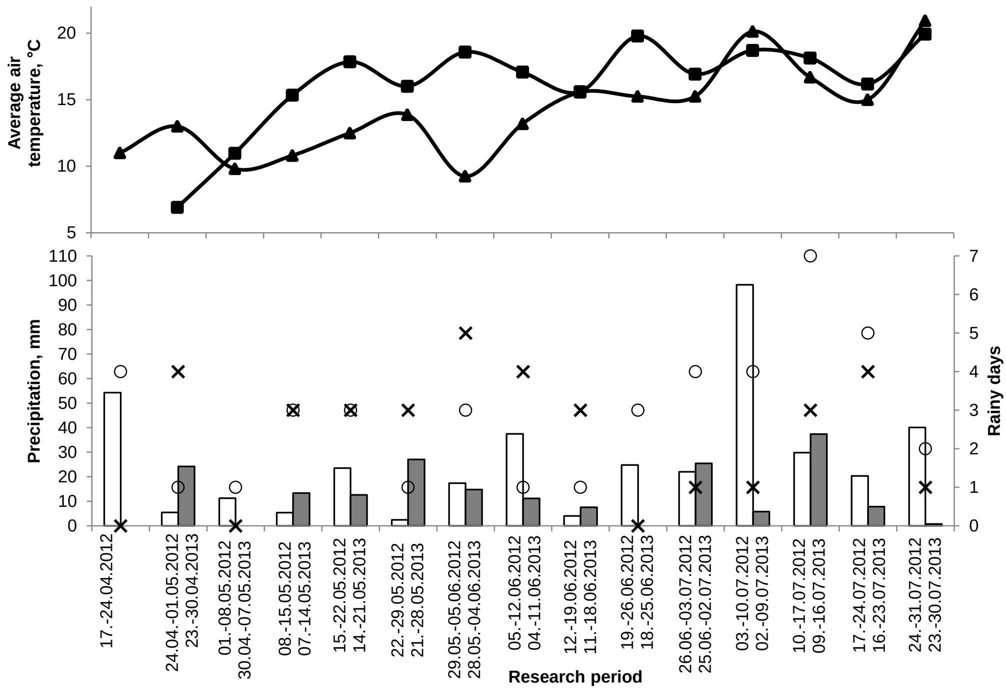

Figure 2. Meteorological conditions in studied winter wheat fields during vegetation season of 2013 and one year earlier (—▲— – air temperature in 2012; —■— – air temperature in 2013; □ – precipitation in 2012; ■ – precipitation in 2013; ○ – rainy days in 2012; × – rainy days in 2013).

In this paper, biodiversity of ground beetles is expressed as reciprocal Simpson's index (*$1/D_s$*). This value was calculated for each of 120 pitfall traps. Total number of ground beetle individuals collected throughout the vegetation season was used for the calculations. Interconnections among biodiversity and both agro-ecological factors were assessed calculating Spearman's rank correlation coefficients (r_s) with two-tailed significance test using SPSS 17.0. Correlation was calculated for six different variants: between the biodiversity and soil tillage independently from pre-crop (1), between the biodiversity and pre-crop independently form soil tillage (2), between biodiversity and pre-crop in harrowed soil (3), between biodiversity and pre-crop in ploughed soil (4), between biodiversity and soil tillage if pre-crop was winter wheat (5) and between biodiversity and soil tillage if the pre-crop was spring rapeseed (6). The strength of correlation was estimated after Green et al. (2000):

$|r_s|$ = 0.00-0.19 – very weak correlation;
$|r_s|$ = 0.20-0.39 – weak correlation;
$|r_s|$ = 0.40-0.59 – moderate correlation;
$|r_s|$ = 0.60-0.79 – strong correlation;
$|r_s|$ = 0.80-1.00 – very strong correlation.

RESULTS AND DISCUSSION

Composition of ground beetle species assemblage

In total, 60,024 ground beetles from 57 species were observed in studied winter wheat fields in 2013, but not all species were present in all sample plots. The number of species varied from 39 until 48 in differently managed fields (Table 1).

Table 1. List of ground beetles and number of their individuals caught in differently managed winter wheat fields during 2013. Species are grouped according to their habitat preferences after Barševskis (2003) and listed in descending order according to their frequency in each ecological group. (H – non-inverse tilled (harrowed) soil, P – ploughed soil; pre-crops: W.W. – winter wheat, S.R. – spring rapeseed. * – number of individuals caught in 20 traps; ** – number of individuals caught in 40 traps; Hab. – habitat preferences: G – generalists, species inhabiting open habitats and forests; Oh – open habitats; F – forests; x – xerophilous; m – mesophilous; h – hygrophilous)

Species	H.* W.W.	H.** S.R.	P.* W.W.	P.** S.R.	Hab.
Pterostichus niger (Schaller, 1783)	1,019	4,999	1,170	5,331	G, h
Pterostichus melanarius (Illiger, 1798)	1,824	3,540	892	3,083	G, h
Poecilus cupreus (Linnaeus, 1758)	877	2,160	992	2,154	G, m
Loricera pilicornis (Fabricius, 1775)	375	1,317	306	1,016	G, h
Trechus quadristriatus (Schrank, 1781)	36	348	138	560	G, m
Bembidion lampros (Herbst, 1784)	94	261	66	203	G, m
Bembidion properans (Stephens, 1828)	88	161	34	113	G, h
Clivina fossor (Linnaeus, 1758)	22	77	40	59	G, h
Amara plebeja (Gyllenhal, 1810)	120	30	19	29	G, x
Blemus discus (Fabricius, 1792)	15	38	25	60	G, h
Carabus cancellatus Illiger, 1798	10	70	8	43	G, m
Bembidion quadrimaculatum (Linnaeus, 1761)	10	28	22	27	G, m
Amara similata (Gyllenhal, 1810)	9	17	3	16	G, m
Acupalpus meridianus (Linnaeus, 1761)	7	11	2	11	G, h
Poecilus versicolor (Sturm, 1824)	6	3	4	14	G, m
Notiophilus palustris (Duftschmid, 1812)	9	7	5	7	G, h
Notiophilus aquaticus (Linnaeus, 1758)	5	6	6	7	G, h
Notiophilus germinyi Fauvel, 1863	6	4	2	2	G, h
Badister sodalis (Duftschmid, 1812)	6	-	6	4	G, h
Amara ovata (Fabricius, 1792)	3	6	1	5	G, m
Carabus granulatus Linnaeus, 1758	-	7	-	3	G, h
Carabus nemoralis O.F.Müller, 1764	-	6	-	3	G, m

Table 1 (continued)

Amara aenea (DeGeer, 1774)	1	3	3	1	G, m
Amara familiaris (Duftschmid, 1812)	-	1	-	5	G, m
Amara nitida Sturm, 1825	-	5	-	1	G, m
Badister bullatus (Schrank, 1798)	-	-	-	2	G, m
Patrobus atrorufus (Ström, 1768)	-	-	-	1	G, h
Pterostichus nigrita (Paykull, 1790)	-	1	-	-	G, h
Harpalus rufipes (DeGeer, 1774)	3,020	6,452	3,169	6,969	Oh, x
Bembidion guttula (Fabricius, 1792)	680	1,379	677	1,573	Oh, h
Anchomenus dorsalis (Pontoppidan, 1763)	98	237	81	265	Oh, m
Bembidion obtusum Audinet-Serville, 1821	37	180	36	123	Oh, x
Harpalus affinis (Schrank, 1781)	66	79	95	97	Oh, x
Notiophilus aestuans Dejean, 1826	12	30	6	35	Oh, x
Asaphidion flavipes (Linnaeus, 1761)	3	9	4	9	Oh, x
Pterostichus macer (Marsham, 1802)	3	6	9	7	Oh, m
Chlaenius nitidulus (Schrank, 1781)	1	6	1	10	Oh, h
Amara eurynota (Panzer, 1796)	5	3	-	4	Oh, x
Calathus ambiguus (Paykull, 1790)	1	4	3	4	Oh, m
Calathus fuscipes (Goeze, 1777)	2	4	1	3	Oh, m
Amara littorea Thomson, 1857	3	3	1	-	Oh, x
Amara apricaria (Paykull, 1790)	5	-	2	-	Oh, m
Harpalus calceatus (Duftschmid, 1812)	-	-	-	6	Oh, x
Synuchus vivalis (Illiger, 1798)	-	2	-	3	Oh, m
Harpalus distinguendus (Duftschmid, 1812)	-	1	-	2	Oh, x
Microlestes minutulus (Goeze, 1777)	1	-	2	-	Oh, x
Cylindera germanica (Linnaeus, 1758)	-	-	1	-	Oh, m
Anisodactylus signatus (Panzer, 1796)	-	-	-	1	Oh, h
Harpalus signaticornis (Duftschmid, 1812)	-	1	-	-	Oh, m
Microlestes maurus (Sturm, 1827)	-	-	1	-	Oh, x
Agonum gracilipes (Duftschmid, 1812)	-	-	1	-	Oh, h
Nebria brevicollis (Fabricius, 1792)	4	159	8	65	F, m
Pterostichus vernalis (Panzer, 1796)	15	46	4	17	F, h
Platynus assimilis (Paykull, 1790)	-	7	1	4	F, h
Pterostichus oblongopunctatus (Fabricius, 1787)	-	4	-	2	F, m
Leistus piceus Frölich, 1799	1	-	-	-	F, h
Stomis pumicatus (Panzer, 1796)	-	-	-	1	F, m
Total species	39	45	41	48	
Total individuals	8,499	21,718	7,847	21,960	
Average individuals per trap	425	543	392	549	

Overall in 2013, general species richness in studied fields was similar to the species richness observed in other studies in cereals in Latvia and elsewhere in Europe. For instance, 41 ground beetle species were observed in wheat fields in Eastern Latvia (Bukejs & Balalaikins, 2008). In general, all observed ground beetles are creating typical species assemblage inhabiting arable land. Almost all species mentioned in Table 1 are reported as more or less common faunistic elements of different agrocenoses and other open habitats across the Europe (Basedow et al., 1976; Hellenius et al., 2001; Purvis et al., 2001; Irmler, 2003; Tamutis et al., 2007; Bukejs et al., 2009; Kosewska et al., 2014; Kazlauskeitė et al., 2015).

In studied winter wheat fields, general composition of species assemblage differed between vegetation season of 2013 and one year earlier. Sixteen species observed in 2012 (Gailis & Turka, 2014b) were not present in the studied winter wheat fields during 2013, and seven species observed in 2013 were not established in the fields one year earlier (Table 2). Overall, this should be considered as an unimportant difference of species assemblage that was not caused by studied agro-ecological factors. All species observed in just one vegetation season were subrecedents and represented by only few individuals in the species assemblage. Mostly, infrequent species with low population density are observed in certain area due to their migration fortuities, therefore one species, observed in 2012, can avoid pit-fall traps one year later. For example, *Dolichus halensis* was observed in studied sample plots in 2012, but not in next year. However, in 2013, this species was observed at the edge of arable field located circa 50 meters apart from studied sample plots (Gailis & Turka, 2014b; Telnov et al., 2016).

Table 2. List of ground beetle species observed in studied winter wheat fields during only one vegetation season

Species observed in 2012 only	Species observed in 2013 only
Carabus arcensis (Herbst, 1784)	*Leistus piceus* Frölich, 1799
Dyschirius aeneus (Dejean, 1825)	*Cylindera germanica* (Linnaeus, 1758)
Dyschirius politus (Dejean, 1825)	*Patrobus atrorufus* (Ström, 1768)
Harpalus luteicornis (Duftschmid, 1812)	*Anisodactylus signatus* (Panzer, 1796)
Harpalus tardus (Panzer, 1796)	*Harpalus distinguendus* (Duftschmid, 1812)
Stenolophus mixtus (Herbst, 1784)	*Amara littorea* Thomson, 1857
Demetrias monostigma Samouelle, 1819	
Agonum sexpunctatum (Linnaeus, 1758)	
Pterostichus diligens (Sturm, 1824)	
Pterostichus strenuus (Panzer, 1796)	
Dolichus halensis (Schaller, 1783)	
Amara communis (Panzer, 1797)	
Amara convexior Stephens, 1828	
Amara spreta Dejean, 1831	
Amara fulva (O.F. Müller, 1776)	
Amara aulica (Panzer, 1796)	

One revision also must be done for the list of ground beetles observed in studied winter wheat fields in 2012 (Gailis & Turka, 2014b). Three misidentifications of species were recognized after repeated checking of ground beetle material collected in 2012. Firstly, all *Badister sodalis* specimens were incorrectly identified as *Badister dorsiger*. Secondly, one *Microlestes minutulus* individual was incorrectly identified as *Microlestes maurus*, thus both *Microlestes* species were observed in studied sample plots. Thirdly, some untypical *Bembidion guttula* specimens were misidentified as *Bembidion mannerheimii*. All these misidentifications are acknowledged as minor mistakes, because both *Microlestes* species and *B. sodalis* were subrecedents, and only 34 *B. guttula* individuals from in total 6,237 ones (circa 0.5%) were incorrectly identified as *B. mannerheimii*.

Species dominance structure in differently managed winter wheat fields

In 2013, five species – *Harpalus rufipes*, *Pterostichus niger*, *P. melanarius*, *Poecilus cupreus* and *Bembidion guttula* – were the most frequent species in all studied winter wheat fields. One more species – *Loricera pilicornis* – joined the group of subdominant species in all fields except those with ploughed soil and winter wheat as pre-crop (Fig. 3). *H. rufipes* noticeably over dominated all other species, especially in previously mentioned fields with ploughed soil and winter wheat as pre-crop, where *H. rufipes* reached eudominant status.

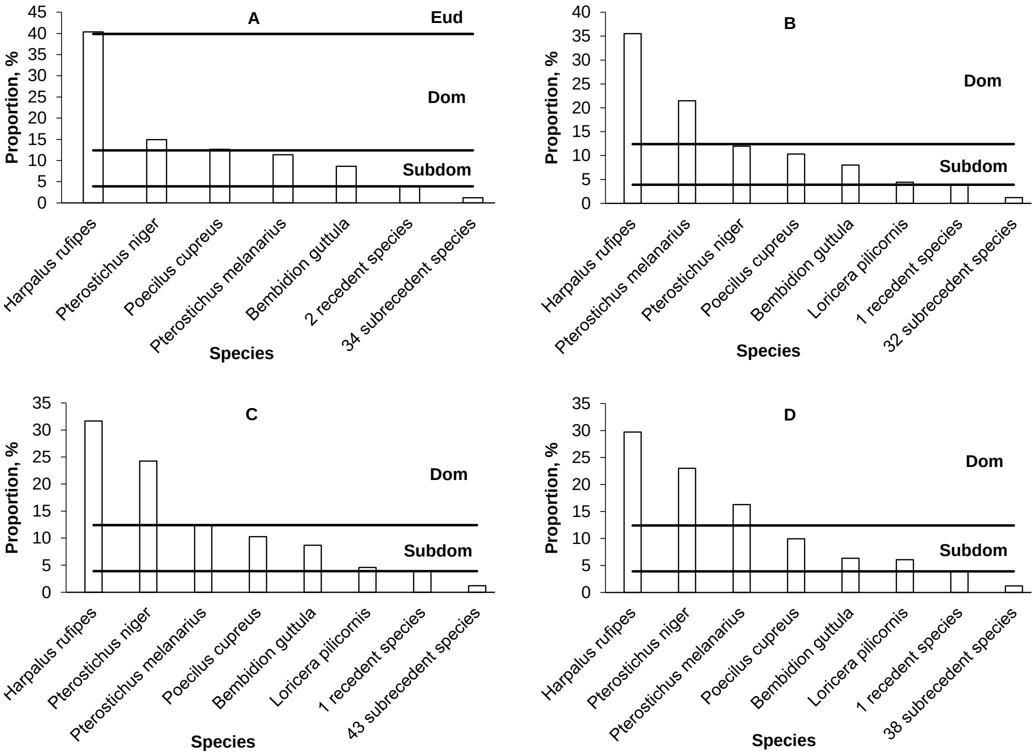

Figure 3. Dominance structure of ground beetles in differently managed winter wheat fields in the season of 2013 (A – ploughed soil, winter wheat as pre-crop; B – harrowed soil, winter wheat as pre-crop; C – ploughed soil, spring rapeseed as pre-crop; D – harrowed soil, spring rapeseed as pre-crop; Eud – eudominants, Dom – dominants, Subdom - subdominants).

In fields with all management types, ground beetle dominance structure observed in the growing season of 2013 noticeably differed from one observed a year earlier. In 2012, *Bembidion guttula* and *Loricera pilicornis* over-dominated other species, but the proportion of *H. rufipes*, *P. cupreus* and both *Pterostichus* was significantly smaller. However, all these species reached dominant or subdominant level in species assemblage in almost all studied winter wheat fields. Two more species – *Bembidion obtusum* and *Amara plebeja* – were among subdominant ground beetles in some fields in 2012, as well (Gailis & Turka, 2014b). In 2013, these species were comparably frequent, but their proportion did not reach at least subdominant level in the fields with any type of management.

Such significant changes of species dominance structure may be explained by noticeably changed activity density of the most frequent species. The activity density of *L. pilicornis*, *B. guttula* and other *Bembidion* species significantly decreased in the studied winter wheat fields in the 2013 comparing with the 2012, and it happened despite more than twice-bigger total density of ground beetles in the growing season of 2013. On the contrary, activity density of other commonest species such as *H. rufipes*, *P. melanarius*, *P. niger* and *P. cupreus* significantly increased in 2013. Between the years, fluctuations of ground beetle populations are common occurrence (Irmler, 2007). It could be explained by several ecological factors, e.g. weather and variation of reproduction cycle which also may depend on climatic conditions. Meteorological situation was significantly different in both years of the research. The growing season of 2012 was cooler and rainier than the season of 2013 (Fig. 2). Irmler (2007) found out that the activity density of *P. niger* and *H. rufipes* negatively correlates with the amount of precipitation, but some *Bembidion species* (e.g. *B. lampros*) increase their activity density if the weather is rainier. *P. cupreus* and some *Harpalus* species activity density correlates with the environmental temperature – temperature increase by 1ºC promotes the increase of beetle's activity density for 6–7 percentage points (Honěk, 1997). Meteorological conditions also can significantly affect presence of food resources for the ground beetles. For example, more precipitation (as it was in 2012) can enhance abundance of springtails (Collembola) by almost 50% (Wu et al., 2014). These soil arthropods are primary food source for *L. pilicornis*, but they are also used as the secondary food by *Bembidion* species (Mundy et al., 2000). *P. niger* and *P. melanarius* are species with varying reproduction cycle. Typically, they are autumn-breeding univoltine species, but their reproduction cycle may become biennial due to unsuitable environmental conditions for individual development (Matalin, 2006; Irmler, 2007; Trushitsyna & Matalin, 2016). Higher population density of those species may be expected in the year after comparably cool summers, and it was observed also in our study. Overall, facts mentioned above explain why the dominance structure of ground beetles was so noticeably different in each vegetation season.

In 2013, proportions of some dominant and subdominant species were noticeably affected by studied agro-ecological factors. In some cases, these effects were similar as in 2012, but in other cases, these effects differed between the years. In 2013, proportion of *P. melanarius* was noticeably bigger in harrowed soil than in ploughed soil. Independently from the pre-crop, *P. melanarius* reached dominant status in harrowed fields while in ploughed soil, this species was subdominant (Fig. 3). This fact disagrees with results of some other researches reporting that *P. melanarius* more often is affected by pre-crop, but not by soil tillage in arable land. It usually prefers agrocenoses without or with minimal crop rotation (Lövei, 1984; Lübke-Al Husein, 2000). It was also evident in our previous research performed in 2012, when *P. melanarius* was significantly less abundant and its proportion was significantly smaller in the fields pre-cropped with spring rapeseed than in fields pre-cropped with winter and spring wheat, but its proportion was not noticeably affected by soil tillage regime (Gailis & Turka, 2014b).

The proportion of *P. niger* was mainly affected by pre-crop in 2013. In the fields pre-cropped with spring rapeseed, the proportion of *P. niger* exceeded 23%, and the species was convincingly dominant. In fields pre-cropped with winter wheat, *P. niger* was comparably less frequent – in ploughed soil, it reached dominant level (proportion 14.9%), but in harrowed soil, *P.niger* was subdominant (proportion less than 12%)

(Fig. 3). Similarly with the season of 2013, also in 2012, *P. niger* was significantly less abundant and its proportion was smaller in fields pre-cropped with winter wheat than in fields with other pre-crops (Gailis & Turka, 2014b). Explanation is not clear for this fact, yet.

Soil tillage regime and pre-crop did not noticeably affect proportion of *B. guttula* in 2013. This species reached subdominant status in all studied winter wheat fields (Fig. 3). In 2012, *B. guttula* was significantly more abundant and more dominant in ploughed fields than in harrowed ones. That connectedness was explained with bare soil surface (without straw aggregations) which is more suitable for small-sized ground beetles to move and to notice a prey (Gailis & Turka, 2014b). In Europe, studies on relationships between other *Bembidion* species and soil tillage intensity are done, but those results also are inconsistent. For example, in one study, *B. obtusum* is reported as the species which equally prefer ploughed and minimally tilled agrocenoses (Holland & Luff, 2000), but results of other study show that this species may be significantly more frequent in minimally tilled soil than in ploughed one (Holland & Reynolds, 2003). It means that soil tillage intensity may be significant affecting factor for *Bembidion* species (also for *B. guttula*) inhabiting arable land, but not always.

In 2013, the proportion of *L. pilicornis* had a tendency to be bigger in fields promoting more decaying plant material on the surface of soil and in the upper layer of soil. This species was subdominant in all fields except those with ploughed soil and winter wheat as the pre-crop (Fig. 3). This fact was more evident in the vegetation season of 2012 when *L. pilicornis* was the most dominating species in all harrowed fields, but the second most abundant species in ploughed fields independently from the pre-crop (Gailis & Turka, 2014b). *L. pilicornis* mostly feeds on springtails (Collembola) which are saprophagous elements of epigeic fauna. Intensive soil tillage, e.g. ploughing, significantly reduces abundance of springtails in ecosystem (Sousa et al., 2004; Brennan et al., 2006) causing lesser abundance of their predators.

Biodiversity of ground beetles in differently managed winter wheat fields

In 2013, in cases when both agro-ecological factors – soil tillage intensity and pre-crop – were considered independently from each other, weak, but statistically significant correlations were observed between them and ground beetle biodiversity. They showed tendency that soil harrowing and spring rapeseed as the pre-crop promoted higher biodiversity of ground beetles if comparing with soil ploughing and winter wheat as the pre-crop, accordingly (Fig. 4).

Combined effect of both agro-ecological factors was more noticeable, but not in all cases. In ploughed soil, moderate correlation was observed between ground beetle biodiversity and pre-crop – spring rapeseed promoted significantly higher biodiversity than winter wheat. In harrowed soil, pre-crop did not significantly affect ground beetle biodiversity; however, it was higher in the fields pre-cropped with rapeseed (Fig. 4).

Moderate negative correlation was observed between biodiversity and soil tillage intensity in the fields pre-cropped with the winter wheat. In these fields, harrowed soil promoted significantly higher biodiversity of ground beetles than ploughed soil. In fields pre-cropped with spring rapeseed, soil tillage intensity did not significantly affect biodiversity, however non-inverse soil tillage had a tendency to promote higher biodiversity than soil ploughing (Fig. 4).

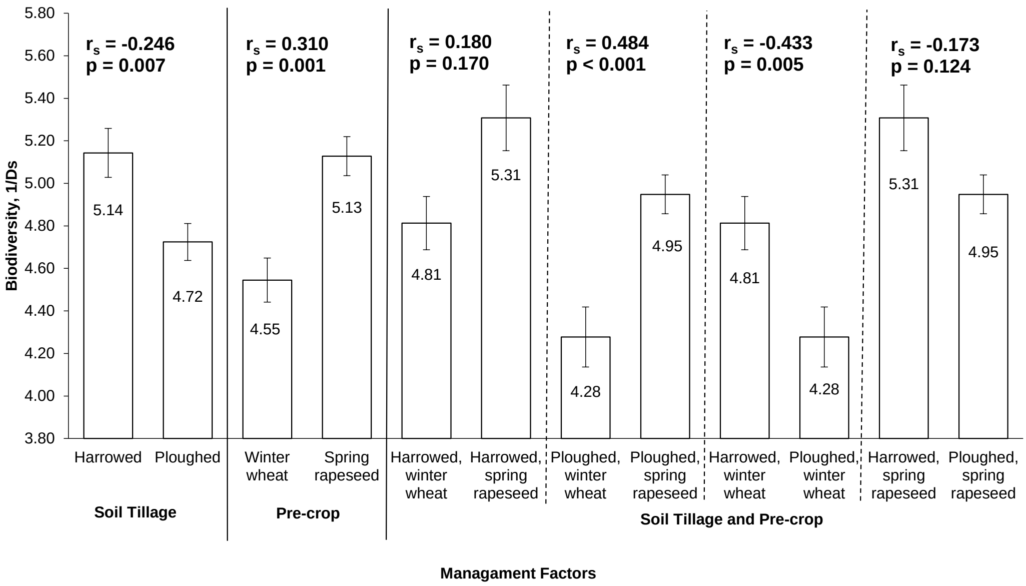

Figure 4. Ground beetle biodiversity's dependence from the soil tillage regime and pre-crop in winter wheat fields in 2013.

Similarly with the species dominance structure, also connectednesses between biodiversity of ground beetles and studied agro-ecological factors were noticeably different between the 2013 and previous season. In 2012, biodiversity mostly positively correlated with the intensity of soil tillage – harrowed soil promoted higher ground beetle biodiversity only in the fields pre-cropped with spring wheat, but in other fields (pre-crops: winter wheat and spring rapeseed), biodiversity was higher in ploughed soil. In harrowed soil, spring rapeseed as the pre-crop promoted significantly lower biodiversity than the wheat as the pre-crop. But in ploughed soil, the highest biodiversity was observed in the fields pre-cropped with the rapeseed (Gailis & Turka, 2014b), and this was the only analogous case in both years.

In general, direct connection between ground beetle biodiversity and soil tillage intensity is still unclear (Holland & Luff, 2000; Roger-Estrade et al., 2010). Many studies show that intensive soil tillage and loosening simplifies species assemblage and reduces biodiversity of ground beetles due to the negative effect to different trophic and body size groups of the beetles. Soil ploughing can reduce population density or fully eliminate *Carabus* and other big-sized ground beetles from the ecosystem (Cárcamo, 1995; Aviron et al., 2005; Cole et al., 2005; Hatten et al., 2007; Skłodowski, 2014). On the contrary, other studies report that soil ploughing and non-inverse tillage have similar influence on ground beetle biodiversity in arable land (Booij & Noorlander, 1992; Andersen, 2003; Belaoussoff et al., 2003; Mason et al., 2006; Twardowski, 2006; Lalonde et al., 2012), or that soil ploughing is more favourable for ground beetles than the reduced tillage (Hole et al., 2005). There are also papers available showing that soil tillage intensity can be affecting factor for some trophic groups of ground beetles. For example, Kosewska et al. (2014) found out that omnivorous species are negatively affected by soil ploughing, but carnivorous species (all body size groups) do not react to different soil tillage intensity. Other study reports that soil ploughing reduced (27%), but non-inverse soil tillage significantly promoted (26%) ground beetle density in arable fields (Thorbek & Bilde, 2004).

More likely, in the harrowed soil, bigger biodiversity of ground beetles can be explained by denser and more diverse weed vegetation that was observed in all harrowed sample plots during 2013, but only in sample plots pre-cropped with spring wheat in 2012. In the arable land, presence of weeds enhances biodiversity of plants, but vegetation that is more diverse enhances biodiversity of invertebrates including ground beetles. Presence of weeds also creates thicker vegetation compensating thin vegetation of wheat promoted by long-term non-inverse soil tillage. Weeds provide more various shelters and additional food resources – attracted phytophagous invertebrates for carnivorous ground beetles and seeds and seedlings for herbivorous ground beetles (Cromar et al., 1999; Norris & Kogan, 2000; Pfiffner & Luka, 2003; Hole et al., 2005; Diehl et al., 2012; Saska et al., 2014). In our sample plots, herbicides were used during the growing season of 2013 just as one year previously. However, weed control was comparably effective only in 2012, when noticeable weed vegetation remained only in harrowed sample plots pre-cropped with spring wheat. During the growing season of 2013, weed density did not significantly decrease or even increased after herbicide application in harrowed sample plots (Fig. 1). Perhaps this is the main reason why higher biodiversity of ground beetles was observed in harrowed soil (all pre-crops) in 2013, but mostly in ploughed soil – in 2012 (excepting fields pre-cropped with spring wheat).

There is one more issue that is complicated. In the season of 2013, biodiversity of ground beetles overall was noticeably lower than one year earlier. The highest biodiversity was observed in harrowed fields pre-cropped with spring rapeseed, but contrary situation was observed in the growing season of 2012, when this combination of soil tillage and pre-crop promoted the lowest biodiversity of ground beetles (Gailis & Turka, 2014b). However, this management regime was the single one promoting similar biodiversity of ground beetles in both study seasons, while in the fields with other management regimes, biodiversity noticeably decreased in the 2013 comparing with previous year. This decrease may be explained with less balanced species assemblages in the greatest part of studied winter wheat fields in the second study year. More suitable environmental conditions for *H. rufipes*, *P. niger* and *P. melanarius* and less suitable conditions for *L. pilicornis*, *Bembidion* and other species promoted a noticeable dominance of one or two species over the other ones in 2013. In the harrowed fields pre-cropped with rapeseed, environmental circumstances enabled *P. melanarius* to increase its proportion until dominant level in 2013, while in 2012 this species was significantly less frequent in the fields with such type of management (Gailis & Turka, 2014b). This factor maintained mainly similarly balanced species assemblage and also similar biodiversity of ground beetles in these fields in both study seasons. Partly such differences of ground beetle species' proportions may be explained by significantly different climatic conditions between study seasons. As it was discussed previously, air temperature and precipitation influence ground beetle breeding cycles, presence of their prey in the agro-ecosystem and possibly other still unknown environmental factors differently affecting densities of various ground beetle populations and also biodiversity. However, our study is still too short to do valid conclusions about environmental factors causing differences of assemblages and biodiversity of ground beetles inhabiting winter wheat fields located at the same place and having the similar management regime in different years.

CONCLUSIONS

In winter wheat fields located at the same place, ground beetle species assemblage may vary between two consecutive vegetation seasons. However, these are minor differences connected with migration fortuities of subrecedent species, but not with studied agro-ecological factors – soil tillage and crop rotation.

Ground beetle species dominance structure may be significantly different in the same winter wheat fields during consecutive years. Species dominating in the first vegetation season may become subdominant in the second season. And contrary –former subdominants may reach dominant or eudominant state during the second season.

Different soil tillage regimes and different crop rotation schemes are affecting the dominance structure of ground beetles inhabiting winter wheat fields. However, this effect may be different between two years and is still unclear. More likely, both studied agro-ecological factors are combining themselves with other environmental factors, and those combinations differently affect proportion of different ground beetle species in the species assemblage.

Soil tillage regimes and different pre-crops may significantly affect ground beetle biodiversity in winter wheat fields. However, the effect of those factors may depend on other agro-ecological factors, e.g., efficiency of herbicide application. If weed control was successful, then ploughed soil in combination with spring rapeseed as pre-crop promotes significantly higher biodiversity than harrowed soil and wheat as pre-crop. Otherwise, significantly higher ground beetle biodiversity is observed in harrowed soil independently from the pre-crop.

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Evaluation and optimization of milking in some Polish dairy farms differed in milking parlours

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Abstract. Farms are continuously growing and modernized in Poland during the last years. The increased dairy herds require also modernization of milking equipment. The aim of this paper was to present the main criteria, which could be used for the solution of principal questions important for the choosing, optimization and evaluation of milking parlours in conditions of Polish agriculture. The research was conducted on three modern dairy farms in Poland to assess effectiveness of different milking parlours use. The first farm with 60 cows was equipped by side by side milking parlour, the second farm with 85 cows was equipped by herringbone milking parlour and the third farm had 80 cows and autotandem milking parlour. The choosing and evaluation of milking parlours parameters were based on the available information and results of previous research in dairy farms in the Poland, using the mathematical model created in the Czech Republic. Time for milking and final specific direct costs were main parameters which enable evaluation and choosing of suitable milking parlour for the dairy farm. The results of measurement and calculation in current farms were compared with possible future enlarged farms to capacity of 200 cows.

Key words: milking parlours, dairy farms, costs, cows, milking process.

INTRODUCTION

Livestock production in countries with intensive agriculture is undergoing big and rapid changes. Number of farms are expanding and increasing the average annual milk production per cow. These factors lead to modernization of milking equipment. European housing systems are steadily changing from tied barns towards loose barns and larger herd sizes (Maton et al., 1985; Bottema, 1992; Hansen, 1999; Bieda & Herbut, 2007; Gaworski et al., 2013; Gaworski & Leola, 2014; Gaworski & Priekulis, 2014; Gaworski & Priekulis, 2015). Due to these changes, many dairy farmers will have to design and build new milking parlour systems.

The milking process is the key operation on dairy farms. The function of milking parlour is one of the factors which affect the efficiency of milk production on the farm. There are many problems which influence the choosing and proper use of milking parlour. Some of them should be solved in advance during the preparation and design of dairy farm.

Modern large-scale farms require appropriate modern technical equipment. Equipment producers want very often to sell farmers the most expensive product which is not always appropriate. Operation is affected e.g. by selected number of milking stalls, by high or low number of milkers, sometimes by incorrectly selected or by choosing insufficient automation equipment. Therefore, it is important to compare different possibilities of milking parlours and try to find the strengths and weaknesses of some proposals. Model calculations allow comparing options and making decision taking into account the accurate and uniform criteria correctly according to the results of calculations.

The same milking parlours have different operating conditions in different countries around the world. Dairy farms in Poland are interesting, because traditional small farms are rapidly growing and milking technology should be modernized. For these farms it seems to be useful to calculate (model in advance) different variants of equipment and operating conditions by precisely selected and uniform criteria. The objective of the paper was to present the main criteria, which could be used for the solution of principal questions important for the choosing, optimization and evaluation of milking parlours in conditions of Polish agriculture.

MATERIALS AND METHODS

Many books, reports and scientific publications present results of research and recommendations focused on the problems of AMS (automatic milking system), usually also including comparison of AMS and milking parlours, in some publications information related to problems of performance and economic analysis (Bottema, 1992; Kic & Nehasilova, 1997; Kic, 1998; Priekulis & Laurs, 2012).

Leading companies producing milking equipment usually offer a variety of constructions of milking parlours recommended for different number of cows on the farm. They also recommend the possible level of automation and number of milkers which should work in the milking process (Brunsch et al., 1996; Dolezal et al., 2000; Chiumenti, 2004). But there are rather big differences in local conditions of the farms according to the production, economic, market and labour situation of the country or province. Although the use of AMS for large farms with a big capacity is developing, the high cost of this solution discourages many farmers. The question for medium and large farms is to currently choose an appropriate type of milking parlour.

It is possible to say that there are two divergent interests and goals in choosing the appropriate type of milking parlour. On the one hand there is interest of manufacturer and dealer who strives for the highest price contract and on the other hand, a farmer who would like to receive the best parlour, but for the price as favourable as possible, i.e. the lowest investment costs.

There are various practical recommendations in the literature, however, there are usually not sub-economic data included which result in a specific numerical data, characterizing the overall result of milking parlour solutions. Some publications (Provolo, 1992; Provolo & Marcon, 1993) present models focused on the choosing of milking parlours, but not in a complete universal approach which could be adapted everywhere. Results of research and basic equations used for calculation of several parameters of milking parlours are presented by Gaworski & Priekulis (2014). Similar calculations, completed with several important economic results which are valid for rotary milking parlours are presented by Ozolins et al. (2012).

The question is which criteria would be suitable to determine the type of milking parlour for each farm. If we know them, according to them can be evaluated different milking parlours, as well as we follow them when consider specific aspects and individual issues which influence the selection of milking parlour for the farm.

For objective assessment and selection of milking parlours there can be used and considered a lot of different aspects, e.g.: animal welfare and ventilation system (Herbut et al., 2012; Herbut & Angrecka, 2015; Herbut et al., 2015), capacity, price, the number of milkers, the complexity and sophistication of the operation, reliability, the dimensions (Gómez et al., 2017) and complicated installation in the building, demand of maintenance and service, and some other aspects like producer satisfaction (Wagner et al., 2001).

Overestimating or underestimating some aspects may result in problems during the normal operation of the milking parlour in practice and thus negatively affect the operation of the farm. In some cases this may lead to unnecessary wastage of finance for investment, without any real benefit to the operation of the farm.

To develop problem concerning evaluation of milking process, three farms typical for current situation in the Polish conditions were included in the investigations. All data used for the calculation were based on the data from modern dairy farms in the Poland.

The Farm 1 with 60 cows was equipped by side by side milking parlour (2 × 5). The Farm 2 with 85 cows was equipped by herringbone milking parlour (2 × 7) and the Farm 3 had 80 cows and autotandem milking parlour (2 × 3). The investigated dairy farms were under milk recording system. The following annual milk yield per cow was identified in particular farms (in kg cow^{-1} $year^{-1}$): 8,400 (Farm 1), 8,700 (Farm 2) and 8,200 (Farm 3). In all farms cow herds weren't divided into technological groups. The Holstein Friesians breed of dairy cattle was kept in all investigated farms.

There were calculated criteria and compared results between current situation and future, when increased herd size of all three farms would be 200 cows.

The first criterion which is important for the function of the farm is the time for milking. The fast milking of all cows enables to have enough free time in which cows have the opportunity to take feed and relax, to go to pasture and so on. The duration of one real milking of all cows can be calculated according to the equation (1).

$$T_{vd} = \frac{N}{Q_{LS}} + T_{pr} \quad (1)$$

where: T_{vd} – the duration of one real milking, min; N – the number of lactating cows on the farm, cow; Q_{LS} – the real capacity of a milking parlour, cow min^{-1}; T_{pr} – the time of working breaks, min.

As regards of a human working process and working operations there is important the total time of duration of one milking including preparatory operations and finishing work after milking, calculated according to the equation (2).

$$T_{cd} = T_{vd} + T_p + T_c \quad (2)$$

where: T_{cd} – the total time of duration of one milking including preparatory operations and finishing work after milking, min; T_p – the time of preparatory work before milking, min; T_c – the time of finishing and cleaning work after milking, min.

When this period T_{cd} is short enough then there is enough time for workers (milkers) to carry out the other activities (feed preparation, cleaning, control of animals etc.). Therefore the time should be a criterion for optimization and the selection of a suitable milking parlour for the farm.

The second decisive criterion for choosing the appropriate milking parlour should be the economic criteria. It is necessary to compare the specific data, which are in this case the final specific direct costs of a milking parlour per cow and year $^uC_{MP}$, which are calculated according to the equation (3) as a sum of specific labour costs of milking per cow and year ^uC_W, specific costs of the milking equipment per cow and year ^uC_P and specific costs ^uC_S of consumed supplies.

These specific costs are sum of many individual parameters, which presentation is not the aim of this paper, mainly because of the extent. Specific labour costs of milking per cow and year ^uC_W are based on the labour need per cow per year and the average salary of milker, specific costs of the milking equipment per cow and year ^uC_P are including the annual percentage of technique depreciation, annual percentage depreciation of parlour building and specific repair costs expressed and calculated by a coefficient of repairs. Specific costs ^uC_S of consumed supplies are including sum of the cost of electricity, water for washing, chemicals for cleaning, disinfectants and towels needed during the milking.

$$^uC_{MP} = {}^uC_W + {}^uC_P + {}^uC_S \quad (3)$$

where: $^uC_{MP}$ – the final specific direct costs of milking parlour, EUR cow^{-1} year^{-1}; ^uC_W – the specific labour costs per cow and year, EUR cow^{-1} year^{-1}; ^uC_P – the specific costs of the milking equipment, EUR cow^{-1} year^{-1}; ^uC_S – the specific costs of consumed supplies, EUR cow^{-1} year^{-1}.

Specific labour costs ^uC_W are calculated on the basis of labour requirements per cow per year T_r (h cow^{-1} year^{-1}) calculated by using equation (4) and average hourly wage of the milker. The labour requirement T_d can be used by equation (5).

$$T_r = \frac{365 \cdot T_d}{60} \quad (4)$$

where: T_r – the labour requirement for milking per cow per year, h cow^{-1} year^{-1}; T_d – the labour requirement during milking per cow per day, min cow^{-1} day^{-1}.

$$T_d = i \cdot \left[\frac{N \cdot (t_{rc} + t_p + t_c) + T_{pr} \cdot n_{ds}}{N} \right] \quad (5)$$

where: i – the number of milking per day, day^{-1}; t_{rc} – the average net labour requirement for milking per cow, min cow^{-1}; t_p – the time of preparatory work before milking calculated per one cow, min cow^{-1}; t_c – the time of finishing and cleaning work after milking calculated per one cow, min cow^{-1}; n_{ds} – the real number of milkers, pers.

Specific costs of the milking equipment $^{u}C_P$ are calculated as specific data of total operating costs of the milking machine converted per one cow. Therefore it includes the amortization of machinery, which is the purchase price of the machine expressed by percentage of machine amortization, further amortization of construction that includes construction costs and percentage of building amortization and the cost of servicing, maintenance and repairs, which are usually expressed as a percentage of planned acquisition costs.

Specific costs of consumed supplies $^{u}C_S$ are calculated as a sum of costs of all necessary operating materials and energy. The consumption of electricity is proportional to the power inputs of motors and all electrical appliances of milking parlour during their operation, water, disinfection etc. All is re-calculated per cow and year (EUR cow^{-1} year^{-1}).

The real number of milkers for the whole farm n_{ds} is the rounded integer n_d. The theoretical required number of milkers n_d is based on the calculation of equation (6).

$$n_d = \frac{Q_{PL}}{W_d} \tag{6}$$

where: n_d – the theoretical required number of milkers per one parlour, pers.; Q_{PL} – the required capacity of the milking parlour, cow min^{-1}; W_d – the working capacity of one milker, cow min^{-1} pers^{-1}.

The maximum reasonable number of milkers per a parlour n_{dm} is a criterion to avoid the idle time or complicated work of milkers. It is calculated by the number of milking stalls m_Z divided by the number of clusters n_s that can operate one milker.

$$n_{dm} = \frac{m_Z}{n_s} \tag{7}$$

where: n_{dm} – the maximum number of milkers per one parlour, pers.; m_Z – the number of milking stalls in milking parlour, pcs; n_s – the maximal number of clusters per milker, pcs.

An important technical parameter is the theoretical number of milking stalls in a parlour m_T, calculated by using equation (8).

$$m_T = Q_{PL} \cdot (t_d + t_v) \tag{8}$$

where: m_T – the theoretical number of cows which are in milking parlour in one moment (this corresponds to theoretical number of milking stalls in parlour), pcs; t_d – the average duration of milking by milking machine per one cow, min; t_v – the average idle time of a cluster, min.

$$t_v = t_n + t_s + t_m \tag{9}$$

where: t_n – the average time for cluster attachment, min; t_s – the average time to remove the cluster, min; t_m – the average time for manipulation with cluster, min.

RESULTS AND DISCUSSION

The results of calculations of current situation at the Farm 1–3, including the abovementioned data on annual milk yield per cow and the others were presented on the Figs 1–3. Two milkers were supposed to work in all variants of milking parlours at all three Farms. There was a standard level of technical equipment in all variants of milking parlours.

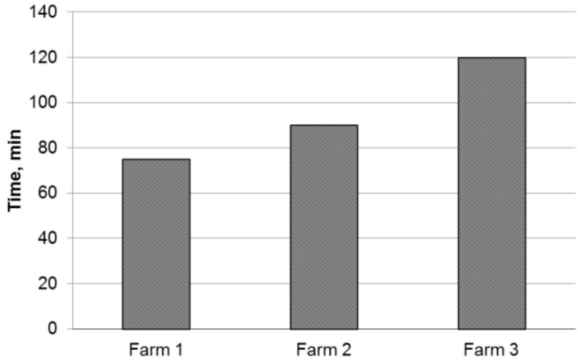

Figure 1. Current time of one milking in dairy Farm 1 (side by side milking parlour), Farm 2 (herringbone milking parlour) and Farm 3 (autotandem milking parlour).

The aim the Fig. 1 was to show the whole time of one milking, which is important for farmer from practical point of view. It is comparable with the results of bigger farm (200 cows) presented in the Fig. 4.

The data concerning time of one milking (Fig. 1) were usable to propose and calculate the index of milking stall load per cow. In order to find the index value, the time of milking was multiplied by number of milking stalls (in the milking parlour) and divided by number of cows in the farm (herd). Differences in the index values for investigated dairy farms were presented in the Fig. 2.

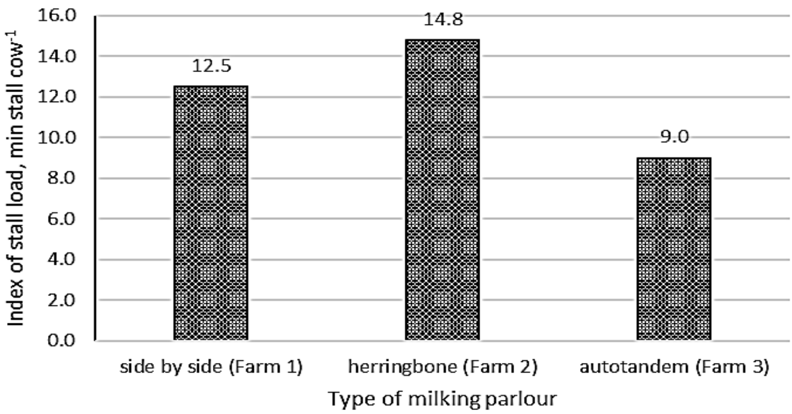

Figure 2. Index of milking stall load for three type of milking parlours in the investigated dairy farms.

The index of milking stall load shows the lowest value for autotandem type of milking parlour. Such result confirms that cow milking in individual stalls like autotanded can reduce time spent by each animal in milking parlour. The side by side and herringbone milking parlours include group milking so as a result it is possible to indicate longer time spent by each cow in milking stall. It can be suggested that higher number of stalls in herringbone milking parlour (14 stalls) decide about higher index of milking stall load in comparison with the index calculated for side by side milking parlour (10 stalls). The lower values of the index of milking stall load can identify shorter time spent by cows in milking parlour and such situation would be recognized as a more comfortable for animals.

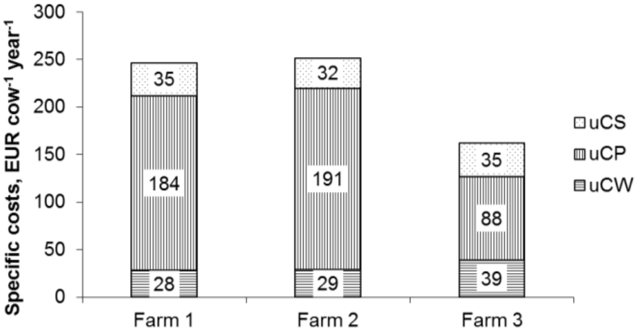

Figure 3. Specific costs of milking in the milking parlours in current situation in dairy Farm 1 (60 cows, side by side milking parlour), Farm 2 (85 cows, herringbone milking parlour), Farm 3 (80 cows, autotandem milking parlour).

The variant in Farm 1 (60 cows) with milking parlour side by side 2 × 5 milking stalls has rather good labour productivity as well as sufficient milking capacity therefore the labour requirements are not too high in this variant and the time of one milking (Fig. 1) is shorter than in the Farm 2 (85 cows) with herringbone milking parlour 2 × 7 and shorter than in the Farm 3 (80 cows) autotandem milking parlour 2 × 3. On the other side milking parlour in the Farms 3 is cheaper (${}^{u}C_{P}$), which results in the lower final specific direct costs of milking parlour ${}^{u}C_{MP}$ (Fig. 3).

The evaluation of current milking conditions is a background for calculations of future situation in all farms with increased number 200 cows. There are calculated and checked some basic principle parameters.

The real values of number of milking stalls in a parlour m_Z and real number of milkers n_{ds}, the theoretical number of milking stalls in a parlour m_T according to the equation (8), the theoretical required number of milkers n_d based on the calculation of equation (6) and the maximum reasonable number of milkers per a parlour n_{dm} according to the equation (7) are presented in the Table 1.

Table 1. Real and theoretical values of main parameters of milking parlours in the Farms 1–3

Parameter	Farm 1	Farm 2	Farm 3
m_Z	10	14	6
m_T	21	18	10
n_{ds}	2	2	2
n_{dm}	1.3	2	1.5
n_d	2.8	2.6	2.6

Explanation: m_Z – the number of milking stalls in milking parlour; m_T – the theoretical number of milking stalls in parlour; n_{ds} – the real number of milkers; n_{dm} – the maximum number of milkers per one parlour; n_d – the theoretical required number of milkers per one parlour.

The theoretical values are calculated with respect to the current duration of one real milking time T_{vd} and the total time of duration of one milking including preparatory operations and finishing work after milking T_{cd}.

Calculated numbers of milking stalls m_T which should be in the milking parlours in farms with future capacity 200 cows are in all Farms 1–3 bigger than real number of milking stalls m_Z in currently used milking parlours. It means that the time of milking will be longer. It is confirmed by results presented in the Fig. 3. Number of milkers n_{ds} should be theoretically higher (n_d), but on the other hand the maximum reasonable number of milkers per a parlour n_{dm} is lower. In the case of the Farms 1 and 2 it is even lower than currently working milkers n_{dm}.

The results of calculations of the farms with increased future herd size to 200 cows are presented on the Figs 4 and 5. The time per one milking is increased and differences between three milking parlours are more obvious (Fig. 4). The variant at Farm 1 with milking parlour Side by Side 2 × 5 milking stalls is thanks to good labour productivity as well as sufficient milking capacity still acceptable as the time of one milking which can be expected (2.5 h) is shorter than in the Farm 2 (3 h) with herringbone milking parlour 2 × 7 and shorten than in the Farm 3 (4.5 h) autotandem milking parlour 2 × 3.

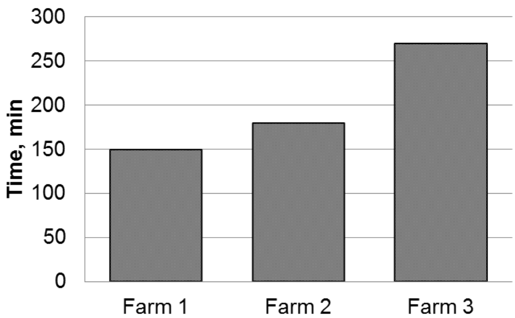

Figure 4. Time of one milking at dairy Farm 1, Farm 2, Farm 3 in future with 200 cows.

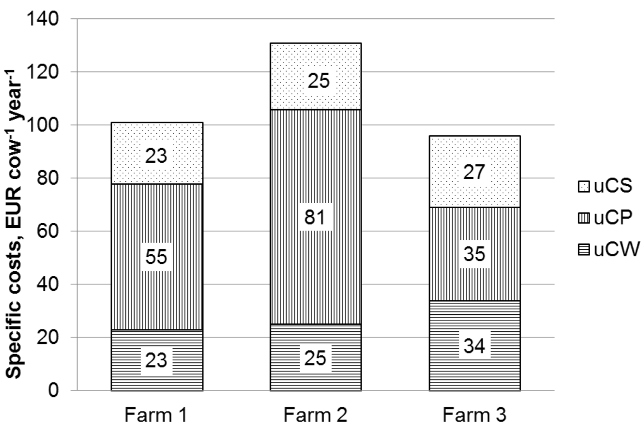

Figure 5. Specific costs of milking at dairy Farm 1, Farm 2, Farm 3 in future with 200 cows.

The final specific direct costs ${}^{u}C_{MP}$ of milking calculated per one cow and year (Fig. 5) are dramatically lower than the current situation in all three farms (Fig. 3). It looks that the best results are achieved in the Farm 1, equipped with the milking parlour Side by Side 2 × 5 milking stalls. Specific direct costs are rather low and the time of milking is the shortest from all three studied farms. The Farm 3 has slightly lower specific direct costs, but time of milking is very long; it could complicate the organization of all technological activities in the farm during the whole day. In the case of increased capacity of farms it is recommendable to modernise the milking parlour and install the new one with bigger capacity. The final decision of choosing the suitable milking parlour will depend on the priorities of the farmer, if he prefers cheaper solution or more expensive variant but with higher capacity and shorter time of milking.

Currently there are a variety of mathematical models, including stochastic models (Nitzan et al., 2006) which can help us to optimize the solution of various functional dependencies. It is always necessary to find appropriate criteria for the decision-making process. Some results of optimization and calculation based on mathematical model focused on the conditions of dairy farms and milking production in Czech Republic presented by Kic (2015a, 2015b) constitute one of the examples to develop some considerations concerning increase in milking effectiveness.

CONCLUSIONS

The time for milking and the final specific direct costs are the main parameters which enable evaluation and choosing of suitable milking parlour for the dairy farm. Both previous mentioned parameters in proposed methodology include the main technical parameters, indicators of labour productivity and economic criteria which can be used for determination of optimal parameters of milking parlour.

Calculation for all evaluated farms showed that the increased capacity from current situation (60, 85 and 80 cows) to the capacity 200 cows brings significantly lower final specific direct costs of milking parlour (reduction about 40% in the Farm 1, about 48% in the Farm and about 41% in the Farm 3). The time of one milking at all dairy farms is significantly increased; the biggest extension of milking time is in the farm equipped with the smallest milking parlour (Farm 3).

It is advantage that the calculation using this model allows, unlike the calculations solved earlier by other authors, to change all basic parameters of the construction and operation of the milking parlour on dairy farms. The preliminary calculations in the preparatory phase before developing a project enable to evaluate (positives and negatives) various solutions of milking parlours. The evaluation of existing milking parlours in the farms can help to improve the milking process and operations from the point of view of either technical improvement or improved activity of milkers, especially for the future development of farms.

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High-temperature effects on seed germination of fourteen Kentucky bluegrass (*Poa pratensis* L.) cultivars

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Abstract. Kentucky bluegrass (*Poa pratensis* L.) is a perennial cool-season grass commonly used for sport and ornamental turfgrasses in transition zones. It is a rather difficult species to establish due to slow germination and the relatively moderate growth rate of seedlings. Early autumn is considered the best time for sowing Kentucky bluegrass in temperate regions. Spring sowing is not recommended as low soil moisture and high temperatures can have a negative impact on germination. However, unavoidable circumstances often force turfgrasses to be sown in spring with high probability of failure. The risk of failure may increase in the near future as a consequence of climate change, so more knowledge is required on the ability of Kentucky bluegrass cultivars to germinate at high temperatures. A laboratory study evaluated the germination response of fourteen cultivars selected among those most used in northern Italy. They were compared in a conditioning chamber under five regimes of alternating temperatures (20/30 °C, 23/33 °C, 26/36 °C, 29/39 °C, 32/42 °C). Germination was recorded weekly starting from sowing. The germination patterns were similar up to 26/36 °C. At 29/39 °C only five cultivars had a germination of over 50%. At the highest temperature regime none of the cultivars had more than 3% germination. It is concluded that only when very extreme high temperatures occur, growers need to pay attention to the choice of cultivars to avoid problems during the germination-emergence phase, but based on the climate change scenario this is likely to happen with greater frequency in the future.

Key words: germination pattern, climate change, germination temperature, turfgrass, establishment.

INTRODUCTION

Kentucky bluegrass (*Poa pratensis* L.) is a perennial cool-season grass native to Europe and Asia widely distributed throughout the cool humid regions where it is commonly used for establishing sport and ornamental turf (Hartley, 1961; Huff, 2003; Raggi et al., 2015). This species is appreciated because it combines high aesthetic value with the ability to recover quickly from damage by means of vigorous creeping rhizomes (Bradley et al., 2010; Bonos & Huff, 2013). Although Kentucky bluegrass can be established vegetatively from rhizomes, the primary method of establishing turfgrass is from seed.

The establishment rate of Kentucky bluegrass is slower than most cool-season species, due mainly to slow germination that takes approximately 2 weeks in soil and low seedling growth rate (Hall, 1996; Brede, 2000; Huff, 2003; Ball, 2009). The slow germination of this species may be due to a higher thermal time (or hydrothermal time) required for the process (Larsen & Bo Martin, 2005). Early autumn is the ideal time for sowing Kentucky bluegrass in transition zones because soil temperature and moisture are generally stable and sufficiently high to ensure germination. Spring sowing is more problematic, especially because soil temperatures vary significantly during the season, however, circumstances often warrant a spring seeding. Due to climate change spring temperatures not favourable for germination of cool-season species are expected to occur more frequently than in the past. This scenario is confirmed by numerous studies that predict an increase in summer days (when daily Tmax is above 25 °C) and tropical nights (when daily Tmin is above 20 °C) for Italy; a lower cumulative precipitation is also expected by simulation models for all Italian regions (Toreti et al., 2008; Medri et al., 2013; IPCC, 2014; Zollo et al., 2015). The Mediterranean region is foreseen to be particularly exposed to possible exceptional temperatures, increasingly frequent extreme weather events and reduced annual precipitation over the coming years (Monai et al., 2010; Medri et al., 2013; Mazdiyasni & AghaKouchach, 2015). The high temperature hardiness of Kentucky bluegrass has been widely studied (Wehner & Watschke, 1981; Minner et al., 1983; Jiang and Huang, 2001; Su et al., 2007), but little information is available on the effects of high temperatures on germination. Larsen et al. (2004) observed that germination percentage of the cultivars Andante and Brodway, assessed in the laboratory, was lower at 25 °C than 10 °C and pointed outsuggested that the optimum temperature for germination is below 25 °C. In a growth chamber experiment Van'T Klooster (2007) found significant differences in temperature germination response among Kentucky bluegrass cultivars under fourteen temperature levels ranging from 5 to 35 °C. Aamlid & Arntsen (1998) tested germination of Kentucky bluegrass under several temperature and continuous light regimes on a thermogradient plate, on paper and in soil in a phytotron, and demonstrated that temperature and cultivar were the main factors driving the germination process.

Since improved cultivars are developed continuously by turfgrass breeders, information on the germination capacity of new materials under high temperature conditions seems to be essential. The objective of this study was therefore to evaluate the germination response to extreme high temperatures of fourteen cultivars of Kentucky bluegrass with the aim of helping turf managers in cultivar selection to successfully establish turfgrasses from seed.

MATERIALS AND METHODS

A study was done at the laboratory of the Agricultural Research Council in Tavazzano (Lodi), Italy, to define the germination response of fourteen Kentucky bluegrass cultivars at five levels of alternating temperatures. The experiment was conducted during spring 2015 in conditioning chambers (mod. CRIOCABIN 2004, Cavallo srl, Milan). The Kentucky bluegrass cultivars compared were 'Balin', 'Barimpala', 'Blue Sapphire', 'Brooklawn', 'Evora', 'Geronimo', 'JumpStart', ‘Marauder’, ‘Mercury’, ‘Moonlight Slt’, ‘Nublue Plus’, ‘Platini’, ‘Right’ and ‘Sobra’. These cultivars were chosen on the basis of their current availability on the Italian turfgrass market. The seeds were pre-chilled at a constant temperature of 5 °C for 5 days according to ISTA rules. Six replicates of fifty seeds for each of the cultivars were then incubated at five levels of alternating temperatures: 20/30, 23/33, 26/36, 29/39, 32/42 °C and photoperiod of 8h darkness/16h light. Irradiance was provided by cold white fluorescent tubes at 880 lux m^{-2} during the higher temperature period. In each replicate, the seeds were placed on filter paper saturated with deionized water (4.0 ml) in glass Petri dishes (11 cm diameter). Additional deionized water was added weekly as needed to keep the filter paper moist. The experimental design was completely randomized. Germinated seeds were counted weekly. The experiment was stopped after 28 days, when germinated seeds were no longer observed.

Data analysis

Germination patterns and t50

The germination pattern was analyzed using a logistic function in the Bioassay97 program (Onofri 2001) as follows:

$$\mathrm{GP} = \frac{M}{1 + e^{a \cdot (\ln(t+0{,}0000001) - \ln b)}} \tag{1}$$

where GP is the germination percentage on total sown seeds, M is the percentage of maximum germination (higher asymptote), t is the time (in days), a represents the slope of the curve, and b the inflexion point.

The goodness-of-fit was evaluated with the model efficiency index (EF; Loague & Green, 1991). The model EF was calculated as follows:

$$\mathrm{EF} = \frac{\sum(Oi - \bar{\mathrm{O}})^2 - \sum(Pi - Oi)^2}{\sum(Oi - \bar{\mathrm{O}})^2} \tag{2}$$

where Pi is the predicted value, Oi is the observed value, and $\bar{O}$ is the mean of the observed values. The value of EF can range from -∞ to 1. For an ideal fit, the EF value equals 1.

The times necessary for the germination of half of final germinated seeds (t_{50}), which in the above equation correspond to the inflexion points (b), were considered statistically different (0.05 probability level) according to the criterion that their respective 95% confidence intervals did not overlap, as already adopted in other studies (Loddo et al., 2012, 2013).

Final germination percentage

Data of final germination percentage (number of germinated seeds on total sown seeds per sample) were transformed by the arcsine of square root transformation to reduce non-normality of the dataset distribution. This result was confirmed by analysing plots of residuals. ANOVA was performed on transformed data using the general linear models module of STATISTICA 7.1 (StatSoft Inc., 2005). Graphs and discussion are based on untransformed data. Fisher’s protected least significant difference test was used at the 0.05 level of probability to identify significant differences between means.

RESULTS AND DISCUSSION

At alternating temperature of 32/42 °C none of the tested cultivars germinated, with the exception of very few seeds of ‘Jumpstart’ and ‘Evora’ (Fig. 1). For all the other cultivars, the 29/39 °C treatment was the highest temperature regime where germination was observed. Germination was high under temperature regimes of 20/30, 23/33 and 26/36 °C, and differences among cultivars were limited. At regime 29/39 °C the cultivars clearly displayed the greatest difference in their ability to germinate, ‘Jumpstart’ showed the highest final germination percentage, while ‘Evora’ and ‘Nublue Plus’ had the lowest (Fig. 1). At 29/39 °C only five cultivars reached at least 50% of germination (‘Balin’, ‘Blue Sapphire’, ‘Brooklawn’, ‘Jumpstart’ and ‘Right’). This result corroborates those of Van‘t Klooster (2007) who observed distinct temperature regimes for germination in various cultivars of Kentucky bluegrass and good germination in some of them at high temperatures (up to 35 °C).

The germination patterns were also similar between cultivars under temperature regimes of 20/30, 23/33, and 26/36 °C (Fig. 2), as confirmed comparing their t_{50} (if their respective 95% confidence intervals do not overlap, they are considered statistically different at $P < 0.05$) (Table 1). The statistically significantly t_{50} was reached later than at the other temperature regimes (95% confidence intervals do not overlap) only under 29/39 °C by the cultivars ‘Moonlight Slt’ and ‘Sobra’. The t_{50} ranged between 4 (at 23/33 °C) and 12 days (at 29/39 °C). For the cultivars ‘Barimpala’, ‘Evora’ and ‘Nublue Plus’ at 29/39 °C there were too few data points for an acceptable non-linear regression, therefore no t_{50} was estimated. EF of the logistic functions were in almost all cases more than 0.9, with just a few exceptions at 29/39 °C; in any case the fitting was satisfactory considering that an EF of 0.5 is suggested as the lower range value for acceptable model prediction (Ramanarayanan et al., 1997).

The different responses observed at the temperature regime of 29/39 °C indicate a real risk of poor germination for several cultivars in the case of spring sowing. A maximum temperature of 39 °C or more in the soil germination layer (0–5 cm) can be expected in temperate climate zones, such as northern Italy, in late spring (June) (Loddo et al., 2015). Due to global changes in climate that are expected to affect not only average, but also extreme temperatures, this critical temperature is also predicted to occur earlier, in early spring. Therefore, in the future, when sowing is planned in either early or late spring, it is reasonable to select cultivars with high germination capacity under high temperatures to avoid possible turf establishment failure.

In the coming years, cultivar selection based on germination rate at high temperatures could be essential to obtain a rapid and regular establishment of Kentucky bluegrass turfgrasses. A sparse stand derived from poor germination due to heat damage has a direct negative influence on turf quality and wear tolerance (Fiorio et al., 2012). Moreover, poor density in the initial phase of establishment may have a negative environmental impact because it leaves gaps for a weed infestation that requires treatment with chemicals.

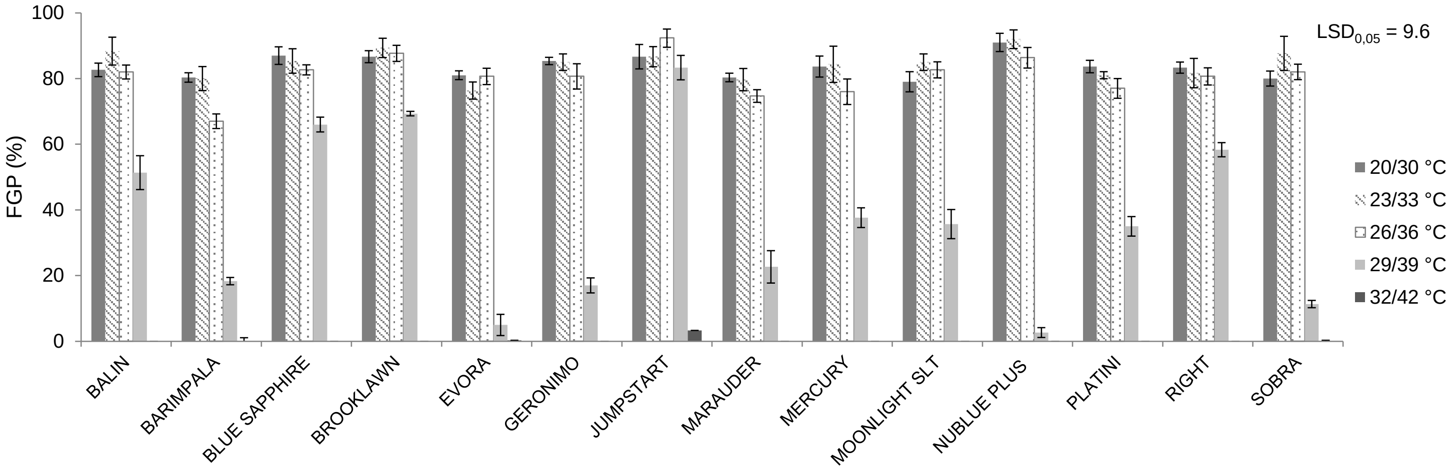

Figure 1. Final germination percentage (FGP) after 28 days in a conditioning chamber under five regimes of alternating temperatures (20/30–32/42 °C) of fourteen Kentucky bluegrass cultivars (bars represent standard error of the mean, LSD value at 0.05 level of significance is reported in figure).

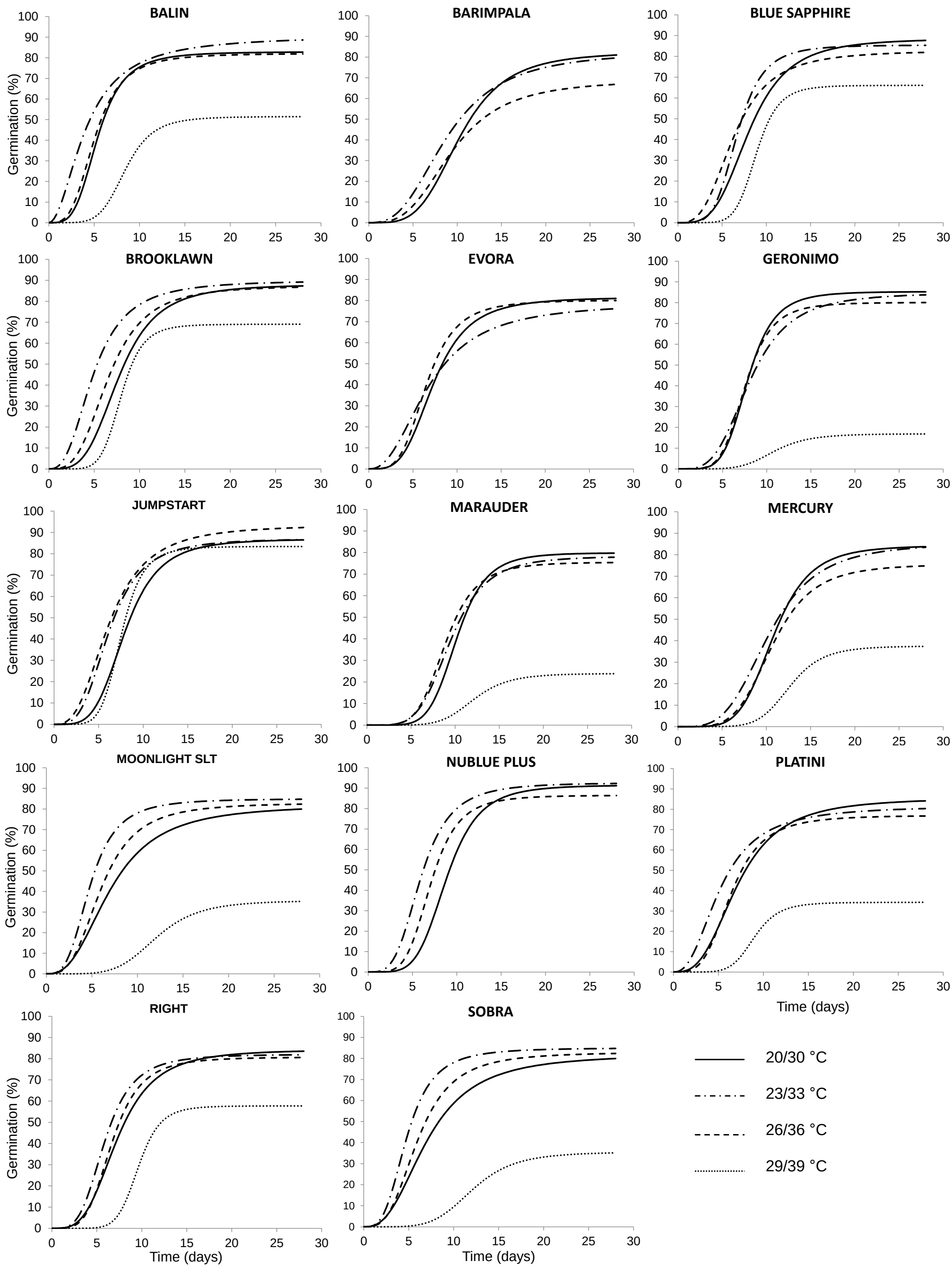

Figure 2. Germination pattern of fourteen Kentucky bluegrass cultivars at four regimes of alternating temperatures (20/30–29/39 °C).

Table 1. Time to reach 50% of germination and efficiency index of the logistic functions for each Kentucky bluegrass cultivar and each alternating temperature regime

Cultivar	Temp. regime (°C)	t_{50} (days)	St. err.	Upper limit (0.95)	Lower limit (0.95)	EF
Balin	20/30	5.39	0.516	6.43	4.35	0.98
	23/33	4.07	1.606	7.37	0.78	0.93
	26/36	5.06	1.150	7.42	2.70	0.96
	29/39	8.44	0.594	9.66	7.22	0.94
Barimpala	20/30	10.28	0.425	11.14	9.42	0.96
	23/33	8.85	0.555	9.99	7.71	0.96
	26/36	9.44	0.460	10.38	8.49	0.97
	29/39	--	--	--	--	--
Blue Sapphire	20/30	8.08	0.165	8.41	7.75	0.99
	23/33	6.73	0.172	7.08	6.37	0.98
	26/36	6.33	0.293	6.93	5.73	0.97
	29/39	8.86	0.598	10.08	7.63	0.97
Brooklawn	20/30	7.73	0.174	8.09	7.38	0.99
	23/33	4.90	0.570	6.07	3.73	0.98
	26/36	6.59	0.311	7.23	5.96	0.96
	29/39	7.96	0.508	9.00	6.91	0.97
Evora	20/30	7.36	0.229	7.83	6.90	0.97
	23/33	6.82	0.918	8.70	4.94	0.86
	26/36	6.55	0.232	7.03	6.08	0.97
	29/39	--	--	--	--	--
Geronimo	20/30	7.84	0.168	8.18	7.50	0.99
	23/33	8.09	0.651	9.43	6.76	0.91
	26/36	7.58	0.297	8.19	6.97	0.97
	29/39	10.85	1.592	14.12	7.59	0.73
Jumpstart	20/30	7.97	0.184	8.34	7.59	0.99
	23/33	6.17	0.393	6.98	5.37	0.95
	26/36	6.14	0.240	6.64	5.65	0.98
	29/39	7.53	0.188	7.92	7.15	0.99
Marauder	20/30	10.16	0.526	11.23	9.10	0.95
	23/33	9.48	0.533	10.57	8.39	0.95
	26/36	8.89	0.371	9.66	8.13	0.97
	29/39	12.14	1.699	15.63	8.66	0.72
Mercury	20/30	10.81	0.338	11.49	10.13	0.98
	23/33	10.28	0.471	11.25	9.32	0.97
	26/36	10.69	0.489	11.69	9.69	0.96
	29/39	12.67	0.505	13.71	11.63	0.97
Moonlight slt	20/30	7.05	0.409	7.88	6.23	0.95
	23/33	4.79	1.402	7.67	1.92	0.96
	26/36	6.04	0.257	6.57	5.51	0.98
	29/39	12.13	0.518	13.19	11.07	0.96
Nublue plus	20/30	8.84	0.201	9.24	8.43	0.99
	23/33	5.95	0.350	6.67	5.23	0.98
	26/36	7.06	0.161	7.39	6.74	0.98
	29/39	--	--	--	--	--

Table 1 (continued)

Platini	20/30	7.13	0.176	7.49	6.77	0.99
	23/33	5.19	0.626	6.48	3.91	0.96
	26/36	6.41	0.237	6.90	5.93	0.98
	29/39	9.00	1.594	12.27	5.73	0.77
Right	20/30	7.27	0.238	7.75	6.79	0.97
	23/33	5.88	0.669	7.26	4.51	0.94
	26/36	6.68	0.219	7.13	6.23	0.97
	29/39	9.61	1.191	12.05	7.17	0.90
Sobra	20/30	7.05	0.409	7.88	6.23	0.95
	23/33	4.79	1.402	7.67	1.92	0.96
	26/36	6.04	0.257	6.57	5.51	0.98
	29/39	12.13	0.518	13.19	11.07	0.96

t_{50} – time to reach 50% of germination; St.err – standard error of t_{50}; Upper and Lower limits (0.95) – 95% confidence limits of t_{50}; EF – efficiency index.

CONCLUSIONS

The results of this study demonstrated good germination of all the Kentucky bluegrass cultivars at the three lower temperature regimes tested (20/30, 23/33, 26/36 °C). There were large and significant differences among cultivars at 29/39 °C with only five reaching almost 50% of germination. At this temperature regime 'Jumpstart' showed the highest germination percentage (83%). Results suggest that breeding for traits such as germination at high temperatures may help develop cultivars that can be sown successfully in the spring. Improved cultivars with these traits will represent an excellent choice for transition zone areas, such as northern Italy, where extreme weather events may become more common as a result of climate change. This study focused on germination, which is certainly a key factor, but it is only a part of the establishment phase that is very critical for growing high-quality durable turfgrass. These results should therefore be validated through a field study not only to compare laboratory tests with field conditions but also to understand the influence of germination temperature response on speed of establishment.

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Biodiesel from tomato seed oil: transesterification and characterisation of chemical-physical properties

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Abstract. The transesterification process of an oil is influenced by four variables: reaction temperature, reaction time, amount of alcohol and amount of reaction catalyst. The cost of production, yield and chemical-physical characteristics are therefore directly dependent on these variables. In this work, tomato seed oil was transesterified and the influence of the quantities of the alcohol (methanol) and catalyst (potassium hydroxide) was tested. The values of total esters, density, kinematic viscosity, iodine value, acid number, linolenic acid, cetane number and residual glycerides in the different biodiesels produced (Bio from A to F), were studied and compared with the current European regulations EN14214: 2014 (Liquid petroleum products - Fatty acid methyl esters for use in diesel engines and heating applications - Requirements and test methods). The six obtained biodiesels yielded between 72.59 (BioB) and 96.8% (BioE) of the total esters. The presence of non-transesterified oil, besides being a yield index, also negatively affects the viscosity at 40 °C of the produced biodiesel. In fact, the only sample with a value within the legal limit was BioE (4.95 $mm^2 s^{-1}$), while the others showed viscosity values higher than the 5.00 $mm^2 s^{-1}$ established by the European regulation. The density, however, always remained within the specified limits, with values between 880 kg m^{-3} in BioE and 891 kg m^{-3} in BioB. The presence of linolenic acid was well below the maximum legal limit in all samples, the iodine value ranged between 119 and 122 g I_2 $100g^{-1}$.

Key words: biodiesel yield, industrial waste, methanol, potassium hydroxide, tomato seed oil, transesterification.

INTRODUCTION

The worldwide demand for energy is increasing day by day, and, consequently, reserves of fossil fuels, which until now have largely satisfied this demand, are becoming depleted. In addition to this problem, there are growing health and environmental concerns, due to fossil fuel emissions. For these reasons, alternative sources of renewable energy are needed (Royon et al., 2007). Some alternatives are different forms of biofuels: liquid or gaseous (Sajjadi et al., 2016) obtained from different types of biomass. Biodiesel is one of the most common biofuels produced from biological resources such as vegetable oils by their transesterification (Kilic et al., 2013). Many studies have been conducted on edible oils: castor oil (Meneghetti et al., 2006), soybean oil (Abrahamsson et al., 2015; Al-Mulla et al., 2015), palm oil (Al-Zuhair et al., 2007; Hameed et al., 2009), peanut oil (Giuffrè et al., 2016a), transformed into biodiesel by transesterification. However, both from an ethical and economic point of view, it would be preferable if biodiesel was produced from material otherwise considered food industry waste. Tomato seeds, obtained from the waste of canning industries, contain around 18– 23% of oil (Giuffrè & Capocasale, 2015), which could be used for biodiesel production (Giuffrè et al., 2015; Giuffrè et al. 2016b).

The transformation of a vegetable oil into biodiesel occurs by a chemical reaction: transesterification, in which the oil is mixed with an alcohol in the presence of a catalyst and is heated. Four variables (reaction temperature, reaction time, amount of alcohol and amount of reaction catalyst) directly influence the conversion of the vegetable oil's fatty acids into their methyl esters (Gupta, 2016).

The most common alcohol used for transesterification is methanol (Demirbas, 2009; Knothe & Steidley, 2009), which substitutes the functional group of a fatty acid with a methylic functional group, with production of three water molecules (Fig. 1). The higher the molar ratio MeOH/oil, the higher the reaction yield.

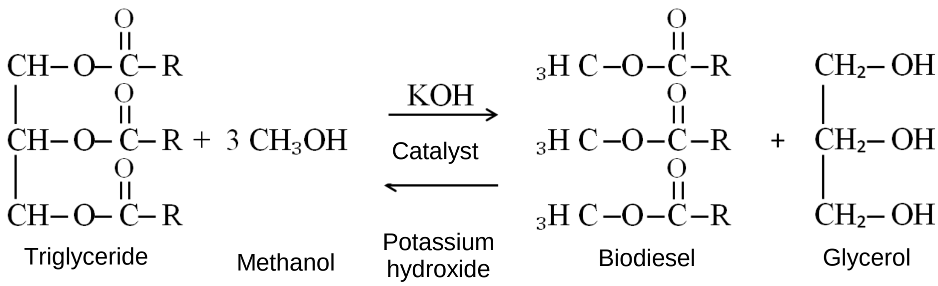

Figure1. Diagram of a chemical reaction of a overall transesterification of a vegetable oil.

Potassium hydroxide is one of the common used catalysts (Macario et al., 2013). It can reduce reaction time and, at the same time, the yield of the reaction. Potassium hydroxide is homogeneous catalyst. Although the removal of homogeneous catalyst after reaction is more difficult than heterogeneous catalysts, use of homogeneous catalyst is gaining attention because of its good catalytic activity, faster reaction rate and moderate reaction temperature conditions, between 50 and 70 °C (Vicente et al., 2007; Georgogianni et al., 2009).

In all chemical reactions, the higher the temperature, the faster the product formation. Furthermore, the variables of temperature and time can influence both the yield and the chemical physical parameters of the final product. For the production of biodiesel, these parameters must stay within those values defined by the European regulation EN14214:2014 (Table 1).

The aim of this work was to study the influence of alcohol and catalyst content on biodiesel production from tomato seed oil by transesterification at a fixed temperature and reaction time. This was both to optimise the conversion parameters from tomato seed oil into biodiesel, and to identify the best product in relation to the current European biodiesel legislation.

Table 1. Parameters of biodiesel described in European regulation EN 14124– 2014 analysed

Characteristics	Unit	Values		Analysis Method
		min	max	
Total Esters	% wt.	96.5		EN 14103
Density 15 °C	kg m^{-3}	860	900	ISO 12185:1996
Viscosity 40 °C	mm^2 s^{-1}	3.5	5.0	ISO 3104:1994
Cetane number		51		EN 5165
Acid value	mg KOH g^{-1}		0.5	EN 14104
Iodine value	g I_2 100 g^{-1}		120.0	EN 14111
Linolenic acid methyl ester	% wt.		12.0	EN 14103
Monoglycerides	% wt.		0.8	EN 14105
Diglycerides	% wt.		0.2	EN 14105
Triglycerides	% wt.		0.2	EN 14105

MATERIALS AND METHODS

Tomato seed oil

The oil used for the experiment was obtained from seeds from a mix of tomato cultivars (*Solanum lycopersicum* L.) discarded after tomato sauce processing in an Italian factory. Oil extraction was conducted by pressing after a complete drying of the seeds. The oil was preserved until its utilisation in dark bottles in cool, dark and dry place.

Chemicals

Methanol, potassium hydroxide, chloroform, acetic acid, Wijis reagent, sodium thiosulphate, starch soluble, ethanol, diethyl ether and phenolphthalein, all of analytical grade; *n*-hexane GC grade ≥ 99.5% were from Panreac (Barcelona, Spain). Sigma-Aldrich (Steinheim, Germany) provided the authentic standard samples of methyl heptadecanoate (purity ≥ 99.0%), N-methyl-N-trimethylsilyl- trifluoroacetamide (TMS) synthesis grade, tricaprin (purity ≥ 99.0%), monolein (purity ≥ 99.0%), diolein (purity ≥ 99.0%), triolein (purity ≥99.0%).

Characterisation of the oil

Each vegetable oil has a characteristic fatty acid composition, which influences its molecular weight. Consequentially, it is necessary to know the composition of these fatty acids to calculate the molar ratio of methanol for esterification. Fatty acid composition was determined by the method reported in CONSLEG 2011 (annex Xa) and is reported in Table 2. The oil's molar mass weight (MW Oil) was calculated using its fatty acid methyl ester (FAME) composition by the following formula (1):

$$MW\ Oil = \frac{\sum_{i=1}^{n} x_i \ \ MWx_i}{100} \tag{1}$$

where: x_i is the mass fraction (in %) of the individual FAME and MWx_i is the molecular weight of the individual FAME.

Table 2. Acid composition and molar mass weight of the tomato seed oil used in the transesterification

Fatty acid	%	Mass weight g mol^{-1}	Mass fraction G mol^{-1}
C14:0	0.11	228.37	0.30
C16:0	14.66	256.42	35.47
C16:1	0.20	254.40	1.08
C17:0	0.07	270.45	0.25
C17:1	0.00	268.43	0.97
C18:0	4.82	284.47	12.94
C18:1	20.44	282.46	54.89
C18:2	57.42	280.44	163.96
C18:3	1.88	278.42	6.24
C20:0	0.34	312.53	1.15
C20:1	0.06	311.51	0.31
Total molar mass weight			277.56

Oil transesterification

The experiment was conducted in a 500 ml Erlenmeyer flask with glass stopper and with the use of a refrigerant. A 50 gram sample of oil was preheated to 55 °C and kept in continuous agitation. For each esterification test different amounts of methanol and catalyst were added (Table 3), the chemical reaction was conducted for one hour in all the tests. The choice of this experimental plan was supported by the literature (Refaat et al., 2008; Argawal et al., 2012) where different combinations of temperature, time reaction, amount of catalyst and alcohol were studied. After transesterification, the obtained methyl esters were separated from glycerol by decantation using a separating funnel, and were subsequently washed with until a neutral pH was reached, and to remove any excess catalyst. The resulting biodiesel was heated at 95 °C until their clarification and to remove any excess water. Each sample was stored in a dark glass bottle at room temperature until analysis.

Table 3. Experimental plan

Sample name	Tomato seed oil / MeOH (molar ratio)	KOH/Tomato seed oil (% wt. ratio)
BioA	1/4	1.0
BioB	1/4	0.5
BioC	1/5	1.0
BioD	1/5	0.5
BioE	1/6	1.0
BioF	1/6	0.5

Acid value

The acid value was calculated according to the EN 14104 method. A sample of biodiesel was dissolved in 50 ml of an ethanol/diethyl ether mixture (1 : 1) and titrated with a 0.1 N potassium hydroxide solution. The results were expressed as mg of potassium hydroxide per gram of biodiesel.

Iodine value

Using the EN 14111 method, a sample of biodiesel was dissolved in 20 ml of glacial acetic acid and 25 ml of Wijs reagent was added. After 5 minutes, a 15 ml of potassium iodide (10% w/v) solution was added and the mixture was titrated with a 0.1 mol l^{-1} sodium thiosulphate solution.

Density

Density was determined by a glass densimeter as described by the ISO 12185:1996 method. The analysis was conducted at 15 ± 0.5 °C and the results were expressed as kg m^{-3}.

Kinematic viscosity

Kinematic viscosity was determined according to the ISO 3104:1994 method by a Ubbelohde viscometer and a thermostatic bath to maintain temperature at 40 ± 1 °C. The kinematic viscosity was expressed as $mm^2\ s^{-1}$.

Cetane number

Cetane number determination is laborious. Freedman & Bagby (1990) and Geller & Goodrum (2004) proposed a method to estimate the cetane number (CN) on the basis of FAME composition of the vegetable oil used. This method was implemented by Giuffrè et al., (2016b) on the basis of the formula (2) proposed by Viola et al. (2011):

$$CN = \sum_{i=1}^{n} x_i\ CN_i \tag{2}$$

where: x_i is the mass fraction of the individual FAME and CN_i is the experimental CN of the individual methyl ester (Viola et al., 2011). It was calculated by the experimental determination of the cetane number of each single FAME following the EN ISO 5165 methods and, then, a relationship between them was found.

Determination of Total Esters and Linoleic acid methyl ester

Gas chromatography-FID analysis was used to determinate the conversion yield after transesterification of each oil sample. The EN 14103 method was applied to verify the yield conversion in Total Esters (TE) in the biodiesel sample and to quantify the single linoleic acid methyl ester. A 250 mg sample of each sample was weighed in a 10 ml glass vial and they were added 5 ml of methyl heptadecanoate in *n*-hexane (10 mg ml^{-1}) was added as an internal standard. A 1 µl sample of this solution was injected in split mode. A Varian GC Thermo 1300 instrument was used, equipped with a fused silica capillary column VARIAN WCOT specific for FAME analysis: length 25 m, ID 0.32 mm and film thickness 0.30 µm. The analytical conditions of the oven were: 140 °C held for 2 minutes, increased by 2 °C min^{-1} to 180 °C held for 5 minutes, increase 5 °C min^{-1} to 230 °C and held for 8 minutes. The injector and detector temperatures were 250 and 280 °C, respectively, with a carrier gas (helium) set at a flow rate of 2.7 ml min^{-1} in constant flow. The TE content was expressed as a percentage and was calculated using the formula (3):

$$TE = \frac{\sum A - AC_{17}}{AC_{17}} \frac{C \times V}{m} 100 \tag{3}$$

where: $\sum A$ is the sum of all the FAME peak areas; AC_{17} is the area of the methyl heptadecanoate; C is the concentration in mg ml^{-1} of the methyl heptadecanoate; V is the volume in ml of the methyl heptadecanoate solution and m is the mass in mg of the sample.

The linoleic acid methyl ester (L) content was expressed as a percentage and was calculated using the formula (4):

$$L = \frac{A_L}{\sum A - AC_{17}} 100 \quad (4)$$

where A_L is the area of linoleic acid methyl ester.

Residual glycerides

The determination of mono-, di- and triglycerides non-transesterified into methyl esters, but residual in the biodiesel sample, was carried out using the EN 14105 method modified as follows: 100 mg of the sample was weighed in a 10 ml glass vial and 200 µl of TMS solution, was added. The vial was hermetically sealed and mixed. After 15 minutes, 2 ml of *n*-hexane were added and 1 µl of the obtained solution was injected in splitless mode into a GC-FID Thermo 1300 trace instrument equipped with a pre-column Restek RXI Guard Column with a length of 5 m, ID of 0.53 mm, and a capillary column Restek Rxi-5HT with a length 15 m, ID of 0.32 mm and film thickness of 0.10 µm. The oven ramp was as follows: 50 °C held for 1 minute, increased to 180 °C at 15 °C min^{-1}, increase to 230 °C at 7 °C min, finally increase to 380 °C at 30 °C min^{-1} and held for 5 minutes. The detector and injector temperatures were 390 °C and 300 °C, respectively, with helium as a carrier gas (4 ml min^{-1} flow rate). The residual glyceride quantity was calculated after injection of mono-, di- and triglyceride standard solution (1,000 mg l^{-1} in *n*-hexane), with tricaprin as an internal standard. This procedure was adopted to calculate the response factor (RF) by the formula (5):

$$RF = \frac{(A_{st} \;\, C_{st})}{(C_i \;\, A_i)} \quad (5)$$

where: A_{st} is the area of the reference standard; C_{st} is the concentration in mg ml^{-1} of the reference standard; C_i is the concentration mg ml^{-1} of the glyceride standard; and A_i is the area of the glyceride standard. RF is necessary to correct the area (A) of the glycerides in the sample and to obtain the corrected area of each peak (A_c) by the formula (6):

$$A_c = A \times RF \quad (6)$$

Statistical analysis

The analyses were conducted in triplicate and the results are reported as the mean ± SD, Excel for Windows software (2010 version) was used. The analysis of variance was conducted by one-way ANOVA to study the significant differences and a Tukey's test was applied at $p < 0.05$ by the software SPSS version 17.0 (SPSS Inc., Chicago, IL, U.S.A.).

RESULTS AND DISCUSSION

Yield of biodiesel

Table 4 shows that the highest conversion into biodiesel was obtained from the sample BioE with a content of total esters of 96.80%, the only sample that gave a value above the minimum limit set by European legislation. The other samples showed a lower rate of conversion. This indicates that methanol values lower than the molar ratio 1/6 and an amount of potassium hydroxide lower than 1% does not yield acceptable conversions, at the temperature and for the time used in this experiment. Refaat et al., 2008 using the same conditions as our best result but using a different vegetable oil (fried sunflower oil) and a different reaction temperature (65 °C) obtained a conversion of 96.10%. A 98.20% yield, from a mix of waste cooking oil, was found under the same transesterification conditions, using only a different temperature of 70 °C (Argawal et al., 2012).

Table 4. Total ester content. Data as expressed as means of three measurement ± DS

	% Total Esters
BioA	76.32 ±1.46 c
BioB	72.59 ± 1.96 d
BioC	87.08 ± 1.45 b
BioD	79.77 ± 1.35 c
BioE	96.80 ±0.12 a
BioF	93.73 ±0.07 a
Sign.	**

Means with different letters are statistically different (**, $p<0.001$).

Parameters of biodiesel quality

Biodiesel quality is characterised by its chemical-physical properties (Table 5). The density ranged from 880 to 891 kg m^{-3}: within the range established by European regulations (Table 1). This parameter varied significantly, in particular it is lower in the samples in which a higher yield was obtained. This is due to the fact that the oil has a higher density than the esters of its fatty acids.

Table 5. Qualitative characteristics of biodiesel samples. Data are expressed as means of three measurements ± DS

	Density kg m^{-3}	Kinematic viscosity mm^2 s^{-1}	Cetane Number	Iodine value g I_2 100 g^{-1}	Acid value mg KOH g^{-1}
BioA	883 ± 1.2 c	7.55 ± 0.16 b	53.2 ± 0.13 c	122 ± 0.1 a	0.14 ± 0.03 a
BioB	891 ± 0.1 a	8.62 ± 0.04 a	53.3 ± 0.12 c	120 ± 1.0 b	0.11 ± 0.01 ab
BioC	881 ± 0.4 d	6.04 ± 0.02 c	53.6 ± 0.09 bc	122 ± 0.4 a	0.11 ± 0.00 ab
BioD	887 ± 0.2 b	7.62 ± 0.07 b	53.6 ± 0.06 bc	119 ± 0.3 b	0.09 ± 0.03 b
BioE	880 ± 0.7 d	4.95 ± 0.03 d	54.2 ± 0.20 a	119 ± 0.3 b	0.06 ± 0.01 b
BioF	886 ± 0.4 b	5.97 ± 0.03 c	54.4 ± 0.15 a	119 ± 0.3 b	0.09 ± 0.03 b
Sign.	**	**	**	**	**

Means in the same column with different letters are statistically different (*, $p < 0.01$; **, $p < 0.001$).

Petrodiesel fuel has a lower density than biodiesel (Lalvani et al., 2015). The density of diesel oil is important because it gives an indication of the delay between the injection and combustion of the fuel in a diesel engine (ignition quality) and the energy per unit mass (specific energy). This can influences the efficiency of the fuel atomization for airless combustion systems.

Kinematic viscosity, expressed as $mm^2\ s^{-1}$, describes a fluid's internal resistance to flow and is influenced by the temperature: an increase in temperature becomes less viscous a liquid. It refers to the thickness of the oil, and is determined by measuring the amount of time in seconds taken for a given measure of oil to pass through an orifice of a specified size (Raj & Sahayaraj, 2010). The viscosity of petrodiesel is often lower than biodiesel (Ramkumar & Kirubakaran, 2016), Kinematic viscosity is the most important property of biodiesel since it affects the operation of fuel injection equipment, particularly at low temperatures when an increase in viscosity affects the fluidity of the fuel (Demirbas, 2009). Moreover, high viscosity may lead to the formation of soot and engine deposits due to insufficient fuel atomization.

BioE was the only sample showing a kinematic viscosity within the legal limit (4.95 $mm^2\ s^{-1}$), significantly lower and different from the other samples. This was due to the presence in the other samples of unconverted oil, which adversely affects the kinematic viscosity.

Another qualitative parameter of a fuel is the cetane number. The cetane number measures the ignition delay of an engine: a lower cetane number produces higher ignition delay. Moreover fuel with low cetane number tend to cause diesel knocking and show increased gaseous and particulate exhaust emissions due to incomplete combustion. Sometimes petrodiesels have lower cetane numbers than biodiesel fuels.

The studied biodiesel samples showed a cetane number from 53.2 to 54.4, therefore all of them were above the minimum limit stated by the EN 14124:2014, which is 51.0.

Different fuels are characterised by a different unsaturated fatty acid composition, which influences some of their important characteristics such as the oxidation stability: the higher the saturation, which means less double bonds, the higher the fuel stability. Moreover, the oxidation stability negatively affects some engine components such as nozzles, piston rings and piston ring grooves because of the formation of deposits. The iodine value is the parameter used to quantify total unsaturation and is expressed by $g\ I_2\ 100\ g^{-1}$ of sample.

The current European legislation states that the iodine value of a biodiesel must not exceed 120 $g\ I_2\ 100\ g^{-1}$ (Table 1). Transesterification significantly affects this parameter. However, tomato seed oil has a high degree of unsaturation, which is then reproduced in biodiesel. In this study, three samples BioD, BioE and BioF did not exceed the legal limit, while the other three were over the legal limit (Table 5).

The acid value is used to evaluate fuel acidity over time. It is expressed as mg of $KOH\ g^{-1}$ of biodiesel. According to EN 14124:2014 the acid value must not exceed 0.5 mg $KOH\ g^{-1}$ of biodiesel (Table 1).

The lowest acid value was found in the BioE sample (0.06%), while the highest was in BioA (0.14%). Therefore, all samples were well below the maximum legal limit (Table 5).

Regarding the iodine value, the presence of linolenic acid increases the instability of biodiesel and thus worsens the biofuel quality, since its three double bonds are easily oxidizable. The legal limit is set at 12% of the total FAME composition, considerably higher than the values found in our samples. The content of linolenic acid was always well below the legal limit (the highest content of 1.88% was found in BioA), as shown in Table 2. This reflects the composition of tomato seed oils. Similarly low figures for tomato seed oils are found in the literature: 1.98% (Zuorro, 2013) and 2.06% (Zuorro et al., 2014).

The vegetable oil was not completely converted into biodiesel. For this reasons, in biodiesel a residual of triglyceride and mono- and diglyceride partially esterified could be found. The presence of these compounds, not only adversely affects the quality parameters of the final product, (i.e. kinematic viscosity and yield), but also causes an engine malfunction because they are not suitable for the diesel cycle. So, the allowed amount of these molecules in biodiesel is regulated, and a maximum limit has been set at 0.8%, 0.2% and 0.2%, respectively, for monoglycerides, diglycerides and triglycerides (Table 1). As reported in Table 6, only BioE respected this statement.

Table 6. Residual of non-transesterified oil. Data are expressed as means of three measurements ± DS

	Monoglyceride %	Diglyceride %	Triglyceride %	Linolenic acid methyl ester %
BioA	2.34 ± 0.03 c	3.16 ± 0.07 c	5.11 ± 0.50 b	1.88 ± 0.05 a
BioB	8.40 ± 0.28 a	5.65 ± 0.52 a	6.29 ± 1.27 a	1.84 ± 0.04 ab
BioC	2.67 ± 0.73 c	1.02 ± 0.33 d	2.22 ± 0.54 cd	1.71 ± 0.10 bc
BioD	4.61 ± 0.21 b	4.46 ± 0.03 b	9.45 ± 1.22 b	1.79 ± 0.09 abc
BioE	0.30 ± 0.01 d	0.11 ± 0.02 e	0.05 ± 0.01 d	1.65 ± 0.03 c
BioF	0.12 ± 0.01 d	0.43 ± 0.02 de	0.15 ± 0.01 c	1.71 ± 0.01 bc
Sign.	**	**	**	*

Means in the same column with different letters are statistically different (*, $p<0.01$; *, $p<0.001$).

CONCLUSIONS

This study showed that the esterification of tomato seed oil could lead to the production of biodiesel. The biodiesel yield very significantly increases both with increasing amounts of methanol and catalyst, and in one case the biodiesel yield complied with the European legislation, which regulates its conversion. However, other combinations of variables - time reaction, temperature reaction, alcohol and catalyst amount -, should be studied to further improve the yield.

Biodiesel Bio E, which was obtained by trans-esterification of tomato seed oil, at 55 °C for one hour of reaction time with an oil/MeOH ratio molar 1/6 and 1% wt. of catalyst in oil, showed the best yield and had the best chemical-physical parameters. These results confirm the possibility that biodiesel could be economically processed from tomato seed oil with characteristics compliant with the European regulation EN14124:2014. Therefore, such biodiesel could be used as an alternative, or in addition, to fossil fuels, which would also help existing energy sources.

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The authors have declared no conflict of interest.

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The effect of immunomodulation composition on systemic immune response and udder health in case of bovine subclinical mastitis

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Abstract. Modulation of mammary gland immune response may offer an alternative to antimicrobial therapy in the treatment of subclinical mastitis. The aim of the study is to investigate the systemic immunomodulating effect and the impact of composition LLG which consists of lysozyme, lactic acid and glycopeptides isolated from *Lactobacillus spp.* on udder health parameters. A total of 10 cows with subclinical mastitis were used in the study – 5 cows (19 udder quarters) in the experimental group and 5 other cows (19 udder quarters) in the control group. The experimental cows received intramammary infusions of LLG, given once per day, 3 times with the intervals of 48 h. Cows from the control group received infusions of sterile 0.15 M NaCl, given in the same way as the treated cows. The following parameters were measured: somatic cell count (SCC), the total number of blood leucocyte, differentiation between banded neutrophils, segmented neutrophils and lymphocytes, peripheral blood mononuclear cells and markers of cell activation. Besides bacteriological culturing was performed. Quarter milk and blood samples were taken several times from the 1st to the 21st day. During the treatment the number of lymphocytes and T helper cells significantly decreased in the blood of the treated group, the CD8+ cells did not change remarkably, the number of CD25+, CD38+, as well as CD69+ and CD95+ cells had diminished during the treatment. On the 21st day a rapid increase of IL-2 receptor bearing cells was detected. A significant elevation of SCC in the treated group was observed but pathogenic bacteria incidence decreased.

Key words: subclinical mastitis; immunomodulation; somatic cell count, lymphocyte subpopulations.

INTRODUCTION

Bovine subclinical mastitis is defined as an inflammatory condition of the mammary gland in response to infection, which is characterized by decreased milk production and altered milk quality but, in contrast to clinical mastitis, is without any visible changes in milk and udder (Harmon et al., 1994; Hillerton & Berry, 2005; Nogueira et al., 2012). The mammary host defence system has evolved into an extremely well-developed, complex, and highly effective barrier against pathogens, integrating both the innate and adaptive immune systems. Modulation of the immune response may offer an alternative to antimicrobial therapy in the treatment of subclinical mastitis. In our chosen strategy of the modulation of host immune response we use a composition of well-known bactericidal protein lysozyme, lactic acid and glycopeptides isolated from *Lactobacillus spp.* (composition LLG). An effective systemic immune reaction is extremely important for a corresponding local response to ensure immune cells in peripheral blood directly involved in resolution of inflammation.

The treatment with immunomodulators has been intensively studied in the past three decades because the induction of systemic immune response could protect cattle from the development of clinical mastitis or lead to self-treating conditions. One of the promising immunomodulation approaches was treatment with cytokines: IL-2, GM-CSF, IL-8. Recombinant bovine IL-2 was used in intramammary treatment to promote T cell immune response and eventually to develop immunological memory (Daley et al., 1992; Erskine et al., 1998; Oviedo-Boyso et al., 2007; Zecconi et al., 2009). Another approach is to use herbal drugs with immunomodulating properties. V.D. Bhatt et al. (2014) obtained results that indicate significant decline of the total bacterial load after treatment with a topical herbal gel. However, the average differences in SCC after treatment were not significant. It has also been reported that intra-mammary administration of platelet concentrate may be useful for a quick resolution of the inflammatory response, which plays a role in tissue damage to the mammary gland and reduces the recurrence rates (Lange-Consiglio et al., 2014).

We have developed and studied the immunomodulation composition LLG which is unique because all the ingredients originate from milk. The aim of the study is to investigate the systemic immunomodulating effect and impact of composition LLG on udder health parameters.

MATERIAL AND METHODS

Study population and design

The clinical trial was conducted from May to June 2015 with lactating Holstein and Latvian Brown dairy cows located in one dairy farm in Latvia. The herd consisted of 320 dairy cows housed in tie-stall arrangement and milked twice a day. The sample of study was subclinically infected cows with somatic cell count (SCC) greater than or equal to 400,000 per mL in cow composite milk. All the cows included in this study had increased somatic cell count at least for two consecutive months. Cows which had received treatment with drugs for any reason within 30 days of the treatment start date were excluded from the study. The study sample consisted of 10 multiparous cows with 8,000 kg average 305-day yield during the previous lactation. Cows were in their 2nd or 3rd lactation, had been lactating for more than 60 days, and were less than 90 days from their expected dry-off dates. All the cows had no history of concurrent disease other than subclinical mastitis, and had insignificant physical examination findings. The study was performed as a simple randomized and placebo-controlled trial.

Immunomodulating composition

For the treatment, a composition of lactic acid, lysozyme and other component from *Lactobacillus spp.* bacteria in 10 mL 0.15 M NaCl solution was applied (composition LLG). The composition was developed in Riga Stradins University, Institute of

Microbiology and Virology (PCT application LV 2015/000005). The sterility of the preparations was checked by inoculation of 0.1 mL from each batch on blood agar, followed by incubation at 37 °C for 48 h, and observation of visible colony forming.

Treatment procedure

The treatment regimen consisted of intramammary infusion in 19 udder quarters of 5 cows of 10 mL LLG, given after milking once per day, 3 times at the intervals of 48 h (day 1, day 3 and day 5). The negative (placebo) control was a 10 mL infusion of sterile 0.15 M NaCl, given in the same way – once per day, 3 times at the intervals of 48 h, administered in 19 quarters of other 5 cows.

Following milking, the teat end was disinfected by scrubbing with a single-use cotton pad saturated with 70% isopropanol. The LLG was then infused through the teat canal in teat cistern using the individual syringe fitted with a sterile J-12 cannula (Jorgensen Laboratories, USA). Following infusion, the mammary gland was massaged upwards briefly to distribute the treatment into the gland. Rectal temperature and milk yield were measured every day to evaluate the effects of treatment on the physiological homeostasis of the animals and on any side effects.

Sampling

Udder quarter milk samples were collected by the project veterinarian (first author) in accordance with Bradley et al. (2012) aseptic technique, from 38 udder quarters five times during the study: day 1 (before treatment), day 3, day 5, day 7 and day 14 after first treatment. Sterile laboratory plastic vials of 40 mL capacity were used for detecting the milk somatic cell count, but sterile no additive tubes of 8 mL capacity (Vacutest Kima, Italy) were used for bacteriological analyses. After collection, milk samples were immediately refrigerated, placed on ice and transported to the laboratory at 4–6 °C. In the laboratory, samples were stored at 4–5 °C while analyses were completed within 12 hours of the milk sample collection on the farm.

Peripheral blood samples were taken by the farm veterinarian from the tail vein of 10 cows four times during the study: day 1 (before treatment), day 3, day 7 and day 21 after the first treatment. Blood samples were collected in tubes containing EDTA as an anti-coagulant (Vacutest Kima, Italy) and immediately transported to the laboratory at 4–6 °C. In the laboratory, analyses were completed within 12 hours of the milk sample collection on the farm.

Evaluation methods

The total number of blood leucocytes was determined with blood cell with 5-part differential haematology analyser (ABX Pentra 60, HORIBA ABX SAS, Japan) according to the standard protocol and differentiation between banded neutrophils, segmented neutrophils and lymphocytes was performed by light microscopy. Peripheral blood mononuclear cells and markers of cell activation were determined using fluorescence-activated cell analysis. CD4+, CD8+, CD14+, CD16+, CD18+,CD19+, CD25+, CD38+, CD69+ and CD95+ cells were detected by a flow cytometer (FACS Calibur, Becton Dickinson, USA) using corresponding mouse antibodies (for example, IgG1 isotope murine monoclonal antibodies, specific for CD4+ and CD8+) according to the manufacturer′s protocol.

Milk SCCs were determined by fluoro-opto-electronic cell counting using a Fossomatic FC (Foss A/S, Denmark) somatic cell counter in accordance with the standard LVS EN ISO 13366-2:2007 ‘Milk – Enumeration of somatic cells – Part 2: Guidance on the operation of fluoro-opto-electronic counters’.

SCC data about days 22, 50 and 83 were obtained from the state institution ‘Agricultural Data Centre’ and they characterise a cow’s composite milk.

Milk samples for bacteriological culturing were serially diluted 3-fold in buffered peptone water (Oxoid, England). Baird Parker agar with egg yolk supplement (Biolife, Italia) for the enumeration of staphylococci was used in accordance with the standard LVS EN ISO 6888-1: 1999/A1:2003 ‘Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (*S. aureus* and other species) – Part 1: Technique using Baird-Parker agar medium – Amendment 1: Inclusion of precision data’. The enumeration of bacteria of the family *Enterobacteriaceae* was carried out on pour plate technique on Violet Red Bile Glucose agar (VRBG, Biolife, Italia) in accordance with the standard LVS ISO 21528-2:2007 ‘Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony-count method’. *E. coli* detection was carried out in accordance with previously described technique (Leininger, Roberson, Elvinger, 2001) but blood agar was used for isolating *Streptococcus* and *Enterococcus spp*. following UK Standards for Microbiology Investigations (UK Standards…, 2014).

S. aureus and *E.coli* was considered as major pathogen while other isolated microorganisms (including coagulase positive and negative staphylococci, environmental streptococci, enterococci, other bacteria of the family *Enterobacteriaceae* instead of coliforms) were defined as minor pathogens in accordance with Reyher et al. (2012).

Statistical analysis

Data were analysed using the GraphPad Prism computer program (Prism 7.0 for Mac, La Jolla, California, USA). Dynamic changes of measured parameters and differences among groups at were assessed on the cow level with ordinary two-way ANOVA (taking into account two main sources of variation – time and treatment) followed by two-stage step-up method of Benjamin, Krieger and Yekutieli or Fisher's LSD test for between-groups comparisons or RM two-way ANOVA followed by Holm-Sidak's multiple comparison test for intra-group comparisons to assess the time factor. Results are expressed as median, more objective measure of central tendency for given data sets, and ± IQR (interquartile range) as dispersion characteristics. Statistical significance was set at $p < 0.05$ for all statistical analyses.

RESULTS AND DISCUSSION

Peripheral blood cell analysis

The infected mammary gland (MG) tissue and milk contain mainly neutrophils, recruated to the site of infection and act as phagocytes (Werfel et al., 1997; Mehrzad et al., 2003). Nonspecific or innate responses are mediated by macrophages, neutrophils, natural killer (NK) cells, cytokines (CK), and complement. Neutrophils are essential cells for innate host defence; the rapid influx of neutrophils with high antimicrobial activity to the foci of infection is the main process that leads to elimination of infection (Mehrzad et al., 2005). If the invading bacteria survive, neutrophil infiltration is replaced by T and B lymphocytes and monocytes (Raihard & Riollet, 2003). Another important cell involved in antimicrobial defence is the monocyte/macrophage expressed CD14 receptor – high-affinity protein for the complex of bacterial LPS and LPS-LBP protein (Nemchinov et al., 2006). Not only does the number of polymorph nuclear neutrophilic leukocytes (PMNL) increase enormously but their defensive responses (e.g., phagocytic activity) increase as well (Targowski, 1983).

No remarkable changes in the total number of leucocytes, neutrophils and CD14+ monocytes in peripheral blood were detected in the treated and the control groups during the observation (3rd to 21st day) so it may be concluded that phagocytic activity does not differ meaningfully between both groups after the treatment with LLG (data not shown). However, the number of lymphocytes was changed significantly in the treated group: during the treatment (on day 3) and two days after the end of the intramammary administration of the immunomodulator (on day 7), the number of lymphocytes had decreased in comparison with the pre-treatment amount ($p = 0.0317$ and $p = 0.013$). Sixteen days after the last treatment (on day 21) the number of lymphocytes increased and was close to the pre-treatment amount (Fig. 1).

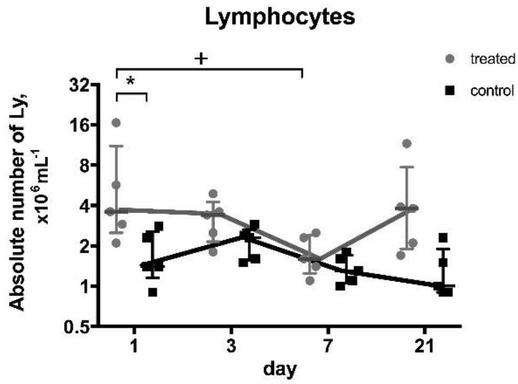

Figure 1. Number of lymphocytes (median ± IQR) in treated (n = 5) and control (n = 5) cows' peripheral blood before the treatment (day 1), during the treatment (day 3) and after the treatment from 7th to 21st day. + P value vs day 1; * P value vs control.

Activation of specific immune defences results in the selective elimination of mastitis-causing pathogens (Paape, 2003). Recognition of pathogenic factors is mediated by several lymphocyte (ly) populations (CD4+, CD8+, CD19+ cells), macrophages, and antibodies (Sordillo, 2009).

CD4+ T helper cells may be involved in protective immunity against the challenge of intra-mammary infection as the main inductor of adaptive immune response. This is supported by the observation that CD4+ cells are increased in the milk of the cattle during mastitis (Banos et al., 2013). CD4+ cells have the ability to secrete certain cytokines, therefore they play an important role in the activation of CD19+ B ly, CD8+ T cytotoxic ly, and macrophages (Sordillo et al., 1997). In mastitis CD4+ ly is predominantly activated by molecular complex recognition, formed between MHC II molecules or by antigen presenting cells, B lymphocytes and macrophages (Ohtsuka et al., 2004; Park et al., 2004). Cytotoxic CD8+ T lymphocytes recognize and eliminate host cells expressing foreign antigens in association with MHC class I molecules. Suppressor CD8+ T ly controls or modulates the immune response during bacterial infection (Oviedo-Boyso et al., 2007).

The number of T helper cells (CD8+) had diminished significantly in the treated group on days 3 (median 850 cells mm^{-3}) and 7 (median 863 cells mm^{-3}) during the study in comparison with day 1 (median 1,176 cells mm^{-3}, $p = 0.0180$ and $p = 0.0066$ respectively); in contrast – CD8+ cells did not change remarkably during the observation (Fig. 2). In the control group an opposite tendency was observed – increasing number of T cells on day 3 followed by decreasing of both subpopulations until day 7 and 21.

The decreased number of CD4+ T lymphocytes in the peripheral blood during the treatment that we observed in our study might be explained by possible faster migration to the local site, induced by composition LLG. On the 21st day the number of CD4+ and CD8+ T lymphocytes was very similar to the pre-treatment number in both groups (Fig. 2).

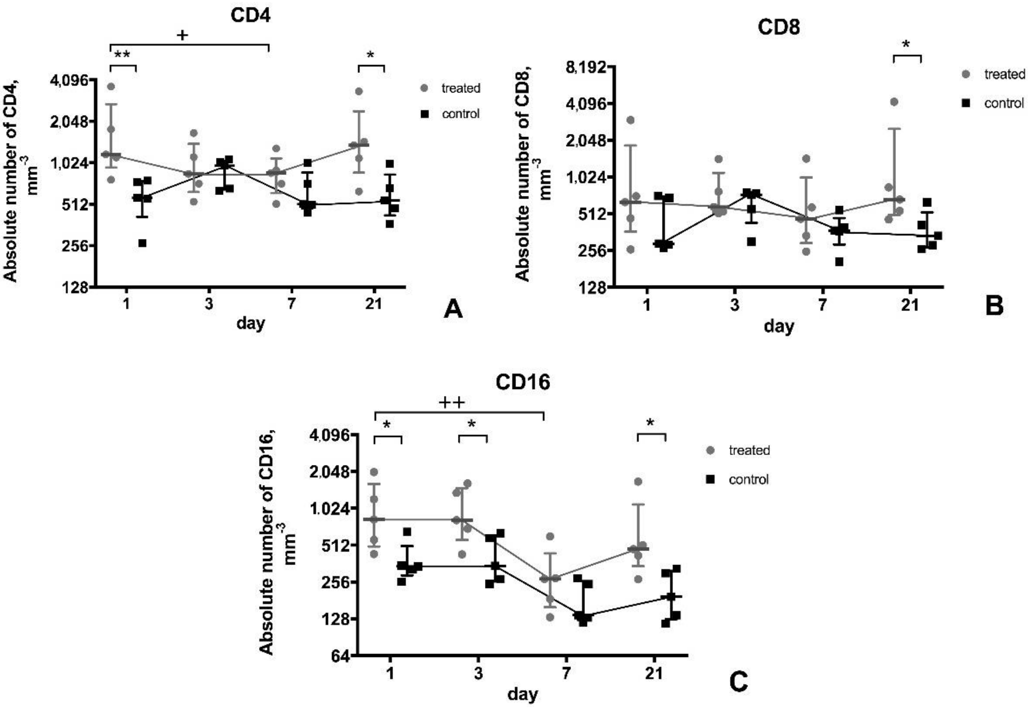

Figure 2. Number of CD4+ cells (A), CD8+ cells (B) and CD16+ cells (C) (median ± IQR) in treated (n = 5) and control (n = 5) cows' peripheral blood before the treatment (day 1), during the treatment (day 3) and after treatment from 7th to 21st day. + P value vs day 1; * P value vs control.

Various surface markers are expressed during the activation of immune cells; some are involved in cell proliferation and signal transduction, some are indicators of cell maturation, some reflect capacity of functional activity. CD69 expression is induced by the activation of T lymphocytes and some NK cells. CD69 is involved in lymphocyte proliferation and functions as a signal-transmitting receptor in lymphocytes (Werfel et al., 1997). CD25 is the alpha chain of a type I transmembrane protein present in activated T cells, some thymocytes, that can act as a high-affinity receptor for IL-2, glycoprotein that stimulates the growth of T cells and provides other biochemical signalling to the immune system (http://www.kingfisherbiotech.com). CD95 (Fas-R) is a cell surface receptor that, when engaged by Fas ligand or specific agonistic antibodies, triggers apoptosis. Fas-R is expressed in blood neutrophils and lymphocytes rendering the cells highly sensitive to apoptosis (Hu et al., 2001).

We observed that NK cells represented by an innate immune response react slower with a decreasing of absolute count in peripheral blood after the last treatment. During the treatment, a stable number of NK cells was detected, but after the end of the treatment on day 7 (median 246 cells mm^{-3}) the amount of NK cells decreased significantly in comparison with the amount of day 1 (median 566 cells mm^{-3}, $p = 0.0048$) (Fig. 3c). No differences were detected in CD16+ in the control group (Fig. 2c).

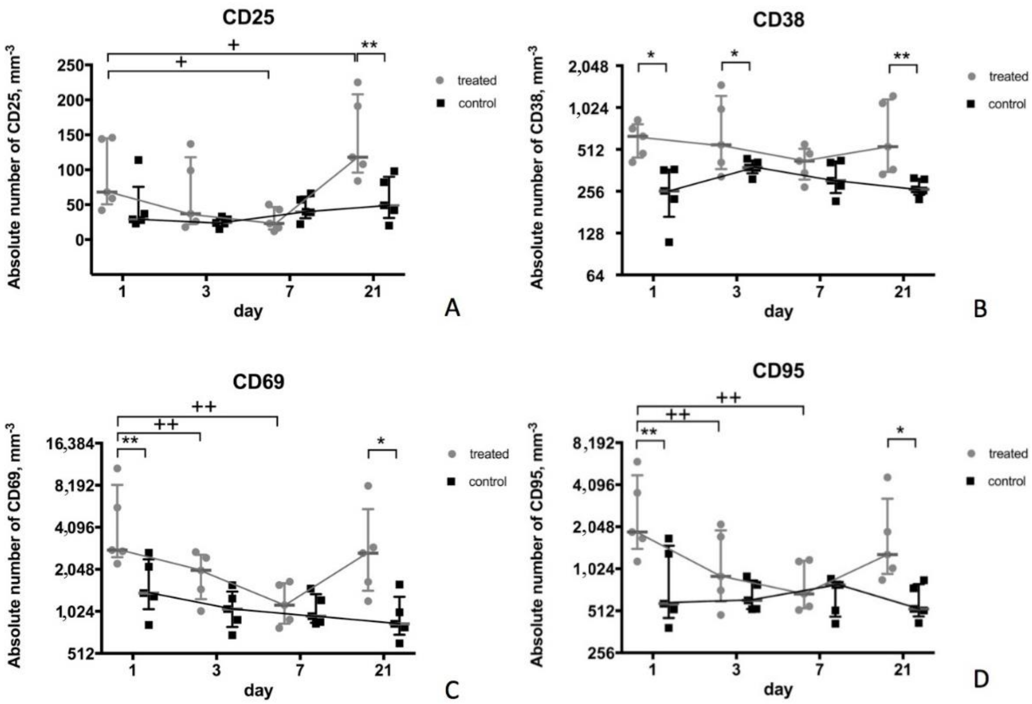

Figure 3. Number of CD25+ cells (A), CD38+ cells (B), CD69+ cells (C) and CD95+ cells (D) (median ± IQR) in treated (n = 5) and control (n = 5) cows' peripheral blood before the treatment (day 1), during the treatment (day 3) and after the treatment from 7th to 21st day. + P value vs day 1; * P value vs control.

It was reported that NK cells can modulate adaptive immune responses via early production of T helper type 1 associated cytokines or interactions with antigen presenting cells (Banos et al., 2013). We can speculate that the NK attempts to boost the function of the T arm of the adaptive immune system in peripheral blood in case of CD4+ ly early migration to the tissue.

The tendency of quantitative changes of CD19+ B lymphocytes was similar to that of NK cells (but not significant) in both groups during the observation (data not shown).

The number of lymphocyte expressed markers of activation belonging to T cells CD25 and CD69 decreased significantly two days after the last treatment (Fig. 3a, c). The common lymphocyte activation marker CD38 bearing cells remained relatively stable, possibly due to the constant number of CD8+ cells during the follow-up period (Fig. 3b). The number of CD25+, CD69+ and CD95+ cells was diminished during the treatment (Fig. 3 a, c, b). On day 21, a rapid increase of IL-2 receptor bearing cells was detected as compared to day 7 (median 118 cells mm^3 and 23 cells mm^3 respectively, $p < 0.0001$) (Fig. 3a). The number of cells expressed CD38, CD69 and CD95 molecules did not differ significantly from the pre-treatment count. No remarkable changes in the number of cells expressed markers of activation was noticed in the control group (Fig. 3b, c).

Milk somatic cell count analysis and bacteriological culturing

We assessed the local effect due to the treatment using quarter milk SCC analysis and bacteriological culturing which are traditional and well-established methods of mastitis diagnosis.

Mastitis is an inflammation predominantly caused by pathogenic bacteria and it is therefore not surprising that mastitis will result in an acute upregulation of the immune system (Sordillo & Streicher, 2002). As a result, a rapid transient increase in immune components in milk can be observed including elevated SCC. The SCC measures all types of cells in milk, including epithelial cells, lymphocytes, macrophages, and polymorph nuclear neutrophilic leukocytes (Kehrli & Shuster, 1994).

We observed no differences in the SCC between the treated and the control groups at the beginning (prior the treatment) of the observation ($p = 0.222$). During the intra-mammary administration of composition LLG a significant increase of SCC was detected in treated group (Fig. 4). Similarly, the infusion of recombinant bovine sCD14 lead to an increase in SCC, due to more rapid recruitment of neutrophils that was accompanied by a faster clearance of bacteria (Erskine et al., 2004).

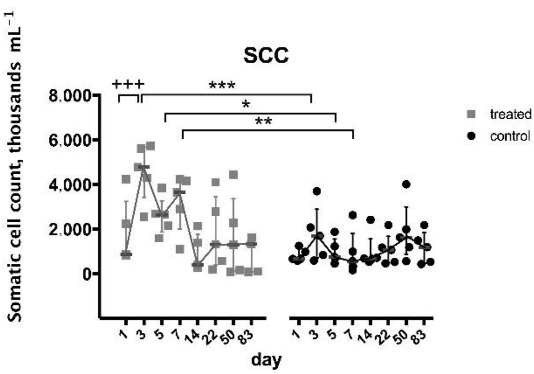

Figure 4. Variation of quarter milk somatic cell count (median ± IQR) in treated (n = 19) and control (n = 19) group before treatment (day 1), during the treatment (day 3 and day 5) and after the treatment from 7th to 14th day in conjunction with cow composite milk somatic cell count in post-trial period (day 22, day 50 and day 83). + P value vs day 1; * P value vs control.

It has also been reported that immunomodulators could improve the capacity of the adaptive immune system to respond to pathogenic challenge, with subsequently lower incidence of clinical and subclinical mastitis (Banos et al., 2013). If the innate immune response is unable to contain the infection, then the adaptive immune system will come into play via T and B lymphocyte responses.

The antibacterial activity of milk lysozyme and lactic acid as part of the unspecific innate defence mechanism is well established fact (Brul & Coote, 1999; Benkerroum, 2008; Ella et al., 2011; Espeche et al., 2012). In this study we found that the composition of LLG reduces the prevalence of pathogenic bacteria in milk, but the incidence of minor mastitis pathogens did not change significantly.

Before treatment, 16% of treated quarter milk samples and 53% of control quarter milk samples were bacteriologically negative; coagulase negative staphylococci were isolated from 47% of treated quarters and 32% from that of control quarters; *S. aureus* (16%) and bacteria from the *Enterobacteriaceae* family were detected only in the treated group (10%); mixed culture were isolated from 10% of treated quarter milk samples and 16% from the control group.

The authors report that major mastitis pathogens *S. aureus* and coliforms are usually considered more virulent and damaging to the udder than minor mastitis pathogens such as coagulase-negative staphylococci (CNS) (Reyher et al., 2012). As we isolated major pathogens only from the experimental group quarter milk samples, several analysed parameters (for example, the number of lymphocytes and CD molecules) significantly differed on day 1 (before treatment) between the control and the experimental group.

The prevalence of minor pathogens (such as CNS) changed insignificantly both in the treated and the control group. Besides we observed a significant decrease of major pathogens in the treated group milk two days post-treatment (day 7) – *S. aureus* were detected in one sample (5%) but we did not isolate bacteria from the *Enterobacteriaceae* family. Thereby prevalence of major pathogens had reduced by 75% in the experimental group milk after the application of LLG.

In total on day 7 53% of the experimental and 63% of the control group udder milk samples were bacteriologically negative. It means that 37% in the experimental group and 11% in the control group udder quarters were bacteriological cured after the application of LLG.

None of the cows in the treated and the control groups showed any abnormal clinical symptoms or any visible local reactions were observed in the areas injected with the control or LLG solution. Feed intake and milk yield remained unchanged during the observation in the treated group, whilst 1 cow from the control group developed acute clinical mastitis 20 days after the beginning of the study.

CONCLUSIONS

Our data show that the composition LLG modulates the T arm of the adaptive immune system and the expression of markers of lymphocyte activation. The proven immunomodulation composition reduced prevalence of pathogen bacteria (*S. aureus* and *Enterobacteriaceae* family) in milk by 75%. Our results suggest that the composition LLG demonstrates immunomodulatory and antibacterial activity in subclinical mastitis and could be used as alternative treatment of subclinical udder infection in cows.

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The use of TOPSIS method in the manufacturing process of clutch plate of agricultural machinery

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Abstract. Rating and determination of factors of importance affecting the pressing process of clutch plates is, in most organizations, an individual and subjective decision of individual employees.
The paper describes the process of relevant material selection for the organization. This process is designed with the aim to use the exact methods in order to replace subjective material selection by objective process. The most appropriate method appears the multi-criteria statistical analysis TOPSIS.
TOPSIS method has been widely applied in many areas with good results as the choice of marketing strategy, optimization of mechatronic systems, safety management, business competitions, management of crisis situations, assessment of trends, planning and many others. This is the first time when TOPSIS method was used in the selection of the most important causes affecting the pressing process of clutch plates.
The aim of this paper is to carry out the multi-criteria statistical analysis TOPSIS which evaluates and determines the order of importance of various factors affecting the pressing process of clutch plate which is a component part of agricultural machinery. On the basis of performed method we can prevent from subjective evaluation and make rational decisions based on the statistical analysis.

Key words: TOPSIS, Multi-criteria Analysis, Statistical Method, Process, Pressing.

INTRODUCTION

Successful searching and determining the factors affecting the process is the main prerequisite for successful improvement of the process whose result is an effective and efficient convert of inputs to outputs. When evaluating factors affecting the process there are two possible ways – subjective way of evaluation and the objective one. Subjective evaluation is affected by individuals and by subjective decision of individual employees.

During the objective evaluation there are used the one-criterion and the multi-criteria statistical methods ensuring the impartiality and objectivity of the final factors evaluation (Kowalski et al., 2009).

Method TOPSIS (Technique for the Order of Preference by Similarity to Ideal Solution) belongs to multi-criteria statistical methods.

In another words, the multi-criteria technique is based on obtaining the alternative that approaches the most ideal alternative. For this purpose is considered the positive ideal alternative and the negative one. It is a technique for order the preference by similarity to ideal solution (Nagyová et al., 2014).

Many multi-criteria analyses are based on the additive concept together with the assumption of independence, however, not all individual criteria are independent (Mateo, 2012; Lestyánszka et al., 2013).

Development of this method by Hwang and Yoon is dated back to 1995. At the beginning, the alternatives were sorted according to their distance from ideal (positive) and inappropriate (negative) solution, it means the best alternative is also the shortest distance from the ideal solution and inappropriate solution with the longest distance from the ideal solution. The ideal solution is identified as the variant with a hypothetical alternative that has the best values for all considered criteria, while inappropriate variant is identified as the hypothetical alternative which has the worst values of the criteria (Sarraf et al., 2013).

It is a method of countervailing aggregation, which compares the complex of alternatives by identification of weights for each criterion, standardized score for each criterion and calculating geometric distances between each alternative and the ideal variant according to the best score for each criterion (Hwang & Yoon, 1995; Cheung & Suen, 2002; Ťavodová, 2006)

The aim of this paper is to evaluate and determine the order of importance of each factor affecting the pressing process of clutch plates, which are a part of agricultural machinery by applying the multi-criteria TOPSIS method. On the basis of performed method, we can prevent from subjective evaluation and make rational decisions based on statistical analysis.

MATERIALS AND METHODS

Before applying the multi-criterial statistical method TOPSIS it is necessary to identify each factor. For identifying each factor is possible to use various tools, for example the brainstorming.

Procedure for brainstorming application:

1. Determining the appropriate place for meeting of larger group of people and determine the date of meeting.
2. Informing the representatives of each involved department in organization (Department of quality, design, manufacturing, marketing, purchasing, sales, tools and warehouse) about the meeting.
3. Selecting the responsible person (moderator) for brainstorming leadership.
4. Keeping the principles and rules of brainstorming.
5. Writing down and process all ideas and comments in the usable form due to creation of Ishikawa diagram in which they will be sorted according to individual causes and consequences resulting from the needs of diagram.
6. Displaying the results of brainstorming into the mind map.

Procedure for TOPSIS method application

Selection of plate was realized on the basis of multi-criterial method TOPSIS by applying the following procedure:

1. Converting minimum criteria to maximum criteria.
2. Constructing normalized criteria matrix $R = (r_{ij})$, where $i = 1,\ 2....m$ (m – number of variants) and $j = 1,\ 2....r$ (r – number of criteria).

$$r_{ij} = \frac{g_{ij}}{\sqrt{\sum_{i=1}^{m} g_{ij}^2}} \qquad (1)$$

where: g_{ij} – the value of i – th row (variants) and j – th column (criteria).

3. Calculating the weighted criteria matrix $W = (w_{ij})$ that each column of matrix R is multiplied by weighted vector (v_j) of corresponding criterion ($w_{ij} = v_j \times r_{ij}$).
4. Determining the distance of ideal variant (d_i^+) and distance of basal variant (d_i^-), by using individual parameters (elements) of matrix W and the equation:

$$d_i^+ = \sqrt{\sum_{j=1}^{r} (w_{ij} - H_j)^2} \qquad (2)$$

$$d_i^- = \sqrt{\sum_{j=1}^{r} (w_{ij} - D_j)^2} \qquad (3)$$

where: H_j – max w_{ij}; D_j – max w_{ij}.

5. Subsequently counting up relative distance indicators of variants from basal variant.

$$c_i = \frac{d_i^-}{d_i^+ + d_i^-} \qquad (4)$$

6. As the last point- arranging the variants according to resulting values in descending order.

RESULTS AND DISCUSSION

Brainstorming method preceded to TOPSIS method performed in order to identify areas which can affect the non-conformities the most and joined issues like claims and financial unprofitability of pressing process of clutch plates.

We invited representatives of all stakeholders (departments) in organization to the brainstorming realization: project department, production department, design department, tool room, warehouse, technology department, quality department, purchasing department, marketing department and sales department.

After explaining the issues and determining the aim of brainstorming and its rules, we recorded all ideas and good points of each participant. Recorded ideas and thoughts were plotted in the graphical form by using the mind map (Fig. 1).

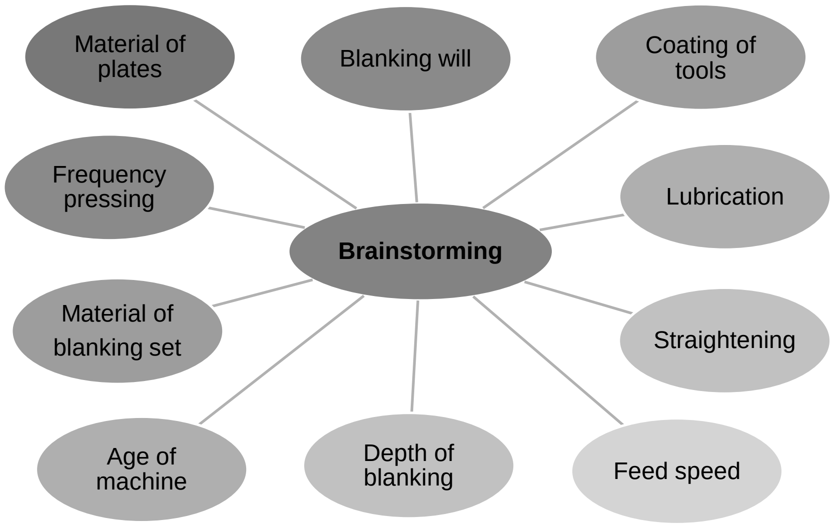

Figure 1. Mind Map from the Brainstorming Group.

The aim of brainstorming was to find factors affecting causes as well as increasing lifetime of press tool, what will lead to the overall effectiveness and efficiency of pressing process of clutch plate.

As could be seen in Table 1, there are the individual factors of brainstorming and the evaluation range of individual factors. Selected four-step range described the dependence of each factor affecting the improvement of pressing process of clutch plates used in agricultural machinery. Professionally qualified members (Deputy of project, production, design, technology, quality department and tool room) were invited to brainstorming meeting and participated in ratings.

To each factor was assigned the degree of dependence by each deputy. Finally were allocated values recorded into Table 1.

Table 1. Brainstorming Factors with the Degree of Dependency

Factors from brainstorming	Non-dependence	Weak dependence	Medium dependence	Strong dependence
Material of plates	1	1	0	4
Blanking will	3	1	2	0
Coating of tools	3	1	0	2
Lubrication	1	1	1	3
Straightening	1	3	2	0
Feed speed	3	2	0	1
Depth of blanking	4	1	1	0
Age of machine	1	3	1	1
Material of blanking set	0	1	0	5
Frequency pressing	4	1	1	0

Individual criteria were transformed by our own marking system:

- Non-dependence = Criterion 1 (C1)
- Weak dependence = Criterion 2 (C2)
- Medium dependence = Criterion 3 (C3)
- Strong dependence = Criterion 4 (C4)

The brainstorming factors were transformed into variants 1–10, succession of factors remained unchanged for better identification after the statistical methods evaluation.

We created a decision matrix with standard values of weights for each criterion with a cumulative sum of weights equal to 1.00 (Table 2). Standard values of weights were set on the basis of Metfessel's allocation, when 100–point scale was allocated among the criteria.

Table 2. Decision Matrix with Values Weight

	C_1	C_2	C_3	C_4
Weight	0.05	0.15	0.3	0.5
Variant 1	0.0063	0.0279	0	0.2673
Variant 2	0.0189	0.0279	0.1732	0
Variant 3	0.0189	0.0279	0	0.1336
Variant 4	0.0063	0.0279	0.0866	0.2004
Variant 5	0.0063	0.0836	0.1732	0
Variant 6	0.0189	0.0557	0	0.0668
Variant 7	0.0252	0.0279	0.0866	0
Variant 8	0.0063	0.0836	0.0866	0.0668
Variant 9	0.0000	0.0279	0	0.3341
Variant 10	0.0252	0.0279	0.0866	0

We calculated a relative distance indicator of individual variants from basal variant (Table 1). Values of these parameters vary between 0 and 1, where 0 represents the basal variant and 1 is the most ideal variant. We sequenced all variants according to calculated values of indicators into Table 3.

Table 3. Arrangement of Factors on the Basis of TOPSIS Method

Rank	Marking of factor	Value
1.	**Material of blanking set**	**0.7546**
2.	**Lubrication**	**0.7182**
3.	**Material of plates**	**0.6507**
4.	Coating of tools	0.4171
5.	Age of machine	0.3819
6.	Straightening	0.3429
7.	Blanking will	0.2621
8.	Feed speed	0.2427
9.	Depth of blanking	0.1905
10.	Frequency pressing	0.1056

On the basis of TOPSIS method results were determined the most relevant factors (those which reached the values of 0.5 and above): material of blanking sets, lubrication and material of plate.

Material selection is an extremely important decision-making process that affects mainly purchasing department of each company (Lukoszová, 2004).

The greater purchasing opportunities, the more suppliers, the more ways to meet the needs of the company but also the more serious and difficult is this decision. Decision-making process is not a simple process. This process must be understood in a broader sense, as a process that precedes:

- identifying the decision problem;
- assessing of situation;
- decision-making criteria in materials selection;
- selecting the methods (Nenadál, 2006).

The most significant advantage of TOPSIS method is that customers can determine their own criteria, which by relevance assign the weight and on that basis is calculated the best variant of solution (Čupić & Suknović, 2003).

CONCLUSION

There are several methods of multicriteria decision that have the same objective. The objective is to consider several variants of solution of the problem according to selected criteria and determination of their order.

The individual methods differ mainly according to determined weight of individual criteria (Garcia-Cascales & Lamata, 2011).

Displayed diversity of input data us points to the wide possibilities of variability of the result process (Nagyová et al., 2013). The right choice of importance of individual factors affect the effectiveness and efficiency of manufacturing process as well as the quality and lifetime of press tool.

After determining the factors affecting the pressing process of clutch plates with using brainstorming, we proceeded to multi-criteria statistical method TOPSIS. On the basis of values taking into account the criteria and the weights we obtained the final order of the factors affecting the pressing process. As the most important factors we determined following: material of blanking sets, lubrication and material of plate with regard to achieve a higher value as determined i.e. 0.5.

Analysis result by TOPSIS method led to the identification of three most important factors influencing the manufacturing process. During monitoring and control of these factors is possible to reduce the subjective assessment of the process and also to increase the objectivity of decision making.

In this paper there was evaluated one of the possibilities for effective and objective evaluation of manufacturing process. TOPSIS method can be applied also in the other manufacturing processes within the particular organization.

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The action of force measurement for the three-point hitch of a tractor

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Abstract. The object of the executed measurements which were carried out was to obtain time courses for the forces and pressures inherent in a hydraulic system for the three-point hitch of a tractor during the process of ploughing with a carrier-mounted plough and also with a semi-mounted plough. It was necessary to obtain experimental data on the basis of which it was then possible to consider and analyse the issue of dynamic loading for the three-point hitch of a tractor during basic agricultural operations. The selected operation was ploughing in random working conditions using, in turn, a carrier-mounted plough and a semi-mounted plough. The aim was to use the results obtained to simulate the loading and full usage of the three-point hitch (TPH) under laboratory condition by means of hydrostatic simulator.

Key words: tractor, three-point hitch, hydraulic system.

INTRODUCTION

At present, agricultural tractors as manufactured are characterised by high rates of standardisation and feature a range of additional attachments which allow the wider use of each tractor and greatly facilitate its operation (Kosiba et al., 2012). The need for tractors and agricultural machinery to be tested from the point of view of their suitability to agricultural use will grow continuously because these machines directly affect agricultural production. This is within the context of rising demands on new tractors in terms of their performance and work productivity rates, while it also relates to increasing requirements for technical lifetimes, and the reliability of individual functions and assembly parts (Majdan et al., 2011; Porteš et al., 2013; Zastempowski, 2013 and Tulík et al., 2014). Last but not least, the introduction of elements which are of brand new designs requires the possibility of their parameters being testing during operation, within reason, due to the need for operational reliability. So the need for the technical parameters and individual properties of newly-designed parts and systems increases (Kučera & Rousek, 2008; Zastempowski & Zastempowski, 2012).

The analysis of forces which serve to influence the three-point hitch of a ploughing tractor was carried out because, when compared with tractor tractive tests, the loading for a ploughing tractor shows characteristic differences. Whilst ploughing, the tractor wheels slide into a furrow on one side of the tractor so that the vehicle becomes inclined to that one side (Čupera & Šmerda, 2010; Hoffmann et al., 2013; Simikić et al., 2014). In combination with the power effects of the plough itself, this inclination results in a different loading for individual wheels and also in changes in the loading for both axles. This results in significant changes not only in the grip of the driving wheels but also in an increased compaction of the soil (Čupera et al., 2011; Manes et al., 2012). This measuring was carried out and completed for the tractor factory, Zetor Tractor a.s. as requested by the factory's owners. As may be known, no such similar measurements have been carried out anywhere in the world up to this date.

MATERIALS AND METHODS

The tractor being tested has to fulfil the specified data requirements in the testing report, has to be used in accordance with manufacturer recommendations which concern standard operations and, before testing, it has to be fully run-in. Hydraulic fluid has to be recommended by the manufacturer and determined by type and viscosity according to the ISO 3448 standard. In order to achieve the goals which have been determined for this issue, the following procedures were selected:

- experiments with a ploughing kit (tractor and PH1-435 plough (four furrows));
- experiments with a ploughing kit (tractor and PH1-422 plough (four furrows));
- processing for measurement results by PC.

In order to be able to obtain the characteristics of TPH operational loading, The mechanical and physical state of the soil was detected by penetrometer and also by an analysis of soil samples. The samples for humidity determination, bulk density, and specific weight were collected during the process of taking measurements and also following the completion of measurements. The physical-chemical properties from the collected samples were as follows:

- weight humidity: 14.3%;
- bulk density of wet soil: 1.45g cm^{-3};
- specific weight of the soil grain: 2.5g cm^{-3};
- porosity: 37%.

On the basic of penetrometer measurements, an average value was determined for soil resistance of about 110 kPa. The soil samples were collected from each 10 m^2 area of soil. The soil type was clay loam. Soil moisture content was at 18%, with no stones. Soil texture was not determined. During the process of taking measurements, a tractor's ploughing tools were used, consisting of the tractor itself and two types of plough, and the technical parameters were as shown below in Table 1. In order to be able to carry out the measurement process, the following parameter ranges were selected:

- ploughing depth: 25 cm and 27 cm;
- tractor speed – engaged speed when in gear: I/2, I/3;
- tractor regulation control: position control (P), draught control (D), and mixed control (M).

The length of each measured section for all measurements was 100 m. Sections were prepared at a length of 25 m. The measurements for a three-point hitch were realised for the purpose of obtaining of the following experimental data:

- time courses for forces in the left-hand lower drawbar (LBD) TPH;
- time courses for forces in the right-hand lower drawbar (RBD) TPH;
- time courses for forces in the left-hand upper drawbar (LTD) TPH;
- time courses for forces in the right-hand upper drawbar (RTD) TPH.

Table 1. Technical parameters of ploughs

Parameters	Carrier-mounted plough	Semi-mounted plough
Type	PH1-435 TP 536 111-198/84	PH1-422 TP 53 611.38.198/84
Working speed	7 km h^{-1}	7.5 km h^{-1}
Transport speed	10 km h^{-1}	10 km h^{-1}
Maximum working depth	24 cm	27 cm
Maximum rated soil resistivity	120 kPa	130 kPa
Engagement width (adjustable)	35 cm	from 30 to 42 cm
Weight	665 kg	2.850 kg

Essential criterion for the selection of measurement results was the maximum loading of the three point hitch. This refers to the maximum loading for the bottom drawbars (horizontal forces) and connecting bars (vertical forces). In order to determine the forces in specific components for the tractor's three-point hitch during ploughing, the three-point hitch was offset by a tensiometer. During the process of conducting the measurements, telemetry apparatus were used and the time courses for the forces employed were analysed using a spectrum analyser. Thanks to this, frequency spectrum and histograms were obtained. From the measurements obtained and following their processing, results were selected for presentation which were important in terms of the designed simulation device for the loading of a three-point hitch.

RESULTS AND DISCUSSION

The prevailing conditions during the ploughing process are shown in Table 2. The main experimental values covering the forces used at the individual drawbars, TPH, as obtained during the measurements process are shown in Table 3.

Table 2. Conditions of ploughing

Number of measurements	Depth of plough, m			Position of gear shift	Control	Working speed		Note
	Required	Real	Standard deviation			m s^{-1}	km h^{-1}	
	h_p	h_r	δ_h			v_p	V_p	
1	0.27	0.283	0.158	I/3/Z	P	1.15	4.14	Plough PH1 – 422
2	0.27	0.305	0.146	I/2/Z	M	1.05	3.79	
3	0.27	0.276	0.285	I/2/Z	D	1.04	3.74	
4	0.25	0.273	0.164	I/3/Z	P	2.08	7.50	Plough PH1 – 435
5	0.25	0.276	0.144	I/3/Z	M	2.10	7.56	
6	0.25	0.296	0.419	I/3/Z	D	2.08	7.50	

Legend: h_s – arithmetic mean of measured ploughing depth, δ_h – standard deviation of ploughing depth, P – position control, D – draught control, M – mixed control.

Table 3. Forces in TPH during ploughing

Number of measurements	Drawbar	Left bottom drawbar Force F_{dl} (F_{sl}), kN min.	median	max.	σΦδλ(σλ) kN	λ Φδπ(σπ) -	Right bottom drawbar Force F_{dp} (F_{sp}), kN min.	median	max.	σΦδπ(σπ) kN	λ Φδπ(σπ) -
1	top	15.04	23.35	33.12	3.74	0.32	0.72	4.14	10.72	2.98	0.56
2		9.33	18.16	28.43	3.21	0.35	1.45	11.01	20.93	2.14	0.39
3		9.33	20.75	35.33	3.56	0.34	0	8.73	22.4	3.22	0.73
4		7.78	18.16	25.9	3.88	0.42	0	9.01	22.4	2.45	0.54
5		9.33	20.75	33.67	3.44	0.33	6.32	14.45	25.79	3.08	0.43
6		10.38	19.21	32.02	2.89	0.3	5.85	15.02	23.79	2.99	0.4
1	bottom	15.97	26.34	36.57	4.42	0.34	12.08	19.52	26.6	2.76	0.28
2		6.21	13.59	22.25	3.32	0.49	9.64	15.85	21.72	2.08	0.26
3		2.87	13.49	23.,4	3.97	0.59	8.48	15.36	22.94	3.18	0.41
4		5.84	10.28	23.37	3.71	0.49	4.15	10.28	17.64	2.36	0.46
5		3.89	11.93	22.88	3.51	0.59	2.59	9.88	19.2	3.15	0.64
6		6.68	14.14	21.42	2.71	0.38	2.59	11.25	20.24	3.43	0.61

The formulas used for arithmetic mean and standard deviations are as follows:

$$\bar{\bar{x}} = \frac{1}{n}\sum x_i \tag{1}$$

$$\delta = \sqrt{\frac{1}{n-1}(x_i - \bar{x})^2} \tag{2}$$

Figs 1–5 show the values which were obtained from Measurement No 6 in order to demonstrate the measurements as a whole. Figs 1 and 2 show the measured time courses for forces in the left-hand lower drawbar, F_{dl}, and in the right-hand lower drawbar, F_{dp}, of the three-point hitch. Fig. 3 illustrates the probable action of forces (F_{dp}), (F_{dl}). Figs 4 and 5 show the normalised power spectral density of these forces during ploughing with an operational speed: v_p = 7.20 km h^{-1}, with force regulation and depth of ploughing included. A tractor was used with a five-carrier-mounted plough, model PH1-435.

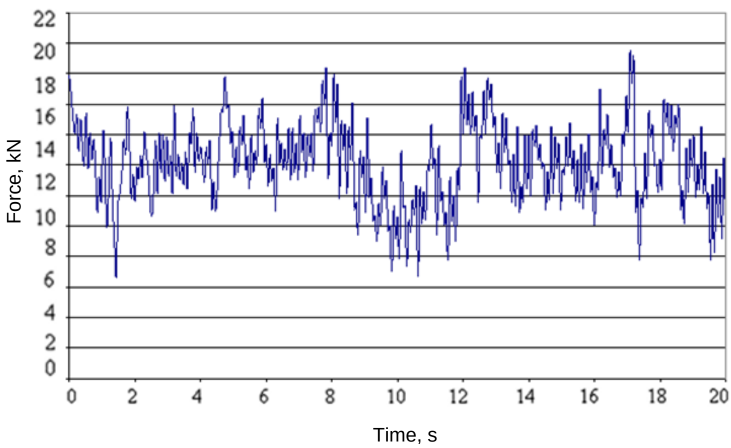

Figure 1. Times courses of forces on the left down drawbar F_{dl}.

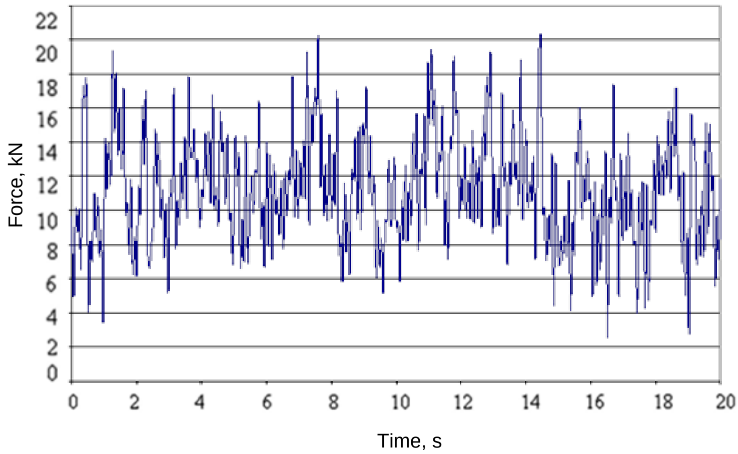

Figure 2. Times courses of forces on the right down drawbar F_{dp}.

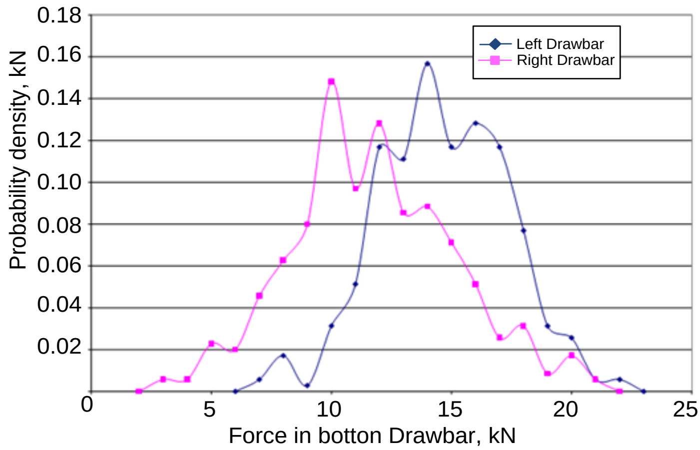

Figure 3. Probable densities of forces in right down and left down drawbars $p(F_{dp})$, $p(F_{dl})$.

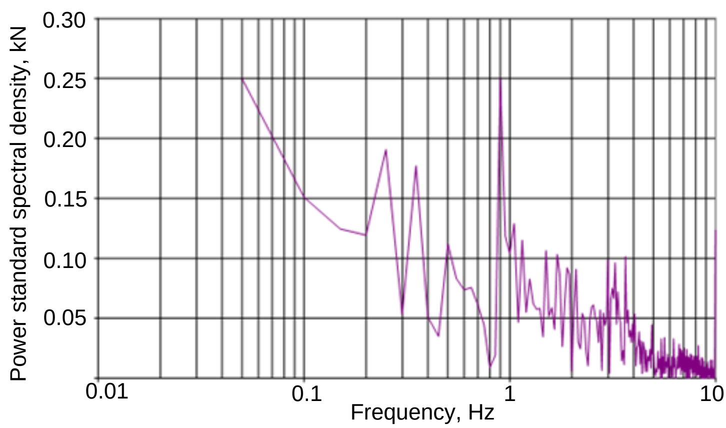

Figure 4. Normalized power spectral density – G_{Fdl}.

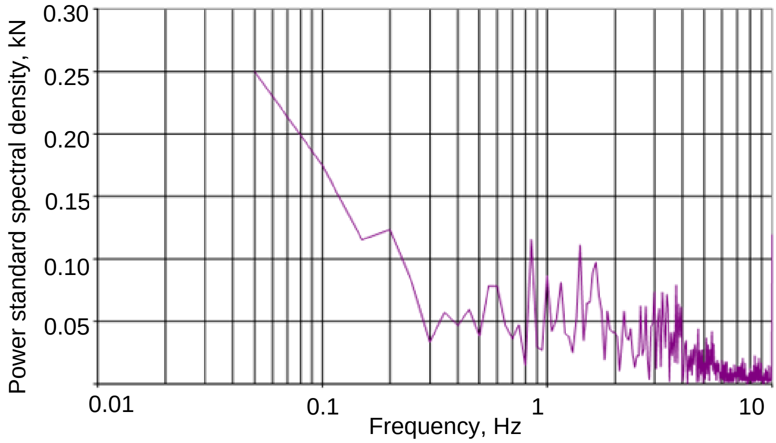

Figure 5. Normalized power spectral density – G_{Fdlp}.

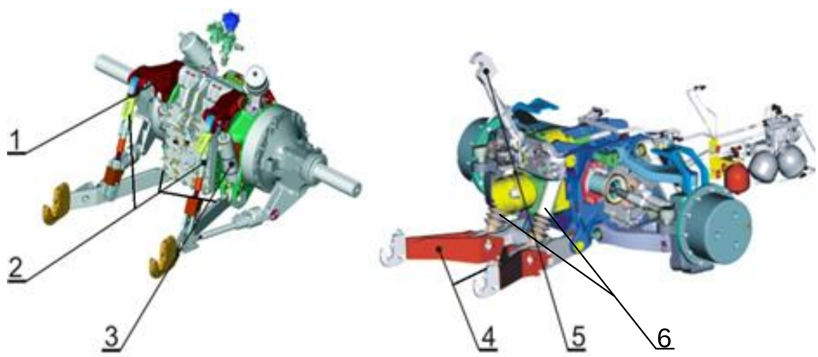

Figure 6. Three point-hitch: 1 – lifting arm, 2 – position of tensiometer, 3 – rectilinear hydraulic motor, 4 – left and right bottom drawbars, 5 – upper drawbar, 6 – connecting drawbars.

CONCLUSION

The results obtained showed the following:

- forces in the lower drawbars (LBD, RBD) were within the phase of the monitored frequency range;
- for LBD, generally higher amplitude of forces were registered;
- significant components in the Fast Fourier Transform spectrum (FFT spectrum) for measured signals, RBD and LBD, did not exceed a value of 5 Hz;
- the maximum values for forces on the RBD and LBD did not reach values of 37 kN;
- forces for the connecting drawbars (LTD, RTD) in the monitored frequency range were practically in antiphase, which means that they were phase-shifted from 160° to 200°;
- on the LTD, the amplitude of forces was higher;
- significant FFT spectrum components for the measured forces, RTD and LTD, did not exceed a value of 5 Hz;

- the maximum value of forces on the LTD and RTD did not reach a value of 36 kN.

The results of experimental measurements for forces and pressures on the three-point hitch of the tractor's ploughing assembly allows the design type and operational loading simulation conditions on a dynamic system to be realised under laboratory conditions. By using only a traditional process of mobile device testing in operational conditions, an excessive increase would be created in demands on the range and the number of repetitions for the verification of constructive modifications. As part of the process of carrying out experimental measurements for a tractor plough assembly, excepting the measurement of forces in the three-point hitch, also measured were the pressures involved in the tractor's hydraulic circuit. These values were compared with obtained values for pressures in the hydraulic circuit of the testing device under laboratory conditions (Majdan et al., 2011; Tulík et al., 2013). By carrying out individual experimental measurements for the tractor ploughing assembly, the necessary data to determine the requirements for the development of a functional design in the hydrostatic simulator of tractor and its control circuits were obtained under laboratory conditions.

On the basis of the results obtained, it can be claimed that the unsteadiness coefficients in terms of operational loading which are expressed in maximum, minimum, and mean values, as specified by most authors, are unstable and are also highly variable (Bentaher et al., 2008, Seyedabadi, 2015). On the other hand, when it comes to the unsteadiness coefficients for the operational loading of a tractor which are specified on the basis of mean square deviation and mean values $\lambda_x = \frac{2.\sigma_x}{\bar{x}}$, these are constant, and accurately express the unsteadiness of the given quantity for courses, as seen in Table 4.

Table 4. Significant values of forces coursed in TPH

Number of measurements	Significant values of spectrum, Hz			
	Bottom drawbar		Connecting drawbar	
	Right	Left	Right	Left
1	0.4	0.45; 1.0*	0.4	0.4
2	0.15; 0.4*	0.15	-	-
3	0.3	0.3	-	0.4
4	0.3	0.2	0.6	0.6
5	0.45; 1.05*	0.35; 1.05*	0.8	0.3
6	0.85; 1.5*	0.25; 0.9*	0.25	0.25

*This status was caused by error during the processing of the measured results.

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Ergonomic intervention programs in different economic sectors: a review article

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Abstract. Unlimited number of hazards can be found in almost every workplace increasingly causing work-related diseases (WRDs) and injuries among workers. In work environment there are various risk factors: physiological, physical or psychological. An awkward and static postures, repetitive movements, high work pace, non-ergonomic tools and poorly organized workstations are most likely causing musculoskeletal disorders. As well inconvenient room temperature, noise, vibration and poor lighting conditions can conduce to additional work discomfort, mental stress, fatigue, injury, or trauma among employees. Ergonomic interventions are coming more popular. Many organizations are trying to find best solutions to avoid musculoskeletal disorders (MSD). The aim of this paper is to describe different ergonomic interventions focused on diminishing of musculoskeletal discomfort and MSDs among workers. This paper gives overview about the most common and effective ergonomic interventions which really have worked in practice. For this research were used three different databases EBSCO, Science Direct and Mendeley. The selection of publications passed three phases of systematic search of literature: the first elimination consist of keywords 'ergonomics, intervention' and year of publication. In the second phase was added a keyword 'work' and in the third phase were eliminated repeated and literature review publications and as well publications which had little sample size or the exploration was not covered with real interventions. The publications (n = 209) of ergonomic interventions carried out in the past five years 2010–2015 were analyzed. Wide spectrum of different ergonomic interventions was found in several economic sectors, whereas the most effective ones were related to well-known ergonomics methods, workstation adjustment, training and exercises.

Key words: ergonomic interventions, ergonomically designed workplaces, musculoskeletal disorders.

INTRODUCTION

Musculoskeletal disorders are most common occupational diseases throughout the industrialized world, in Europe as well in Estonia (Eurostat, 2009; Health Board, 2016). Explorations have shown that feeling pain or discomfort in different parts of musculoskeletal systems is the main reason for unproductive work or even sick leave. Musculoskeletal disorders are still the most often reported occupational diseases causing the lost working hours, increasing economic costs for enterprises (Noroozi et al., 2015).

Work-related musculoskeletal disorders are widespread among every occupation and costly to the health care system (Fabrizio, 2009). There is evidence that inappropriate design of workplaces and work processes contributes significantly to the development and chronicity of common musculoskeletal disorders (Rivilis et al., 2008). Musculoskeletal disorders occur due to many aspects like awkward working posture and poor workstation design. Even office workers in prolonged sitting position can´t mention the psychological risk factors. Also prolong standing leads to physiological discomfort, fatigue and health problems. In agricultural industry workers are required to perform physically demanding jobs that put them at significant risk for developing work-related musculoskeletal disorders. Workers among manufacturing industry are not left untouched by musculoskeletal diseases. Material handling and lifting tasks have to make properly to avoid any disorders, discomforts or injuries. In electronic industry can be found factors for example static postures, work methodology, condition of work environment and not so well designed workplaces which direct to musculoskeletal disorders.

The main focus of health and safety issues is related to upper or lower limb pain. Upper limb regions are neck, shoulders and arms. Lower limb regions are back, hips, knees, ankles and feet. Musculoskeletal disorders can affect the muscles, bones and joints. Most common diagnoses are tendinitis, carpal tunnel syndrome, osteoarthritis, rheumatoid arthritis, fibromyalgia and bone fractures. Discomfort in any body region could lead further to musculoskeletal disorder. This is the reason why is very important to focus on discomfort even if this is not causing a lot of afflictions yet.

Furthermore, evidence that the risk or protective factor precedes the disorder is an important indication that the factor has at least a potential role in causation. Also, there may be a dosage effect, what means the stronger the risk factor, the more disorders (Mrazek & Haggerty, 1994).

To achieve better health in easiest and inexpensive way there comes to the picture ergonomics and ergonomics knowledges. Ergonomics is defined by the International Ergonomics Association as ‘the scientific discipline concerned with the understanding of the interactions among humans and other elements of a system, and profession that applies theoretical principles, data and methods to design in order to optimize human being and overall system’ (International Ergonomics Association, 2000).

To change something by using knowledge of ergonomics are called ergonomics interventions. Ergonomic interventions have become more prominent and are one of many proposed interventions for treatment and prevention of work-related musculoskeletal disorders. The effectiveness of ergonomic principles for preventing and reducing musculoskeletal disorders has led to a recent boom in intervention research (Dempsey, 2007).

Ergonomic interventions have grown as the most popular research method and important part of work place safety and health strategy in the last decade. Prevention is the basic principle of occupational health and safety (OHS) culture to minimize occupational risks and diminish occupational accidents and diseases. Ergonomic intervention is a part of OHS prevention programs. Ergonomic intervention is the use of ergonomics in preventing disability in injured workers and classified as individual, organizational, physical and psychosocial ergonomic interventions (Driessen et al., 2011). An involvement of workers in decision-making, sharing information and rewarding from management to lower levels of organization have seen as participation ergonomics (Institute for Work and Health, 2008). Ergonomic interventions can be divided to: individual ergonomic intervention (IEI), physical ergonomics intervention (PEI), organizational ergonomic intervention (OEI) and combined ergonomic interventions (CEI). A number of OEI studies have proved that the effectiveness of an organization is closely related to a human performance at work, skills and health. The owners of companies have to know that ergonomics intervention may cost in the beginning, but will compensate in the end.

There are a variety of actions that have been applied in the workplace for eliminating or reducing the occurrence of occupational musculoskeletal disorders among employees. One rapid way to prevent musculoskeletal disorders is to expand ergonomic knowledges and use interventions among employees. The purpose of any ergonomic intervention is to improve worker comfort. Till today scientists have made lot of work to figure out the best ergonomic interventions. They have analyzed their impact to health and through this the impact to productivity. Any ergonomic intervention will be preceded by setting up strategies expected to lead to the intended succeed change.

Physical ergonomic interventions are dealing with most important physiological risk factors as lifting loads, physically heavy work, awkward postures, repetitive movements, frequent bending or twisting of elbow, wrist and fingers (Driessen et al., 2011). Adapting of physical loads not always reduce pain prevalence, intensity and/or duration, so it must combine with physical exercises It has been evaluated as an effective preventative intervention method for reducing MSDs, especially in the neck and lower back (Linton & Tulder, 2001).

The goal of psychosocial interventions is to reduce distress and impact of stressful events, minimize symptoms and decrease disability, to improve coping skills and quality of life. Evidence-based psychosocial interventions in use more often in mental health care for measurement whether the patients are receiving high-quality care. Psychosocial interventions apply a positive effect on quality of life and positive mental health but not always with successful outcomes (Forsman et al., 2011). Three types of psychosocial intervention approaches have used in practice: 1) universal prevention (mental health promotion through general health education) targeting healthy older adults in active aging; 2) selective prevention, targeting older age groups not suffering from mental disorders and 3) indicated prevention directed at people with sub-clinical symptoms of depression (World Health Organization, 2004).

The aim of this study is to give a critical analysis of the peer-reviewed literature, to provide a comprehensive summary of effectiveness of ergonomic interventions in improving workers health and productivity.

MATERIALS AND METHODS

Researches about ergonomics interventions are very young field, but the past decade has brought encouraging progress. In spite of huge amount of research have been published, lack of systematic information about methodology of ergonomic interventions has seen yet. For this experiment were used EBSCO, Science Direct and Mendeley databases. In a systematic search of combined keywords ergonomic and intervention it has found together 1,631 articles. Most publications were found in Science Direct, then EBSCO and Mendeley databases. The title and content of article were decisive and main criteria. The general principles for selecting articles were effective, successfully implemented and be well-documented ergonomic interventions.

A framework of eliminating the publications for critical analysis contained of three phases. In first phase were culled out articles, which were not written in English and older than 2010 year published. Eighty four publications of 1,631 were left out because these were in Chinese, French, Persian, Spanish and Portuguese. The first elimination of first phase on flowchart below (Fig. 1) shows, how many articles survived after reading English abstract and language control. After publication year control, altogether in the foreground remained 604 articles.

The first elimination of first phase on graph shows how many articles where survived after language control and second one shows how many articles after publication year control. The amount of articles after first phase is still very big. Altogether in the foreground remained 604 articles.

In the second phase special keyword ‘work’ was included and searched from titles, abstracts and main text. In the end of second phase left on the surface 209 articles. Analyzed were 209 publications all over the world in various fields over the past years. Abstracts from all 209 articles were read.

In the third phase has not taken account of articles, which were repeated in databases, which described review of literature, where participants were less than 50 and if these articles were out of subject. Sample size was selected to 50 persons, because larger number will give better overview of impact of intervention. If the intervention was about tool design, then sample size did not matter. Five articles of 91 described design or change of tool intervention. The first elimination of third phase on graph shows how many articles where survived repeating control, second one shows how many articles after review of literature control, third one after sample size control and forth off the subject control. After the eliminations and systematic research remained 91 publications (Fig. 1).

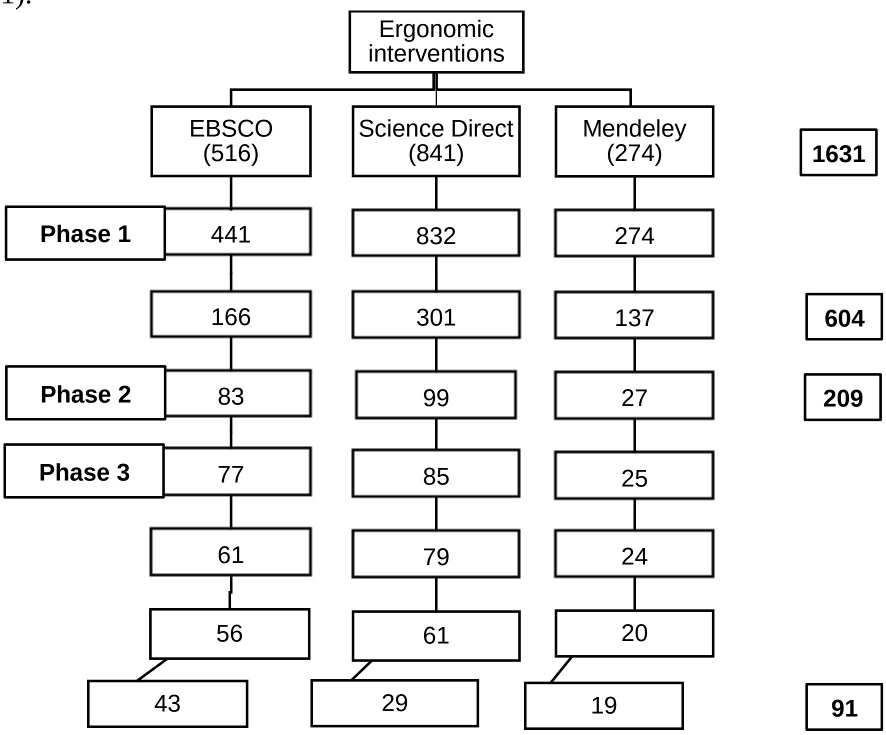

Figure 1. The flowchart of eliminating articles about ergonomic interventions.

In this observed research on ergonomics interventions the area of dissected occupational area was very wide: office workers, administrators, nurses, students, construction workers, carpet weavers, goldsmiths, postmen, school teachers, dentists, sewing machine operators, material handling and workers in industrial or agricultural occupation.

Ergonomic interventions have become a popular way to describe the improvements at workplace and it really has ambivalent understanding. Some publications have named ergonomic intervention as a questionnaire studies that described just results giving overview of current situation. These articles were excluded from this analysis. The studies about ergonomic interventions with positive results were taken under the analysis. The authors concentrated especially on these publications which showed statistically significant difference between the experimental and control group or achieved considerable better health or increased productivity after the intervention.

Ergonomic interventions by the activities were classified as engineering, administrative and behavioral or personal ones. Engineering changes would modify the work station or work environment. Administrative interventions often change the duties of workers by job assignment or rotation or work-rest schedules. Personal interventions mostly implemented changes in work process. There have been used training methods to improve knowledge and physical exercises to diminish prevalence of MSDs among employees.

The regions where the ergonomic intervention projects have carried out were: Iran, United States of America, China and India. Also there were articles from Brazil, United Kingdom, Thailand, Hong Kong, Sweden, Kenya, Canada, Egypt, Netherlands, Indonesia, France and Latvia.

However, in some publications the effectiveness of interventions was unclear. Because of methodological and organizational reasons and poor scientific quality these studies were not accepted.

For better understanding the articles, following questions were considered:

- Which type of interventions have used?
- To whom the intervention was made?
- What exactly have been done?
- Does it have results?
- How was it evaluated?
- Is this result comparable to other studies at this field?

The search strategy was targeted on 91 potential articles on ergonomic interventions, and had calculable scientific value. The different occupations were under the analysis, for instance industry workers, teachers, nurses, household workers, computer workers, dentists or manual material handling operators. Each research concentrates on their field. The main aim was to find out best ergonomics interventions which really have been worked in practice, irrespective of on whom these were implemented.

RESULTS AND DISCUSSION

Wide spectrum of different ergonomics interventions were found in this research. Most of the changes focused on well-known ergonomics methods like:

Rapid Upper Limb Assessment (RULA);

Rapid Entire Body Assessment (REBA);
Ovako Working posture Assessment System (OWAS);
The Quick Exposure Check (QEC);
Strain Index (SI);
Rapid Office Strain Assessment (ROSA);
Occupational Repetitive Actions (OCRA);
National Institute for Occupational Safety and Health Lifting Equation (NLE);
Association of periOperative Registered Nurses (AORN) Ergonomic Tool.

Work processes and tools´ design were the common target topics of ergonomic interventions. The latter involved situations when it was started to utilize new instrument or some modification with old ones or totally new tool was designed or change in work processes, work methods or techniques was implemented. Special auxiliaries categorized as well in this category. All equipment interventions were included in this distribution, too. Usually the questionnaires were used to determine the prevalence of musculoskeletal disorders for pre- and post-intervention period. The Nordic Musculoskeletal Disorders Questionnaire was used most often.

In the observed studies the next questionnaires were used:
Nordic Musculoskeletal Disorders Questionnaire (NMQ);
Copenhagen Psychosocial Questionnaire (COPSOQ);
Corlett and Bishop's body part discomfort scale (BPD);
Work-related upper extremity musculoskeletal symptoms (WUEMSS);
Dutch Musculoskeletal Questionnaire (DMQ);
Ergonomic Questionnaire (EQ).

Workstation improvement and knowledge training were the interventions on the fourth place. Workstation improvements interventions considered the ergonomically well designed workstations, where workplace adjustment and improvement of workstation were under observation. Some analysis used Quick Exposure Check (QEC) to get overview of workplace. Assessment of body posture and anthropometric measurements belonged to this intervention methodology. Training and educational interventions have used by eleven researches and have shown to reduce MSDs among employees. Many publications have concentrated on risk analysis and improvements of work environment. Work environment observations and measurements, ergonomic inspection, analysis, survey, inclusion of ergonomist or occupational therapist and ergonomic counselling were the most often used methods. Biomechanical analysis of repetitive movements was used by some researches. There were also interventions where material handling tasks, physical exercises and health care procedures were used. The categorized ergonomic intervention methods used in the present literature review are shown in the Fig. 2.

The combined interventions were under the analysis as well. A systematic approach to intervention appeared to be more useful.

To reduce or prevent MSDs in one engineering company a professional guidance of occupational therapist or physical therapist was used to control the workstations and this intervention program was successful (Goodman et al., 2005).

Great illustration of tool based intervention was carried out among 105 male assembly workers of a semiconductor in Tehran province. Implemented was a new tool to minimize musculoskeletal discomfort. They started to use magnificent loupes. The standardized Nordic Musculoskeletal Questionnaire (NMQ) was used to determine the prevalence of MSDs. To evaluate body discomfort before and after the intervention Corlett and Bishop's body part discomfort scale (BPD) was used. After ergonomic intervention significant decrease of discomfort was observed in neck, shoulder, upper arm, elbows, lower arm, lower back and whole body discomfort (Aghilinejad et al., 2016).

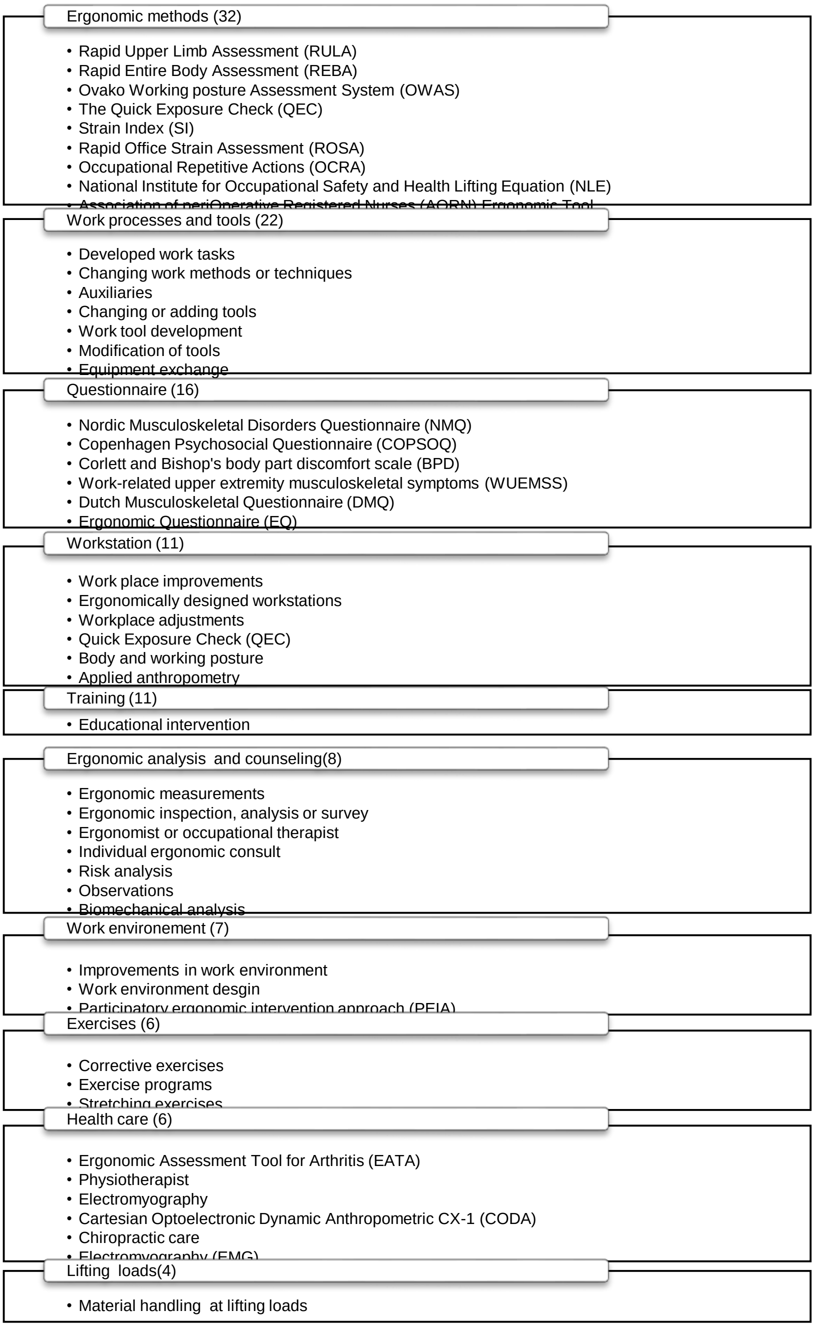

Figure 2. Categorized ergonomic intervention methods.

Educational training (pamphlet, lecture and workshop) in the automobile factory in Iran was implemented in three different ways. Workshop as an ergonomic training method was effective, decreasing the prevalence of neck and shoulders complaints among factory workers (Aghilinejad et al., 2015).

That body posture and workstation layout interventions are successful it has been described among 400 computer workers in United States. After baseline assessment, those in the intervention group participated in a multicomponent ergonomic intervention program including a comprehensive ergonomic training consisting of two interactive sessions, the ergonomic training brochure was divided, and workplace visits with workstation adjustments. Follow-up assessment after 6 months intervention showed significant decrease of work-related upper extremity musculoskeletal symptoms (WUEMSS) decreased significantly in the intervention group compared with the control group. Physical and mental health-related quality of life improved significantly compared to the reference group (Esmaeilzadeh et al., 2014).

It is well-known that poor lighting conditions cause eyestrain and in long perspective vision impairment and decrease of work productivity. Hemphäläa & Eklundb (2012) demonstrated that new lighting system improved the illuminance and light distribution in mail sorting facilities in Sweden. Also, the new acquired personal spectacles diminished eyestrain among the postal workers. Use of the specific type of sorting spectacles among those, who already used progressive lenses privately, improved head postures, alleviating muscular strain and decreasing risk of MSDs. (Hemphäläa & Eklundb, 2012).

Fourteen effective ergonomic interventions are described in the Table 1. There are six of them carried out in industries, four among health care workers and four among officials, office workers and teachers. The articles are categorized yearly.

As we can see in the Table 1, the combined interventions had positive effect among fifty nurses in Hong Kong. The 8-week intervention program consisted of ergonomic training, daily exercise program, equipment modification, computer workstation assessment and typing training. Positive results were found in decreased symptom scores (Szeto et al., 2013).

The combined ergonomic intervention (training, software and sport exercises) was carried out among the gas company staff in Iran and the used activities were compared. The Nordic Musculoskeletal Questionnaire (NMQ) was used for measurement of prevalence of MSDs and rapid upper limb assessment (RULA) for assessment of upper limb posture risks. The results of used activities were compared using McNemar test, t-test, and Chi-square test. Significant decrease of musculoskeletal symptoms was detected in the group after they received the training. McNemar test showed that pain intensity in the lower back, neck, knee, and wrist was significant ($p < 0.05$). The results obtained from the RULA method for evaluation of posture showed an average 25 points decrease in the right side of the body and 20 points decrease in the left side of the body in the group subjected to training. Based on t-test, the decrease was significant. The study demonstrated that majority of the participants accepted interventions and seeking the ergonomic improvements at the workplace. Overall, the findings show that that all three interventions training, chair adjustment, and arrangement in workplace could decrease MSDs and most effectively in the training group (Habibi & Soury, 2015).

Table 1. Successful ergonomic interventions in different fields of economic sectors (Abbreviations: IEI – individual ergonomic intervention; PEI – physical ergonomics intervention; OEI – organizational ergonomic intervention; CEI – combined ergonomic interventions)

Authors	Year	Location	Subjects	Intervention type	Control group	Dependent measures	Statistics	Results	Comments
Aghilinejad, M., Azar, N., Ghasemi, M., Dehghan, N. & Kabir-Mokamelkhah, E.	2016	Iran	Assembly workers N = 105	IEI: Using a magnifying loupes	Pre- and post-intervention results	NMQ (BPD)	$p < 0.05$	Decreased discomfort in neck, shoulder, upper arm, elbows, lower arm, lower back	Decreased whole body discomfort. There was no reference group, but results were good
Abdollahzade, F., Mohammadi, F., Dianat, I., Asghari, E., Asghari-Jafarabadi, M. & Sokhanvar, Z.	2016	Iran	Nurses N = 147	PEI: Working postures, educational programs.	No	REBA	Exercise ($p = 0.048$), Experience ($p = 0.003$), Shifts num. ($p = 0.006$)	Working posture can consequently lead to promotion of health and well-being	No reference group, but effective results
Habibi, E. & Soury, S.	2015	Iran	Industry workers N = 75	PEI: Training, exercise, and software	No	NMQ RULA	McNemar test: pain in low back, neck, knee, and wrist $p < 0.05$	Training, chair adjustment, and arrangement in workplace could decrease musculoskeletal disorders	No reference group, but effective results
Ghanbary, A., Habibi, E. & Darbandy, A.A.	2015	Iran	Household workers N = 100	CEI: Posture analysis Training	No	NMQ OWAS	Work experience ($p < 0.01$)	The prevalence of MSDs had significant correlation between work experience	Worker´s work experience has influence to disorders

Table 1 continuing

Aghilinejad, M., Kabir-Mokamelkhah, E., Labbafinejad, Y., Bahrami-Ahmadi, A. & Hosseini, H.	2015	Iran	Automobile factory workers N = 503	OEI: Ergonomic training (pamphlet, lecture, workshop)	Yes	NMQ Chi-square and Mann-Whitney tests	Recent week ($p = 0.002$) year ($p = 0.02$)	Workshop helped to decrease the prevalence of neck and shoulders complaints	Large amount and three different training interventions.
Esmaeilzadeh, S., Ozcan, E. & Capan, N.	2014	United States of America	Computer workers N = 400	CEI: Body posture and workstation layouts	Yes	Ergonomic Questionnaire WUEMSS	Body posture ($p < 0.001$) workstation layout ($p = 0.002$) physical ($p < 0.001$), mental $p = 0.035$)	WUEMSS decreased significantly	Large amount and combined interventions.
Jian, S., Pengying, Y., Liping, L., Fengying, L. & Sheng, W.	2014	China	School teachers N = 350	PEI: Training	Pre- and post-questionnaires	DMQ NMQ	$p < 0.001$	The awareness rate, attitude and health behavior improved	Interventions had positive effect.
Kumar, B.A. & Begum, S.H.	2014	India	A pedal operated maize sheller	IEI: Designed tool	Yes (manually operating)	Collection efficiency through put rate	150 kg h^{-1}	This tool help to 5.5 times more than hand operated sheller	Good design and good analysis
Szeto, G.P., Wong, T.K., Law, R.K., Lee, E.W., Lau, T., So, B.C. & Law, S.W.	2013	Hong Kong	Nurses N = 50	CEI: Training Exercises Work processes and tools Workstation	Yes	NMQ NPQ CODI DASH	$p < 0.05$	Positive results in decreased symptom scores	Combined interventions showed positive effect

Table 1 continuing

Rafeemanesh, E., Jafari, Z., Kashani, F. & Rahimpour, F.	2013	Iran	Dentists N = 58	PEI: Improvement of job postures	No	NMQ REBA	Prevalence of MSD 75.9% for the neck, 58.6% for the shoulders, 56.9% for the upper back, 48.3% for the lower back and 44.8% for the wrist	Overview of musculoskeletal disorders among dentist	Concluded that work postures of dentists need to be improved
Hemphäläa, H. & Eklundb, J.	2012	Sweden	Workers in mail sorting N = 75	OEI: Work environment and auxiliaries	Yes	EMG	Eyestrain. 44% -> 32%	Eyestrain decreased and productivity increased	Better work conditions improve health and productivity
Bernardes, J.M., Wanderck, C. & Moro, A.R.	2012	Brazil	Manual material handling operators N = 500	OEI: Ergonomic analysis rede-signed the assembly line's layout.	No	NIOSH equation	$p < 0.05$	Lift index > 1.0	Low back pain was totally eliminated
Ma, C., Szeto, G.P., Yan, T., Wu, S., Lin, C. & Li, L.	2011	China	Computer workers N = 72	PEI: Training	Yes N = 3	EMG	$p < 0.05$ Cervical erector spinae muscle, bila-teral upper trapezius	Pain reduced significantly more in the biofeedback group	Training had positive effect
Nader, R., Effat, B. & Fadya, R.	2010	Iran	Industry workers N = 91	PEI: 8-week corrective exercise program	No	NMQ	($p < 0.05$); low back (26.3 %), shoulder (18.9%) knee (17%)	Exercise program was effective to decrease work-related disorders	No reference group, but effective results

Physical activity has good effect on health. Very often corrective exercises have been used for individual ergonomic intervention. The experimental study was carried out among 91 workers in Teheran Loabiran industry. The corrective exercise program consisted of three sessions per week; each session extended 45 to 90 minutes). After the intervention it was concluded that corrective exercise program was effective and it was recommended to decrease risk of prevalence of MSDs among industrial workers (Nader et al., 2010).

Participatory ergonomic intervention was aimed to reduce lower back pain in the dispatch department of a catalogue and e-commerce retail company in Brazil. Based on the findings of the ergonomic analysis the company's own employees redesigned the assembly line's layout. Two job tasks of manual material handling were eliminated and more control over the jobs was given to the employees responsible for moving boxes from the end of the assembly line to pallets on the ground. The results demonstrated that participatory ergonomic interventions were effective – the revised NIOSH equation showed lower risk for lifting-related low back pain (Bernardes et al., 2012).

The study of 147 operating room nurses in Tabriz was conducted to evaluate working postures using a questionnaire and the Rapid Entire Body Assessment (REBA) checklist. The data were analyzed using t-test, Pearson correlation coefficient and analysis of variance (ANOVA) tests for univariate analysis and the linear regression for multivariate analysis. The mean (±SD) of REBA score was 7.7 (±1.9), showing high risk of working postures for nurses. There was significant relationship between daily regular physical exercises ($p = 0.048$), work experience ($p = 0.003$) and number of shifts per month ($p = 0.006$). The findings highlighted the need for ergonomic interventions and educational programs to improve working posture among nurses, promoting health and well-being of this group (Abdollahzade et al., 2016).

Training intervention among computer workers in an outpatient physiotherapy clinics and a local hospital was carried out in China. Activity of muscles 72 computer workers were measured by electromyographic (EMG) method. Post intervention, average pain was reduced significantly more in the biofeedback group than in the other 3 groups not exposed to training. This training intervention maintained for 6 months. Significant post intervention reduction of electric activity in cervical part of *m. erector spinae* and bilaterally in *m. trapezius superior* was measured in the biofeedback group (Ma et al., 2011).

The intervention where muscle activity was measured by using questionnaire before and after the intervention is widely used in the studies. One more of this was made in United States of America in dairy industry. Surface EMG was sampled continuously during the entire work shift while workers performed milking parlor tasks (Roscerance & Douphrate, 2012).

In 2015 was made successful case study, which proved that worker´s age and work experience have influence to inchoative musculoskeletal disorders (Ghanbary et al., 2015). This means if worker have to be long time in not suitable work environment that will increase health issues.

All above mentioned interventions have showed statistically significant reduction of MSDs in all body regions of observed occupations, except one study among 165 call center operators. This one-year prospective study passed four workplace interventions: ergonomics training, trackball and ergonomics training, forearm-support board and ergonomics training and forearm-support board, trackball and ergonomics training. No any significant changes were found with change in neck–shoulder or left upper-extremity pain after the interventions (Krause et al., 2010).

Majority interventions focused on decreasing shoulder, neck and low back pain. Wrist, ankles, hips, knee and feet regions were less or not used.

Other important results

Often is believed that is too late to make any ergonomic interventions. Reasons are diverse: workers are too old or too sick, not enough time or money. In this systematic research several articles of ergonomic interventions which were applied on people with disabilities have found. These were not taken into account of the further analysis, but still were good examples. For instance an experiment which was carried out among workers who have arthritis is great example. In the total sample (n = 89) contained of 38% with rheumatoid arthritis and 62% with osteoarthritis. The work place ergonomic intervention group reported less arthritis symptoms than the others (Baldwin et al., 2012).

Ergonomic interventions can be used also on people who already have musculoskeletal disorders, named as case studies. In United States of America was in 2010 made experiment with administrative assistant who had work-related lateral epicondylitis. The worker received ergonomic and behavioral interventions to treat her injury that included modification to her work environment and education on modifying behaviors that would decrease stress and excessive work. Results were that client reported decreased headaches with improved lighting and increased tolerance to typing with the addition of a keyboard tray (McCormack, 2010).

The minus of this research is that it was made only with one person, but still had positive effect. Another similar case study was made with 26-year-old woman with right upper-extremity and neck pain. This report again demonstrates the importance of examining the work habits and work-related postures. Providing an ergonomic intervention in concert with traditional physical therapy may be the most beneficial course of treatment (Fabrizio, 2009).

A 41-year-old woman presented with hand weakness and numbness along the medial aspect of her right forearm and the three most medial fingers. Chiropractic treatment consisting of manipulation, soft tissue mobilizations, exercise, and education of workstation ergonomics appeared to reduce the symptoms of ulnar nerve compression symptoms for this patient. Over a series of 11 treatments, her symptoms resolved completely and she was able to perform work tasks without dysfunction (Illes & Johnson, 2013).

People with disabilities may allow ergonomists to develop specific solutions for successful intervention and improve their functional capacity. Being too sick is not the excuse to not implement ergonomic interventions. Even these ergonomic interventions have shown positive effect.

Even young people like school children could have musculoskeletal disorders. Ergonomically design furniture and ergonomic awareness reduce discomfort and pain. In Pakistan among 229 school children in 2nd Grade (age 8 years) and 5th Grade (age 11 years) were implemented ergonomic interventions. Weight of the bag, Rapid Upper Limb Assessment (RULA) score and Ergonomic Quiz were used as main indicator to analyze the effectiveness of programs to reduce ergonomic risks (Ismail et al., 2010). In consequence the age is also not a factor.

The economic benefits of ergonomic interventions may easily overwhelm the costs because of productivity and quality with improved health. To the allegation that there is not enough money against this there are also objections. There is possible to make calculations before interventions. Cost-benefit analysis (CBA) can help to justify an investment in ergonomics interventions. A predictive CBA model would allow practitioners to present a cost justification to management during the planning stages, but such a model requires reliable estimates of the benefits of ergonomics interventions. Cost-justifying ergonomics interventions prior to implementation may support secure management support for proposed changes (Goggins et al., 2008).

CONCLUSIONS

Very wide spectrum of different ergonomics interventions focused on well-known methods. Work processes and tools are also very common ergonomic interventions. Workstation improvements, trainings, various ergonomic analysis, work environmental modification, exercises or using guidance of physiotherapist were the most effective interventions diminishing risk of MSDs among employees. The questionnaires were most often used to determine the prevalence of MSDs pre- and post-intervention program.

Based on the successful interventions described in this research it can be concluded that most commonly used ergonomic interventions were primary and secondary, Among them was most often used PEI – physical ergonomic intervention (6 times), then CEI – combined ergonomic interventions have used three times and OEI – organizational ergonomic intervention and finally IEI – individual ergonomic intervention (2 times). Primary physical ergonomic interventions were improvements of working postures or training exercise programs. Primary organizational ergonomic interventions have focused on the problems of organizational level for instance redesigning assembly lines layouts or work environmental interventions. Primary individual ergonomic interventions concentrated on re-design of work place or use of new tools or auxiliaries. Combined interventions use several interventions altogether. Among industrial workers for example there was implemented as individual as far as organizational interventions. In medical field the corrections of working posture, physical and combined ergonomic interventions were used. Among computer workers body posture and workstation corrections were used to decrease risk of MSDs.

In general ergonomic interventions are successful to reduce MSDs at work. Standardized ergonomics methods (RULA, REBA, OWAS, ROSA and OCRA) are most often used interventions. Ergonomic specialists are best suited to develop cost-effective interventions that can be easily implemented. Effective ergonomic interventions used large size of samples, compared the results with the control groups, measured the results in pre- and post-intervention period and these interventions were comparable with other researchers' results. There is no any excuse like illness, money or age not to implement ergonomic interventions.

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PID control for sprayer sections under laboratory conditions

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Abstract. The objective of this study was to develop and test a PID controller to adjust the height of bum sections of sprayer booms. This study was conducted under laboratory conditions using an experimental frame representing the boom sections of the sprayer. The boom section was operated using an electro-hydraulic system driven by a hydraulic power pack. Ultrasonic distance sensors were used to adjust the height of the boom tips based on the set height values. During the on-off control, the sudden openings of the hydraulic valve conduits caused oscillations, resulting in unstable operation. In the PID control, increased gain up to 100% resulted in unstable operations and resembled the response of on-off control. With the use of low gain values, oil flow delivered to the hydraulic actuator was adjusted accurately using the proportional valves and smoother boom section motion was possible. The effects of different disturbances on the system dynamic responses were presented graphically using PID control.

Key words: field sprayer, active boom equalization, electro-hydraulic control, PID control.

INTRODUCTION

In modern field sprayers, boom sections are controlled for levelling using two different types of systems: passive and active balancing systems. In passive balancing system, the boom sections become parallel to the ground plane by themselves. For this purpose, passive balancing systems with different designs are used. In the active control system, the boom sections are forced to maintain their horizontal balance using sensors (Çilingir & Dursun, 2009).

Jamming in the cylinders which ensure the vertical parallelism of the boom sections causes increased vibrations in section tips. The most important factors related to jamming are the type of flow rate valves in use and the electronic system controlling the valves. For an electronic control system to perform successfully, the electro-hydraulic system needs to be compatible. It is more appropriate to use a hydraulic system designed for proportional valves or servo valves for precise speed and pressure control (Anthonis et al., 2000; Kartal, 2007).

If the valve controlling a cylinder is turned on and off abruptly while the cylinder operates, the cylinder and the load on it vibrate. This vibration often leads to high levels of noise and impact, resulting from hydro-mechanical resonance. These vibrations can be prevented using servo valves or proportional valves.

Today, there are spraying machines with an working width up to 50 meters and more in different countries. However, increasing working width created the problem of balancing the vibrations at operation. The spraying machines which operate at a high width are equipped with sensors and other relevant equipment to prevent the vibrations from adversely affecting the quality of pest control (Güler et al., 2010).

Deprez et al. (2003) developed a mathematical model which can follow the inclination of the field so that the field sprayers will not be adversely affected by effect of slopes on the ground. The structure of the model was examined in trials on four different suspension systems. Analytically developed models were verified by the same researchers. For a slow and active suspension, a control mechanism was developed to filter out the ground plane ripples. A good performance was achieved despite the use of a low-power accelerator.

O'Sullivan (1988) reported the results of experiments carried out to verify the accuracy of mathematical models used for active and passive versions of a pendulum boom sections and concluded that the mathematical models could be used to design pendulum suspensions.

Koç & Keskin (2011) developed a system for developing and modelling a mechanism to ensure parallelism to the ground plane in oscillating agricultural machines. For the trials of the system developed, a prototype machine equipped with a boom with 5 m sections were embedded in an articulated way.

Pontelli & Mucheroni (2012) equipped each boom section with three position sensors 4, 8 and 12 meters away from the centre of the boom to apply PID and fuzzy logic control. As a control strategy, coefficients associated with the distance from the centre of the boom to the sensors and the weighted average of the information obtained from the sensors was used. A control equation was developed to maintain the height of the boom 50 cm above the ground plane. Tahmesebi et al. (2012) used PID simulations to control spray booms using intelligent active force control. PID control is utilized not only for section control in spray booms but also in tank level and mixture control in agricultural sprayers (Kushwaha & Giri, 2013) since the output can be generated in a short time with minimal overshoot and small error (Bartelt, 2001).

The aim of this study was to use PID as a control method on a sprayer section to reduce the instabilities that were experienced in on-off control method in the laboratory setting.

MATERIALS AND METHODS

The control system used in this study consists of a controller, electro-hydraulic system, and distance sensors. Prototype boom sections were made in the laboratory with short (1.5 m long) boom sections attached to the both sides of the representative sprayer frame. Ultrasonic distance sensors were fixed at both ends of the section tips where each boom section is controlled using a hydraulic cylinder (Fig 1).

Closed-loop block diagram to control the hydraulic cylinder is given in Fig. 2. Depending on the distance information collected from the sensors, the amount of fluid passing through the proportional valve was controlled by the PID and the cylinder speed was adjusted by the proportional valve. The control system consisted of a technological

CPU, a proportional valve for each cylinder, an ultrasonic sensor for height/distance measurements, and the hydraulic power pack.

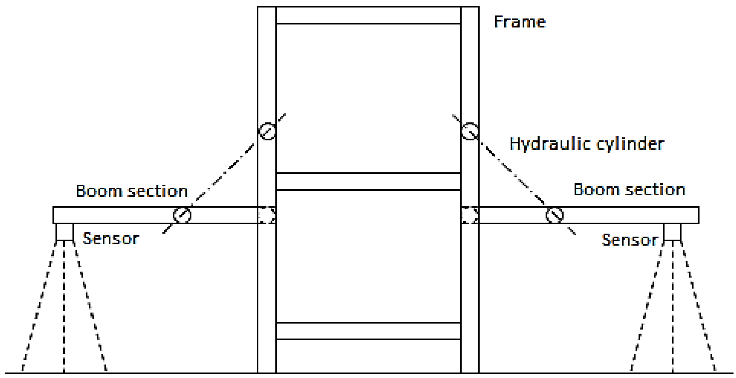

Figure 1. Schematic of the boom sections used in the study (Karadöl & Arslan, 2014).

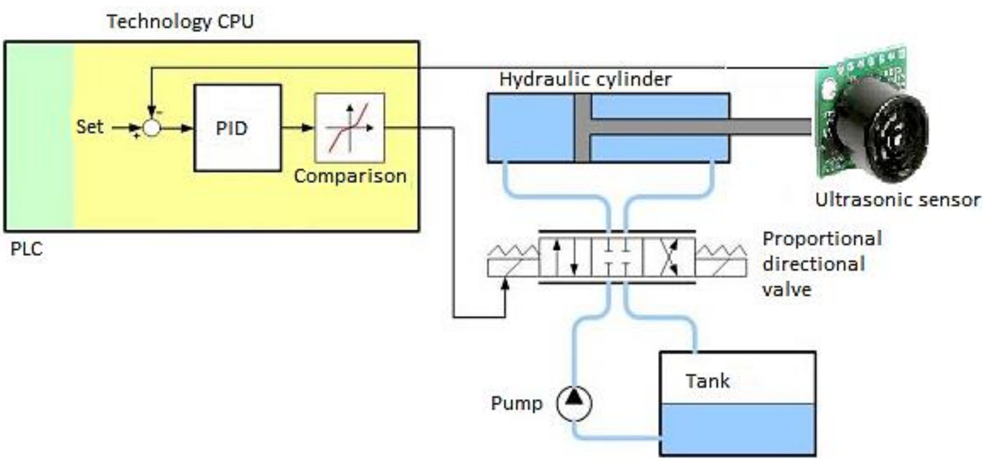

Figure 2. Cylinder control with a PID controller (Siemens, 2015).

The feedback signals that the sensors produced, depending on the distance to the ground plane, were applied to the PLC (Programmable Logic Controller) analogue input channels. Depending on the error signal, the analogue signal in the 0–10 V range produced from each PID analogue output was processed and the cylinders were controlled using the proportional direction control valve. PID controller generates an output based on the error signal and the error signal is sent the proportional valve card to control the hydraulic cylinder.

PID control was done using the signal output of the distance sensors for feedback, which was explained by Eq. 1.

$$c(t) = K_p e(t) + K_i \int_0^t e(\tau)d\tau + K_d \frac{de(t)}{dt} \tag{1}$$

where c (t) is control signal, e (t) is error signal, Kp is proportional gain, K_i is integral time constant, and T_d is derivative time constant. The block diagram of the commonly used closed-loop PID controller system is shown in Fig. 3 (Kaçar et al., 2013).

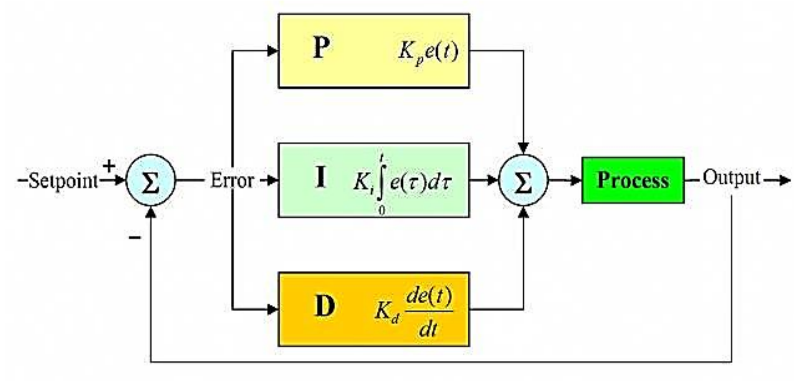

Figure 3. PID controlled closed-loop system.

Proportional valves were controlled by special electronic cards generating PWM (Pulse-Width Modulation) outputs with a variety of duty cycles. PWM pulse width was determined by applying an analogue input within the range of 0–10 V to the control card and the fluid path was opened proportionally to the analogue signal fed in the control card. Theoretically, 0 V input meant that the fluid path was completely sealed whereas 10 V input meant that the fluid path was completely open.

The distance sensor was able to determine the objects from 15 to 646 cm distance and could produce 9.766 mV output signal for each 2.54 cm difference in distance starting from 15 cm. In this study, ultrasonic distance sensors (LV Maxsonar EZ1) were used. The stable operating voltage and the frequency of the sensor were 5 V and 42 kHz, respectively.

Eq. 2 was used to measure the distance between the sensor and the ground.

$$V_i = (V_{cc}/512) \tag{2}$$

where V_{cc} is the supply voltage whereas V_i is the voltage value produced by the sensor.

For the supply voltage value of 5 V, the analogue voltage value produced by the sensor within 80 cm distance was calculated as follows.

For 2.5 cm; V_i = 5.0 V/512 = 9.766 mV

For 80 cm; V_i = 9.766·80/2.54 = 307.6 mV

The distance from the sensor and the ground was varied by 10–20 cm with the introduction of disturbances and the system's response was observed under different operating conditions.

RESULTS AND DISCUSSION

The distance sensors were attached to the tips of boom sections and were able to produce outputs with 2.54 cm precision. It was estimated that it could operate with 3% error margin for a height setting of 80 cm in field conditions. The measured voltage values were correlated with boom height with $R^2 = 0.99$ and the sensor output signal could be measured up distances much greater than the boom heights in real applications.

When the analogue signal from a sensor was applied to the analogue input of PID controller (Input_PER), the controller generated a signal from the output channel (Output_PER) within the 0–10 V range in accordance with the error value (Fig. 4). The analogue signal produced by using the amount of error change in the output of the PID block is applied to the control card of the proportional valve. Based on the signal applied to the control card input, a PWM signal is produced in the card output. By adjusting the duty cycle of the PWM signal in response to the 0–10 V value, the oil flow rate passing through the proportional valve was adjusted precisely. The oil flow rate delivered to the cylinders increases as the system drifts away from the set value (as the error value increases) and decreases in the opposite scenario, thus enabling to reach the set value smoothly. The openness rate of the PID controller's output channel was tracked online and by switching this value to the manual mode the output rate was brought to the desired level (Fig. 5).

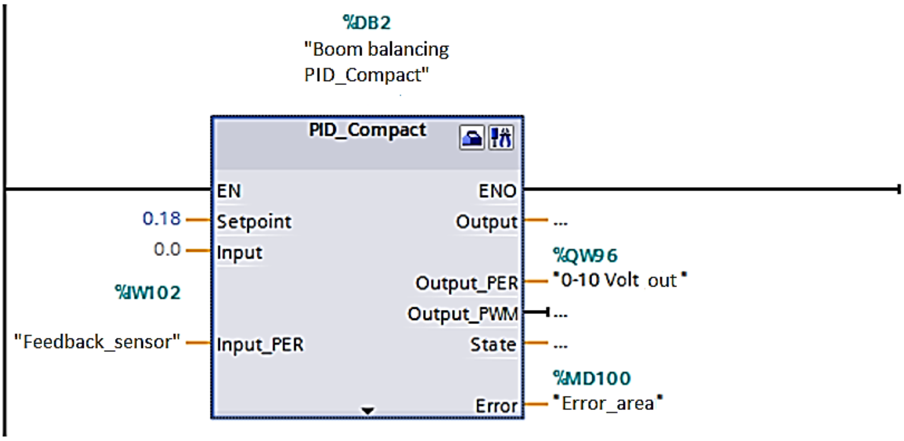

Figure 4. The structure of the PLC-PID block.

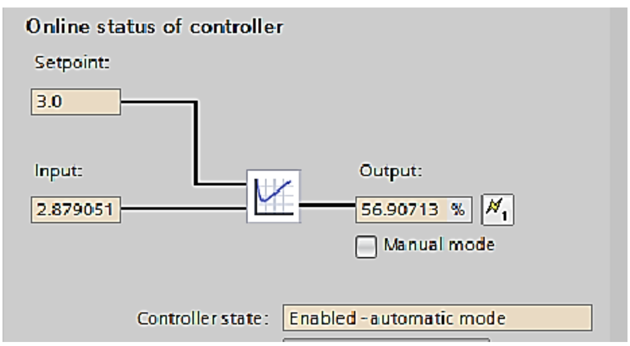

Figure 5. PLC-PID controller input and output statues – values on the chart were amplified using an op-amp in this trial.

During control operation, the input, output, and set values were tracked graphically online, shown by the red, green, and blue lines in Fig. 6, respectively. In this figure, the disturbance generated a voltage value greater than the set point because the distance between the boom section and the ground was increased.

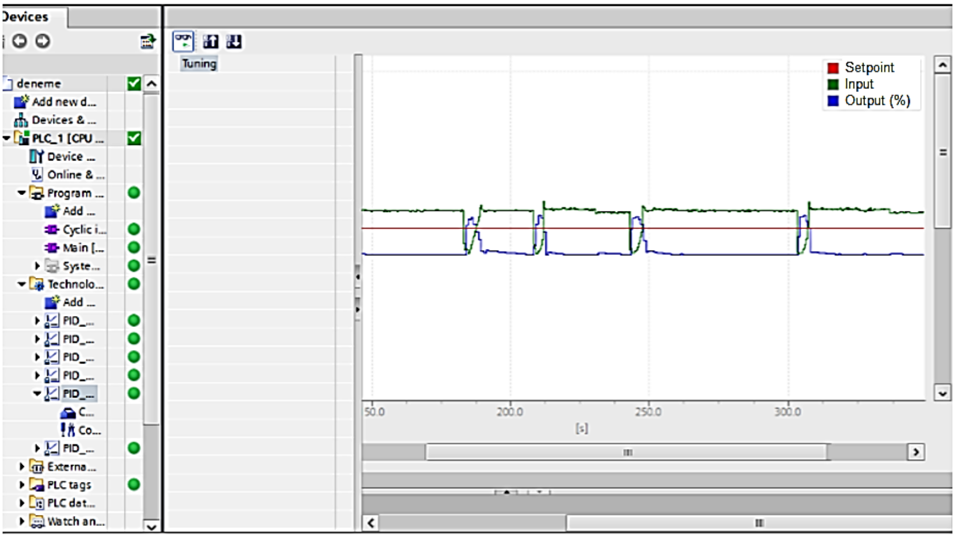

Figure 6. Tracking the set point, input, and output signals in an ongoing process.

In an ongoing operation with no disturbances, all three lines were at the set value (0.180 V corresponding to about 47 cm) with zero feedback as seen in the leftmost part of Fig. 7. When a disturbance was introduced between the sensor and the ground, the magnitude of the measured signal voltage reduced proportionally to the distance between the sensor and the disturbance. Output voltage signal was increased based on the feedback signal, allowing the proportional valve to open to compensate for the input so that the boom section height could be maintained at the initial height setting (Figs 7–8). The system quickly responded to short (1 s) and long (10 s) disturbance signals and brought the boom section to balance. It was demonstrated that the control system was able to return to the set point as a response to continual disturbances by generating an immediate output signal based on a disturbance signal (Fig. 8). However, the response should be improved when the disturbance is introduced successively between the ground and the sensor (Fig. 7). Although the output signal could be generated in response to disturbance signal, the set point could be reached with an error from 2.5 to 5 cm until the next disturbance was introduced. However, the system behaviour was better when mixed disturbances were used at lower and higher locations from the set point (Fig. 8). Successive disturbances were off set successfully by the control system both at a constant height and varying heights, corresponding to varying levels of disturbance signals. The system optimizes its dynamic response to the disturbances through the automatic tuning function that is used for obtaining the best gain value during operation. More tests under laboratory and field conditions can help improving the system response.

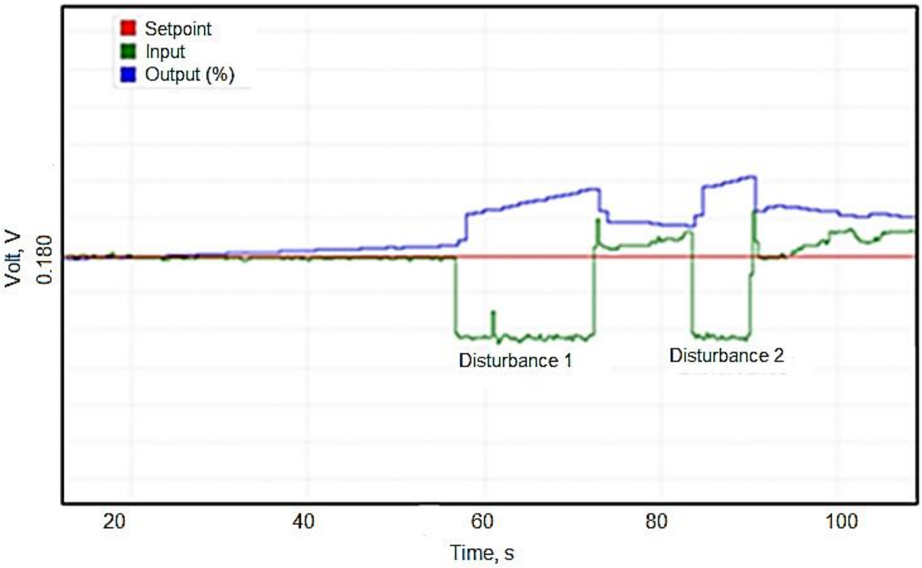

Figure 7. The status chart of the ongoing system at set values against disruptive factors.

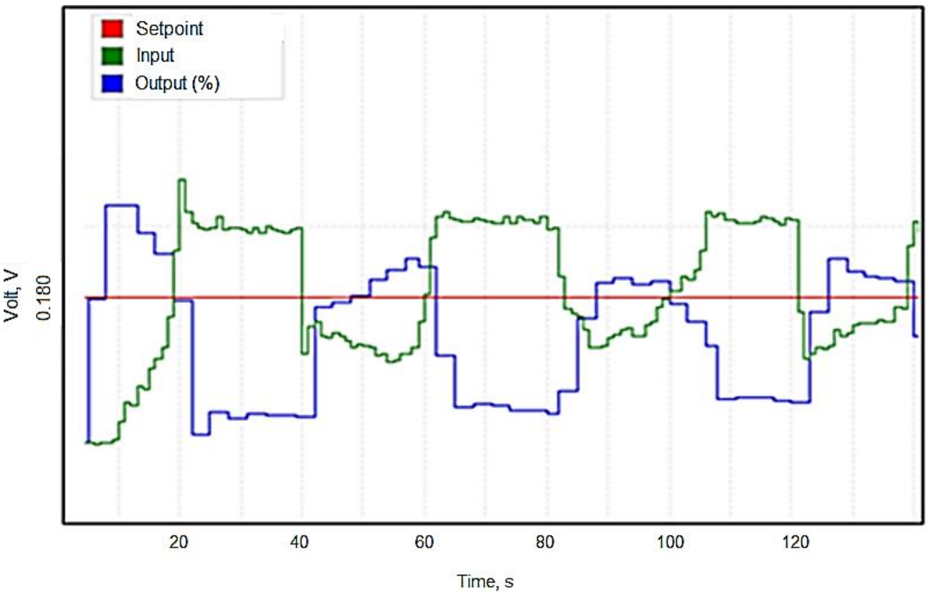

Figure 8. The status chart of the system against continual disturbances.

To draw a comparison between PID and the on-off control, the PID gain (K) value was set very high and the operation of the system was simulated as an on-off controller. In this case, the proportional valve was fully open and lead to occasional oscillations in the system by giving 100 percent output on all error values (Fig. 9). This behaviour was similar to the dynamic behaviour observed in on-off control experiments where the signal fluctuated before reaching the set point (Karadöl & Arslan, 2014). In this case, the control system tries to bring the boom section to the set value, but surpasses it, changes direction and draws closer to the set value or reaches it. Due to this operation model, the set values cannot be reached smoothly but may be possible through several back and forth movements. Subsequently, an oscillating control was achieved.

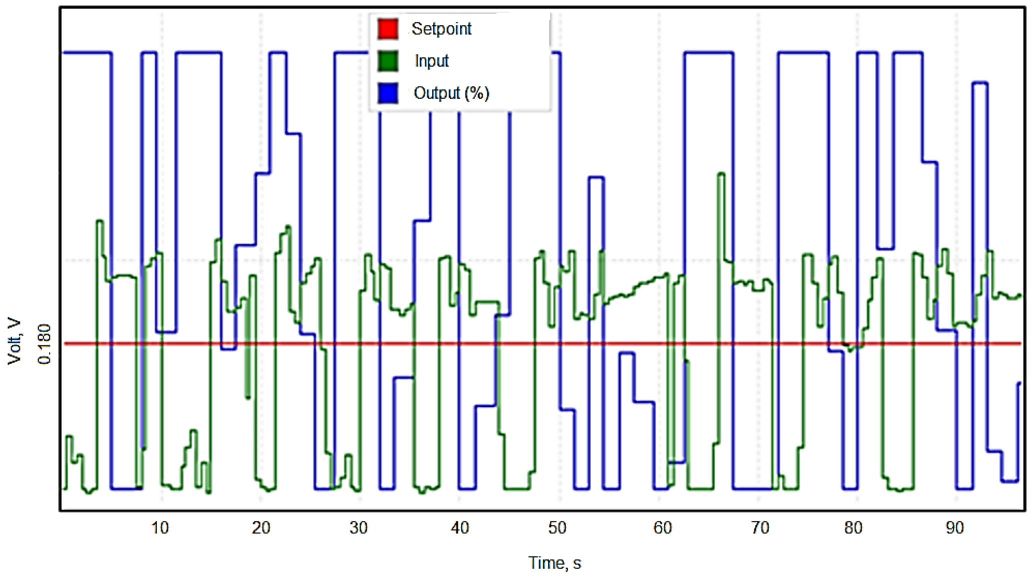

Figure 9. The status chart similar to on-off control due to a very high proportional gain value.

Consequently, the experimental findings showed that when the on-off control was used to balance the boom section, the balance point was reached through a series of intermittent movements. In the case of PID control, a proportional controller was used and the section balance disrupted by the disturbance was restored to the set value with a smooth transition. As the gain was increased by the PID, the system behaviour was similar to that of the on-off system. In order to adapt these findings obtained in laboratory conditions to the boom levers of an actual spraying machine, a few small adaptations may be needed in the controller that was developed.

CONCLUSIONS

PID controller with a technological CPUenabled smooth boom section movements when disturbances were introduced up to 20 second durations. The gain can be optimized by executing the software program developed under various operating conditions. Increased gain resulted in an operation similar to on-off control, which is not favourable. More appropriate gain values should be determined using the results from both laboratory and field tests. In on-off control to adjust the boom section heights, the solenoid valves fully open the conduits resulting in unwanted oscillations in the system and results in an intermittent control process.

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Nutrient status of the American cranberry in Latvia (2005–2016)

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Abstract. The American cranberry (*Vaccinium macrocarpon* Aiton) is an evergreen groundcover plant native to North America. Nowadays cranberries are successfully cultivated in Latvia with total plantings of more than 125 ha. Being a native wetland plant, cranberries are considered as nutrients low requiring crop, however, balanced mineral nutrition is one of the key factors that determine plant growth and yield development. Surveys were carried out to determine the actual status and trends in mineral nutrition of American cranberries in Latvia during 2005–2016. Together 190 plant samples were collected from different cranberry producing sites in Latvia over 3 periods: 2005–2007, 2008–2011 and 2012–2016. Cranberry tissue analyses were used as diagnostics method to control plant nutrient (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B) status. The obtained results revealed notable nutrient imbalance in the years of the study. In general, our results suggest that only about 50% of plant tissue nutrient indices were in the sufficient range. Deficiency of N P, S, Fe, Cu, and Mo, and high levels of Mn was found in the majority of samples analyzed. In general, diverse tendencies were stated for the nutrient supply of cranberries from 2005 to 2016: positive trend in nutrient status of cranberry crop were found for N, K, Ca, while mean concentrations of S, Fe and Mo, as well as frequency of optimal indices decreased. The small count of nutrient indices in high till toxic range suggested on environmentally sound way of cranberry fertilization practices in Latvia.

Key words: *Vaccinium macrocarpon* A., plant analysis, mineral nutrition.

INTRODUCTION

American cranberry (*Vaccinium macrocarpon* Aiton) is evergreen, perennial groundcover plant native to eastern and central North America including the eastern territories of Canada (Trehane, 2004). Commercial production of cranberries in the United States of America has existed for close to two centuries (Eck, 1990).

American cranberry has significant commercial value and offers important health benefits. Cranberry fruits are rich sources of bioactive compounds, such as phenolics, organic acids, anthocyanins, proanthocyanidins, flavonol glycosides and sugars (Reed, 2002; Szajdak & Inisheva, 2016). Cranberry juice and fruits are widely reported to demonstrate a number of health advantages including: inhibition of development and progression of cancer and cardiovascular diseases, antimicrobial activities and prevention of urinary tract infections and stomach ulcers, cholesterol reduction and the reduction of biofilm formation (Vattem et al., 2005; Chi-Hua Wu et al., 2008; Blumberg et al., 2016; Maki et al., 2016; Das et al., 2017). Berries are also valued for their fresh taste as well as their potential for being processed. Today, an increasing demand for healthy ingredients by the food industry and changed consumer consciousness provide great opportunities for further progress of cranberry production.

In general, about 98% of global production comes from the United States of America and Canada alone. However, with more than 125 ha of commercial plantings, Latvia is the fifth major cranberry producing country (worldatlas.com). The cultivation of cranberries in Latvia is comparatively recent – the first experimental plantation was established in 1985, but commercial cultivation of American cranberries started in last 20 years (Osvalde & Karlsons, 2010). Latvia is a country with abundant peat resources and intensive peat production. The total area of peatlands covers 10.7% of the entire territory and raised bogs occupy more than 41% of the peatlands. At present, about 9% of Latvia's raised bogs (about 70,000 ha) are affected by peat extraction, 20,000 ha are nearly exhausted (Osvalde et al., 2010; Silamikele et al., 2011). Therefore, the need of recultivation of more than 17,000 ha abandoned and excavated high bogs, along with moderate climate, sufficient freshwater supply, and high market demand provide excellent cranberry growing conditions.

Overall, cranberry growth and development are affected by many internal (plant physiology, genetics) and external factors such as light, temperature, water availability and quality. The cranberry plants are adapted to sandy (North America), nutrient-poor, low pH soils and its nutritional requirements are low compared with many other perennial fruit crops. However, proper mineral nutrition is one of key factor which can affect cranberry yield amount and quality (Roper, 2009; DeMoranville, 2015). While cranberry nutrient management and status in the North America has been studied in considerable detail, mineral nutrition problems of cranberry crop in Latvia and other European countries where production is recent have not been elucidated sufficiently (Osvalde & Karlsons, 2010; DeMoranville, 2015). In the United States and Canada cranberries are grown in beds layered with sand, peat, and gravel located in bogs which were originally formed as a result of glacial deposits. Applying 0.6 to 2.5 cm of surface sand to cranberry beds every 3 to 5 years is a common practice in commercial cranberry production for the majority of cranberry plantations in North America. As a result rooting zone typically contains about 95% sand (Davenport & Schiffhauer, 2000). Mostly sand is applied to cover old runners to stimulate new root development and upright production, as well as to control weeds and pests (Eck, 1990; Sandler et al., 2014). Also, roughly 90% of growers use a flood to protect the plants from desiccation in the winter and to harvest the fruit in the fall (DeMoranville, 2008). These cultivation conditions and technologies are considerably different from those in Latvia where cranberry plantings are mostly developed in raised bogs and flooding technique is not used. Therefore, direct application of nutrient recommendations from N. American production areas is limited. Considering that average yield in Latvia (2.3 t ha^{-1}) is significantly lower to compare with the United States of America (23.2 t ha^{-1}) and Canada (27.9 t ha^{-1}) research on mineral nutrition as one of potential limiting factors of reduced yield of American cranberries in Latvia are critically important (factfish.com). Over the last few years, several investigations into optimal cultivation technologies of cranberry crop grown in raised bogs were made. These studies are only in preliminary stage and reports concerning the mineral composition of cranberry plants grown in acid sphagnum peat as well as nutrient concentrations in this specific substrate are still scarce (Osvalde & Karlsons, 2005; Osvalde & Karlsons, 2010). A survey was carried out to determine the status and main trends in mineral nutrition of American cranberries in Latvia over 3 periods: 2005–2007, 2008–2011 and 2012–2016. Plant tissue analysis was used to evaluate the availability of nutrients (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo and B) in cranberry production in Latvia.

MATERIALS AND METHODS

Together 190 cranberry plant samples were collected from 10 different commercial cranberry producing sites in Latvia over three time periods 2005–2007 (44 samples), 2008–2011 (72 samples) and 2012–2016 (74 samples). All plantations were developed on extracted raised bogs and annually fertilized. For each plant sample 200 upright tips of current season were collected from locations representative of the planting. For analysis of nutrients, dry plant material was finely ground using a laboratory mill. Samples were dry-ashed in concentrated HNO_3 vapors and re-dissolved in 3% HCl for K P, Ca, Mg, Fe, Cu, Zn, Mn, B and Mo detection. Wet digestion was used for for N (in H_2SO_4) and S (in HNO_3) determination in plant samples. The levels of Ca, Mg, Fe, Cu, Zn, and Mn were measured by atomic absorption spectrophotometry (AAS) *AAnalyst 700* (PerkinElmer, Singapore), acetylene-air flame (Page et al., 1982; Anonymous, 2000). K was detected with the flame photometer *JENWAY PFPJ* (Jenway Ltd, Gransmore Green, Felsted Dunmow, Essex, UK). The contents of P, Mo, N, S, and B were determined by colorimetry: P – by ammonium molybdate in an acid reduced medium, Mo – by thiocyanate in reduced acid medium, N – by Nesler's reagent in an alkaline medium (modified Kjeldal method), B – by hinalizarine in sulphuric acid medium, S – by turbidimetric method by adding $BaCl_2$ with a spectrophotometer *JENWAY 6300* (Barloworld Scientific Ltd., Gransmore Green Felstad, Dunmow, Essex, UK) (Rinkis et al., 1987; Karlsons et al., 2008; Osvalde, 2011). The statistical analysis of the results was carried out using *MS excel 2016* software. Standard errors (SE) were calculated in order to reflect the mean results of chemical analysis. T-test 'Two-Sample Assuming Unequal Variances' at $P < 0.05$ was used for testing the differences between study periods. Evaluation of the mineral nutrition status of American cranberry was done on the basis of tissue standards developed by Nollendorfs (1998) for Latvia.

RESULTS AND DISCUSSION

To characterize the nutrient status of American cranberries in Latvia the levels of 12 essential nutrients were estimated in plant tissue samples. The acquired results – mean and range of macro and micronutrient concentrations in cranberry leaves, as well as tissue standards developed by Nollendorfs (1998) for American cranberries in Latvia are shown in Table 1. Our results suggest that from 2005–2016, only 50% of plant tissue nutrient indices were in sufficient range (Fig. 1). Percentage of plant samples with low, optimal and excessive amount of nutrients clearly revealed main cranberry mineral nutrition problems in Latvia (Table 2). Overall, mean macronutrient concentrations in plant tissue could be characterized as optimal, with the exception of low N (2005–2007; 32% optimal), slightly decreased P (2005–2016; 16–38% optimal) and S (2008–2016; 15–43% optimal). Different trends in macronutrient status of cranberries were found between analyzed time periods from 2005 to 2016, depending on the particular nutrient. Optimal mean values increased for nitrogen from 0.78 (2005–2007) to 0.91% (2012–

2016). Percentage of samples in the optimum range also increased from 32% to 38% accordingly (Table 2). Despite the fact that mean N concentrations for time periods 2008–2011 and 2012–2016 were similar, only 29% of indices were in optimal range for 2008–2011. It is explainable by high dispersion of the N concentration in cranberry tissue samples: from serious deficiency (0.45%) to abundance (2.85%).

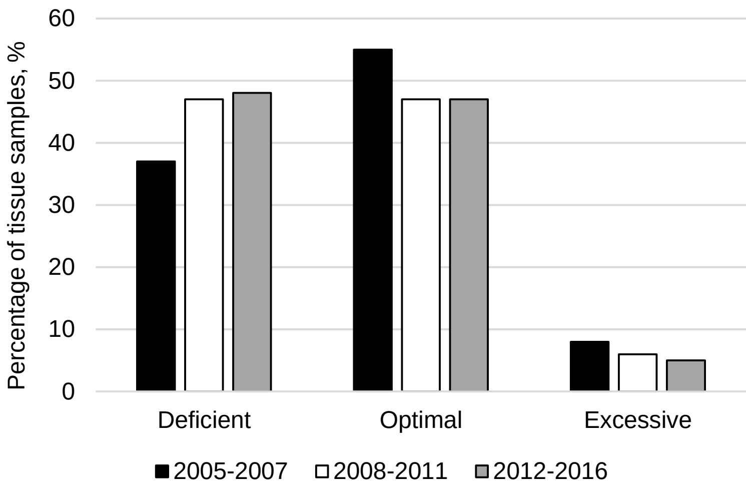

Figure 1. Distribution of total nutrient indices in different nutrient supply levels for cranberry tissue samples in Latvia, 2005–2016.

It should be noted that nitrogen is one of a key element in cranberry nutrition and adequate fertilization is necessary to maintain renewal growth, crop production, and flower bud development for next year's crop (Davenport, 1996; DeMoranville, 2015). The results obtained by Osvalde et al. (2011) on macronutrient concentrations in peat samples from different cranberry producing plantings in Latvia established on extracted raised bogs demonstrated serious N deficit in almost all of the peat samples, as well as insufficient mean concentrations of S.

Although a decrease of mean concentration of K was found in 2012–2016, in comparison to previous time periods, leaf K status for cranberries in Latvia could be characterized as optimal till high, as compared to sufficiency levels.

In general, a high range of macronutrient N, K and S concentrations was found: the ratio of the highest/lowest concentration for N-6, K-14 and S-9. Decreased S levels and a broad range of concentration are likely associated on the one hand with naturally low S concentrations in peat and increased leakage promoted by anionic properties, and on the other hand – with standard application of sulphate containing fertilizers.

Table 1. Nutrient concentrations in cranberry plant samples in Latvia, 2005–2016

Element	Concentrations in dried tissue						Optimal levels
	2005–2007		2008–2011		2012–2016		
	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	
Macronutrients, %							
N	0.40–1.35	0.78 ± 0.04a*	0.45–2.85	0.88 ± 0.04b	0.56–1.90	0.91 ± 0.03b	0.90–1.50
P	0.09–0.28	0.15 ± 0.01a	0.06–0.30	0.15 ± 0.01a	0.07–0.3	0.14 ± 0.01a	0.17–0.35
K	0.33–0.94	0.59 ± 0.02b	0.15–2.1	0.61 ± 0.03b	0.27–0.89	0.52 ± 0.02a	0.35–0.75
Ca	0.21–1.27	0.73 ± 0.04a	0.33–1.56	0.72 ± 0.03a	0.39–1.13	0.71 ± 0.02a	0.55–0.95
Mg	0.16–0.44	0.25 ± 0.01a	0.13–0.55	0.24 ± 0.01a	0.13–0.41	0.24 ± 0.01a	0.17–0.35
S	0.07–0.35	0.13 ± 0.01a	0.04–0.36	0.12 ± 0.01a	0.06–0.18	0.11 ± 0.01a	0.13–0.30
Micronutrients, mg kg^{-1}							
Fe	20–116	52.2 ± 3.2b	24–160	50.9 ± 2.8b	20–55	37.2 ± 1.2a	60–200
Mn	34–500	159.6 ± 15.1b	38–850	199.9 ± 20.5c	18.4–320	103.6 ± 8.5a	35–140
Zn	11–42	29.5 ± 1.0b	9–52	31.6 ± 1.1b	18.6–42	26.6 ± 0.6a	25–90
Cu	2.2–104	13.2 ± 3.2b	0.4–78	6.9 ± 1.2a	1.8–90	25.2 ± 3.9c	6.0–15.0
Mo	0.05–1.6	0.38 ± 0.05b	0.1–1.5	0.41 ± 0.03b	0.1–0.7	0.29 ± 0.01a	0.5–5.0
B	14–92	35.6 ± 2.1c	10–72	29.7 ± 1.5b	9–62	24.5 ± 1.2a	25–70

*Means with different letters in a row were significantly different (t-Test, $p < 0.05$, a< b < c).

Table 2. Distribution of cranberry plant tissue samples in different nutrient supply levels in Latvia, 2005–2016

Element	Percentage of samples (%) in different nutrient supply levels								
	2005–2007			2008–2011			2012–2016		
	Low	Optimum	Excessive	Low	Optimum	Excessive	Low	Optimum	Excessive
N	68	32	0	65	29	6	59	38	3
P	62	38	0	57	43	0	84	16	0
K	2	85	13	10	71	19	7	86	7
Ca	29	57	14	19	65	15	14	80	7
Mg	0	98	2	8	81	11	3	96	1
S	45	53	2	56	43	1	85	15	0
Fe	73	27	0	85	15	0	100	0	0
Mn	0	57	43	0	46	54	0	89	11
Zn	9	91	0	25	75	0	31	69	0
Cu	60	20	20	71	17	13	59	11	30
Mo	72	28	0	82	18	0	99	1	0
B	18	80	2	39	58	3	41	59	0

The levels of micronutrients in plant tissue were of particular interest as, with the exception of Zn and B, a serious imbalance in the microelement supply was found (Table 1). Insufficient levels of Fe and Mo, as well as increased concentrations of Mn, were found in in the vast majority of plant samples. In the years 2005–2011 only 15–27% of the samples had tissue Fe at concentrations ≥ 60 mg kg^{-1} which is optimal for cranberry growth, but in time period 2012–2016 already 100% of Fe indices were in insufficient range. The vast majority of samples tested (72–99%) were also insufficient in Mo (below 0.50 mg kg^{-1}). On contrary, there were no samples tested deficient in Mn. Moreover, about 43% (2005–2007), 54% (2008–2011) and 11% (2012–2016) of them were in the high range (above 150 mg kg^{-1}). Our study suggests that the increased Mn concentrations reflect high Mn availability in a low-pH cranberry soils (Osvalde & Karlsons, 2010) and possible application of Mn containing fertilizers.

At the moment the relationship between Mn and Fe in plants from acid soils has been little studied. In Nova Scotia, Canada, (Lockhart & Langille, 1962) measured Mn/Fe ratios in the leaves of lowbush blueberry (*Vaccinium angustifolium* Ait.), an acidophilic Ericaceous plant like cranberry, indicating possible interference of Mn with the uptake and utilization of iron at low pH values. The interaction between Mn and Fe, low Fe concentration in growing media, as well as insufficient fertilization are likely main reasons for dramatically low Fe supply in cranberry leaves. Low Fe supply in growing medium and accordingly in plant tissue are fundamental distinction compared Latvia and North America. In USA bog environments typically have abundant concentrations of soluble iron and other metals that may be even toxic to non-adapted plant species (Siebach et al., 2015).

According to recommendations based on first studies on cranberry nutrition in Latvia foliar supply of micronutrients (Fe, Cu, B, Mo) should be applied to correct specific deficiencies found in tissue tests (Osvalde & Karlsons, 2005). Our study demonstrates that only Cu status becomes more correspondent to tissue standards.

On, average 50% of the samples had tissue Cu levels below the optimal value of 6 mg kg^{-1}, while 20% to 30% were in high range (above 15 mg kg^{-1}). We suggest that considerable heterogeneity of Cu concentrations found in all study periods are linked with: a) excessive Cu levels were caused by contamination from foliar fertilizers and the use of fungicides with high Cu content, and b) insufficient Cu concentrations – by extremely low levels of Cu in the growing medium and lack of appropriate fertilization. In general, the results presented in our study differ from those of Roper & Combs (1992) for the nutrient status of Wisconsin cranberries where, with the exception of Zn, almost all cranberry producing beds had adequate conditions of mineral nutrition.

CONCLUSION

The main goal of cranberry fertilization is to remove limitation to yield and quality by supplying the plants with all nutrients in optimal concentrations. Plant tissue analysis revealed serious imbalance in American cranberry providing with essential mineral elements in Latvia during 2005–2016. Deficiency of N P, S, Fe, Cu and Mo was stated for vast majority of samples tested. Consequently, particular attention should be paid for decision making about fertilizer application rates and methods, as well as timing for these nutrients in cranberry nutrient management plan. Significant changes were found in the average nutrient concentrations during the past 12 years, showing a trend towards increase in nutrient status of N, K and Ca. Our research also reflects the decrease in concentration level and frequency of optimal indices for S, Fe and Mo. The present investigation suggests that environmentally wise cranberry fertilization in plantings mainly established on cutover peatlands is a site-specific decision and should be based on the precise analytical basis not only to obtain high and qualitative yield but also to prevent bog contamination.

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Air quality mapping using an e-nose system in Northwestern Turkey

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Abstract. A gas sensor based electronic nose system is developed for monitoring air quality dispersion in and around livestock barns. The mobile system can be used in various applications under laboratory and field conditions. The system consists of 10 metal oxide Figaro gas sensors and a temperature/humidity sensor integrated with custom made circuits and data acquisition software. The sensors are sensitive to major odorous compounds. The e-nose system provides an easy, cost effective and user friendly tool for air quality monitoring. There is a relationship with sensor responses and gas concentrations are linear. Therefore, instead of calculating concentrations using statistical methods such as PCA and ANNs raw sensor data is used to monitor air quality. In order to monitor spatial distributions of sensor responses Kriging method is applied. Interpolation maps for each sensor response are developed. In order to visualize the areas where air quality problems occur, response of an air quality module is used as reference. Results showed the effectiveness of the developed system and method.

Key words: Gas sensors, electronic nose, environmental quality, livestock housing, air quality.

INTRODUCTION

Odour generated from livestock buildings is considered to be one of the most important air quality problems in agricultural production systems. Air quality problems are not only an issue of nuisance it can also have effects on human/animal health by direct irritation or psychopathologic mechanisms (Schiffman & Williams, 2005). Therefore, measurement or evaluation of air quality problems associated with livestock odour is highly critical. However, there are still science-based approaches needed to evaluate air quality and related control technologies (Zhang et al., 2001). One of the most common techniques used to evaluate air quality related odour concentration is olfactometry. The olfactometers are used to gauge odour detection thresholds of substances. This technique employs human panellists in laboratory settings. The results are subjective to the panellists' senses (Powers & Bastyr, 2004).

Livestock odour is a mixture of various compounds and intensity of overall odour is not simply sum of all compounds since they interact with each other making the air quality assessment more complicated and complex (O'Neill & Philips, 1992; Schiffman et al., 2001; Zahn et al., 2001¸ Pan & Yang, 2007). Most of the odorous compounds are monitored with highly expensive systems. It is possible to conduct well comparable and controlled measurements with these technologies. However, it is not possible to monitor spatial distributions of air pollutants. Measurement of concentrations of only some components is not always enough to assess the air quality problem (Kaur et al., 2007).

There have been a great deal of effort to develop cost effective and mobile air quality monitoring devices that employ low-cost gas sensors integrated with navigation devices and mobile phones (Elen et al., 2012). There are applications of mobile devices used to acquire urban air quality data at a high temporal resolution. Also, use of mobile devices in such applications makes it possible to assess spatial variations of pollutants for short term studies which are not possible with stationary measurements (Westerdahl et al., 2005; Peters et al., 2012).

Electronic nose systems have been used as an alternative for non-invasive online monitoring of air quality issues especially related to biological processes (Bachinger & Haugen 2002). They can provide a signal that could be used to obtain information on various compounds (Romain et al., 2004). An e-nose also could be used to better understand odour release (Nicolas et al., 2001) because of their mobility and compactness. However, the cost of commercial and sophisticated e-nose systems is still high (Yin & Zhang 2016). Also, importing these systems to developing countries such as Turkey increases the cost considerably. Another shortcoming of using commercial systems is their easiness of use. They generally employ software or data management tools that are not user-friendly. Hence, there is a strong need to develop mobile and cost-effective (Jasinski et al., 2015), and user-friendly systems that is integrated with software written in native language, in our case Turkish.

In the development of e-nose systems gas sensors are used because of their high analytical performance and reasonable costs (Nenov & Yordanov, 1996). These sensors generate a current response signal that is proportional to ambient gas concentration. The relationship between sensor response and gas concentration is linear (Kızıl et al., 2000; Jasinski et al., 2015). In this study it was aimed to develop a mobile device and method that is applicable to on-site air quality monitoring based on the principle of this linear relationship. Development of e-nose system explained and a case study was conducted to evaluate the performance of the system.

MATERIALS AND METHODS

Study site

Aşağıokçular village (Fig. 1) is located in the North-western coastal province of Çanakkale, Turkey at 40° 3’N and 26° 27’E. The village is 14 km from the Çanakkale province, and 8 km from Kepez district. The economy is mainly based on agriculture. The province and districts’ population have been increasing in recent years threatening the Aşağıokçular’s agricultural land. There are poultry operations located nearby the village causing odour problems. Odour from a poultry operation housing total of 75,000 broilers in three deep-litter houses assessed. Rice hull, capable of absorbing moisture, is used as bedding and litter material. Charcoal is used within the heating system. Fan-pad evaporating cooling system is used in air conditioning.

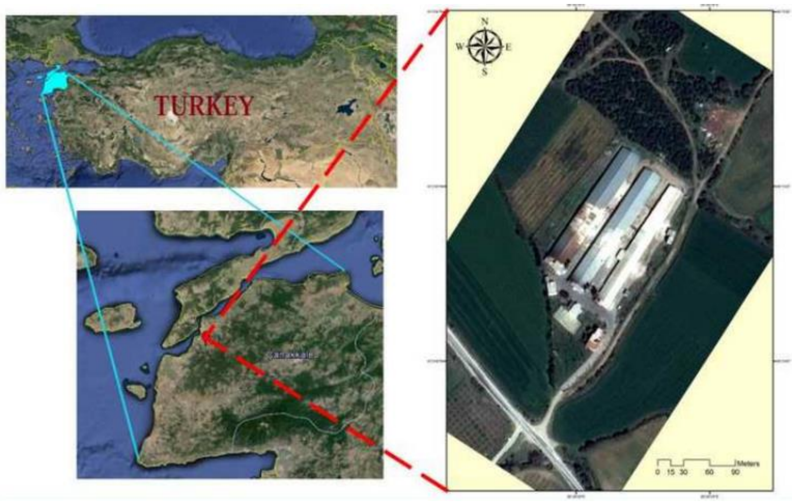

Figure 1. Study area and poultry operation.

The e-nose system

Kızıl et al. (2015a) developed an e-nose system consisting of main body (housing the sensor array, circuits and associated electronics), a desktop computer (software), a sample container, and purge gas unit. The system was designed to identify *Salmonella enterica* in poultry manure under laboratory conditions, and it was starting point for us to develop a new version to be used in monitoring of outdoor air quality. Both old and new versions are equipped with metal-oxide gas sensors (Figaro Engineering, Inc., Osaka, Japan). The metal-oxide gas sensors have low electrical conductivity in clean air. As they are exposed to odorous compounds their resistance changes resulting in more electrons to flow. By monitoring the change in the conductivity, concentrations of the odorous compounds can be evaluated. In its first version the e-nose system wasn't mobile and capable of being used in odour identification. Detailed technical information about sensors and other electronics is provided in Kızıl et al (2015a). The new version was developed to evaluate the potential use of this system in the assessment of air quality.

The system employs a sensor array requiring 5 V power for each sensor. Depending on the chemical characteristics of ambient air, output signals of each sensors range from 0.1 to 5 V. The sensor responses were acquired and released using a PIC16F877A microprocessor (Microchip Technology Inc., Chandler, Arizona, USA). Initially, we used two 18650 type batteries to power up the sensor array and data acquisition system, and three of same type batteries to power up micro air pump (Xavitech Intelligent Pumps, Härnösand, Sweden). This unregulated power unit was causing quick discharge of batteries. Then, two 18650 type power banks that provide regulated current were integrated to the system. These power banks are comprised of four 18,650 type special batteries with a circuit to control power flow. The output current-limiting protection avoids possible damages when overloaded. They operate with a charging input of 5V / 1A, and output of 5V / 3A. A DC to DC step-up converter was used with one of the power banks since the micro air pump requires 12 V power supply. The new version of the e-nose system is shown in Fig. 2.

Figure 2. Modified e-nose system [A: sensor array, B: power bank, C: micro pump control unit, D: micro pump, E: sample air inlet, F: sample air outlet (reference gas inlet if needed), G: reference gas outlet (if needed), H: USB 4 port to tablet PC, I: sensor array power switch and USB charging port, J: power indicator LEDs, and K: micro pump power switch and USB charging port].

The e-nose has one main air inlet. The micro pump purges the odorous air sample from air inlet (E) to the sensor compartment. Air sample then leaves the system through outlet (F) due to the pressure difference caused by the micro pump. In some cases, especially in laboratory applications, a reference gas should be purged into the system. In such cases, outlet (F) serves as reference gas inlet. The reference gas will leave the system through outlet (G). Depending on the application, pump flow rate can be adjusted via control unit (C) by changing the pump frequency. The block diagram of the system is provided in Fig. 3.

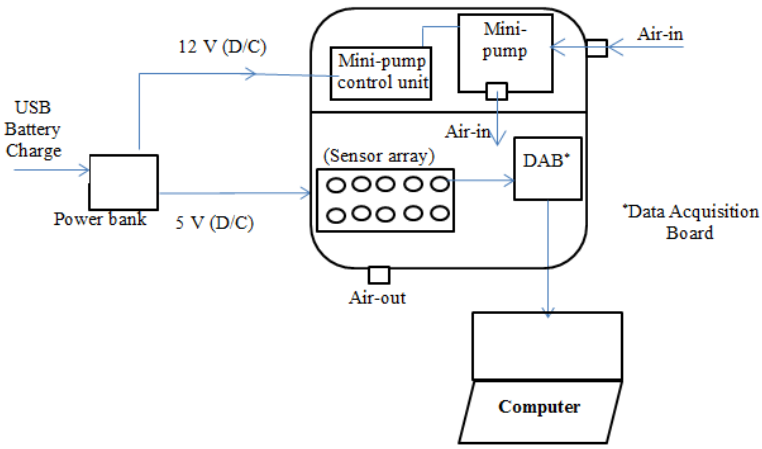

Figure 3. Block-diagram of the e-nose system.

In the graphical user interface (GUI) folder management and sampling options was handled. Once the sampling frequency, total number of sampling, and name and the location of reading file determined, software and micro air pump can be started simultaneously. The GUI allows monitoring real time sensor responses. The reading ends when the entered number of readings has been taken.

Experimental procedure

In order to verify the effectiveness of the e-nose system a field experiment was conducted. The e-nose was operated to determine the air quality conditions within a poultry operation where there are several odour sources such as three barns, two outdoor manure piles, an incineration pit, a chemical barrel and charcoal ash storage areas. An aerial image of the area was obtained from Google Maps application to determine the possible reading points. In the monitoring of wind velocity a handheld anemometer (Trotec GmbH&Co. KG, Heinsberg, Germany) that is capable of sensing ambient air temperature was used. Field measurements were conducted at 50 reading points. The readings were not collected simultaneously and the total duration of the experiment was about 3.5 h. Experiment was conducted in May. The coordinates of the reading points were recorded to be later used in GIS environment by a Garmin GPSMap 60CSx model GPS device (Garmin International Inc., Olathe, KS, USA). While at each point, e-nose readings, ambient air velocity and temperature, and coordinates were recorded. All the data collected by e-nose, GPS unit, and anemometer were used to develop GIS database in ArcGIS 10.3.1 software (ESRI, Redlands, CA, USA).

In air quality, especially odour, studies a reference method is used to evaluate the performance of the used or developed technique/method such as olfactometer or gas chromatography (GC). In this study, a Figaro AM-1-2600 (Figaro Engineering, Inc., Osaka, Japan) model air quality sensor module was used as the reference method. This module uses an air contaminant gas sensor and a microcomputer to measure the actual contamination levels. The air quality module was integrated with TD-200 (Paradox Security Systems, Istanbul, Turkey) dual tone, multi-frequency signalling system (DTMF) (Kızıl et al., 2015b).

Sensor responses under non-odorous, clean air conditions were determined using above mentioned air quality module. The microprocessor within the air quality module receives the output signal from the sensor and creates a benchmark level. In this study air quality conditions in a well ventilated non-odorous room was considered to be reference benchmark for each sensor. Both air quality module and e-nose system operated in a well ventilated room as the non-odorous conditions maintained. Response of each sensor within the e-nose was monitored and base-line non-odorous conditions were determined for all sensors.

Data processing

As the e-nose starts operating, it collects pre-determined number of readings at an entered frequency. At total of 50 points e-nose responses were recorded around the poultry operation. The data consisted of date and time of sensor readings, sensor responses in Volt, and temperature and relative humidity of the sensor compartment in MS Excel format. Initially, in e-nose systems a reference condition is obtained by exposing the sensor to a reference air (clean, non-odorous air). Non-odorous reference condition is a typical measurement in non-odorous ventilated room. Then, the sensors are exposed to odorous air or headspace gas of the sampled material. This creates a sensor response curve for each reading. In this study we used raw sensor response data. The major goal of this study was to spatially monitor the response of gas sensors that are reactive to certain air pollutants instead of measuring exact gas concentrations. The reason for this approach is that relationship between the sensor response and gas concentration is linear, as explained above. At each point the e-nose recorded 20 readings with a recording interval of 5 seconds. In order to monitor spatial distribution of air quality, a unique value representing each point for each sensor required. Plot of sensor readings showed that there are only minimal fluctuations observed during recording period at each point due to the change of wind conditions. Sample plots of all sensors will be given in the following section of the study. Average of 20 readings at each point was considered to be sensor response. Of those 10 gas sensors only 6 responded sampled air. Those sensors and corresponding target gases are; TGS 813 (CH_4, C_3H_8), TGS 822 (volatile organic compounds), TGS 825 (H_2S), TGS 826 (NH_3), TGS 2600 (H_2, CO), and TGS 2602 (NH_3, H_2S). Remainder yielded either extreme fluctuations or no response to odorous air samples. Polar compounds such as water vapour may cause these fluctuations (Balasubramanian et al., 2004). Once the averages of each sensor responses at each point were calculated, a point shape file overlaying on the aerial image of the experimental area was created in ArcGIS for further spatial analysis (Fig. 4). In the figure, green points represent the e-nose reading locations.

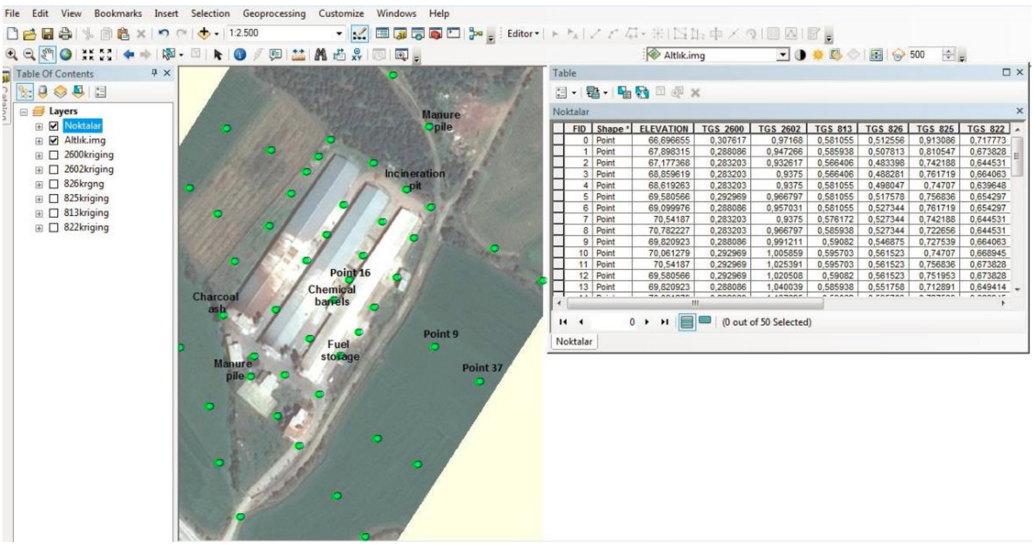

Figure 4. GIS database.

In the initial evaluation, 4 reading points which are within varying distances from the barns were selected (Fig. 4). Point 16 was selected in between 2 barns where there are ventilation fans operating, and protected from the prevailing wind. Point 9 is also close to barn where concentrations of odorous compounds are less than point 16 due to its location. Point 37 was located about 100 m east of the barn. In order to spatially visualize overall sensors' response, Kriging method was applied. This method is more applicable in terms of monitoring spatial distribution of air quality data (Ball et al., 2008). In this method, a value is predicted based on a trend that all values of known points follow and an additional element of variability (Kizil & Tisor 2011). Kriging uses a semi-variogram model to express the spatial dependence of each point. A semi-variogram model can be expressed as follows (Delhomme, 1983).

$$\lambda(d_{mn}) = \frac{1}{2N}\sum_{1}^{N}[(r_m - r_n)^2] \quad (1)$$

where; $\lambda(dmn)$ is the semi-variogram for the points P_m and P_n sensor readings r_m and r_n, d is the lag distance, and N is the number of pairs of reading points. Spherical, circular, exponential, and Gaussian, models are some of the mostly used semi-variogram models (Christakos, 1984). In this study, these models were applied to generate raster interpolation of each sensor response. Once the Kriging interpolations are developed, the best model that yields the smallest root-mean-square error (RMSE) was chosen as the semi-variogram model. Entire experimental study and data processing steps are illustrated in Fig. 5.

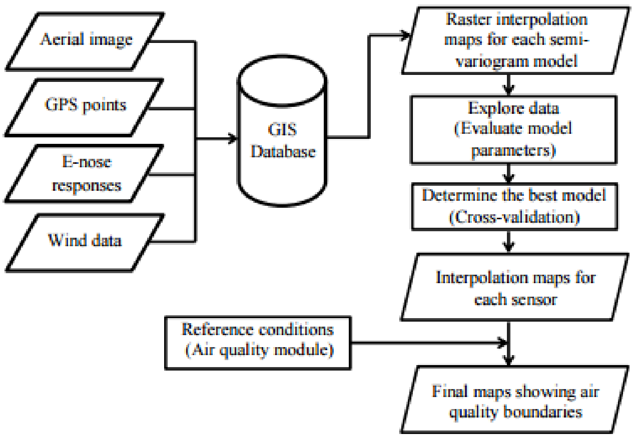

Figure 5. Schematic representation of experimental study and data processing.

RESULTS AND DISCUSSION

The sensors employed within the e-nose system are sensitive to chemical classes like alcohols or general combustible gases (Romain et al., 2004) that make each sensor sensitive to a variety of chemical compounds. Spatial distributions of each sensor response were monitored as a method to assess the surrounding air quality rather than monitoring overall odour which is a subjective method. Once the database was created responses of each sensor were compared. Readings form 3 different locations that are within various distances from the barns were collected (Fig. 4). Responses of each sensor at these locations were plotted in Fig. 6. As it was expected, sensor responses get larger values as the sampling location gets closer to barns. Considering the fact that there is a linear relationship between sensor responses and gas concentrations, e–nose readings can be used in air quality monitoring.

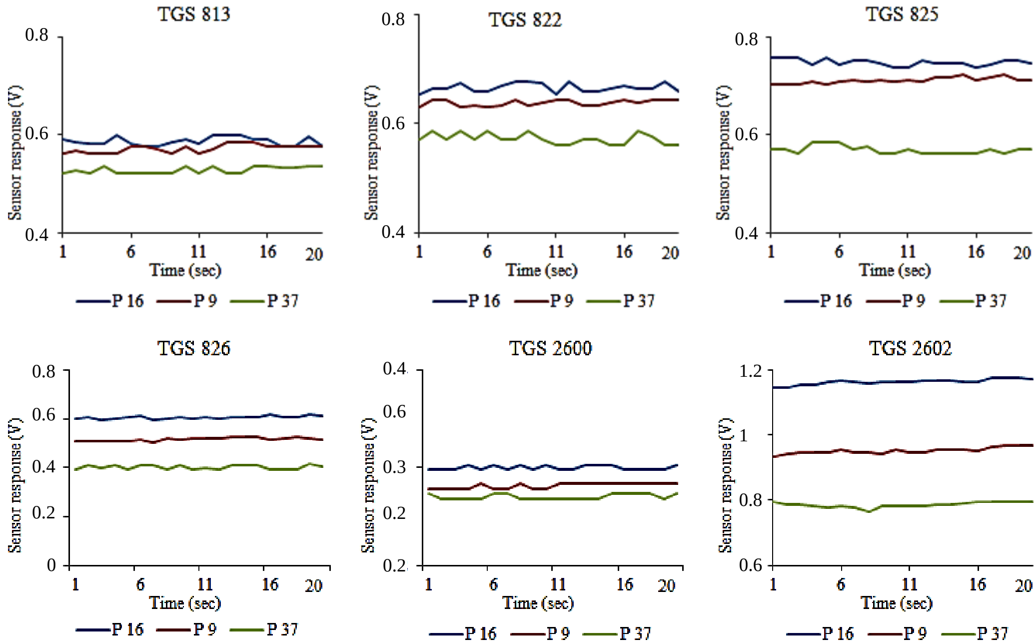

Figure 6. Sensor responses at 3 different locations.

Interpolation maps showing the spatial distributions of each sensor responses were created. In raster interpolation Geostatistical Analyst extension of ArcGIS software was used. The e-nose and GPS data containing sensor responses and coordinates of each reading point were used. Root–mean–square errors of spherical, circular, exponential, and Gaussian semi– variogram models were compared via cross-validation (Davis, 1987; Barton et al., 1999; Kizil & Tisor, 2011). This method is used to determine the best model that predicts the sensor response of unknown points in the creation of interpolation maps. The model predicts the sensor response value of a known point using the entire dataset and then compares the predicted value with actual value yielding a RMSE. The cross-validation results including RMSEs and other statistics are shown in Table 1. The cross-validation results show that of 4 models Gaussian is the best with lowest RMSE for all sensor responses.

Table 1. Cross – validation results for all sensors

		TGS 813		
Error	Spherical	Circular	Exponential	Gaussian
M	0.00097	0.00104	0.00143	0.00091
RMS	**0.01406**	**0.01067**	**0.01075**	**0.00946**
AS	0.01538	0.01506	0.01773	0.01196
MS	0.03838	0.04027	0.04900	0.05885
RMSS	0.67420	0.70530	0.50160	0.88450

Table 1 (continued)

		TGS 822		
Error	Spherical	Circular	Exponential	Gaussian
M	0.00271	0.00222	0.00274	0,00193
RMS	**0.02154**	**0.02173**	**0.02167**	**0.02167**
AS	0.02542	0.02490	0.02887	0.02333
MS	0.06385	0.05129	0.05657	0.05311
RMSS	0.86900	0.90400	0.75510	1.00500
		TGS 825		
Error	Spherical	Circular	Exponential	Gaussian
M	0.00352	0.00402	0.00488	0.00325
RMS	**0.04200**	**0.04307**	**0.04256**	**0.04130**
AS	0.04996	0.04904	0.05659	0.03900
MS	0.04063	0.04744	0.05079	0.06532
RMSS	0.85200	0.89310	0.74490	1.18400
		TGS 826		
Error	Spherical	Circular	Exponential	Gaussian
M	0.00365	0.00315	0.00415	0.00323
RMS	**0.03219**	**0.03300**	**0.03397**	**0.03071**
AS	0.04690	0.04602	0.05395	0.04381
MS	0.04857	0.04310	0.04821	0.05055
RMSS	0.72010	0.74920	0.63660	0.77290
		TGS 2600		
Error	Spherical	Circular	Exponential	Gaussian
M	0.00057	0.00052	0.00055	0.00055
RMS	**0.00652**	**0.00662**	**0.00639**	**0.00648**
AS	0.00757	0.00759	0.00774	0.00751
MS	0.05307	0.04885	0.04551	0.05299
RMSS	0.89150	0.90220	0.85040	0.90170
		TGS 2602		
Error	Spherical	Circular	Exponential	Gaussian
M	0.00581	0.00642	0.00685	0.00534
RMS	**0.05466**	**0.05578**	**0.05587**	**0.05309**
AS	0.08701	0.08658	0.09739	0.08423
MS	0.04144	0.03155	0.04419	0.04372
RMSS	0.66760	0.68120	0.61580	0.69570

M: mean; RMS: root-mean-square; AS: average standard; MS: mean standardized; RMSS: root-mean–square standardized.

The next step in the study was to visualize the areas where the sensor responses are below the reference threshold values. As explained above, response of AM–1–2600 air quality module under non-odorous, clean air conditions were used as the threshold values. It was observed that under non-odorous conditions sensor responses remain similar within a minimal deviation range depending on the temperature and humidity conditions. Average responses of each sensor under these conditions were determined as given in Table 2. All sensor responses above these values considered to be odorous conditions.

Table 2. Threshold sensor values for non-odorous conditions

Sensor	Reference sensor responses (V)
TGS 813	0.56
TGS 822	0.63
TGS 825	0.67
TGS 826	0.49
TGS 2600	0.27
TGS 2602	0.88

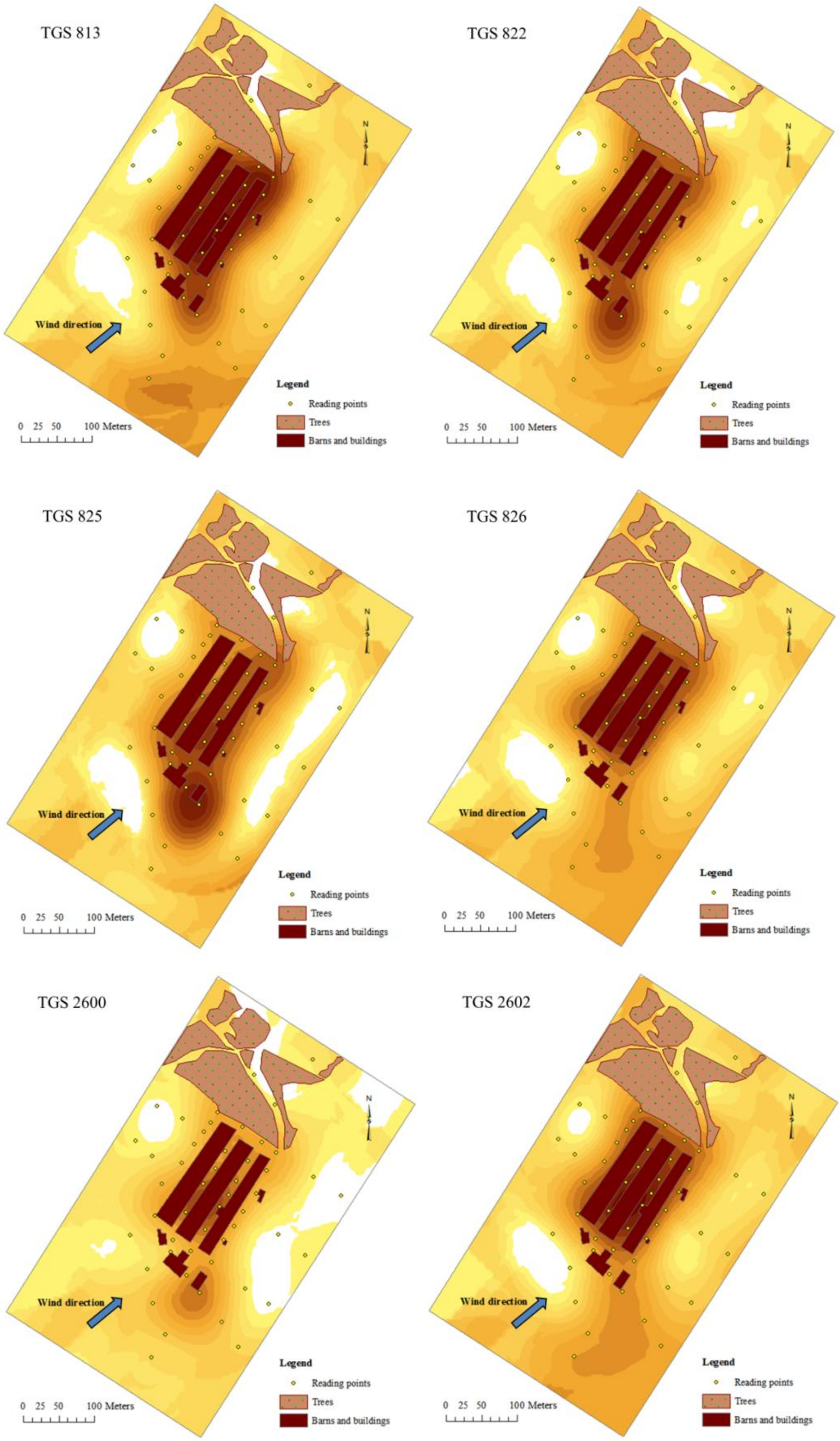

Figure 7. Air quality dispersion over the study area.

Finally, on the Kriging interpolations, areas where the sensor responses are below the threshold values were determined to be non-odorous areas. In Fig. 7, e-nose reading points, buildings, trees, and the interpolated responses of each sensor are represented. As expected, the sensor responses get larger values as they get closer to odour sources. The larger values were indicated with darker colours. On the interpolation maps areas where the sensor responses are below threshold values white-coloured. As it is clearly seen there are two white spots denoting non-odorous conditions on west-side of the barns and odour sources. Even though they are on downwind direction there are white areas on NE direction of the operation. This is because the trees that function as windbreak reducing the odour dispersion. White areas on the west of operation can be explained by the topography of the study area. Elevations of those areas are above the operation. The wind blows the odorous air in between the hill and barns creating a passage that moves odour beyond the operation. It was reported in ÇCAAP (2014) that the most frequent wind direction in study area is north-northeast (NNE) with a frequency of 35 to 50% during warm seasons. They also noted that, in the cold seasons of the location prevailing wind direction are southwest (SW) and south-southwest (SSW) which was observed during the study. Therefore, it could be concluded that depending on the season dispersion of odour will change shifting the direction of problem. The meteorological data of the study area is provided in Table 3 and Fig. 8 (MGM, 2016).

Table 3. Meteorological data of the study area

	Max.	Min.	Average
Temperature (°C)	39.0	-11.8	15.0
Wind speed (m s^{-1})	139.3		3.9

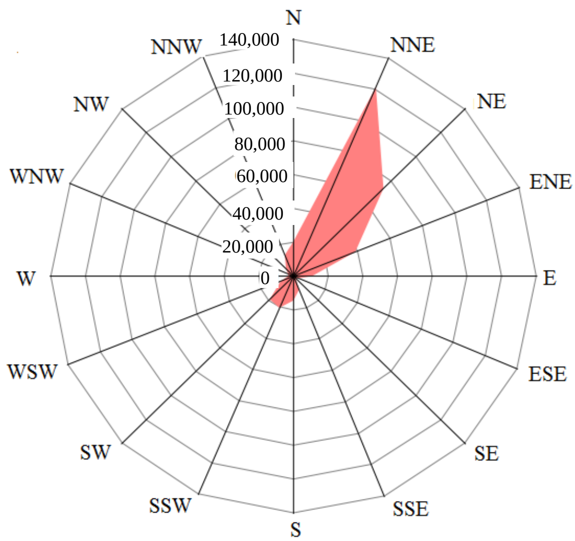

Figure 8. Wind rose of the study area.

CONCLUSIONS

Air sample readings were collected at poultry operation in Aşağıokçular village of Çanakkale, Turkey that included 3 deep-litter houses. In order to evaluate the potential use of an e-nose in air quality monitoring an e–nose containing metal-oxide gas sensors was developed. The major advantage of the e-nose system was its cost. The system employed a custom-made software and data acquisition board to acquire process and store the air quality data that made it cost-effective. The mobile e-nose system provides a user-friendly technique that could be used in various areas including air quality monitoring. With the e-nose system it was possible to visually monitor air quality dispersion within an area. It should be noted that e-nose readings were not collected simultaneously. Thus actual sensor responses at a given time may vary. Considering the fact that during the study meteorological conditions didn’t vary a lot results were still a good representation of aerial conditions. In the current version of e-nose system reading locations are acquired via a separate GPS unit. In the next version a GPS sensor will be integrated with the hardware and software components of the system. The results demonstrated that gas sensors associated with relevant software can be used to monitor air quality within an area. However, sometimes concentrations of aerial pollutants must be identified. For such cases, it is possible to calibrate employed gas sensors to monitor odorous gas concentrations. In the next version it also planned to calibrate gas sensors for this purpose. Finally, it can be concluded that with current technology it is possible to monitor and evaluate air quality problems caused by livestock operations. Then, further action can be taken to eliminate and/or limit the air quality problems.

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The prevention of harmful gases and odours dispersion by biofiltration in the animal farm

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Abstract. Animal farms are have to be controlled regarding to environmental issues beacuse of their waste's effects. Dispersion of harmful gases and odour is some most important effect of animal waste. Decomposition of animal waste may cause dispersion of harmful gases such as ammonia, methane, carbon dioxide, hydrogen sulfide etc. and odours. Harmful gases and odours impact on human and animal welfare negatively. Biofiltration is a technique used to prevent the dispersion of harmful gases and odour on animal farms. Especially some animal production types such as swine and poultry farms may cause great problem in terms of harmful gases and odour, so biofiltration has been seen as an effective method treating polluted air in these farms. The process of biofiltration is conducted based on biological degradation of pollutants. The bed materials such as litter, mulch and woodchips etc. are used in biofiltration to ensure microbiological environment. In this study, it was purposed to give information about the biofiltration, its basic principles and usage on animal farms based on conducted researches.

Key words: animal waste, biofilters, treatment, environmental problems.

THE BASIC PRINCIPLES OF BIOFILTRATION IN ANIMAL FARM

Biofiltration is an effective method to treat ventilation air from mechanically ventilated livestock buildings. The ventilation air flows through a bed of biological material. Harmful gases are absorbed by cultures of microorganisms that grow within the bed. Two main design configurations for biofilters are that flat-bed type and a vertical biofilter. The flat-beds are easier to construct and cost less, but they occupy more space than the vertical biofilters. Vertical biofilters are more difficult to construct and biological material can settle, then it causes leaking problem. Vertical biofilters can be designed in multiple layers to reduce the effects of settling (Harmon et al. 2014).

Several technical factors should be considered in the designation and operation process of biofilter systems. These factors are biofilter media, moisture content, microorganisms, oxygen, temperature, pH, medium depth and pressure drops, nutrients, load of contaminant, toxic and inhibitory by products removal, dust and grease of contaminated air (Armeen, 2006).

Selecting suitable biofilter media has great importance to reach success in the biofilter systems. Desirable media properties include suitable environment for microorganisms depend on nutrients, moisture, pH and carbon supply, large surface area to maximize attachment area and sorption capacity, stable compaction properties to resist media compaction and channeling, high moisture holding capacity, and high porosity to maximize empty bed residence time (EBRT) and minimize pressure drop, low bulk density to reduce media compaction potential (Williams & Miller, 1992; Swanson & Loeher, 1997; Chen & Hoff, 2009). Comparison of media types is given in Table 1.

Table 1. Comparison of Media Type (Edwards & Nirmalakhandan, 1996; Devinny et al., 1999; Armeen, 2006)

Media Type	Advantages	Disadvantages
compost/peat	• high population of microorganisms • suitable for low concentration volatile organic compounds • low cost • high to medium nutrients • life time 2 to 4 years • high absorption of water	• compaction and channeling • limited buffer capacity • low biodegradation capacity
granular activated carbon packed bed	• high adsorption • good biomass adhesion • fast start up (adsorption) • suitable for high contaminant concentrations • high biodegradation capacity • life time more than 5 years	• high cost • difficult to clean due to adhesion • no nutrients
pelletized ceramic	• easy to clean • less expensive than activated carbon • high biodegradation capacity	• more expensive than compost or peat
perlite, and other inert materials	• high surface area • life time more than 5 years	• medium cost • no availability of nutrients

Generally organic materials such as bark mulch, woodchips, litter, wood shavings and compost are selected as biofilter media. These materials are inexpensive and can be easily supplied (Chen & Hoff, 2009). Also, some alternative media materials such as pine nuggets, lava rock, cedar chips and natural zeolite have been investigated for longer service and higher porosity (Luo & Lindsey, 2006; Janni et al., 2009; Akdeniz et al., 2011).

Media moisture content is a critical parameter for biofiltration systems. Researches indicate that 40% to 65% of moisture content are sufficient depend on media materials (Nicolai & Janni, 2001; Chen & Hoff, 2009). Temperature and pH should be controlled to optimize microbial activity in the media. The ideal operation temperature is between 30 and 40 °C (Yang & Allen, 1994). Williams & Miller (1992) suggested that the optimal pH is between 6 and 7.

Air pollutants are degraded by several groups of microorganisms. These are bacteria, fungi and actinomycetes. Bacteria have ability to degrading air contaminants faster. Fungi are more tolerant than bacteria to low moisture content and low pH of media, but they are more susceptible of low oxygen concentrations in the environment. Actinomycetes are similar to fungi, but they are small in size and are technically classified as bacteria. They have more tolerance to low moisture condition than bacteria. However, they have a low tolerance for acidic conditions. Fungi and actinomycetes are more active in the conditions of biofilters contain organic media with low moisture content (Metcalf & Eddy, 1991; Atlas & Bartha, 1993; Armeen, 2006).

Pressure drop should be considered when the biofilters are designed. Energy consumption is increased in the condition of high pressure drop (Yang et al., 2007). The biofilter media depth and air flow rate are primary factors to affect pressure drop and removal efficiency. Depths ranging from 0.3 to 1 m with most between 0.3 to 0.75 m have been generally used for on-site biofilters. Higher media depth can give good results based on removal efficiency but it may causes higher pressure drop. The media depth of 0.25 to 0.50 m has been recommended as optimal for agricultural biofilters (Chen & Hoff, 2009).

Some indicators such as empty bed residence time (EBRT), removal efficiency (RE) and elimination capacity (EC) are used to describe effectiveness of biofilter systems. EBRT can be calculated by dividing the volume of the biofilter media by the air flow rate. This indicator depends on the media depth, airflow rate, cross sectional area, porosity, physical properties of the media, mass loading and degradability of the odourants (Devinny et al., 1999; Armeen, 2006). 3–5 seconds of EBRT is sufficient for livestock facilities (Janni et al., 2011). The performance of a biofilter is often assessed by RE and it is defined as the fraction of contaminant removed. RE can be calculated by dividing the difference of inlet and outlet gas concentration by inlet concentration (Oliver, 2015) EC is the mass of contaminant that is degraded per unit volume of filter media per unit time (Devinny et al., 1999; Armeen, 2006).

THE OVERVIEW OF RECENT LITERATURES IN BIOFILTRATION

Martens et al. (2001) intended to determine the potential reduction of microbial bioaerosol, odour and ammonia emissions by biofilters in a pig facility. Five different media materials (Biochips, coconut-peat, wood-bark, pellets-bark and compost) were used in this study and the results showed obvious differences among media materials. Numbers of airborne cultivable bacteria were decreased by ca. 70 to 95% and the total counts of bacterial cells from ca. 25 to 90%. The total amount of fungal cells was reduced by at least 60%. Airborne endotoxins and MVOC (Microbial Volatile Organic Compounds) were effectively reduced by all filter materials to at least 90%. The mixture of chopped bark and wood had best performance compare than other media materials based on airborne endotoxins reduction and also the mixture of pellets and bark had best performance based on MVOC. The average odour reduction was between 40 and 83%. However, only Biochips slightly affected (8.4%) in the reduction of ammonia emissions.

Hong & Park (2004) investigated the influence of wood chip biofilter properties and the depth of biofilter on ammonia emission from composting manure. It was pointed out that ammonia emission was affected by the depth of biofilter media. Besides, optimum biofilter media depth was determined as 40 cm in a closed wood chip filter for allowable ammonia emissions.

Armeen (2006) aimed to determine designed a treatment system (a combination of biosrubbler and biofiltration) to reduce the NH_3 and H_2S compounds of polluted air from animal facilities and the effect of NH_3 and H_2S on biofilter performance. It was indicated that the best biofilter performance was reached by a mixture of polystyrene (75% by volume) and peat moss (25% by volume). It was pointed out that the removal efficiency was different between the bioscrubber with dilute sulfuric acid and the bioscrubber without acid. Sulfuric acid had positive effect on biofilter performance based on elimination of ammonia. Also, the ammonia concentrations significantly affected the EC, RE, and pH of the biofilters ($p < 0.05$). It was pointed out that the odour concentration was reduced 50% by bioscrubber and 72% by combination of bioscrubber and biofilter with no NH_3 injection

Luo & Lindsey (2006) assessed the effectiveness of biofilters, which contained different sizes of crushed pine bark or a mixture of zeolite and crushed bark to treat the exhaust gases from direct-fired meal dryers. Biofilter odour-removal efficiencies were measured between 80% and 99%. It was mentioned that the fine crushed bark biofilter generally is more effective than the coarse bark biofilter in reduction of odour concentrations. The additions of zeolite to the bark medium in the biofilter causes a very small decrease in odour-removal performance.

Lau & Cheng (2007) assessed the performance of a pilot-scale biofiltration system for treating odours from the exhaust air streams of a commercial duck farm building. The average odor removal efficiency of the biofilter system was found as 95 ± 3%.

Chen et al. (2008) developed a pilot-scale mobile biofilter contained two different types of wood chips (western cedar and hardwood) to reduce odour emissions from a deep-pit swine finishing facility in central Iowa. Volatile Organic Compounds were analyzed by a multidimensional gas chromatography-mass spectrometry-olfactometry system. As a result of this study, average reduction efficiency were found as between 76–93%. It is indicated that both type of chips had good performance for average reduction. Also, they had significant reductions in p-cresol, phenol, indole and skatole., On the other hand, it was pointed out that maintaining proper moisture content is more important than media depth and residence time.

Chen et al. (2009) tested two different types of wood chips (western cedar and hardwood) as media material to treat odor emissions from a deep-pit swine finishing facility in central Iowa. The results indicated that reduction of odour concentrations (average 70.1 and 82.3% for HW and WC, respectively) were considerably achieved by both types of chips. As a result of this study, average reductions of H_2S concentrations were found as 81.8 and 88.6% for HW and WC, respectively and of NH_3 concentrations were found as 43.4 and 74% for HW and WC, respectively.

Akdeniz et al. (2011) evaluated gas reduction efficiencies and gas reduction efficiencies of two alternative biofilter media (pine nuggets and lava rock) at three empty bed contact times (1, 3 and 5 s) and two moisture levels (82% and 90% relative humidity). It was pointed out that pine nuggets and lava rock can be accepted as alternative media types at %90 relative humidity and 5 s empty bed contact time to reduce hydrogen sulfide, total reduced sulfur, ammonia and greenhouse gas emissions. Also, they have lower pressure drop than wood chips biofilter. It was observed that odour was reduced up to 48% but it was not consistent.

Lei (2011) determined the optimal residence time of biofilters to ensure high reduction ammonia emission and low nitrous oxide production. Also, it was aimed to determine interaction between ammonia removal and nitrous oxide production. In this study a biofilter system was built and installed in front of a group of exhaust fans inside a broiler house. It was indicated that there was a linear relationship between the removal efficiency of ammonia and residence time ($p < 0.05$). While the average of nitrous oxide production rate were found as 3.92 ± 1.14 mg hr^{-1} at 20 sec residence time in low NH_3 concentrations, the average of nitrous oxide production rate in high NH_3 concentrations were found as 1.50 ± 0.40 mg hr^{-1} at 20 s residence time. At the end of the study, 50 sec residence time was recommended for high ammonia conditions.

Akdeniz & Janni (2012) evaluated biofilter media characteristics and NH_3, H_2S, SO_2, CH_4 N_2O reduction efficiencies from eight biofilters on four animal feeding operations. The biofilters were located on a dairy, a swine nursery, and two swine finishing farms. The result of study showed that the deep bed biofilters at the dairy farm, had the most porous media and lowest unit pressure drops and there was no N_2O generation in this biofilter. The highest H_2S, SO_2, NH_3 and CH_4 reduction efficiencies were measured from a flat-bed biofilter at the swine nursery farm. Whereas, the highest N_2O generation (29.2%) was also measured from this biofilter. This flat-bed biofilter media had the lowest porosity.

Oliver (2015) investigated spatial and temporal fungal dynamics in full-scale woodchip biofilters. It was pointed out that dynamics and potential abilities of fungi in biofilters treating livestock production emission can be used to guide the connection between fungi and biofilter function. It was mentioned that this relationship has potential to improving biofilter performance and better protect air quality.

CONCLUSIONS

The studies showed that contaminated air could be treated by biofilters at the high rate. But also, there are a number of subjects that could be improved in this system with regards to designation criteria, bed materials and relationship between microorganisms and the system.

In conclusion, biofiltration should be considered as a part of animal production facilities based on environmental impacts, human and animal welfares.

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The impact of biological agents on properties of heavy-textured soil and productivity of organically grown crops

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Abstract. Soils with high content of particles φ ≤ 0.02 mm usually have nonfavourable physical and mechanical properties. In order to determine the impact of biological agents on properties of heavy-textured soils in organic farming systems, in the years of 2007–2009 an on-farm scale field experiment was carried out in Budziszewo, Pomeranian province in Poland. The farm was organically managed for 20 years and was characterized by proper management (an appropriate crop rotation and a high stand of dairy cows). Nevertheless, in spring there were problems with soil drying, which used to delay sowing of spring cereals by ca 2 weeks, which in turn resulted in low yielding. Therefore, the following biological agents, i.e. beneficial microorganisms, were applied in 3 consecutive years 3 times each year. Produced on-farm (biodynamic horn preparation) and the others produced by industry under the trademark of: effective microorganism (EM), Humobak and UGmax. In 2007 silage maize was grown, in 2008 – winter spelt wheat and in 2009 – spring common wheat. The soil samples to study soil properties were collected from 5–15 cm soil layer during the vegetation period. The following soil properties were analysed: granulometric composition, bulk density of dry soil, total porosity, soil humidity and air content during sampling, organic matter content, soil pH, content of macroelements (P, K, Mg), soil aggregation based on dry and wet sieving. Moreover in 2009 additional soil samples were taken from an adjusting neighboring field of the conventional farm with the aim of comparing soil physical properties developed both under organic and conventional management.

The authors concluded that the application of above mentioned biological agents did not affect significantly soil properties. The applied agents did not affect crop productivity, with the exception of Humobak which decreased yield of silage maize and spring common wheat in the range of 41, and 26% respectively. A proper organic management as opposed to application of biological agents has positive effect on soil physical properties.

Key words: biological agents, crop productivity, heavy-textured soil, organic farming, soil properties.

INTRODUCTION

Soils with high content of particles φ ≤ 0.02 mm usually have unfavourable physical properties. Nevertheless, besides this fraction there are other factors which affect soil physical properties, like the content of silt (φ 0.002–0.05 mm) and clay (φ < 0.002 mm). Moreover, physical soil properties are modified by chemical soil properties, like content of Ca and other elements (Bronick & Lal, 2005). All these factors affect the soil structure, including the formation of water-proof aggregates. The soil aggregation in turn has a strong impact on soil porosity and possibility of water and gases exchange in the soil.

In organic farming great importance is attached to continuous improvement of soils. The application of organic fertilizers, legumes and green manures cultivation in a crop rotation, lead to an enhancement of the content of soil organic matter. Soil organic matter influences the improvement of numerous chemical, biochemical and physical properties of soil (Rychcik et al., 2006; Romaneckas et al., 2016). Studies on the organic soil management on the physical properties of soils are scarce (Papadopoulos et al., 2014). Organic farming practices usually results in the increase of the content of organic carbon in the soil and of the total soil porosity, as well as in the decrease of bulk soil density. In the study of Papadopuolos (2014) it was proved except the mentioned features, also enhanced aggregate stability. The researches emphasize though that the positive effects of organic farming on soil structure are not necessarily constant over time and are scale dependent.

Producers and dealers of different biological agents often state that the preparations not only improve microbial processes in soil, but also enhance crop's health and growth, soil chemical (increase humus content, nutrients availability) and physical properties (soil structure and water retention) (Koepf et al., 1976; Higa, 2003; Wistinghausen et al., 2007). These studies were focused on soil physical properties, but chemical ones and crop yields were also determined.

The study was done on the farm located in Budziszewo near Jablonowo Pomorskie, on east part of Chelminskie Lakeland (Kondracki, 2000). The farm was organically managed for 20 years and was characterized by a proper management (an appropriate crop rotation and a high stand of dairy cows of 1.8 LU per ha, so farmyard manure was produced and applied on regular basis in high rates). Nevertheless, besides the proper crop rotation and the high rates of farm yard manure (FYM) there were still problems with soil structure, so in the spring time water was stagnating on field surface, and there were problems with too slow soil drying, thus field cultivation and the sowing time were delayed. Sowing of spring cereals was delayed by ca 2 weeks, which in turn resulted in lower yielding. The aim of the study was to evaluate the effect of some biological agents on agrochemical and physical properties of heavy soil and productivity of crops under organic management.

In Poland few biological agents to improve soil biological and physical properties are being used both on organic and conventional farms. An on-farm produced biodynamic horn preparation is obligatory used on biodynamic farms. On both organic and conventional farms industrially produced biological agents were used. EM were commonly used all-over Poland. Humobak was also applied all-over Poland but it was far less common. UGmax was quite commonly used in northern Poland. Since all the above–mentioned biological agents were used on organic farms the authors decided to test their effectiveness.

The authors' main hypothesis states that the use of biological agents can substantially improve soil properties resulting in quicker soil drying after winter, and hence they enable earlier soil tillage and sowing, which in turn could enhance crop yields. The additional hypothesis was that proper organic management has a greater effect on soil physical properties than application of biological agents.

MATERIALS AND METHODS

In September 2007 field and laboratory examination were made to describe soil type of the experimental field on the above mentioned organic farm in Budziszewo, Poland. Hence soil pits were made to determine soil morphology and systematic position. The authors found that soil texture of the top layer (0–30 cm) is L – loam (according to the USDA soil texture classes). The skeletal fraction (φ > 2.00 mm) content is 0%, the sand fraction (φ 2.0–0.05 mm) content is 46%, the silt (φ 0.05–0.002 mm) content is 37% and the clay (φ < 0.002 mm) content is 17%. In the sub-soil layers (30–150 cm) the content of silt and clay fractions are higher, the average silt content is 52%, and the clay content is 35%, and the texture is classified as SiCL – silty clay loam. The soil is classified as *Gleyic Stagnic Eutric Cambisol* (*Loamic*, *Drainic*) according to IUSS Working Group WRB (2014).

In the experimental field silage maize (cv. Reduta) was grown in 2007, in 2008 – winter spelt wheat (cv. Schwabenkorn) followed by white mustard (as green manure) and in 2009 – spring common wheat (cv. Bombona). Silage maize was fertilized with 35 t ha^{-1} of farm yard manure (FYM). Tested biological agents were applied in three consecutive years, 3 times each year: the first time in autumn after harvest of the proceeding crop on its residues, the second time in spring during soil tillage and the third time in full vegetation. The following biological agents were applied: 1) control treatment (CT) (water only), 2) effective microorganism (EM) 3 L ha^{-1}, 3) biodynamic horn preparation (HP) 0.35 kg ha^{-1}, 4) Humobak (HB) 120 L ha^{-1} and 5) UGmax (UG) 3 L ha^{-1}.

In CT plots biological agents were not applied. The biodynamic horn preparation is internationally known as used by biodynamic farmers (Koepf et al., 1976); EM is also quite commonly used (Higa, 2003). Humobak and UGmax are produced and applied in Poland. The first one is a solid, dry microorganism preparation, in which selected microorganism are multiplied on coconut shells. UGmax is also produced in Poland. Composition of applied agents and application methods are given in Table 1. The data on composition of the agents, given by their producers are usually very general (Martyniuk & Ksiezak, 2011), incomplete or lacking. All these soil conditioners are on the positive list as being acceptable to be applied on organic farms in Poland. The online continuously updated list is provided by the Institute of Soil Science and Plant Cultivation in Pulawy, Poland (http//www.iung.pulawy.pl).

The experiment was laid down in completely randomized design in four replications. The experimental field had an acreage of 1.6 ha and a single plot of 800 m^2. Traditinal soil tillage practices were applied. Already in October 2006 25 t of farm yard manure (FYM) per ha was applied and ploughed in. In March 2007 the field was harrowed for the first time and on 26 April the biological agents were applied and on the same day the field was cultivated with cultivator. After 3 days silage maize was drilled and 3-times harrowed in a week and then hoed twice. After harvesting of silage maize in the end of September ploughing was done followed by soil harrowing and drilling of winter spelt wheat. During the vegetation spelt was harrowed twice to control weeds and destroy crusting. In 2008 after spelt harvesting a shallow ploughing was done followed by harrowing and on the same day white mustard for green manure was drilled. In late autumn (November) green manure crops were cut by a disc harrow. After 4 days when green manure was dryer, the field was ploughed. In 2009 in spring time common wheat was drilled. The wheat was harrowed twice.

Table 1. Composition of biological agents applied in the experimental field (2007–2009)

Biological agent	Macro- and microelements, g kg^{-1}					Microorganisms	Application methods
	N	P	K	Mg	Mn		
Effective microorganism (EM)	-	-	-	-	-	Photosynthetic bacteria, *Lactobacillus* spp., yeasts, *Azotobacter* spp.	Mixed and sprayed in 400 L ha^{-1}
Biodynamic horn preparation (HP)	-	-	-	-	-	-	Mixed and sprayed in 400 L ha^{-1}
Humobak (HB)	-	-	-	-	-	-	Spread mechanically, 120 L ha^{-1}
UGmax (UG)	1.2	0.5	3.5	0.1	0.0003	*Lactobacillus* spp., Photosynthetic bacteria, *Azotobacter* spp., yeasts, *Actinomycetes* spp.	Mixed and sprayed in 400 L ha^{-1}

– no data available.

During the vegetation period in July of 2007, 2008 and 2009 soil samples to study physical soil properties were collected from 5–15 cm soil layer, in order to determine soil aggregation. During samples drying alive and dead crop remains (residues) were removed by hand picking. The material after air drying was sieved by a set of sieves of 30.0, 10.0, 7.0, 5.0, 3.0, 1.0, 0.5 and 0.25 mm mesh. From each fraction aggregate sub-samples of 25 g were taken for a wet sieving. The wet sieving was conducted with the use of modified Baksheiev apparatus (so-called soil aggregate separator) constructed in the Institute of Agrophysics of the Polish Academy of Sciences in Lublin, Poland (Walczak & Witkowska, 1976). The modification consisted in adaptation to simultaneous determination of 3 samples instead of one sample. All fractions (excluding fraction below 0.25 mm) obtained after dry sieving were also used for wet sieving. The same set of sieves was used for wet sieving.

Moreover, the soil samples were collected to steel cylinders of 100 cm^3 every year to determine the following physical parameters: the bulk density of dry soil, the total soil porosity, the soil humidity and the air content during soil sampling. The physical soil properties were determined by commonly used methods (Lityński et al., 1976; Ostrowska et al., 1991). The soil texture was determined by aerometric method of Bouyoucos, modified by Casagrande and Prószyński (Lityński et al., 1976) and the sand fraction was determined by the sieving method.

To evaluate quality of the soil structure and the water resistance of soil aggregates, a set of indices was used:

AI_d – aggregation index, dry – a numerical index of aggregation calculated on the basis of ‘dry sieving’;

AI_w – aggregation index, wet – a numerical index of aggregation calculated on the basis of ‘wet sieving’.

The above mentioned indices were elaborated and tested in the Institute of Agrophysics in Lublin, Poland (Dobrzański et al., 1975; Domżał & Słowińska-Jurkiewicz, 1988). While elaborating them, it was assumed that the impact of soil aggregates on soil quality depends on aggregates diameter. Therefore, the indices weights were used – that means, calculating aggregation indices their shares were multiplied by the weights. It was assumed that the most valuable are crumb aggregates of 1–3 mm, and for them the weight 10 was established. The other aggregates have lower weights and aggregates < 0.25 mm and > 10 mm have no weight. The diameters of soil aggregates (mm) and their weights: 7–10 – weight 1, 5–7 – weight 3, 3–5 – weight 8, 1–3 – weight 10, 0.5–1 – weight 5, 0.25–0.5 – weight 3.

The other indices which were used to evaluate the soil quality structure after dry sieving of soil aggregates are as follows: quality structure index – QSI (according to Rewut, 1980) (Eq. 1):

$$QSI = (\varphi\ 0.25\text{–}7.0\ \text{mm}) / (\varphi < 0.25 + \varphi > 7.0\ \text{mm}), \tag{1}$$

where φ is the content of aggregates of a given diameter; clods index – CI (according to Rewut, 1980, cited after Walczak & Witkowska, 1976) (Eq. 2):

$$CI = (\varphi > 10.0\ \text{mm}) / (\varphi < 10.0\ \text{mm}), \tag{2}$$

where φ is the content of aggregates of a given diameter.

In 2009, after elaboration of the 2007–2008 results, the authors decided to take additional soil samples from a neighbouring conventionally managed field of the same soil type. The soil aggregation of that soil was evaluated via dry sieving method.

Besides soil physical parameters, every year soil samples were collected to determine soil chemical properties. The representative soil samples were obtained from the ploughing level (0–20 cm) by the aid of Egner's cane. The taken material was dried to the state of air dry, ground and was sieved by a sieve of 1 mm mesh. Such prepared samples of soil underwent a chemical analysis and there were measured: pH – potentiometrically in suspension of 1 mol KCl dm^{-3} solution and in H_2O; content of available forms of P and K by Egner-Riehm method; content of available forms of Mg by Schachtschabel method; content of organic carbon by Turin method (Lityński et al., 1976; Ostrowska et al., 1991).

The content of organic matter (humus) was calculated by multiplying the content of organic carbon by a conventional factor 1.724. The content of nutrients in the soil was established in a dry matter (a sample dried in temperature of 105 °C).

Every year crop productivity was determined as the last and the most important parameter of soil productivity. It is obvious that crop yields are influenced not only by applied biological agents, but also by weather conditions. The meteorological conditions in vegetative period of 2007, when silage maize was grown, were favourite for this crop it was warmer than usual and the rainfalls were also higher (Table 2). Favourite weather conditions, similar to those in 2007, were also in 2008, when winter spelt wheat was grown. Only in 2009 rainfalls were lower than multi-year average, but their distributions were good, so no negative effect on spring common wheat was found (dry conditions were in harvesting time of cereals).

Table 2. Air temperatures and rainfalls in vegetation periods in 2007–2009

Months	Air temperatures, °C				Rainfalls, mm			
	means	means			sums	sums		
	1961–2000	2007	2008	2009	1961–2000	2007	2008	2009
April	7.9	7.3	7.8	9.7	35.4	26.8	24.2	3.7
May	12.5	13.7	12.5	12.2	57.6	79.7	93.2	89.6
June	15.8	17.5	16.0	14.7	69.5	60.8	83.5	133.1
July	17.2	17.5	21.0	18.9	81.6	176.0	27.1	82.2
August	16.8	18.2	17.3	18.5	75.2	81.0	141.7	25.7
September	12.6	12.6	15.7	14.7	57.1	65.4	105.6	15.6
April–September	12.9	14.5	14.3	14.8	430.4	489.7	509.6	349.9

The silage maize yields were established after weighing of harvested biomass. The common wheat yields were established after weighing of harvested grains after their collecting by a cereals combine harvester. The spelt ears after collecting by cereals combine harvester were hulled in a special machine and then grain yields were weighted.

ANOVA complete randomized design (CRD) was used. To compare differences between means, the significant difference according to Tuckey's *t*-test was calculated at $p = 0.05$.

RESULTS AND DISCUSSION

Soil chemical properties have impact on soil aggregation; The examination of soil chemical properties revealed that the soil had 2.46% of organic matter, which is not much for such a heavy soil, but at the same time much higher than the average for Polish soils (Table 3).

Table 3. Soil chemical properties of the experimental field, 2007 and 2009

Chemical properties		Treatments					Means	$HSD_{0.05}$
		CT	EM	HP	HB	UG		
Organic matter %	2007	2.56	2.49	2.40	2.42	2.44	2.46	n.s.
	2009	2.55	2.50	2.42	2.44	2.43	2.47	n.s.
pH_{KCl}	2007	6.72	6.86	6.44	6.58	6.64	6.65	n.s.
	2009	6.56	6.85	6.38	6.50	6.41	6.54	n.s.
pH_{H2O}	2007	7.32	7.52	7.18	7.25	7.27	7.31	n.s.
	2009	7.23	7.65	7.08	7.17	7.25	7.28	n.s.
P mg kg^{-1}	2007	46.3	48.7	40.3	42.6	45.6	44.7	n.s.
	2009	46.7	48.2	40.8	40.7	46.5	44.6	n.s.
K mg kg^{-1}	2007	148.0	138.8	155.9	152.2	143.0	146.2	n.s.
	2009	142.8	134.5	151.8	150.2	146.1	145.1	n.s.
Mg mg kg^{-1}	2007	60.9	65.7	64.5	67.1	59.5	63.5	n.s.
	2009	60.9	66.3	68.7	67.5	61.8	65.0	n.s.

CT – control treatment, EM – effective microorganisms, HP – biodynamic horn preparation, HB – Humobak, UG – UGmax; n.s. – not significant differences between treatments and years.

The soil pH_{KCl} on the individual experimental plots stood at 6.38 to 6.86, i.e. was close to neutral. Considering though the beneficial influence of calcium ion on the structure quality of heavy soil, it should be stated, that such a soil ought to be periodically limed. It is considered that heavy soils of the pH_{KCl} less than 6.6 should be limed (Fertilizing recommendations, 1985).

The content of crop available phosphorus was at the border of low and medium. Taking into account the heavy texture of the soil, the content of crop available forms of potassium and magnesium should be considered as medium (Fertilizing recommendations, 1985). The authors concluded that the soil chemical properties were favourable to develop a good aggregate structure. After the three vegetation periods in which 3 times each year tested biological agents were applied, soil samples were taken once again and the same chemical analyses were done. The authors did not find any significant effect of agents on the content of soil organic matter, soil pH, the content of available forms of P, K and Mg (Table 3). Wielgosz et al. (2010) on a loamy soil in Lublin province and with rainfall very similar to those in Budziszewo, found initial decrease in soil pH shortly after EM application and then an increasing trend. In the same study EM application initially caused inhibition of soil enzyme activity followed with increasing trends. The same variation pattern (an initial drop followed by increasing trend) was found in the number of physiological groups of bacteria. This may correspond to the opinion of Martyniuk (2011) that the very small number of microorganisms applied to soil (comparing to the number of autochthonic microorganisms living in the soil) and lack of free niches for newcomers results in a very low probability to cause long-term changes in the soil biotope, thus rather short lasting variations may be expected.

The data from the soil physical properties analyses in the first year of investigations (2007) as well as the last one (2009) were presented in this paper. In Table 4 the impact of biological agents on share of aggregate size classes established after dry sieving was shown.

Among the presented aggregate classes the two biggest ones ($\varphi > 10$ mm) indicate a bad soil structure, having unfavourable effect on crops germination and growth. The same holds true for the smallest class ($\varphi < 0.25$ mm), which in fact causes soil dustiness (no aggregate structure). The most valuable soil aggregates are those of diameter between 1 and 5 mm (Dobrzański et al., 1975; Domżał & Słowińska-Jurkiewicz, 1988).

After the 3 years of investigations the authors found no statistically proven impact of the tested biological agents on soil aggregate structure both in the first year of investigation (2007) as in the last one (2009) (Table 4). What can be seen is a tendency of worsening of aggregate structure in the last year of experiment, regardless of applied soil conditioners. This is clearly visible in the biggest aggregates ($\varphi > 30$ mm), so-cold clods. Their share between 2007 and 2009 was more than doubled, although still is not high on that type of soil.

Table 4. The impact of biological agents on soil structure – share of aggregate size classes established after dry sieving, 2007 and 2009

Diameter of aggregates	Year	Treatments					Means	$HSD_{0.05}$
		CT	EM	HP	HM	UG		
>30 mm	2007	3.84	3.40	3.63	3.47	3.69	3.60	n.s.
	2009	4.91	8.58	5.17	11.93	9.43	8.00	n.s.
	mean	4.38	5.99	4.40	7.70	6.56	5.80	-
30–10 mm	2007	22.80	20.69	21.46	22.02	21.91	21.78	n.s.
	2009	14.41	21.04	25.27	19.23	18.03	19.60	n.s.
	mean	18.51	20.87	23.37	20.63	19.97	20.69	-
10–7 mm	2007	9.81	9.25	9.27	9.08	8.27	9.14	n.s.
	2009	8.00	9.85	9.32	9.34	9.26	9.15	n.s.
	mean	8.91	9.55	9.30	9.21	8.77	9.15	-
7–5 mm	2007	7.33	8.80	7.74	8.37	8.47	8.14	n.s.
	2009	9.01	7.92	8.28	6.54	8.68	8.09	n.s.
	mean	8.17	8.36	8.01	7.54	8.58	8.12	-
5–3 mm	2007	7.44	8.22	8.05	7.64	7.43	7.76	n.s.
	2009	8.61	7.53	6.73	6.53	7.26	7.33	n.s.
	mean	8.03	8.88	7.39	7.09	7.35	7.55	-
3–1 mm	2007	21.26	22.79	19.21	21.12	19.57	20.79	n.s.
	2009	29.02	24.33	23.55	26.14	25.25	25.66	n.s.
	mean	25.14	23.56	21.38	23.63	22.41	23.23	-
1–0.5 mm	2007	12.82	13.88	12.67	12.55	13.43	13.07	n.s.
	2009	11.69	9.49	9.75	9.74	10.53	10.24	n.s.
	mean	12.26	11.69	11.21	11.15	11.99	11.66	-
0.5–0.25 mm	2007	6.40	6.09	7.86	6.30	7.84	6.89	n.s.
	2009	6.33	4.66	4.63	4.28	4.69	4.92	n.s.
	mean	6.37	5.38	6.25	5.29	6.27	5.91	-
<0.25 mm	2007	8.30	6.88	10.11	9.45	9.39	8.83	n.s.
	2009	8.02	6.60	7.30	6.27	6.87	7.01	n.s.
	mean	8.16	6.74	8.71	7.86	8.13	7.92	-

CT – control treatment, EM – effective microorganisms, HP – biodynamic horn preparation, HB – Humobak, UG – UGmax; n.s. – not significant differences between treatments and years.

The reason for such a tendency is the crop sequence. Normally on the farm in which the experiments were carried out the following crop rotation is practiced: white clover grass – white clover grass – white clover grass – winter wheat followed by white mustard for green manure – spring wheat – silage maize – winter spelt wheat undersown with clover-grass. In such rotation we have two phases: first of building soil structure and fertility (clover-grass) and second of utilizing (depleting) both soil structure and its fertility (Kiley-Worthington, 1981). In the experiment the rotation was changed to three annual crops in a row, to enable the authors to observe whether applied biological agents could extend the phase of the high good soil structure and to take samples. As was stated before, the soil structure was worsening in time, so the biological agents were not able to stop the process. The question of the role of crop species and fertilization /management practices will be continued towards the end of the article when comparing soil physical properties on organic and conventional farm.

A soil structure quality can be described in the form of soil structure indices. One of them is an aggregation index calculated on the basis of dry sieving (AI_d). The authors found no statistical difference between the control treatment and the treatments with biological agents on AI_d (Table 5). The same applies for the aggregation index calculated on the basis of wet sieving (AI_w).

The values of QSI were not statistically different in the compared treatments. Moreover, a tendency for better values was noticed in the control treatment in the last year of the study. The CI illustrates the ratio of so-cold clod aggregates ($\varphi > 10$ mm) to all the other aggregates ($\varphi < 10$ mm). The CI index had also a positive tendency of lowering in the control treatment in the last year of the study (Table 5).

Table 5. The impact of biological agents on some indexes of soil structure, 2007 and 2009

Soil structure indexes	Year	Treatments					Means	$HSD_{0.05}$
		CT	EM	HP	HB	UG		
Aggregation index (dry sieving), AI_d	2007	365	417	361	388	383	382	n.s.
	2009	471	399	386	404	413	415	n.s.
	mean	418	408	374	396	398	399	-
Aggregation index (wet sieving), AI_w	2007	2 999	2 832	2 831	2 532	2 887	2 816	n.s.
	2009	3 654	3 356	3 179	3 470	3 259	3 384	n.s.
	mean	3 327	3 094	3 005	3 001	3 073	3 100	-
Quality structure index (QSI)	2007	1.23	1.57	1.38	1.27	1.31	1.35	n.s.
	2009	1.83	1.17	1.12	1.14	1.29	1.31	n.s.
	mean	1.53	1.38	1.25	1.21	1.30	1.33	-
Clods index (CI)	2007	0.36	0.29	0.33	0.34	0.34	0.33	n.s.
	2009	0.24	0.42	0.44	0.45	0.38	0.39	n.s.
	mean	0.30	0.36	0.39	0.40	0.36	0.36	-

CT – control treatment, EM – effective microorganisms, HP – biodynamic horn preparation, HB – Humobak, UG – UGmax; n.s. – not significant differences between treatments and years.

In the case of heavy soils, the bulk density of dry soil should be low. The lower density means the better soil structure. Although some difference between experimental treatments could be seen, they are not statistically significant. The only clear difference is growing of the bulk density between 2007 and 2009 (Table 6). It has nothing to do with the applied treatment, being just a result of soil structure deterioration caused by the increased distance elapsed from clover-grass cropping. The parameter of volume density is connected with total soil porosity. The higher bulk density is the lower soil porosity and the worse conditions for crops growing. The parameter of soil porosity followed the same pattern as bulk density. The same applies to another parameter – the index of soil porosity.

Table 6. The impact of biological agents on some soil physical properties, 2007 and 2009

Physical of soil properties	Year	Treatments					Means	$HSD_{0.05}$
		CT	EM	HP	HB	UG		
Bulk density of dry soil,	2007	1.414	1.323	1.388	1.337	1.367	1.366	n.s.
g cm^{-3}	2009	1.428	1.423	1.409	1.403	1.416	1.416	n.s.
Total soil porosity,	2007	44.50	48.85	49.94	44.77	46.50	46.91	n.s.
% of volume	2009	43.95	44.90	45.67	44.50	44.61	44.73	n.s.
Index of soil porosity,	2007	0.81	0.96	0.89	0.90	0.87	0.89	n.s.
ε	2009	0.79	0.82	0.84	0.81	0.80	0.81	n.s.
Soil humidity,	2007	19.53	15.56	15.79	18.47	17.09	17.29	n.s.
% of volume	2009	29.83	28.56	31.92	29.53	31.25	25.08	n.s.
Air content during	2007	25.12	31.17	35.15	29.71	29.41	30.11	n.s.
sampling, % of volume	2009	14.12	16.34	16.75	16.30	13.36	15.37	n.s.

CT – control treatment, EM – effective microorganisms, HP – biodynamic horn preparation, HB – Humobak, UG – UGmax; n.s. – not significant differences between treatments and years.

The soil humidity was higher in 2007 than in 2009, but in a heavy soil usually air content is the growth limiting factor, rather than humidity. Both parameters are connected, so in 2009 there was higher soil humidity and lower air content. Nevertheless, even the lower air content (on average 15%) was higher than the minimum for good crop growth (10%) and crops development was not limited by oxygen deficit in soil. So, one can conclude that the above discussed parameters were not limiting crop growth (Letey, 1985; Łachacz et al., 2013).

In the literature predominate papers are on the effect of biological agents on soil microbiological properties and crop yields, whereas studies of soil physical properties are scare and contradicting. In the studies of Kaczmarek et al. (2007; 2008) found some contradicting results. In the study of 2007 EM application on light-textured soil resulted in grown soil infiltration, which is a negative effect that might increase drought consequences to crops. In heavy soil infiltration was lower, resulting in slower drying of soil surface after rainfalls, so water can stagnate on fields, postponing spring soil tillage practicing and delaying sowing time. Whereas in the study of Kaczmarek et al. (2008) carried out on different soils, EM application increased infiltration on heavy soil and decreased it on light soil, which is a desired effect.

In our study soil sample examination revealed that although the soil structure was not perfect, but keeping in mind the soil type it was rather good. After a thorough consideration of the soil structure data, the authors came to the conclusion that probably the structure was close to the best that could be achieved on this type of soil. To check this hypothesis it was decided to evaluate the soil structure quality of a neighboring conventional field. The conventional farmer grew crops in the following rotation: silage maize – winter wheat – spring barley – winter oil seed rape – winter wheat. Silage maize was fertilized with 30 t of FYM per ha. The other crops in the rotation were fertilized exclusively with mineral fertilizers. Therefore, the soil samples were taken and a dry sieving was done. It was found that the neighboring conventional field had quite a different soil structure than the organic one (Table 7).

Table 7. Comparison of soil structure – share of aggregate size classes established after dry sieving on organically and conventionally managed fields, 2009

Aggregate diameter, mm	Type of field management		$HSD_{0.05}$
	organic*	conventional	
> 30	4.9	48.3	11.55
10–30	14.4	12.2	n.s.
7–10	8.0	6.4	n.s.
5–7	9.0	5.0	2.00
3–5	8.6	10.3	n.s.
1–3	29.0	8.0	5.50
0.5–1	11.7	5.1	2.79
0.25–0.5	6.3	2.6	1.27
< 0.25	8.1	2.1	1.36
$LSD_{0.05}$	2.44	5.11	–

* – data from the control plots were shown, n.s. – not significant.

The participation of clods (φ > 30 mm) was much higher on the conventional than the organic field (Table 7). The index of soil clods of conventional field was 1.20 that means it was 5-times higher than that of the organically managed soil – 0.24 (data not shown). The other big difference was 3.6 times lower participation of aggregates of diameter 3–1 mm in the conventionally managed fields. At the same time the aggregation index based on dry sieving (AI_d) was two times lower on the conventionally managed field: 471 versus 232 (data is not presented). The above mentioned facts indicate that only this proper organic management (rotation with fertility and soil structure building crops as clover-grass and green manures, fertilization with FYM, non-use of synthetic N) already improved soil structure to probably the best possible level. In that context one may conclude that biological agents could not improve structure of organically managed soil, because it already had the best possible one.

Each year crop productivity was determined as the last and most important parameter of soil quality. In 2007 silage maize was grown giving on average 76.8 t ha^{-1}. Although some variation in yields between the experimental object is visible, statistically lower yields were found only in the object treated with Humobak (Table 8).

Table 8. The impact of biological agents on yielding of crops grown in 2007, 2008 and 2009

Crop production	Treatments					Means	$HSD_{0.05}$
	CT	EM	HP	HB	UG		
Silage maize green mass, t ha^{-1}	81.5	91.5	79.0	48.5	83.5	76.8	13.6
Winter wheat spelt grain*, t ha^{-1}	3.94	4.40	3.97	4.00	4.08	4.08	n.s.
Spring common wheat grain, t ha^{-1}	4.24	3.92	4.13	3.14	4.08	3.90	0.428

* – hulled grain; CT – control treatment, EM – effective microorganisms, HP – biodynamic horn preparation, HB – Humobak, UG – UGmax; n.s. – not significant.

In 2008 winter spelt wheat was grown and high grain yields were harvested. This year no statistical yield difference was found. In 2009 spring common wheat was grown. The lowest yields were harvested on the plots treated with Humobak. To summarize, crop productivity in the three year investigation period, no crop reacted positively on any biological agents applied. At the same time two crops shown negative effect on Humobak application. Lower crop yields were not detected only in 2008. The authors think that the lowering of yields in 2007 and 2009 was caused by the increased microorganism growth, which were blocking availability of nitrogen to crops. In 2008 the nitrogen blocked in 2007 was released, resulting in no yield drop.

The Humobak application resulted in yield lowering also in another study. Babik et al. (2008) in the study done in Skierniewice (Central Poland) on fertile loamy soil used for vegetable growing for many years and similar rainfalls as those in Budziszewo, found 38.8% yield reduction in broccoli due to the Humobak treatment. In the case of chicory and carrots not only Humobak, but also EM caused a yield reduction by ca 13%. Pisikier (2006) found an increase in spring wheat yield, but the results were not confirmed statistically. The wheat was grown on a sandy soil in Pomeranian province in the very similar climate conditions to those in Budziszewo. It is worth mentioning that Humobak was decreasing potato infestation by Colorado Potato Beetle *Leptinotarsa decelineata* (Nowacki et al., 2011). The experiment was done in Jadwisin ca 30 km from Warsaw. The soil was sandy and the climate was little bit milder than in Budziszewo and rainfalls ca 10% lower.

As it was mentioned in our study no effect of UG on crop yield was found. Nevertheless there is also information on positive effect of UGmax application on potato yielding. Zarzecka and Gugała (2012) in the field experiment carried out on a sandy soil in Jadwisin (the same one as in the study of above-cited Nowacki et al., 2011) in 2009–2010 with different potato cultivars found 30–47.9% yield increase. Unfortunately in the paper there is only information about chemical weed control and no information is given whether this high yield increase had something to do with potato infestation by Potato Colorado Beetle as of the above-cited work of Nowacki et al., 2011.

Although in bibliography one can find both positive and negative opinions on effectiveness of biological agents both on soil structure and crop productivity, the negative opinions are more common (Van Vliet at al., 2006; Mayer et al., 2008; Martyniuk, 2011; Martyniuk & Ksiezak, 2011).

Among different biological agents EM was studied in the highest number of experiments. Sulewska & Ptaszyńska (2005) on a sandy soils in the province of Great Poland (little bit milder and dryer climate than in Budziszewo) applied EM in maize growing without yield gain. Just to quote the latest experiments: Rychcik & Sadowski (2011) found no effect of EM application in organic growing of potato and cereals. Sometimes the research findings are contradicted: e.g., Kuś et al. (2013) in the same experiment found no positive effect on wheat yields and 7% yield gain in potatoes and silage maize. The case of maize is very special – although the yield was 7% bigger, the share of cobs in the yield (the most valuable part of the crop) was lower.

CONCLUSIONS

The research hypothesis was that use of biological agents (biodynamic horn preparation, effective microorganism, Humobak and UGmax) can improve a soil quality and enhance crop yields however an application of the above stated biological agents did not affect significantly soil chemical and physical properties. The applied biological agents did not affect crop productivity, with the exception of Humobak which decreased the yield of silage maize and spring common wheat by 41% and 26%, respectively.

A proper organic management (a proper crop rotation with clover-grass and green manure crops, a high stand of livestock and regular farm yard manure fertilization) has much greater positive effect on soil physical properties, as opposed to an application of biological agents.

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Evaluating thermal performance of experimental building solutions designed for livestock housing: the effect of greenery systems

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Abstract. The thermal performance of a greenhouse-type building provided with a living plant canopy was evaluated in Northern Italy during summer. Four reduced scale buildings with different types of covering were tested. The first type was the reproduction of a gable roof covered with 40 mm-thick sandwich panels (SAND), a widespread solution for dairy barns in temperate climates, used as control. Two roofs were reproductions of a Venlo-type greenhouse covered with a 0.2 mm-thick transparent EVA film equipped with either a reflective shading screen with 70% shading level (TRA+SHA) or with a living plant canopy (TRA+PLA). The last type of roof consisted of the living plant canopy alone (PLA). Plant canopies were made up of climbing plants (*Trachelospermum jasminoides*) with an average LAI of 1.39 m^2 m^{-2}.
Data were analysed with mixed linear models for repeated measures. Fixed effects tested were roof type and the interaction of roof type and time of the day. Internal temperature in TRA+SHA (22.60 °C) was higher than PLA (21.28 °C; $p > 0.001$), SAND (21.53 °C; $p = 0.026$) and TRA+PLA (21.68 °C; $p = 0.036$), with no significant differences among the latter three. Differences were larger during the hottest hours of the day (from 09:00 till 17:00) while, during the night, internal temperature did not differ among types of roof.
Results indicate that greenhouse-type buildings with conventional shading systems may not be adequate for housing livestock in warm climates. However, the employment of greenery systems such as a plant canopy may effectively reduce internal temperature. Further research is deserved to develop suitable building solution for livestock farming.

Key words: livestock housing, greenhouse, green roof, greenery systems, thermal performance.

INTRODUCTION

Heat stress in livestock housing is a major concern and, since the global temperature is likely to increase (IPCC, 2013), the magnitude of this issue will continue to grow. In dairy cows, heat stress can compromise milk production and reproductive performance, modify behavior, cause serious health problems and even lead animals to death (Jordan, 2003; West, 2003; Vitali et al., 2009; Tao & Dahl, 2013). In many cases, natural ventilation alone is not adequate to support the high production level of modern dairy cows (Berman et al., 1985; Armstrong, 1994). For this reason, several studies have been focused on active cooling techniques such as water sprinkling, forced ventilation and evaporative cooling even though their application considerably increases energy consumption (Ecim-Djuric &Topisirovic, 2010; Barbari et al., 2011; Legrand et al., 2011; Honig et al., 2012).

In the last years, significant efforts have been made to improve energy efficiency in the building sector (Týbl & Kic, 2016). Researches on residential and commercial buildings showed that there are many passive solutions which can reduce the energy requirement for cooling purposes. The use of various types of plants as part of the building enclosure has been shown to improve its thermal performance (Saadatian et al., 2013; Pérez et al., 2014). Due to their capacity to intercept solar radiation, plants provide effective shade but, due to evapotranspiration, they also have an air-cooling effect (Jaffal et al., 2012). Both roofs and walls can be provided with vegetation. These techniques are commonly known as green roofs and green façades.

Green roof mainly refers to a roof covered with herbaceous and/or woody plants which are planted in a layer of soil or other growing medium. Many studies have been focused on thermal performance of these systems. In a literature review, Saadatian et al. (2013) found that green roofs can absorb 60% of the solar radiation and decrease the surface temperature of the roof up to 60 °C, thus reducing the internal building temperature up to 20 °C and the cooling load by 32 to 100%. In term of economics, Blackhurst et al. (2010) reported that green roofs can be an economical option in the long term but their initial cost is three to six times higher than a conventional roof.

Green façades are vertical greenery systems that involve virtually any way to set plants in a building façade or wall. Traditionally, green façades consist of climbing plants or shrubs which grow directly along building walls or along supports such as frames and wires. In warm temperate climate, green façades can reduce the external surface temperature of the building façade wall by 12 to 20.8 °C in the summer period and by 5 to16 °C in autumn, with a reduction in energy consumption for cooling between 5% and 50% (Pérez et al., 2014).

Planting and growing trees near the building is another simple and effective mean of reducing building solar loads and thermal regulation requirements in summer. Tree shade can decrease wall surface temperatures by up to 9 °C and external air temperatures by up to 1 °C (Berry et al., 2013). Balogun et al. (2014) found that tree shade sensibly lessens the energy requirement for cooling in a school building. Since greenery systems have the potential to improve the thermal performance of buildings and decrease the energy consumption for active cooling they could be profitably employed in dairy barns. However, literature about the use of these passive systems in livestock housing is almost lacking.

Another solution that has been spread in recent years is the use of greenhouse-type structures for livestock housing (Galama et al., 2011). Greenhouses typically have automatic vents and shading systems which appear to allow a better control over the internal microclimate compared with traditional shelters (Sethi & Sharma, 2007; Vanthoor et al., 2011). Moreover, greenhouses are considered to provide a more natural environment for the cows mainly due to improved natural lighting. Natural lighting also contributes in drying and thus reduces the amount of bedding materials needed, especially during winter (den Hollander, 2014). The use of greenhouse-type buildings for housing dairy cows is mostly spread in temperate areas with moderate summer temperatures. In warmer climates, the characteristics of the materials typically used for greenhouse cladding could pose challenges in keeping adequate internal temperature during summer months.

According to the information available, greenery systems for buildings and greenhouse-type structures can have a role in future development of housing systems for dairy cows. Although there are some issues concerning their employment, they have the potential to lower production costs, improve animal welfare and reduce environmental impact of dairy farming. Despite that, literature about the use of these solutions in housing for dairy cows and other livestock is still sparse. The aim of this study is to evaluate the effect on internal temperature of different roof configurations combining greenery systems and greenhouse-type coverings in warm weather conditions.

MATERIALS AND METHODS

The research was carried out by comparing the thermal performance of 4 different types of roof. Four identical reduced-scale buildings were built (Fig. 1). Each one was 2 m long, 1 m wide and 1 m high. Experimental buildings had a wooden-frame structure made up of 40 x 40 mm timbers. Floor and curtain walls were built using 40 mm thick polystyrene sheets. To allow natural ventilation, a 1 m x 0.33 m opening was created in both sidewalls of the experimental buildings. Since all the buildings were oriented with the ridge running East-West, the opening of the South sidewall was provided with a sloped overhang shading made up of a reflective cloth (ILS 70 F Revolux, Svennson, Kinna, Sweden). One of the four types of roof was applied to each experimental building.

Figure 1. A view of the experimental site with the reduced-scale buildings (originally 6 structures were built but 2 of them were excluded from the study).

The first type of roof was the reproduction of a gable roof with continuous ridge vent, which is a widespread solution for dairy barns in temperate climates and was used as control. The covering consisted of sandwich panels (SAND) with 40 mm-thick polyurethane foam core and 2mm-thick aluminum skins. The thermal transmittance (U) of the panels used in SAND was 0.54 W m^{-2} K^{-1} (Isocop Granite, Isopan, Verona, Italy). To assess performance of different greenhouse-type coverings, 2 roof frame structures that reproduced the shape of a Venlo greenhouse with fixed continuous ridge vents were assembled using 40 x 40 mm timbers. Both greenhouse-type roofs were covered with a 0.2 mm-thick transparent EVA film (PATILUX, P.A.T.I., Treviso, Italy) but were equipped with different internal shading systems. The first consisted of a single shading screen with 70% shading level (TRA+SHA). The screen had an open structure to allow ventilation and its top surface was reflective (ILS 70 F Revolux, Svennson, Kinna, Sweden). The second greenhouse-type roof was provided with a living plant canopy (TRA+PLA). The plant canopy was made up of a metal net that sustained climbing plants trained and tied to create a consistent layer. Eight potted Star Jasmine plants (*Trachelospermum jasminoides*) were used. The pots had a capacity of 4 l each and were fixed to the metal net along the sidewalls of the experimental building. To keep the plants alive and physiologically active during the test, they were irrigated by an automatic drip system which was set to deliver 0.05 l h^{-1} in each pot. In order to evaluate performance of the plant canopy alone, it was also applied to an experimental building with a completely open roof (PLA). Cross sections of experimental buildings with the different types of roof are reported in Fig. 2.

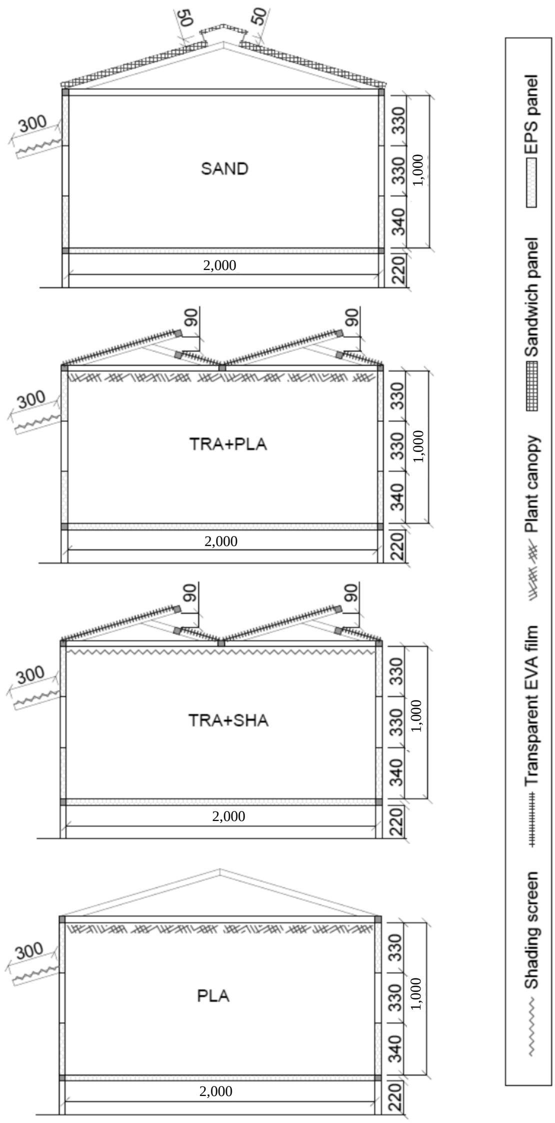

Figure 2. Cross sections of the reduced-scale buildings with the different types of roof tested (dimensions are in mm).

To evaluate the shading level of the plant canopy, the leaf area index (LAI) was measured (Jonckheere et al., 2004). Ten plants were randomly selected among those available and the number of leaves per plant was counted. Twenty randomly selected leaves were plucked from all the plants available to evaluate their dimensions. To determine the leaf's area, length and width, a photo of leaves on graph paper was taken. Leaves were kept flat by placing a glass above them. The pictures were then imported in AutoCAD (Autodesk, San Rafael, California, USA) and scaled using the paper grid as reference. The leaf area was measured using a minimum of 20 points to better approximate the round perimeter. The LAI was calculated by multiplying the average one-sided leaf area by the average number of leaf per plant and by the number of plant used in each canopy. The value obtained was then divided by the internal area of each experimental building. The thickness of the plant canopies was measured in 5 randomly selected points.

To avoid any external source of shading, all the experimental buildings were placed in a field with no trees or constructions nearby and at a minimum distance of 4 m among them. In the experimental area, grass was mown before the beginning of the trials. During the whole experiment, dry bulb temperature was measured outside and inside the experimental buildings. The probe for external temperature was placed 1 m above the ground while the probes for internal measurements were placed inside each experimental building at 0.1 m above the floor. The external probe was provided with a radiant screen. All the sensors used in the experiment were Pt100 resistance thermometers (DMA672-1, Lsi Lastem, Milan, Italy) and they were connected to a 16-bit data logger (E-log, Lsi Lastem, Milan, Italy). Both internal and external temperatures were recorded continuously every 15 minutes. Trials were carried out in Mantua (Italy) during August 2013 (13-27/08/2013). Experiment lasted 15 days but 2 days had to be removed from the data set due to a technical issue with data recording. Final data set included observations collected over 13 full days.

Statistical analysis

Statistical analysis was carried out using R (R Core Team, 2016). Data collected at 15-min intervals were averaged to obtain hourly observations. Descriptive statistics for external air temperature during the experimental period and plant canopy characteristics were computed. Results are reported in text as mean±SD. A mixed model for repeated-measures was built to assess the effect of different types of roof on internal air temperature, which was the response variable. Roof type and the interaction of roof type by time of the day was included as fixed effects. Day was included as random repeated effect. An autoregressive (ar1) correlation structure was used to take into account for autocorrelations of subsequent observations. Model was fitted with ML. Normality and homoscedasticity of variance were visually evaluated using residual plots. Least squares means and SEM were computed and reported in text and figures as LSmeans±SEM. Tukey's method was used for pairwise comparisons of LSmeans. Significance of differences was declared at $P<0.05$ while a tendency was reported at $P < 0.10$.

RESULTS

During the whole experimental period (13-27/08/2013) weather was predominantly sunny. The average, maximum and minimum temperatures were 21.96 °C, 33.15 °C and

11.87 °C, respectively. Highest air temperatures were recorded during the day at 16:00-17:00 while lowest occurred at 5:00-6:00, just before sunrise (Fig. 3).

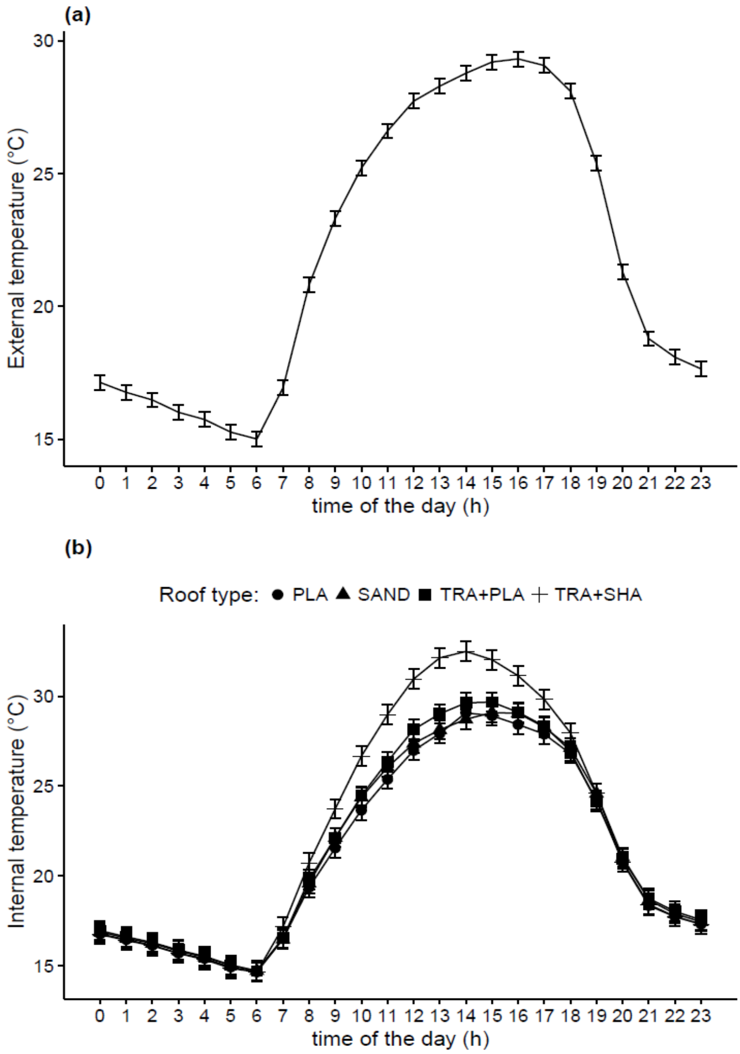

Figure 3. External temperature (a) and temperature measured inside the reduced-scale buildings provided with different roof types (b).

Plant canopy

Plants used in the present study (*Trachelospermum jasminoides*) to make the canopies had 484±91 leaves. Since the area of the experimental building was 2 m^2 and 8 plants were used in each canopy, average leaf density was estimated to be 1,936 leaves m^{-2}. Leaves were 57 ± 6 mm long and 19 ± 4 mm wide. The one-sided area of a single leaf was 720 ± 166 mm^2. On average, the LAI of the plant canopies used in this study was 1.39 m^2 m^{-2}. The vegetation layer was 6.1 ± 5.6 cm thick.

Roof type

Mixed model analysis indicated that both the main effect of roof type and the interaction of roof type by hour of the day significantly affected internal air temperature. Temperature in TRA+SHA (22.60 °C) was higher than PLA (21.28 °C; $p > 0.001$), SAND (21.53 °C; $p = 0.026$) and TRA+PLA (21.68 °C; $p = 0.036$), with no significant differences among the latter three. Interaction with hour of the day showed differences that were larger during the hottest hours of the day while, during the night, internal temperature did not differ among types of roof. During the interval from 9:00 and 17:00, the roof with TRA+SHA configuration produced significantly higher temperature than PLA, SAND and TRA+PLA (Fig. 3). During the same period, no significant differences were detected among PLA, SAND and TRA+PLA, even though PLA tended to have the numerically lowest internal temperature. During the remaining parts of the day (from 00:00 to 08:00 and from 18:00 to 23:00) all roof types produced similar internal temperatures with no significant differences.

DISCUSSION

External air temperature measured during the course of the experiment can be considered representative of the summer period in Northern Italy and potentially reduce welfare and performance of dairy cows as well as other farm species, such as swine and poultry (Jordan, 2003; West, 2003; Barbari & Sorbetti Guerri, 2004; Cândido et al., 2015; Santos et al., 2015). The experimental buildings with greenhouse-type coverings and artificial shading net (TRA+SHA) had higher internal temperature than all other configurations tested. During the hottest period of the day, the difference between TRA+SHA and SAND reached 3.4 °C. Results indicate that, in hot weather conditions, the use of conventional greenhouses, if applied for livestock housing, could result in increased heat stress. However, it has to be considered that cladding materials used in this study were not specifically developed to reduce thermal load. Researches on greenhouse cooling techniques showed that by employing semi-transparent or reflective materials the internal temperature can be reduced (Kumar et al., 2009; Lamnatou & Chemisana, 2013). On the other hand, a semi-transparent cladding would limit heat gain also during winter when, in the case of livestock housing, it would be useful to dry the bedding and reduce its consumption (Leso et al., 2013; den Hollander, 2014).

Temperatures inside the buildings with plant canopies (PLA, TRA+PLA) did not differ from that measured in SAND, despite they were not provided with any insulating material. These results confirm that plants have the capacity to effectively reduce the thermal load of buildings in hot weather conditions. Although difference was not statistically significant, the roof type made up of the plant canopy alone (PLA) produced the lowest internal temperature among all types of roof tested. That was probably due to the open structure of the plant canopy, which may result in a higher air exchange rate. The type of greenery system used in this experiment was not properly a green roof nor a green façade since it was horizontal but there was not a continuous layer of growing medium on the roof (Saadatian et al., 2013; Pérez et al., 2014). For this reason, findings obtained in the present study cannot be directly compared with those reported in most of the relevant literature. Nevertheless, Koyama et al. (2014) used a greenery system for similar purposes. They examined a technique to cool a livestock building by covering its south wall and roof with kudzu vine (*Pueraria lobata*). Findings shown that the internal temperature of a building with 43.9% plant coverage was 3.44 °C lower than that in a bare one. They also found that temperature reduction increased with the percentage of building covered.

In many researches regarding greenery systems for energy saving in buildings, characteristics of the vegetation, such as plant species, canopy density and fractional coverage, have been regarded as the most significant parameters affecting thermal performance (Dvorak & Volder, 2010; Cameron et al., 2014). In the present study, the percentage of the roof surface covered by plants was not measured directly. However, it can be considered to be almost fully covered, since canopies were specially prepared by tying plants to form a uniform and consistent layer. The LAI of the canopies was 1.39 that also indicates a complete covering, though many studies on green roofs and green façades reported higher LAI (Kumar & Kaushik, 2005; Hunter et al., 2014). Higher LAI are also typical for many other planted environments. In forests the maximum LAI varies from 6 to 8. In agricultural fields LAI varies from 2 to 4 for annual crops with a mean LAI for grassland of 2.5 (Asner et al., 2003) According to the information available, increasing LAI in greenery systems for energy saving in buildings would result in improved thermal performance (Jaffal et al., 2012).

Results obtained in the present study showed that, in hot weather conditions and high sun load, providing greenhouses with plan canopies allows obtaining the same internal temperature as with traditional insulated coverings. The plant tested in the present study (*Trachelospermum jasminoides*) is evergreen and was selected for its wide availability and relatively low cost. However, using deciduous plants as shading in greenhouse-type buildings designed for livestock housing could really allow maintaining an adequate temperature during summer and benefit from direct solar radiation during winter. In order to fully evaluate costs and benefits of this kind of housing systems for dairy cows and potentially other farm species, future studies should be carried out during a year round period. Afterwards the trials could be replied in real-scale buildings with animals inside.

Both deciduous and evergreen plants should be taken into account as well as semi-transparent cladding materials. Furthermore, the ventilation properties of a plant canopy would deserve further investigation. Encouraging results obtained with PLA led to think a roof combining plant canopy with retractable roof systems may provide adequate temperature during hot weather and improve natural ventilation.

CONCLUSIONS

Results confirm that the employment of climbing plants as part of the building enclosure improves its thermal performance and has the potential to reduce energy consumption for cooling purposes. In hot weather conditions, greenhouse-type covering provided with plant canopies had the same internal temperature as an insulated roof. Compared with an artificial shading net, plant canopy produced a significantly lower internal temperature.

Outcomes of the present study suggest that greenery systems for energy saving in buildings and greenhouse-type structures can have a role in future development of housing systems for dairy cows and other farm species. Further studies could be required to verify the environmental conditions in real-scale livestock buildings with animals kept inside.

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Digital elevation models as predictors of yield: Comparison of an UAV and other elevation data sources

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Abstract. Topography usually plays an important role for yield variability assessment. This study provides insight into the use of surface models from different sources for agriculture purposes: unmanned aerial vehicle imagery, LiDAR data and elevation data acquired from a harvester. The dataset from an aerial vehicle was obtained in the form of ortho-mosaics and digital surface model using casual camera. The LiDAR data was provided by the State Administration of Land Surveying and Cadastre in the form of Digital Terrain Model of the 4th and 5th generation. The data of yield together with its coordinates were gained from a combine harvester in the form of a regular grid. Yield data was interpolated by kriging geostatistical method. Position data including an altitude was used for modelling the last digital surface model. All gained surface models were correlated with the spring barley yield. Results show correlation similarity across all tested models with the yield; no significant differences were sighted. Free available coarser scale data is able to predict a yield sufficiently. The study indicates less effectivity of using very detailed scale data sources due to its time-consumption or expensive data gathering and processing process.

Key words: Unmanned aerial vehicle, structure from motion, spatial resolution.

INTRODUCTION

Elevation data can be acquired from three main sources: ground surveys, existing topographic maps and remote sensing techniques (Ouédraogo et al., 2014). Imagery acquisition using unmanned aerial vehicles (UAV) is very popular elevation data gathering technique within the last years. Besides other advantages, consumer grade cameras can perform high spatial resolution and high temporal frequency imagery. It is possible to get sufficient-accuracy ortho-mosaic and elevation model of large areas. UAV-based data became a promising tool for many agronomic applications during last few years (Schmale et al., 2008; Zhang & Kovacs, 2012; Gómez-Candón et al., 2014). These systems become an effective complement for conventional agricultural approaches, especially in precision agriculture or site-specific management respectively (Primicerio et al., 2012; Honkavaara et al., 2013; Rokhmana, 2015). UAV could be less expensive and more practical in contrast with satellite and airborne systems for high resolution remotely sensed data (Zhang & Kovacs, 2012). That is why it is possible to use UAV for the creation of topography model for agricultural purposes. Moreover, it is possible to capture actual micro-topography in any time using UAV. The Digital Elevation Model (DEM) is a stable factor compared to other variables (Schmidt & Persson, 2003), and it is generally known that spatial variability of yield can be explained by topography as one of several variables (Zhang et al., 2002). For example, Kumhálová & Moudrý (2014) used RTK-GPS, harvester yield monitor with DGPS and Airborne Laser Scanning (ALS) in their study. Using aerial systems, high spatial scale data are gained. Use of low-cost cameras and specialized software solutions make the generation of ortho-mosaic and elevation models quite easy. UAV based models usually reach resolutions within centimetres. On the other hand, there is still the question of justification of accurate digital surface models in comparison with free available coarse datasets.

The aim of this study was to discuss the effectiveness of Digital Elevation Models from different sources with different spatial resolution for explanation of yield on large agricultural plots.

MATERIALS AND METHODS

The experimental field is located near to Vendoli in Eastern Bohemia (49°43' 47.94"N, 16°24' 14.21"E) and its size is 26.4 ha large. A 15.55 ha section of the field was chosen for our experiment. The terrain of the plot is undulated with an average slope of approximately 6%. The elevation ranges from 555.3 to 571.6 m above average sea level (565.4 m on average). The soil can be classified as modal cambisoils lying on calcareous sandstone. Some parts, on sloped terrain especially, are strongly eroded. The average precipitation is 700 mm per year and the average temperature is between 6–7 °C. Conventional arable soil tillage technology based on ploughing and crop rotation system based on wheat, barley and oilseed rape crops alternation were applied on the plot.

The topographic data were obtained from four sources. The first data set was obtained from perpendicular images taken by an unmanned aerial vehicle using the photogrammetry approach. Aerial photographs were taken on September 11, 2015 by a fixed 16 mm focal length lens at consumer-grade RGB camera Sony NEX5. The camera was mounted on the Falcon 8 V-form octocopter platform manufactured by Ascending Technologies GmbH, Germany. The aerial system and the camera were managed manually by a pilot. Photoscan software solution (version 1.2.6., Agisoft LLC, Russia) was used for aligning imagery and dense cloud generation. Images were aligned using 74 ground control points, which were measured by real time kinematic GPS method using Trimble device with VRS Now corrections. Digital elevation model with its final spatial resolution of 0.05 m was created from 285 overlapping images using Structure from Motion method (Fig. 1a). More than 80 million dense cloud points were gained by this approach. The next sources of elevation data, Digital Terrain Model of the Czech Republic of the 5th generation (DMR 5G) and Digital Terrain Model of the Czech Republic of the 4th generation (DMR 4G), Airborne Laser Scanning data sets were kindly provided by the State Administration of Land Surveying and Cadastre. Both models represent natural man-modelled terrain in digital form from the year of 2013. DMR 4G was distributed in a grid of 5×5 m with total mean elevation error of 0.3 m in open areas, while DMR 5G was distributed in a grid of 2 × 2 m with a total mean elevation error of 0.18 m (Brázdil & Dušánek, 2010; 2012).

Yield and the fourth terrain model has been measured by axial combine harvester New Holland CR9080. The harvester was equipped with a yield monitor and differential GPS receiver. The precision of this system is ± 0.1 to 0.3 m horizontally and ± 0.2 to 0.6 m vertically. The yield and elevation data were stored with the coordinates every second. The yield values of spring barley were corrected using a common statistical procedure; all values that exceeded the range defined as mean ± 3 standard deviations were removed. Because of the large amount of data for every year studied (more than 18 thousand), the MoM (Method of Moments) was used to compute the experimental variograms. Experimental variograms of yield were computed and modelled by weighted least squares approximation in GS+ (Gamma Design Software LLC, USA). Ordinary punctual kriging was done using the relevant data and variogram model parameters for yield data visualization. For detailed description of the data sets see Table 1. All spatial data were processed using ArcGIS solution (version 10.3.1., ESRI, USA).

Table 1. Summary of statistics for data sets used (m)

Source	Yield harvester	DEM harvester	DEM UAV	DEM DMR 4G	DEM DMR 5G
Count	18,537	18,537	62,188,439	6,118	38,811
Resolution			0.05 × 0.05	5 × 5	2 × 2
Mean	4.049	566.8	566.2	565.7	565.1
Median	4.111	567.0	566.7	566.0	565.0
Std	1.377	3.178	3.797	2.994	3.064
Minimum	0.204	557.0	554.0	556.6	556.0
Maximum	8.733	578.0	573.3	571.6	571.0
Skewness	-0.025	-0.310	-0.432	-0.458	-0.449

Statistical data was counted in R free software (version 3.2.2., R Core Development Team, Austria). The number of 23 random sampling points were created for the plot. At each point, the yield and altitude from all four digital elevation models were estimated. The yield spatial autocorrelation was verified by Moran's Index where presence of autocorrelation was not revealed. The estimated altitude from each model in each point was then tested for correlation with yield. R-squared error was also determined by fitting individual linear models for each digital elevation model as predictor of yield. A Hot Spot map of yield was finally created by using the Getis-Ord Gi* statistic for supporting our results.

RESULTS AND DISCUSSION

The results of the evaluation are shown in Table1. DMR 4G and DMR 5G models had similar median and also minimum and maximum values. Slightly different values can be observed in the digital model obtained by UAV (Fig. 1a). This is due to a better resolution which can capture different local roughness. Standard deviation is also slightly higher in UAV (4.15) compared to DMR 5G (3.43) and DMR 4G (3.34). The elevation models used are highly correlated (between ≈0.98 and ≈1.00, Pearson R), see Fig. 2. To evaluate differences in the models, we provide Tests of significance for correlations (r.test). The results show that input models are equivalent as predictor of yield with probability of ≈ 100%. For a better understanding of heterogeneity of yield at the field we have created a hot spot map where statistically significant high (red colour) and low (blue colour) yields can be observed, Fig. 1b. It also reveals relative homogeneity of field yields.

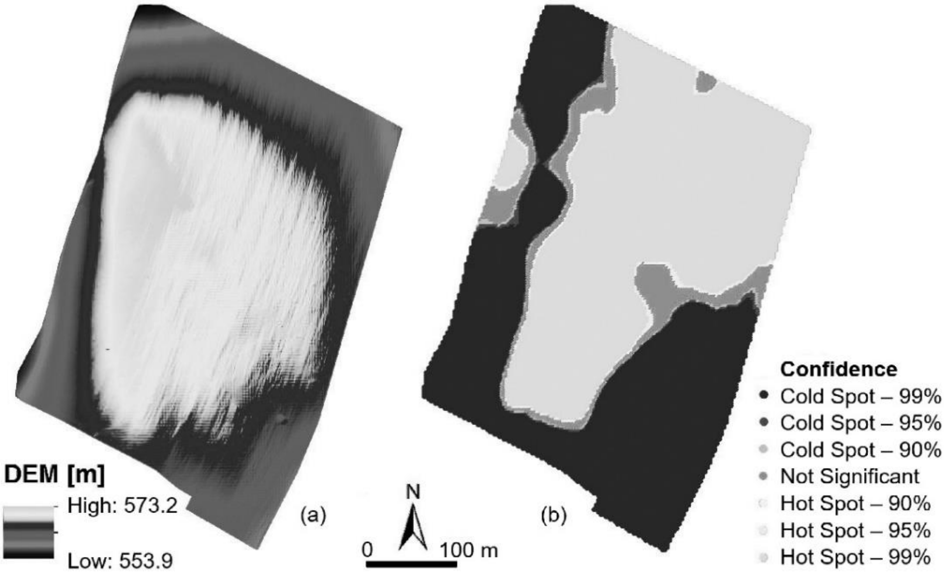

Figure 1. Elevation model using photo-reconstruction methods (a) and yield hotspot map (b) of the field study.

Elevation models were compared according to yield data using the correlation method (Table 2). The best model for yield prediction was DMR 4G explained 22.08% of yield variation followed by DEM from the UAV and DEM from the combine harvester. But all models can equally predict yield. The ability for predicting yield varies from 19% to 22% depending on the model.

Table 2. Statistics of correlation between models; yield and amount of variability in yield

Source	DEM	DEM	DEM	DEM
	harvester	UAV	DMR 4G	DMR 5G
Pearson's correlation coefficient	0.480	0.502	0.477	0.506
Correlation significance (p-value)	0.020	0.015	0.021	0.014
Adjusted R-squared	0.194	0.217	0.221	0.191

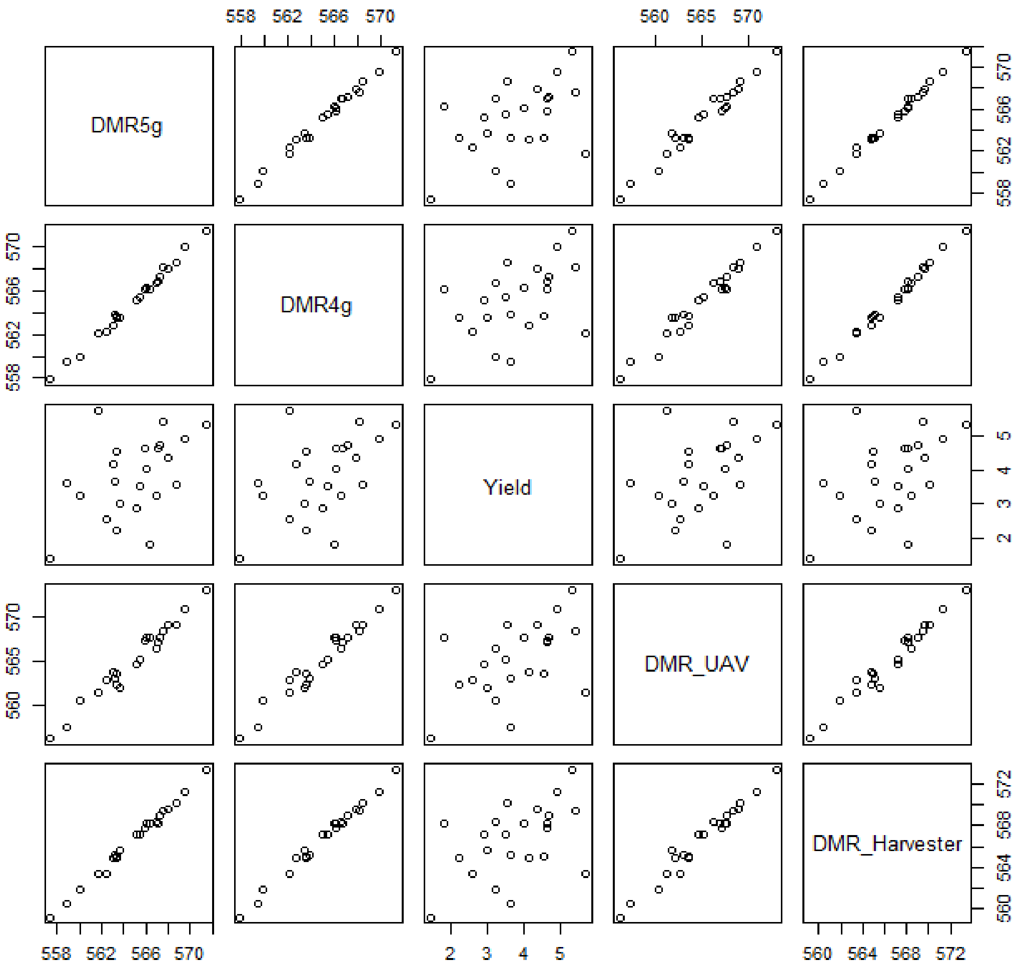

Figure 2. Matrix of input scatterplots showing dependence of input variables.

There is an effort in recent studies (Ristorto et al., 2015; Rokhmana, 2015) to use the most accurate data with the finest resolution as possible. As we show in this study, a field's yield can be relatively homogenous (Fig. 1b). In fact, using the finest resolution for prediction of yield does not necessarily bring additional information and furthermore, can have similar information value as models with coarse resolution. Uysal et al. (2015) discussed in their study the advantages of UAV systems utilization, such as low-cost, real time, high temporal or spatial resolution data. These conclusions are in accordance with our study. The UAV campaign was planned to early spring after sowing the spring barley, when the soil was bare. Belka et al. (2012) stated that the Airborne Laser Scanning was made during the spring or autumn. A large part of the Czech Republic was scanned regardless of vegetation on the fields. The flexibility in time is why the UAV possibility is suitable for monitoring the agriculture plot in different time.

Comparatively, acquisition of DEM from UAV is quite time consuming. To benefit from accurate UAV based DEM, 74 ground control points were necessary in our study. All of the points had to be measured by accurate GPS method. Moreover, special software has been used for computation of DEM from acquired photos. As we can see from the results, the ability to predict yield is similar across our models. In this point of view, the free available DEM models (DMR 5G or DMR 4G) could be better due to less time consumption. The digital model acquired by harvester is also a better choice than UAV in this case; nevertheless, some interpolating technics have to be made in GIS software to achieve final DEM.

The explained variability of yield reached at maximum only 22% in the DMR 4G model. It can be assumed that we could obtain similar results with other predictors, i.e. amount of soil meter, fertilization distribution, distribution of water, solar radiation etc. Using coarse data for predicting future yield or plant health could bring similar information value as the more accurate ones.

CONCLUSION

In this study we compare different digital terrain models obtained from different sources. Despite the fact of different resolution and accuracy of the data (from course 5×5 m to 0.05×0.05 m UAC model), the ability of models to predict the final yield were almost the same. We did not observe any statistically significant difference between input models.

As our results show, to use the most precise data is not necessary in every case. Less accurate, free available data could be equally sufficient to data with high costs or high time consumption. UAV based data can be used for DEM generation as a low-cost and real time source.

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Biogas and hydrogen production from glycerol by *Enterobacter aerogenes* and anaerobic microbial communities

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Abstract. Biological hydrogen production by anaerobic fermentation of widely available renewable resources is a promising and advantageous area. Using microbiological hydrogen production from crude glycerol biodiesel-derived waste was utilized by obtaining renewable energy carrier. The purpose of this research was to study biogas and hydrogen production by *Enterobacter aerogenes* MSCL 758 and by natural microbial communities. Growth medium was supplemented with analytical grade, technical grade or crude glycerol. Inoculants from old municipal landfill, manure and lake sludge were also used. Biogas production was analyzed using Automatic Methane Potential Test System II. Part of the experiments were carried out in serum bottles and evolved gases were tested using mass-spectrometry. Fluorescence *in situ* hybridization was used for bacterial population dynamic determination. Optimal concentration for crude glycerol was found to be six grams per liter. Amount of hydrogen was significantly higher and amount of nitrogen gas was lower in case of analytical grade glycerol usage in comparison to crude glycerol fermentation. *E. aerogenes* acted in synergy with landfill substrate and manure in biogas production from technical grade and analytical grade glycerol. It was not the case for crude glycerol usage. Addition of *E. aerogenes* increased overall amount of produced hydrogen. Obtained results showed potential of *E. aerogenes* for use in bioaugmentation purposes for fermentation of glycerol. Lake sludge inoculum contained microorganisms necessary for the production of hydrogen as well as biogas from glycerol. Clostridia and Gammaproteobacteria were predominant in the inoculum. Cultivable bacteria *Bacillus licheniformis, Burkholderia cepacia*, *Hafnia alvei* and unidentified *Clostridium* species were found to be predominant after six days of fermentation.

Key words: bacteria, bioaugmentation, fermentation, inoculum.

INTRODUCTION

Biological hydrogen production by bacterial anaerobic fermentation of widely available renewable resources is a promising and advantageous area. By microbiological hydrogen production from crude glycerol not only renewable energy carrier is obtained, but it is done by utilizing biodiesel-derived waste (Yazdani & Gonzalez, 2007).

There are two ways of glycerol metabolism during anaerobic fermentation – oxidative and reductive (Biebl et al., 1999). In the reducing pathway, glycerol or 1,2,3-

propanetriol is converted to 1,3–propanediol. In the oxidative pathway, glycerol is firstly converted to dihydroxyacetone, then dihydroxyacetone is phosphorylated and the phosphorylated product is metabolized through glycolysis. Pyruvate may be further converted to various end-products depending on the microorganism (Sarma et al., 2012). Fermentation products such as 1,3-propanediol, butanol, formic acid, propionic acid, succinic acid and ethanol as well as gases H_2 and CO_2 are produced (Yazdani & Gonzalez, 2007; Viana et al., 2012). Various bacterial strains are considered promising for glycerol utilization because of possibility to ferment crude glycerol, and H_2 is one of the end-products of this process (Ito et al., 2005). Hydrogen production comparing to production of 1,3-propanediol is more valuable, hydrogen has higher energy content (142.9 kJ g^{-1}) and it results in higher yield and productivity (Sarma et al., 2012). Ability to metabolise glycerol is possible for following genera of anaerobic and facultative anaerobic bacteria: *Klebsiella, Citrobacter, Enterobacter, Clostridium*, *Lactobacillus, Bacillus, Propionibacterium* and *Anaerobiospirillum* (Yazdani & Gonzalez, 2007; Markov et al., 2011). Most productive microorganisms that grow anaerobically on glycerol as the sole carbon and energy source are *Citrobacter freundii, Clostridium butyricum, C. pasteurianum, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Pantoea agglomerans*, *Rhodopseudomonas palustris* and *Thermotoga neapolitana* (Ito et al., 2005; Sarma et al., 2012). Facultative anaerobe *Enterobacter aerogenes* and anaerobe *Clostridium* species are the most commonly used (Mangayil et al., 2012). *E. aerogenes* is a mesophile and has a temperature optimum of 30–37 °C (reviewed in Fanning et al., 2016). Advantage of *E. aerogenes* in comparison with clostridia is uninhibited H_2 production by high H_2 pressures (Tanisho et al., 1987). However, improved yield of H_2 has been reported after removal of CO_2 because high CO_2 concentration can favour the production of fumarate or succinate, which contributes to consumption of electrons, and therefore decreases hydrogen production (Tanisho et al., 1998). Recently, Patil et al. (2016) discovered a perspective bacterium *Anaerobium acetethylicum*, representing a new genus within the order *Clostridiales*, which degrades glycerol via glyceraldehyde-3-phosphate and produces mainly ethanol and hydrogen and therefore is a future candidate for bioethanol and biohydrogen production.

Glycerol may be used in biogas production to improve both hydrogen and methane yield. Crude glycerol has been added to the anaerobic fermentation as a co-substrate (Yang et al., 2012). Fountoulakis & Manios (2009) reported increase of methane yield 2.6 times after addition of 1% of crude glycerol to fermentation medium. Bruna et al. (2010) have reported production of hydrogen from crude glycerol with anaerobic sludge. Fermentation of crude glycerol has a problem in comparison with analytical grade and technical grade glycerol because different impurities are known to inhibit growth of microorganisms (reviewed in Sarma et al., 2012). Despite the variations in the proportion of components, samples of crude glycerol were shown to contain glycerol, soap, methanol, fatty acid methyl esters, water, glycerides, free fatty acids, and ash (Hu et al., 2012). Glycerol content varies from 40% to 90% (Mane & Rode, 2013). For example, Ito et al. (2005) used crude glycerol with 41% glycerol content, Mangayil et al. (2012) with 45%, Priscilla et al. (2009) with 70% but Jitrwung & Yargeau (2015) with 81% glycerol content. Application of mixed microbial cultures has been proposed as strategy for improved hydrogen production (Sarma et al., 2012). Yield of 0.86 mol H_2 mol^{-1} glycerol was reported by Jitrwung & Yargeau (2015) and 6 moles H_2 mol^{-1} glycerol

(75% of theoretical, 8 moles H_2 mol^{-1} glycerol) was reported by Sabourin-Provost & Hallenbeck (2009).

The aim of this study was to investigate possibilities of Enterobacter aerogenes to produce biogas and hydrogen from crude, technical grade and analytical grade glycerol and to evaluate mixed natural microbial populations as potential inoculants for hydrogen production from glycerol.

MATERIALS AND METHODS

Fermentation experiments

This study focused on biogas and hydrogen production by pure culture of *Enterobacter aerogenes* MSCL 758 and by mixed anaerobic microbial communities in batch experiments. Natural microbial inoculants from old municipal landfill substrate, manure and lake sludge were used in different experiments and they were added to sterile growth media. Ten grams of landfill substrate and manure were suspended in 90 mL of sterile water and activated 18 h at temperature of 20 °C. After, 1 mL of the suspension as an inoculant was added to 400 mL of culture medium in 600-mL reactors or for the serum bottle experiments – 88 μl to 35 mL of culture medium. One mL of lake sludge was added to 35 mL culture medium in serum bottles. Cultivation was carried out triplicate with each sample in sterile microbiological growth medium consisted from tryptone (Fluka Biochem, Switzerland) 1.0 g L^{-1}, yeast extract (SIFIN, Germany) 2.5 g L^{-1} and cysteine hydrochloride (Sigma-Aldrich, Germany) 0.5 g L^{-1}, pH 7.5. Growth medium was supplemented with glycerol source 3–12 g L^{-1} (w/v): crude glycerol with glycerol content 40%, technical grade glycerol with glycerol content 82% (Balteko Ltd., Latvia) or analytical grade glycerol (Stanlab, Poland).

Part of the anaerobic cultivations were in 600-mL reactors with 400-mL working volume according to the instruction of the Automatic Methane Potential Test System (AMPTS) II kindly provided by Bioprocess Control AB, Sweden (Strömberg et al., 2014). Data were recorded by AMPTS II software. In experiments with *Enterobacter aerogenes*, media were supplemented with $NaHCO_3$ 5.5 g L^{-1} and solution of microelements (Angelidaki et al., 2009) 10 mL L^{-1}. Initial pH value was adjusted to 7.5 with solution of HCl. Reactors were flushed with argon gas (AGA Ltd., Latvia) to remove oxygen. Other part of experiments were performed in 50 mL serum bottles (Supelco Analytical, USA) with a working volume of 35 mL. Bottles were sealed with butyl rubber stoppers (Gotlands Gummifabrik, Sweden) and aluminium crimps (Supelco Analytical, USA), and flushed with argon. Cultivation time differed between experiments from 24 h to six days. Cultivation was maintained at temperature of 37 °C and 20 °C.

Analytical methods

Gas production was analyzed in two ways. Automatic system AMPTS II was used for detection of biogas volume. Qualitative analysis of evolved gases from the headspace of bottles in amount of 10 cm^3 were collected by syringe from the test system and injected into the RGA Pro-100 mass spectrometer with input node (HyEnergy, Setaram, France). Data were assessed by SR Residual Gas Analyzer with RGA 3.0 software.

The pH values were measured using a pH meter AD 1405 (Adrona Ltd., Latvia).

Microbiological analyses

The amount of culturable microorganisms was determined by serial dilution of broth or inoculant samples by obtained dilution spreading on agar plates and counting of colony-forming units (CFU) after incubation. Serial dilutions were plated in duplicate on the R2A (Becton & Dickinson, France). For each spread plate 0.1 mL of each dilution was used. R2A plates were incubated at 37 °C or 22 °C for 2–7 days aerobically or for seven days anaerobically (GasPak Anaerobe Pouch, Becton & Dickinson, USA), results were expressed as CFU per mL. Based on colony and cell morphology, predominant cultures (morphotypes) were isolated from the highest dilutions and purified using streaking method. Isolated cultures were identified biochemically with BBL® Crystal™ Gram-Positive ID kit, Enteric/Nonfermenter ID kit and Anaerobe ID kit (Becton & Dickinson, USA). Genera of the isolated fungi were identified using macroscopic and microscopic appearance and keys (Kiffer & Morelet, 2000).

Fluorescence *in situ* hybridization (FISH) and epifluorescence microscopy were used for determination of bacterial population dynamics. Basic method described by Pernthaler et al. (2001) and Fuchs et al. (2007) was used. Samples were air-dried, fixed in 4% para-formaldehyde for 1.5–2 hours. After drying, samples were dehydrated in ethanol (50, 80, and 96%). The 16S rRNA-targeted oligonucleotide probes were purchased from Eurofins MWG Operon (Germany). Probes LGC353B (Felske et al., 1998), GAM42a (Manz et al., 1992) and Chis150 (Franks et al., 1998) were marked at their 5'-end with Cy3, but ARC915 (Stahl & Amann, 1991) was marked with RGR. Epifluorescent microscope DM 2000 (Leica Microsystems, Germany) was used for examination of hybridization results.

Statistical analysis

Statistical analysis was performed using R version 3.2.3. One-way analysis of variance (ANOVA) was used to determine statistical significance ($P < 0.05$) of differences between experimental groups. The strength of association between two groups was determined by calculation of Pearson correlation coefficient.

RESULTS AND DISCUSSION

Experiments with *Enterobacter aerogenes*

Some authors have shown that hydrogen production was not affected by the impurities (Mangayil et al., 2012) but other experiments demonstrated that optimal concentration of crude glycerol as a co-substrate is, for example, 1 g L^{-1} (Fountoulakis et al., 2010) or 3–6% (Amon et al., 2004). Methanol can be removed by evaporation through autoclaving (Denver et al., 2008) as it also could be the case for this study. Optimal concentration of crude glycerol obtained from local producer Balteko Ltd. was found to be six grams per liter (Fig. 1). Although concentration of six g L^{-1} gave the highest volume of hydrogen and total gas volume, there was no significant difference between concentrations of three, six and 12 g L^{-1} ($P > 0.05$). A strong positive correlation was found between evolved gas volume and volumetric fraction of hydrogen ($r = 0.8240$, $P = 0.0005$) after 24 h of fermentation at 37 °C.

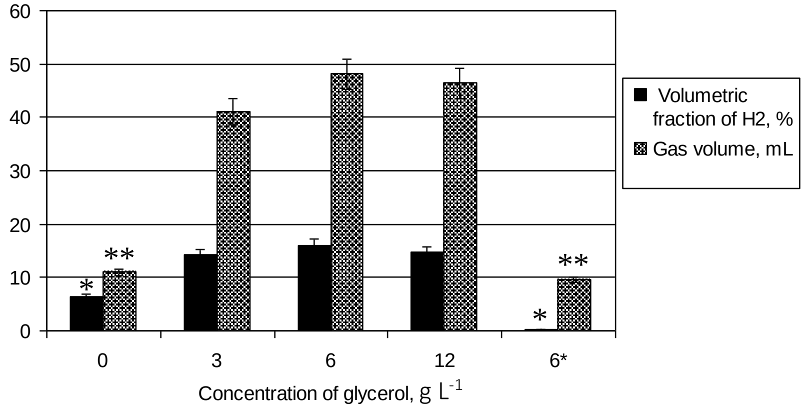

Figure 1. The volumetric fraction of hydrogen produced by *E. aerogenes* in the gas phase of growth medium without crude glycerol (0), with crude glycerol (3, 6 and 12 g L^{-1}) and crude glycerol in water (6*; 6 g L^{-1}) after 24 h of incubation at 37 °C. * – volumetric fraction of H_2 significantly different ($P < 0.05$) from other variants; ** – gas volume significantly different ($P < 0.05$) from other variants.

Although pH effects on the growth of *E. aerogenes* was not studied, as well as pH was not affected during experiments, the pH value was measured at the beginning and after 24 h of cultivation (Fig. 2). Increase of initial pH value was observed together with increase of initial concentration of crude glycerol, especially in the case of glycerol solution in water instead of solution in the growth medium. Apparently, the medium had buffering properties. The broth became more acidic during incubation in all variants, up to pH 4.5 (with glycerol 12 g L^{-1}) and together with increase of glycerol concentration. Tanisho et al. (1989) showed that the most suitable pH for cell mass productivity of *E. aerogenes* is around pH 7.0, and as pH decreases below pH 7.0, the cell weight measured after cultivation also decreases. However, *E. aerogenes* is able to multiply in pH range 4.4–9.0 (reviewed in Fanning et al., 2016). Decrease of pH value during incubation is due to the formation of metabolism products such as succinic acid and propionic acid (Clomburg & Gonzalez, 2013). Certainly, results with values of pH below 5 are too low for good growth and productivity and pH should be corrected and maintained near optimal level. Tanisho et al. (1989) showed that the evolution rate and the yield of hydrogen have maximum values at a pH of approximately 5.8 but Lin & Lay (2004) reported maximum output occurred at pH values between 6 and 7.

Landfill substrate and manure as inoculants with or without inoculation of *E. aerogenes* were used to compare analytical grade, technical grade and crude glycerol as a co-substrate for gas production (Fig. 3). Cumulative gas volume was calculated in AMPTS II after four days of incubation. *E. aerogenes* together with landfill substrate and manure gave 55%, 42% and 8% higher gas volume than landfill substrate and manure without bacterium correspondingly from analytical grade, technical grade and crude glycerol. Parallel experiments with measurements of gas volume and composition were performed in the serum bottles (Fig. 4) because AMPTS II can only distinguish between CO_2, which is captured in the CO_2 trap, and 'not CO_2', which is assumed to be CH_4. The system is not capable of differentiating between CH_4, N_2, O_2 or other gases (Strömberg et al., 2014) including argon used for initial flushing of reactors. Obtained results showed that methane was not produced in this experiment, but AMPTS II records reflected the volume of argon, hydrogen and nitrogen. Gas volume obtained from technical grade glycerol in AMPTS II did not significantly differ ($P > 0.05$) from gas volume obtained from analytical grade glycerol with one exception. Significantly higher gas volume was obtained from analytical grade glycerol than from technical grade glycerol in variant with *E. aerogenes* together with inoculants obtained from landfill substrate and manure (Fig. 3). Increase was based on the presence and action of *E. aerogenes*. Landfill substrate and manure inoculum acted in synergy with *E. aerogenes* in fermentation of technical grade and analytical grade glycerol. Synergy was not observed in case with crude glycerol ($P > 0.05$).

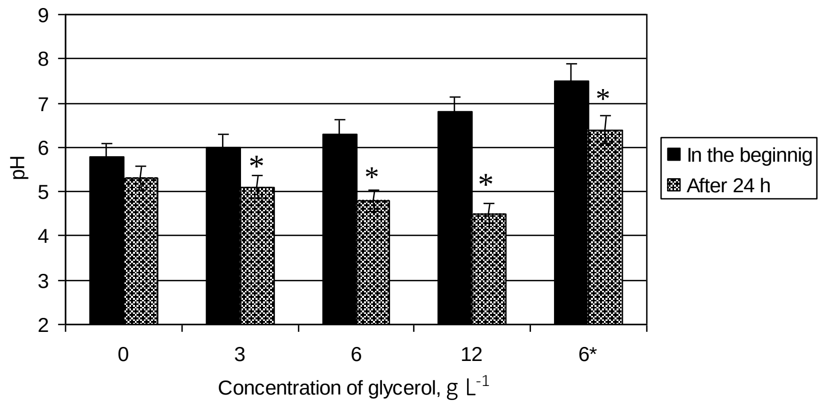

Figure 2. Values of pH of *E. aerogenes* growth media without crude glycerol (0), with crude glycerol (3, 6 and 12 g L^{-1}) and crude glycerol in water (6*; 6 g L^{-1}) in the beginning of the experiment and after 24 h of cultivation at 37 °C. * – significant difference ($P < 0.05$) between beginning and 24 h of incubation.

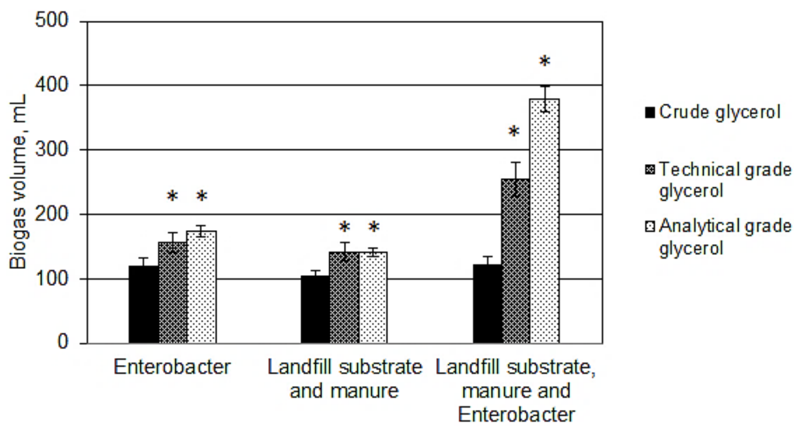

Figure 3. Biogas volume detected in AMPTS II after four days of incubation of growth medium containing glycerol source (10 g L^{-1}) and landfill substrate and manure with or without inoculation of *E. aerogenes* depending on the substrate and type of glycerol. Glycerol concentration: crude glycerol – 40%; technical grade glycerol – 82%; analytical grade glycerol – 99.5%. * – significant difference ($P < 0.05$) between variant with crude glycerol in the same substrate and inoculant group.

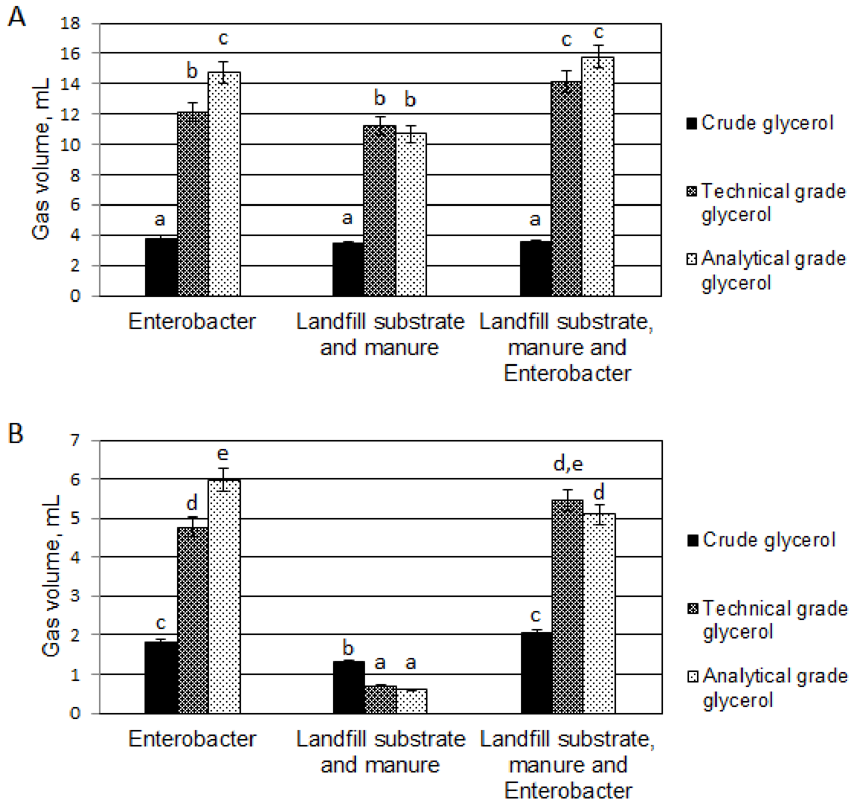

Figure 4. Carbon dioxide (A) and hydrogen (B) gas volume produced in serum bottles parallel with AMPTS II experiment after four days of incubation of growth medium containing glycerol source (10 g L^{-1}) and inoculum of landfill substrate and manure with or without *E. aerogenes* depending on the substrate and type of added glycerol. Glycerol concentration: crude glycerol – 40%; technical grade glycerol – 82%; analytical grade glycerol – 99.5%. Means sharing the same letter are not significantly different ($P > 0.05$) from each other in the corresponding part A or B.

Amount of hydrogen also was significantly higher in the case of analytical grade glycerol usage in comparison with crude glycerol fermentation. Nitrogen gas in amount of 0.4–0.7% in the headspace was detected only in variants with crude glycerol. Landfill substrate and manure gave small quantity of hydrogen, not exceeding 1.3 mL per one serum bottle. Additional inoculation with *E. aerogenes* increased the amount of hydrogen 1.6, 7.8 and 8.6 times in variants with crude glycerol, technical grade glycerol and analytical grade glycerol respectively (Fig. 4B). However, synergy was not found. These volumes were not significantly higher than without landfill substrate and manure, i.e. they were similar to variant with *E. aerogenes* alone. That means that landfill substrate and manure did not contain enough number of microorganisms appropriate for hydrogen production at least in these conditions. Unfortunately, no information about chemical composition of landfill substrate and manure was obtained. It is known that hydrogen production is sensitive to both various inhibitors and the ratios of C : N and C : P and, for example, low nitrogen concentrations also require low phosphorous contents for high productivity (Argun et al., 2008). In the same time, *E. aerogenes* increased the amount of produced carbon dioxide 1.3 and 1.5 times in variants with technical grade and analytical grade glycerol (Fig. 4A). Amount of CO_2 increased 1.1 times in comparison with *E. aerogenes* alone (statistically significant only in the case of technical grade glycerol). There was a strong correlation (r = 0.8235, P = 0.0064) between gas volume and volumetric fraction of hydrogen as well as between gas volume and volumetric fraction of carbon dioxide (r = 0.9762, P = 0.00001).

Acs et al. (2015) carried out bioaugmentation of biogas production by hydrogen-producing *Enterobacter cloacae*. This lead to the altered microbial community and increased biogas production, and authors proposed development of syntrophic relationships between polymer-degrading and H_2-producing Clostridia and *E. cloacae*. Results of this study showed potential of *E. aerogenes* for use in bioaugmentation purposes particularly for fermentation of glycerol.

Experiments with mixed natural microbial communities

Lake sludge inoculum was used in crude glycerol fermentation experiments in the serum bottles with mixed natural microbial communities during six days at temperature of 20 °C and 37 °C. Carbon dioxide was the most produced gas at both temperatures, especially at 37 °C. The highest volume of hydrogen was found after two days of incubation at 37 °C (Fig. 5). Volume of hydrogen was 1.6 times lower at 20 °C than at 37 °C. Methane was detected in one case, i.e. it occurred in amount of 0.45 mL after six days at 37 °C simultaneously with decrease of volume of hydrogen. This suggests that hydrogenotrophic methanogens had begun operating. Homoacetogenesis and aceticlastic methanogenesis are more pronounced at lower temperatures (reviewed in Schink & Stams, 2013). FISH analyses confirmed increased amount of Archaea in this case (Fig. 6). Cultivation of Archaea was not attempted, however, results show that number of aerobically growing bacteria remained higher than number of anaerobically growing bacteria during all experiment regardless of the temperature with exception of two days at 20 °C (Fig. 7).

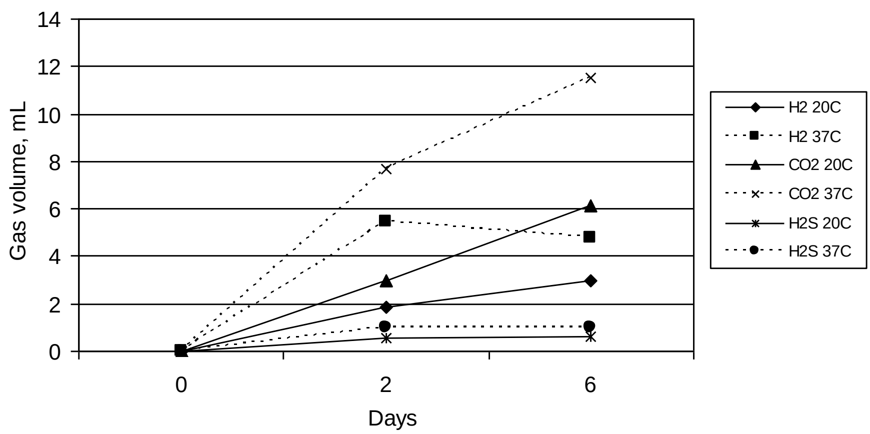

Figure 5. Cumulative volume of gases produced during anaerobic incubation of medium containing lake sludge inoculum and analytical grade glycerol 10 g L^{-1} at temperature of 20 °C or 37 °C.

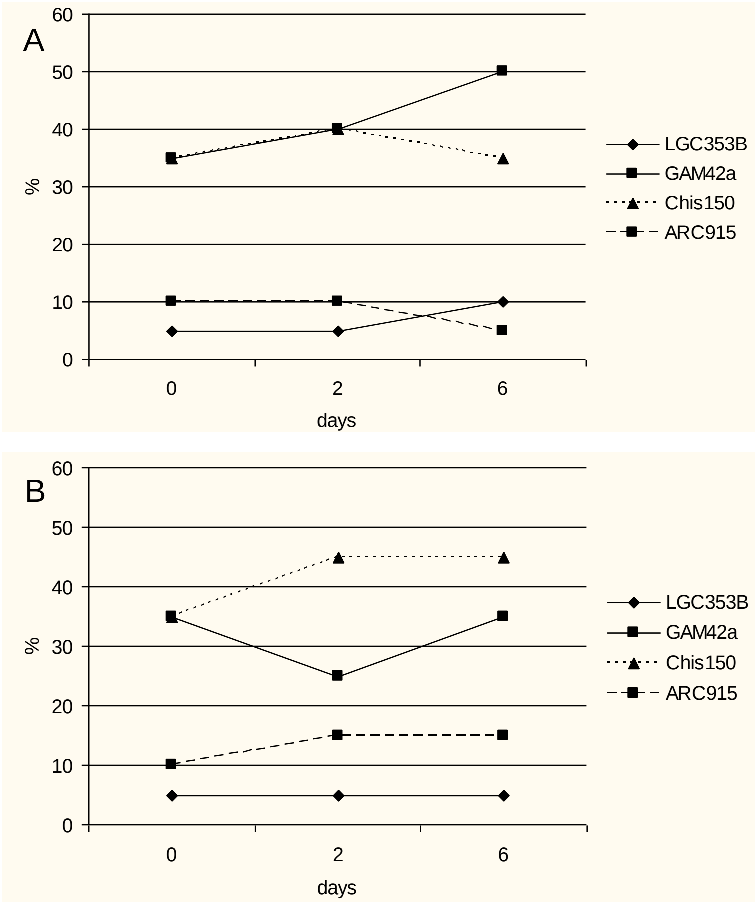

Figure 6. Bacterial groups determined by FISH in fermentation liquid in the beginning and after two and six days of anaerobic incubation at 20 °C (A) and 37 °C (B). Results are expressed as a percentage of all observed cells and been rounded to 5%. LGC353B – probe for *Bacillus* spp.; GAM42a – probe for Gamma-Proteobacteria; Chis150 – probe for *Clostridium* clusters I and II; ARC915– probe for Archaea.

FISH analyses indicated presence of all four studied groups: *Bacillus* spp., Gamma-Proteobacteria, *Clostridium* clusters I and II, and Archaea (Fig. 6). Clostridia and Gammaproteobacteria were predominant in the lake sludge inoculum. Several species were isolated: *Clostridium butyricum* and *C. hastiforme* from class Clostridia, and *Pantoea agglomerans* and *Serratia plymuthica* from class Gammaproteobacteria. Amount of Bacilli and Archaea did not exceed 10% during the experiment (Table 1). However, *Bacillus licheniformis* was isolated and found to be between the predominant after two and six days of incubation at both temperatures. *B. licheniformis* is a widespread facultatively anaerobic bacterium, which reduces nitrate to nitrite and utilizes glycerol (reviewed in Logan & De Vos, 2015). Already Kalia et al. (1994) and Porwal et al. (2008) isolated hydrogen-producing *B. licheniformis* strains from cattle dung and Porwal et al. (2008) also from contaminated food. This facultative anaerobic bacterium produced 0.5 ml H_2 mol^{-1} glucose (Kumar et al., 1995). Unidentified

Clostridium species were recovered after six days of incubation regardless of temperature. Microbial metagenome analyses of biogas reactors usually detect a large number of reads with unidentified microbial origin, indicating that anaerobic degradation process may also be conducted by up to now unknown species (Rademacher et al., 2012).

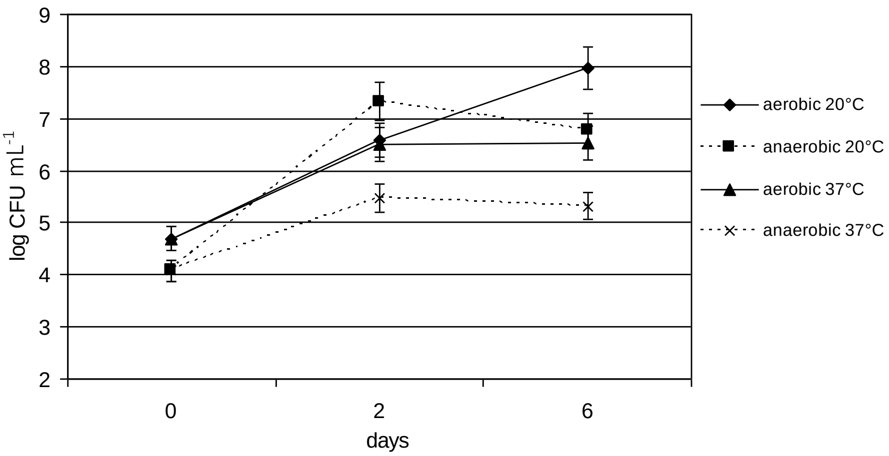

Figure 7. Amount of CFU of aerobically and anaerobically growing bacteria during anaerobic incubation of medium containing lake sludge inoculum and analytical grade glycerol 10 g L^{-1} at temperature of 20 °C or 37 °C.

Enterococci and *Enterobacteriaceae* were the most identified microorganisms which persist after anaerobic digestion of cattle manure (Resende et al., 2014). *Burkholderia cepacia* was found between predominant species in the sludge inoculum and in the fermentation broth after six days of anaerobic incubation at both temperatures (Table 1). Also Resende et al. (2014) identified *B. cepacia* in 8.87% of samples. *B. cepacia* is commonly found in natural materials, particularly soil and it is extremely versatile from the metabolic standpoint (reviewed in Palleroni, 2015). It is a strict aerobe and does not perform nitrate reduction. However, Hutchison et al. (1998) demonstrated that without visible growth during anaerobic incubation, viable *B. cepacia* could be cultured after seven days.

Lake sludge inoculum contained a little amount of fungi. There was *Penicillium* sp. about 1 CFU mL^{-1} (Table 1). Fungi were not detected after two and six days of incubation. Also loss of viability of fungus *Trichoderma asperellum* during anaerobic incubation was previously observed (Nikolajeva et al., 2015). Other fungi belonging to order *Neocallimastigales,* which are anaerobic and are usually found in the digestive tracts of herbivorous animals are recognized as promising candidates for improvement of degradation of fibrous material in fermentation reactors (Dollhofer et al., 2015).

Table 1. Predominant species and class of microorganisms isolated from fermentation liquids and identified in the beginning (0 days) and after two and six days of anaerobic incubation at 20 °C and 37 °C

Species	Class	0 days	2 days 20 °C	2 days 37 °C	6 days 20 °C	6 days 37 °C
Agrobacterium sp.	Alphaproteobacteria	X				
Bacillus cereus	Bacilli	X				
Bacillus licheniformis	Bacilli	X	X	X	X	X
Bacillus sp.	Bacilli			X		
Burkholderia cepacia	Betaproteobacteria	X		X	X	X
Clostridium butyricum	Clostridia	X	X	X		
Clostridium hastiforme	Clostridia	X				
Clostridium spp.	Clostridia				X	X
Hafnia alvei	Gammaproteobacteria				X	X
Pantoea agglomerans	Gammaproteobacteria	X				
Penicillium sp.	Eurotiomycetes	X				
Serratia fonticola	Gammaproteobacteria		X			
Serratia plymuthica	Gammaproteobacteria	X				
Sphingobacterium multivorum	Sphingobacteria	X				
Sphingomonas paucimobilis	Alphaproteobacteria	X				

CONCLUSIONS

Microbiological production of biogas and hydrogen by anaerobic fermentation of glycerol requires further investigation. *Enterobacter aerogenes* is a facultatively anaerobic bacterium that can be used for glycerol fermentation purposes.

In lab-scale experiments *E. aerogenes* produced hydrogen from all types of glycerol source. *E. aerogenes* acted in synergy with landfill substrate and manure inoculum for biogas production from technical grade and analytical grade glycerol. Synergy was not observed in the case of crude glycerol.

Lake sludge inoculum unlike landfill substrate and manure inoculum supported hydrogen production from glycerol. Inoculated mixed microbial populations from lake sludge changed during fermentation. Increase of proportion of Clostridia and Archaea was observed at 37 °C while increase of Gammproteobacteria and *Bacillus* spp. was observed at 20 °C, this temperature was too low for successful fermentation process.

Additional studies are necessary to confirm the potential of *E. aerogenes* and lake sludge inoculum for use in bioaugmentation purposes for mesophilic fermentation of glycerol as a co-substrate together with common feedstocks.

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Do young consumers care about ethics? Influence of DEAR and GDI on buying preferences – A pilot study

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Abstract. These days' consumers can express their concern about ethical behaviour of companies by means of ethical buying and consumer behaviour. The purpose of this study was to investigate how DEAR (Development Education Awareness Rising) and GDI (Global Development Issues) knowledge affects consumers preferences among higher education students in the Czech Republic. The pilot survey covered 136 students out of total 488 from Faculty of Tropical AgriSciences (CULS Prague) and was conducted from February to March 2014. Data collection was done through online survey. Collected data were categorized, coded and analysed in a statistical programme Statistica 10. In the study we examined DEAR impacts on buying preferences and therefore revealed current preferences, intentions connected with knowledge background and practices among selected university students regarding ethical and local consumption. Our results show a positive correlation ($\rho = 0.664$, $\alpha = 0.005$) between examined factors – knowledge of specific terms (effects of GDI and DEAR) with socially responsible consumers' behaviour. If consumers are well informed, positively influenced and have access to ethical products, they act as socially responsible consumers. Therefore, there is proven importance of education and access to information as a key component for conscious behaviour. These days Global Development Education and Development Education Awareness Rising should be considered not only alternatively in education, but become more common parts of educational process. This paper is a pilot study to be followed by in-depth research covering representative samples of students at Czech HEIs which have incorporated DEAR in their study curricula.

Key words: DEAR, Global Development Issues, Ethical products, Ethical Consumption, Consumers Social Responsibility.

INTRODUCTION

Currently, DEAR (Development Education Awareness Rising) is a vital part of studies at many European universities. It aims at involving students in discussion about development issues while including GDE (Global Development Education) and knowledge of GDI (Global Development Issues). The EU higher education institutions (HEIs) have integrated DEAR into their curricula based on the long-term cross-sectoral European Strategy for Development Education, awareness rising and active global citizenship stated in the Declaration of the European Parliament on development education and active global citizenship from 5th July, 2012. Nowadays, consumers can express their concern about ethical behaviour of companies by means of ethical buying and consumer behaviour (Shaw & Shiu, 2003; Carrigan et al., 2004; Ojasoo & Leppiman, 2016).

Doane (2001) defined ethical consumption as the purchase of a product that concerns a certain ethical issue (human rights, labour conditions, animal welfare, and environment) and is chosen freely by the consumer. It is important to notice that ethical consumption or consumerism is a burgeoning social movement (Cherrier, 2007; Carrington et al., 2014). Mainstream consumers increasingly express their concerns about the ethicality and impact of their consumption choices upon the environment, animals and society (Carrington et al., 2014; Shaw & Shui, 2002; Ladhari & Tchetgna, 2015; Kamińska et al., 2016). There is increasing criticism about globalization of agriculture production among consumers (Zander & Hamm, 2010), which also questions economic, environmental and social consequences of global trade and highlight ethical consumption (Raynolds, 2000). Generally growing contribution of social responsibility is unquestionable (Stanislavská et al., 2010).

Consumers living in the world market economies, mainly in developed countries, enjoy a great selection of goods, often for a 'reasonable price' which are produced in different continents. However, it is essential to realize that this reasonable price is very often reached through production ignoring environment protection or decent working conditions (Macak et al., 2014). There are several dimensions of ethical consumer behaviour. Some forms benefit environment (environment friendly products, legally logged wood, animal well-being, local food); while others benefit people (products free from child labour, Fair Trade products). The economy of social and environmental sustainability plays already an inalienable role in the European Union, where accounts for more than 10% of the European economy (in terms of GDP) with more than 11 million workers (Becchetti et al., 2014). Nevertheless, in the field of ethical consumerism, an established and widely accepted theoretical framework for the decision making of ethical consumers has to be still developed (Fukukuwa, 2003 Deng, 2015). Consumerism and ethical thinking are both growing trends worldwide and continued to expand during the last decade (Ojasoo & Leppiman, 2016), because moral responsibility is an important buying motivation among various consumer groups (Shaw & Shiu, 2003; Carrigan et al., 2004). Czech consumers have also started to be increasingly concerned about the safety of their foods and environmental and social implications of food production as well (Zagata 2012; Hejkrlík et al., 2013). Despite embracing the values of ethical consumption, most consumers rarely support their beliefs at the check-out counter (Auger & Devinney, 2007; Szmigin et al., 2009). However, a critical viewpoint is becoming to be an integral part of lifestyle of young and educated people (Stanislavská et al., 2010). HEIs play an important role in shaping the future of the world society in terms of sustainable development and related issues; mainly by generating new knowledge as well as contributing to the development of appropriate competencies and raising awareness (Rieckmann, 2012). The ethical consumption, psychology, social psychology and consumer behaviour domain variously articles, but they do not explain the intention-behaviour gap (Bagozzi, 2000; Szmigin et al., 2009). In case of organic food evidenced by Zagata (2012) there are some beliefs and behavioural intentions bringing prediction of the behaviour of the Czech organic consumers. However, it is important to realize that ethical attributes go beyond conventional standards and increase production costs, usually having negative impacts on competitiveness (Zander & Hamm, 2010). The crucial point is whether consumers are willing to compensate additional production costs caused by keeping ethical principles. It is also essential to mention the neoclassical theory of sustainable consumption as it is a connected vessel with our topic. The neoclassical tradition is often seen as reliant for its authenticity on a presumption of human avarice. Therefore, we refer to Saunders (2014) who examines the question of whether the neoclassical theory can provide keys to deeper understanding of sustainable consumption instead; further, to the study of Illge & Schwarze (2009) who focus on the description how economists think about the issues of sustainability and economics.

This paper aims at investigating the linkage between GDE and DEAR influencing consumers' preferences and furthermore purchasing habits of young consumers. This paper is a pilot study to be followed by in-depth research covering representative samples of students at Czech HEIs which have incorporated DEAR in their study curricula.

MATERIALS AND METHODS

The online survey was conducted among 136 B.Sc. and M.Sc. students selected by convenience sampling out of total 488 students of Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague in the Czech Republic from February to March 2014. It was expected that the target group was familiar with GDI and DEAR through their study programmes. Data was collected through online questionnaire sent via email and social networks to the respondents. The questionnaire consisted of three main parts; i) Characteristics of respondents ii) Respondents' knowledge about specific terms iii) Respondents' purchasing habits and their attitude. Asking awareness, dichotomous yes/no measures were used, for measuring the attitude either single or multiple choice nominal scales were used. Respondents decision making was measured either by Likert scale or by multiple choice scale. Collected data were analysed in a statistical programme Statistica 10. Due to the nature of data (not fitting in a normal distribution) nonparametric Spearman´s correlation coefficient (ρ) was used to detect possible relation between knowledge and consumers' preferences.

RESULTS AND DISCUSSION

Characteristics of respondents

Out of 136 respondents 34% were males and 66% females. The respondents were divided into two age categories 18–28 years old (97%) and 29–39 years old (3%). With respect to the university environment, 39% of respondents finished secondary education, 52% tertiary education of B.Sc. level and 9% tertiary education of M.Sc. Ethnicity of respondents was mainly European (90%), then African (5%) and Asian (5%). More than half of respondents (63%) reported their subjective economic status as average, 20% below average, 12% above average and 5% of respondents did not answer the question. According to Zhao (2012) and Camfield & Esposito (2014) subjective status is a relevant factor for ethical product purchasing. However, in our study differences between the variable '*subjective economic status*' and the variable '*importance of selected factors on acting as socially responsible consumer*' were not proven as significant.

DEAR and its effects on consumer's care

Undoubtedly GDE and DEAR belong to the essential and fundamental missions of educational system in various countries (Farahani, 2014). The Czech Republic undertook development of less favourable countries and contribute to the global poverty reduction after entry into OECD and the European Union. According to the Ministry of Foreign Affairs of the Czech Republic the state can meet this requirement only with the support of public opinion. The necessity of DEAR is also essential because of undignified labour conditions in some countries. In the Czech Republic GDE is one of the topics in primary and secondary educational system; however, it was added quite recently (Ministry of Foreign Affairs, 2011). GDE has started to permeate the Czech educational system since 2000, mainly due to the activities of non-governmental organizations. Finally, in 2011 the National Strategy of GDE for 2011–2015 was created by the cooperation of three Czech Ministries (Ministry of Foreign Affairs, Ministry of Education Youth and Sports and Ministry of the Environment) with representatives of academics and non-profit sector. This opened the way for implementation of GDE at pedagogical faculties and further educational institutions. DEAR offers students to see development issues rather broadly and will make them ready to behave according to their best consciousness and to think about global issues as sustainable development and/or international trade with all their aspects. GDE and DEAR should provide knowledge development and understanding of the issues like social equality, environment protection, international law, citizenship role in international scale, rising awareness of global issues like migration, trade, consumer´s rights, human rights, awareness of factors influencing sustainable development and awareness of labour conditions in countries of the Global South during goods production. In addition, GDE and DEAR should provide students with skills like critical thinking, tolerance, understanding ability, participation and cooperation ability, ability to evaluate different global issues, ability to lead dialogue and have logical decision making and many others. Last but not least, GDE and DEAR should enable students of creating unique values and attitudes (Federal Ministry for Economic Cooperation and Development, 2008).

Our survey showed quite good knowledge of Global Development Issues (GDI) among respondents as 65% of them are familiar with this term, 29% partially and only 6% are not familiar with this term at all. If we consider full and partial knowledge of GDI together, we are at the rate of 94%, which may indicate already essential absorption of these issues. Knowledge of respondents is empowered by the aim and activities of Faculty of Tropical AgriSciences; such as involvement of courses about ethical trade and sustainable agriculture practices in the countries of Global South in curricula, support of interest of students about issues of Global South also through activities of students' organisation focused on promotion and dissemination of information about ethical products. It is essential to think about HEIs as hubs of change, because capacity building for sustainable development education has been targeted over the last decade (Hansen & Lehmann, 2006). If we target on specific terms (Fig. 1), we can see that 40% of respondents know about issues of child labour and undignified working conditions, 33% are aware of dismemberment to the countries of Global South and countries of Global North, and 27% know the term 'socially responsible consumption'. It is essential to realize that universities and their broad spectrum of responsibilities is rapidly growing in importance in globalized, knowledge-based society (Zilahy et al., 2009). Therefore, it is important to cope with an array of challenges in the 21st century including teaching of GDI.

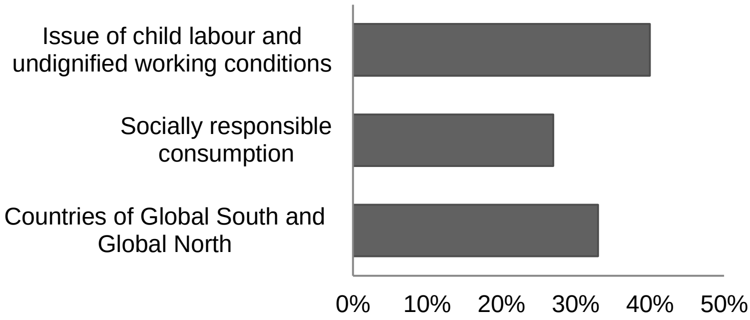

Figure 1. Knowledge of specific terms by respondents (N = 136).

Consumer's habits and attitude

Ethical consumers have different motives for purchase such as political, religious, spiritual, environmental, social or other motives (Carrington et al., 2014; Ladhari & Tchetgna, 2015) in comparison to those of conventional consumers. The overview of our findings about consumer´s habits is shown in Table 1. For 78% of the respondents ethical history of purchased product or environmental aspects of its production are important and play role in their consuming habits. Ethical consumption also serves as a medium for ethical/moral action based on subjective moral judgments applied to individual products/brands across the production, consumption and disposition cycle (Brunk, 2010).

Table 1. Consumer's consumption habits (N = 136)

	Are the factors (ethical history of purchased product or environmental aspects of production) important to you in acting as socially responsible consumer? (%)	Do you buy local production? (%)	Do you buy Fairtrade certified products? (%)	Do you buy products with other ethical certifications? (%)
Yes	78	83	88	63
No	11	3	9	9
I don´t know	11	14	3	28

Another factor is connected with preference of local production, also as socially responsible consumption habit. In general, consumers' interest in local production has steadily increased last decade (Feldmann & Hamm, 2015). In our case 83% of the respondents buy local production, 3% does not and 14% do not care. The majority of respondents (Table 2) buy local production every week (39%) or every month (32%). These results show the trend among certain university students moving towards local production purchasing. If we move to the Fairtrade certified products, 88% of respondents buy these products, the rest (12%) does not or does not know.

Table 2. Frequency of products purchasing (N = 136)

	How often do you buy each product?		
	Local production (%)	Fairtrade certified products (%)	Other ethical certified products (%)
Every day	3	3	0
2–4 times per week	26	10	17
Every week	39	19	35
Every month	32	68	48

The surveyed respondents buy Fairtrade certified products every month in 68% of cases; every week in 19%; then 2–3 times per week in 10% and every day in 3% which might be caused by a newly installed machine with Fairtrade certified products at the building of Faculty of Tropical AgriSciences giving them such opportunity. On the other hand, reasons for not buying Fairtrade certified products (9% of respondents) were various: high price (53.3%), poor accessibility (26.6%), intrust in the product (6.7%), quality does not correspond with price (6.7%) and 'because it is just a trend' (6.7%). Remaining 6.7% (of those who do not buy fair trade products) claim to buy certified products in future. Related to other ethically certified products 63% of respondents buy such products, 9% do not and 28% do not know and do not pay attention to it. Frequency of purchasing other certified products is every month (48%) followed by every week (35%) (Table 2). Respondents showed awareness of the follows ethical certifications BIO (27.0%), KLASA (16.2%), Rainforest Alliance (16.2%), GMO free (8.1%), UTZ (5.4%), products without use of child labour (5.4%), FairWear (2.7%), Forest Stewardship Council (2.7%), EZA (2.7%), GEPA (2.7%), certified natural cosmetics (2.7%), Madeta (2.7%), not tested on animals (2.7%) and vegan products (2.7%). It is essential to notice, that respondents recognize some local certifications (as KLASA) as ethical. It also shows not so clear borders between consumers' recognition of brands (like GEPA and Madeta) and labels (like Fairtrade or KLASA). Such (non)recognition of certifications, brands and labels proves how the orientation is difficult for consumers in the current ethical/non-ethical market. Padel & Foster (2005) came to similar findings in Great Britain as well.

Influence of DEAR on buying preferences

A few researchers and studies moved even beyond formation of cognitive intention to gain insight into the translation between consumers' intensions and actual behaviour (Carrington et al., 2014). Carrigan & Attalla (2001) revealed that social desirability bias plays a significant role in respondent´s ethical intention-behaviour gap. Auger & Devinney (2007) extent these findings by estimating individuals' willingness to pay for social attributes and by researching if what consumers say really matters and consumers' misalignment of preferences with their ethical intensions. Some of the most recent studies tend to assume that some ethical intentions are authentic; however internal and external factors affect actual purchase decisions (Carrington et al., 2014). This is justification of searching for the relationship between DEAR and ethical consumption behaviour. Furthermore, as concluded in Riivits-Arkonsuo et al. (2016) quality labels have the function in extending when consumers are aware of them, understand them and therefore use them in their decision-making. Our results show a positive correlation between examined factors ($\rho = 0.664$, $\alpha = 0.005$) such were knowledge of specific terms, GDI and DEAR with the importance of the factors to the respondents in acting as socially responsible consumers (meaning purchasing products with ethical history or environmental aspects of production). Such results indicate the connection between knowledge about specific issues and consumers' habits. If young consumers (university students) are well- informed, positively influenced and have got an access to willed products they act as socially responsible consumers. Therefore, there is a proven importance of education and access to information as key components for conscious behaviour. It confirms the importance of knowledge about GDI and proper DEAR, as the main linkage between knowledge and consumers habits and their socially responsible behaviour in connection with shopping preferences. There is prioritization of ethical consumption core values of consumers, which should be integrated into the consumer's lifestyle based on consumption enactments or shopping habits. When we think about ethical consumption intentions we should consider primary or secondary models of consumption (aligned or misaligned); however, both are bringing advantages to the producers. The primary model has a greater importance for us, as it is a primary intension of consumers and it is based on knowledge and long-term preferences. Carrington et al. (2014) showed in their study the translation of ethical consumption intentions into actual behaviour, this ethical intention–behaviour gap can be described with core motivational hierarch as following: i) separated into prioritisation ii) integration (plans, habits and willingness to sacrifice) and iii) consumption enactment (pre-mediated). The study of Carrington et al. (2014) also reveals a motivational hierarchy that divide ethical consumption concerns over three levels (ethical consumption core values at the base, then the integration of ethical consumption values into consumer lifestyles, and finally consumption enactments through different modes of shopping). In the case of misaligned behaviour, it is unplanned during integration and there is no willingness to commit sacrifice and during consumption enactment it is only spontaneous act and often it is random. As many consumers profess to want to avoid unethical offerings in the marketplace yet few actually act so (Eckhardt et al., 2010).

Ethical consumption and its prospects (at Czech University of Life Sciences Prague, Czech Republic)

The current study shows quite satisfying results such as knowledge of GDI (at least partial knowledge has been shown by 94% of the respondents); however, attention to the wider context must be still paid. It encourages us to compare results with further studies and partly predict the current state in the Czech Republic. Recently, based on the Western model, Czech consumers have also started being increasingly concerned about further aspects of ways of production; mainly its environmental and social implications (Zagata, 2012; Hejkrlík et al., 2013). These findings support the idea of an increasing impact on ethical consumption and its significant role in shaping the market structure. Nevertheless, in the Czech Republic only few studies on such a topic are available. Further factors influencing consumers' attitude to the products with ethical history may also be socio-economical and geographical, as showed also in study done by Hejkrlík et al. (2013) that there is higher willingness to buy ethical products (with Fairtrade mark) within people from Prague or cities with more than 100,000 inhabitants and consumers with higher income. This leads us to presumption of higher ethical consumer's behaviour in major Czech cities, but we cannot predict the university students´ attitude. However, growing interest in Corporate Social Responsibility (CSR) among Czech universities (Czech University of Life Sciences Prague, University of Economics Prague and Charles University organized together the first CSR conference among universities) and two Fairtrade certified faculties (Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague and Faculty of Economics, University of South Bohemia) show a move towards ethical thinking. Currently modern communication media (including the internet) enhance the opportunities for consumers to influence through the ethical behaviour impacts on producers (Glazer et al., 2010), but only if they are well-informed (Folkes & Kamins, 1999). However, information distributions are highly dependent upon other circumstances like access to relevant information, connections and others. Similar conclusion was formulated in Spain by Vázquez et al. (2012).

CONCLUSIONS

These days Global Development Education and Development Education Awareness Rising should be considered not only as an alternative approach in education, but a common part of curricula. These issues deserve higher attention paid at international and global levels. In this pilot study we investigated Czech university students at Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague with the aim to find out their preferences and basics about their intentions connected with knowledge background and consequently buying preferences. Our results revealed the relationship between DEAR and ethical consumption behaviour; a positive correlation ($\rho = 0.664$) between examined factors: knowledge of specific terms, GDI and DEAR. This confirms the importance of knowledge about GDI and proper DEAR, as the main linkage between knowledge and consumers habits and their socially responsible behaviour in connection with shopping preferences. This pilot study will be followed by in-depth research covering representative samples of the Czech university students.

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Effectiveness of seed treatment against *Fusarium* spp. and *Cochliobolus sativus* of spring barley in different conditions

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Abstract. Effect of fungicide seed treatments on initial growth of spring barley (*Hordeum vulgare* L.) was evaluated in greenhouse trials. The soil collected from minimum tillage fields where spring barley, spring wheat (*Triticum* L.) and oilseed rape (*Brassica napus* L.) have been cultivated in previous growing season were used in trials. Eight fungicide seed treatments and untreated seed as the control were evaluated. Root rot severity and seedling emergence rate were assessed at growth stages 20–22. In addition the incidence of seed-borne *Fusarium* spp. and *Cochliobolus sativus* and germination were assessed in treated and untreated spring barley seeds in laboratory condition. Fungicides prothioconazole and tebuconazole significantly reduced incidence of seed-borne *Fusarium* spp. Seed treated with fludioxonil and tebuconazole more effectively decreased root rot infection in soil from minimum tilled barley field, fludioxonil + difenoconazole in soil from minimum tilled spring wheat field and prothioconazole mixes with tebuconazole or fluoxastrobin in soil from minimum tilled oilseed rape field. This study brings out the pre-crop and seed treatment interaction effect on control of root rot in spring barley.

Key words: barley; fungicide; root rot disease; germination; emergence rate.

INTRODUCTION

Common root rot and seedling blight are early season diseases that occur shortly after germination and have been attributed to infection of cereal seedlings by fungi including *Fusarium* spp. and *Cochliobolus sativus*. These pathogens live in the soil and are a serious threat to spring barley causing yield losses (Kumar et al., 2002). Seed-borne pathogen *C. sativus* contributes to the development of brownish roots and coleoptiles resulting in seedling blight. A numbers of conidia of *C. sativus* varies greatly in soils from the various rotations, the highest number being in barley stubble (Piening & Orr, 1988). Sources of inoculum for *Fusarium* spp. include seed, crop residue and soil (McGee, 1995). *Fusarium* spp. that causes crown rot can infect any tissues but often invade the coleoptile as it emerges (Smiley et al., 2013). Increased levels of crop debris influence the incidence and severity of plant diseases (Bailey & Lazarovits, 2003; Paulitz, 2006; Matusinsky et al., 2009). There are larger microbial populations in the upper layers of soil in reduced tillage conditions (Krupinsky et al., 2002).

Fungicide seed treatment is widely used in grain crops for disease management because positive impact on early plant growth (Pike et al., 1993; Bradley et al., 2001; Cook et al., 2002). Fungicides form a protective zone around germinating seeds and reduce diseases caused by soil-borne pathogens (Galperin et al., 2003). Seed treatment is an advanced and economic delivery system to protect the seed from the moment of sowing. The specific action of the fungicide seed treatment on the interaction between *Fusarium* spp. and seed treatment fungicides is limited. The studies done with barley, wheat (Jones, 2000) and maize (Munkvold & O'Mara, 2002) have determined that most seed treatments are effective against soil-borne infections of *Fusarium* spp.

The objective of the study was to investigate the efficacy of different fungicides on *Fusarium* spp. and *C. sativus* on barley and evaluate germination and seedling emergence ability in different conditions.

MATERIALS AND METHODS

Studies were carried out in autumn 2012 in Estonian Crop Research Institute. Three greenhouse experiments were done with spring barley variety 'Barke'. The soil collected from minimum tillage fields where spring barley, spring wheat and oilseed rape have been cultivated in previous growing season were used in trials. The soil from minimum tilled barley field further in the text will be named as barley pre-crop soil, from spring wheat field as wheat pre-crop soil and from oilseed rape field as oilseed rape pre-crop soil. Eight seed treatment fungicides and untreated control were compared for control of root rot in spring barley. The trials were conducted in boxes for plant cultivation containing different pre crop soils using four replicats. (Table 1). The soils used in trials were collected after the harvest and soil cultivation from top 10 cm layer of minimum tillage spring barley, spring wheat and oilseed rape fields. The soils contain also stubble and straw from the crop. A mix containing field soil and sand in ratio 90:10 (%) was prepared for trials. Complex fertilizer Kemira $N_{10}P_{23}K_{14}$ was used at the rate of 20 g per box (60 x 40 cm², 8 cm deep). The boxes were kept 7 days in warm and moist conditions to propitiate pathogen mycelium growth. Boxes were maintained under cool and moist conditions for 10 days in temperatures 4–8 °C to predispose the infection and delay the seed germination. The plants were then grown in a greenhouse at 23 ± 2 °C with supplemental lighting for 14 h day^{-1} during the test period from leaf development to tillering (GS 10–22 according Zadoks et al., 1974). Root rot disease was measured using the sub-crop internode index (Ledingham et al., 1973): 1– no symptoms of disease; 2–25%; 3–50%; 4–75% of tissue with disease symptoms. 100 plants from each variant were individually scored at shooting stage (GS 20–22). These scores were transformed to root rot index (RRI) and were expressed as a percentage of the root rot by using the formula:

$$RRI = \frac{\sum 100nv}{NV} \qquad (1)$$

where *n* – number of plants with the respective infection level; *v* – infection level 1–4 as described above; *N* – the number of scored plants; *V* – a maximal level of the infection.

Incidence of seed-borne *Fusarium* spp. and *Cochliobolus sativus* was assessed in laboratory conditions using one sample of 100 seeds from each treatment. All 100 fungicide treated seeds were plated onto potato-dextrose agar medium supplemented with streptomycin solution in Petri dishes and incubated at 20–22 °C, 12 hr day^{-1} 12 hr night for seven days. All seeds checked under the microscope. Incidence of both seed-borne fungal genera was calculated as the percentage of seeds infected with that genus.

Seed germination was assessed in laboratory conditions. Treated and untreated seeds were placed in Petri dishes on moist filter paper in four replicates per treatment, 25 seeds per replicate in randomized design. The tests were performed under laboratory conditions at 20 °C and natural room light and monitored daily. Seeds were considered germinated upon radicle emergence. Germination was counted at 4, 7, 11 and 14 days after the start of the trials and germination expressed as a percentage by number of normal seedlings.

Plant vigour was estimated by counting seedlings of each treatment from each pre-crop soil. The emergence percentage is calculated by dividing the number of normal seedlings per 100 seeds obtained at each treatment in the greenhouse. The emergence rate is calculated by dividing the number of days for emergence in the greenhouse (Maguire, 1962).

The Agrobase (release 20; Winnipeg, Canada) software package was used for statistical analysis. Factorial analysis of variance (ANOVA) and one-way ANOVA were applied to test the results also coefficient of determination was founded. The level of statistical significance was set at $P < 0.05$.

Table 1. Product names, active ingredients and doses used in greenhouse trials

	Product	Active ingredients per litre	Dose l t^{-1}
1	Premis® 25 FS	25 g triticonazole	2.0
2	Kinto®	20 g triticonazole + 60 g prochloraz	2.0
3	Bariton® 075 FS	37.5 g fluoxastrobin + 37.5 g prothioconazole	1.25
4	Lamardor® 400 FS	250 g prothioconazole + 150 g tebuconazole	0.2
5	Raxil® 060 FS	60 g tebuconazole	0.5
6	Maxim® 025 FS	25 g fludioxonil	2.0
7	Maxim Extra® 050 FS	25 g fludioxonil + 25 g difenoconazole	2.0
8	Maxim Star® 025 FS	18.8 g fludioxonil + 6.3 g cyproconazole	1.5
9	Untreated control	–	–

RESULTS AND DISCUSSION

Effect of fungicide seed treatment on plant emergence

Fungicide seed treatments had a significant effect on emergence of spring barley in different pre-crop soil (Table 2). Fludioxonil + difenoconazole treated seeds had significantly faster emergence rate in barley pre-crop soil and triticonazole treated seeds slower emergence rate when compared with untreated seeds. The proportion of emerged plants increased by using active substances tebuconazole, triticonazole and fludioxonil, when compared with untreated seeds.

In wheat pre-crop soil emergence rate of tebuconazole and fludioxonil treated seeds was faster when compared with untreated seeds. The emergence was most delayed in seeds treated with triticonazole + prochloraz. Significantly highest emergence rate was observed in barley seeds treated with triticonazole + prochloraz and fludioxonil + difenoconazole. Compared with untreated seeds lower emergence rate values had seed treated with tebuconazole, fludioxonil and prothioconazole + tebuconazole.

In oilseed rape pre-crop soil the emergence rate differences between treated and untreated seeds were very small. Emergence rate was significantly higher in treatments with fluoxastrobin + prothioconazole, fludioxonil + difenoconazole and fludioxonil, when compared with untreated seeds. Treatment with fluoxastrobin + prothioconazole had significantly higher emergence % when compared with untreated seeds. Other fungicides had lower values compared with those of fluoxastrobin + prothioconazole treated seed.

The effectiveness of seed treatment fungicides are usually evaluated by emergence used as an indication of plant vigor (Munkvold & O'Mara, 2002). Speed of seed emergence and seedling growth has been widely accepted as main parameter to monitor growth responses (Briggs & Dunn, 2000). In our trials it was observed at all pre-crop soils that fungicidal treatment affects seed germination and seedling vigor. Seed treatment fungicidal compounds had different decelerate effects of barley emergence. It depends on the plant protection products used. According to Platz et al. (2001) most triazole fungicides have indicated possible effect on emergence. Our results demonstrated decelerate effect on seedlings grown from seeds treated with triticonazole and mixes with prochloraz. Seedlings grown from seeds treated with tebuconazole, mixes with prothioconazole and fludioxonil indicated faster emergence rates in all pre-crop soils. In oilseed rape pre-crop soil, only slight differences were observed in emergence rates between treated and untreated seeds.

Table 2. Seed treated spring barley seedlings emergence from different pre-crop soil in greenhouse trials

Fungicide	Barley pre-crop soil		Wheat pre-crop soil		Oilseed rape pre-crop soil	
	emergence (%)	emergence rate (days)	emergence (%)	emergence rate (days)	emergence (%)	emergence rate (days)
Triticonazole	94	15.7	86	14.3	75	10.7
Triticonazole, prochloraz	84	14.0	92	15.3	80	11.4
Fluoxastrobin, prothioconazole	86	14.3	85	14.2	92	11.5
Prothioconazole, tebuconazole	86	12.3	83	11.9	83	10.3
Tebuconazole	95	11.9	81	11.6	78	9.8
Fludioxonil	93	13.3	82	11.7	83	11.9
Fludioxonil, difenoconazole	82	11.7	92	13.1	81	11.6
Fludioxonil, cyproconazole	90	12.9	91	13.0	80	10.0
Untreated	89	14.8	86	14.3	88	11.0
Average	89	13.4	86	13.3	82	10.9
$LSD_{0.05}$	1.02	0.35	1.19	0.50	1.26	0.43

Effect of fungicide seed treatment on root rot infection

Root rot infection caused by *Fusarium* spp. and *C. sativus* was observed in all pre-crop soils (Fig. 1). In untreated spring barley seedlings 10–11% of roots and crowns were colonized with root rot. All treatments, except tebuconazole in wheat and oilseed rape pre-crop soils and triticonazole in oilseed rape pre-crop soil reduced mean root rot index. Least root rot symptoms was observed in seedlings grown in wheat pre-crop soil from seeds treated with fludioxonil + difenoconazole and in barley pre-crop soil from seeds treated with fludioxonil. However no differences between pre-crop soils were observed in case of seeds treated with prothioconazole + tebuconazole. Seed treated with fluoxastrobine and prothioconazole or prothioconazole and tebuconazole worked more effectively in oilseed rape pre-crop soil. Obtained results demonstrate that minimum tillage pre-crop soils have influence to soil-borne infection of *Fusarium* spp. when no above ground symptoms are evident and the management of early season barley diseases with fungicide seed treatments can result in reduction of root rot. Fungicide active ingredients form a protective barrier around the seed against soil-borne pathogens and have a limited period of activity (Charnay et al., 2000). Systemic fungicides are able stop the progress of *Fusarium* infections (Boyacioglu, 1992). Triticonazole has been tested as seed treatment fungicide in wheat, barley and other small grains protecting against *Fusarium* spp. and other soil-borne fungi (Biradar et al., 1994). In previous studies tebuconazole selectively (Simpson et al., 2001) and prothioconazole (Suty-Heinze & Dutzmann, 2004) highly controlled *Fusarium* spp. Fungicides include fludioxonil reduce damage caused by common root rot.

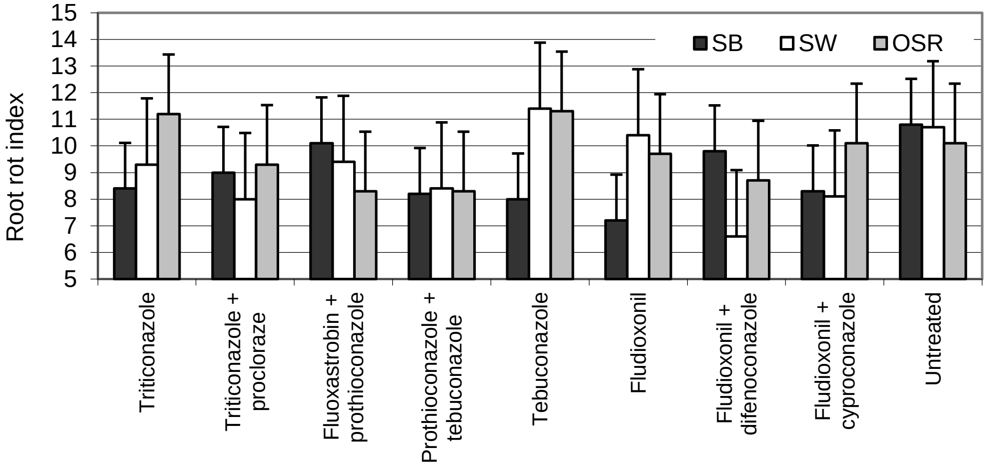

Figure 1. A percentage root rot index in seed treated spring barley seedlings grown in different pre-crop soil in greenhouse trials: SB – barley pre-crop soil; SW – wheat pre-crop soil; OSR – oilseed rape pre-crop soil. LSD0.05 = 1.72 (SB), 2.49 (SW), 2.24 (OSR).

Presence of seed-borne *Fusarium* spp. and *C. sativus* in fungicide treated barley seed

The choice of seed treatment fungicide had significant impact on the incidence and proportion of pathogenic fungi on grains. The evaluation of barley grain showed, that more prevalent fungus in treated seeds was *C. sativus* (range 33–69%) (Fig. 2). Although *Fusarium* spp. was not found so frequently as *C. sativus*, the incidence of infections by *Fusarium* spp. ranged 11–37%. *Fusarium* species was found in rate of 37% in tebuconazole treated seeds, followed by triticonazole + prochloraz (36%), fludioxonil + cyproconazole (35%) and fludioxonil + difenoconazole (34%). These treatments did not show any control of this species, when compared to 32% infections of untreated seeds. It is on contrary with results obtained by Galperin et al. (2003) where prochloraz completely suppressed seed-borne *Fusarium* in maize. According to Rodriguez-Brljevich et al. (2008) triazole active ingredients were more effective against seed-borne *Fusarium* than strobilurins. In our experiment most effective were triazoles containing active ingredients prothioconazole and tebuconazole. In untreated control 84% of seeds were contaminated with *C. sativus*. This pathogen had the high incidence of infection in seeds treated with triticonazole + prochloraz (69%) and with triticonazole (66%).

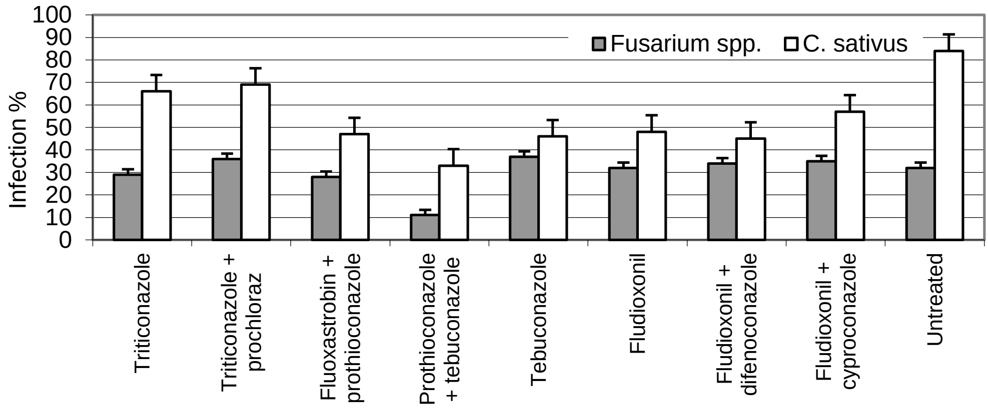

Figure 2. Incidence (%) of *Fusarium* spp. and *Cochliobolus sativus* in seed treated spring barley grains under laboratory conditions: LSD0.05 = 4.99 (*Fusarium* spp.), 15.57 (*C. sativus*).

Effect of fungicide seed treatment on germination rate

The germination of untreated spring barley seeds under laboratory conditions was 80% (Fig. 3). Nearly all seed treatment fungicides increased initial germination in days 4 compared with untreated seeds. Only treatment with fludioxonil delayed germination in all assessed days. In addition, initial germination was somewhat delayed in seeds treated with triticonazole + prochloraz and this effect was slightly observed after 14 days. Fungicide seed treatments increased the germination of barley seeds when compared with untreated seeds in use of fludioxonil + difenoconazole, fludioxonil + cyproconazole and prothioconazole + tebuconazole. A few days difference in time of germination may significantly affect seedling development. The evaluation of barley grain showed, that the germination was more uniform in seeds treated with prothioconazole + tebuconazole and fludioxonil + cyproconazole. This is in agreement with the findings of Querou et al. (1997) where the germination of wheat seeds was positively influenced by the treatment with triazoles. On the contrary to our results, the findings of Rufino et al. (2013) have shown in wheat germination test that the seed treatment with fungicide fludioxonil presented better results than without treatment.

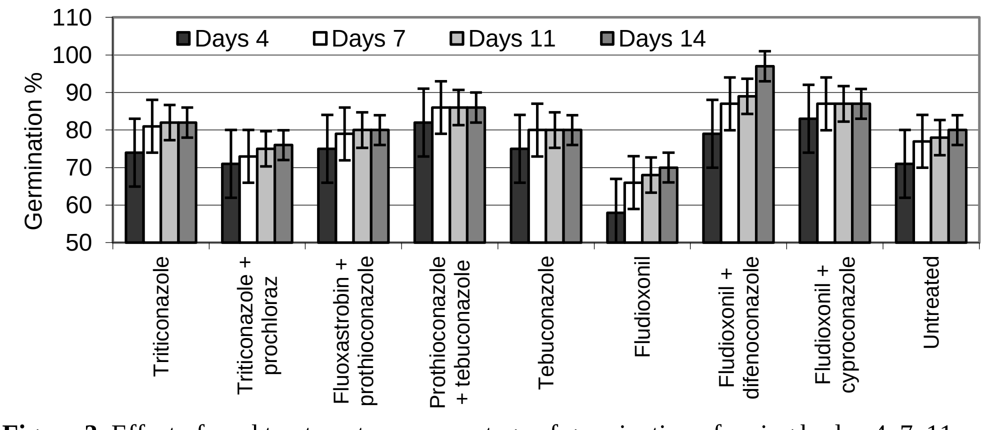

Figure 3. Effect of seed treatments on percentage of germination of spring barley 4, 7, 11 and 14 days after starting: LSD0.05 = 9.01 (days 4), 7.02 (days 7), 4.71 (days 11), 3.98 (days 14).

Results of ANOVA verified that the impact of the pre-crop soil had the biggest influence on the emergence rate and germination (Table 3). The fungicide seed treatment was major factor determining the infection level with root rot disease. The coefficients of determination indicate that environmental and fungicidal factor's contribution to the emergence rate was 70% (R^2 = 0.70). The occurrence of root rot was less dependent on environmental and fungicidal factors (R^2 = 0.65). The rest is related to some other factors.

Table 3. Mean squares of ANOVA of infection data of root rot disease, germination and emergence rate

	Root rot			Germination			Emergence rate		
	MS	*F*-ratio	*P*	*MS*	*F*-ratio	*P*	*MS*	*F*-ratio	*P*
Treatment (T)	49.4	14.07	0.000	2.8	0.37	0.019	14.4	8.52	0.000
Pre-crop soil (P)	44.0	12.5	0.000	51.3	6.82	0.932	73.0	43.23	0.000
T x P	3.1	0.9	0.587	15.6	2.08	0.018	1.6	0.96	0.512
R^2	0.6520			0.4302			0.7049		

MS - mean square, R^2 - correlation between trial factors.

CONCLUSIONS

This experiment brings out the pre-crop and seed treatment efficacy interaction and their compatibility with reduced tillage soil. The results of the study show that the benefit of fungicide seed treatment in reduction infection and colonization of spring barley by *Fusarium* spp. and *Cochliobolus sativus* is evident at seedling growth stage. Prothioconazole and tebuconazole are capable to significantly reduce and suppress seed-borne inoculum of *Fusarium* spp. The seed treatments with fludioxonil, tebuconazole or prothioconazole showed best results in increasing plant stand and vigour and reducing seedling root rot in minimum tillage soil where spring barley was used as preceding crop. This is an important contribution to the epidemiology of soil-borne *Fusarium* spp. and root rot infection.

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PCR–based fingerprinting and identification of contaminative fungi isolated from rye breads

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Abstract. Fungi are the most frequent cause of microbial spoilage in baked products, including rye bread. As the baking process destroys fungal spores in bread, the post–processing is the main source for mould contamination. Rapid detection methods are needed to track down the origin of the contamination source. In the present research we used a combined molecular approach consisting of PCR–fingerprinting with an M13 primer and further identification of each genotype by amplification and sequencing of the Internal Transcribed Spacer region, the β–tubulin gene and the D1/D2 region of the large subunit of the 28S rDNA. Different rye breads from five bakeries were stored plastic–packed for one month and the fungal colonies with unique morphology were isolated from the bread surfaces. Based on random amplified polymorphic DNA analysis using M13 primer 50 fungal isolates were clustered into eight groups and identified as *Aspergillus chevalieri*, *Aspergillus flavus/oryzae*, *Aspergillus niger*, *Aspergillus tubingensis*, *Penicillium citrinum*, *Penicillium corylophilum*, *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus* species. Sequencing of the β–tubulin gene and the ITS region showed an equal efficiency for the identification of *Penicillium* species, whereas only the sequence of the β–tubulin gene allowed us to identify most isolates from the genus *Aspergillus* including closely–related black–spored *Aspergillus* species. Yeasts were identified at the species level based on the sequences of the Internal Transcribed Spacer region and the D1/D2 region.

Key words: Mould identification, Yeast identification, PCR–fingerprinting, ITS region, D1/D2 region, β–tubulin gene, rye sourdough breads.

INTRODUCTION

Fungi are the most frequent cause of microbial spoilage in baked products (Legan, 1993; Saranraj & Geetha, 2012). According to Malkki & Rauha (2000) up to 5% of the total bread produced yearly worldwide is lost due to fungal deterioration. Fungal spores are killed by heat treatment during the baking process and thus, contamination occurs post baking through the air and by direct contact with processing equipment during cooling, slicing, and packaging (Knight & Menlove, 1961; Legan, 1993).

Dough acidification is an important step in traditional rye bread technology. Sourdough, a mixture of water and flour fermented by lactic acid bacteria (LAB), is routinely used as acidifier (Kulp & Lorenz, 2003). Low pH (3.5–4.8) of classical rye breads combined with metabolites produced by LAB during fermentation process bio–

protect against some bacterial spoilage (Schnürer & Magnusson, 2005; Gerez et al., 2009; Dalie et al., 2010). Nevertheless, rye breads can be contaminated by a range of different moulds. Species from the *Penicillium* and *Aspergillus* genera seem to predominate (Lund et al., 1996). *Penicillium verrucosum*, *Aspergillus ochraceus*, *Aspergillus bombycis*, and many other representatives of these two genera are well known mycotoxin producers, including aflatoxins B1, B2, G1, G2, and ochratoxin A (Schmidt, 2003; Varga et al., 2011). Dich et al. (1979) found an aflatoxin producing *Aspergillus flavus* in spoiled rye bread and ochratoxigenic *A. ochraceus* was isolated from mouldy bread in Italy (Visconti & Bottalico, 1983). Thus, in addition to economic losses, public health problems cannot be excluded (Legan, 1993; Bento et al., 2009; Duarte et al., 2009; Gerez et al., 2014).

Only a few papers describing contaminative fungi isolated from rye breads have been published and all of them are based only on morphological identification of fungal isolates (Dich et al., 1979; Spicher, 1985; Lund et al., 1996). However, recent studies revealed that phenotypic–based classification of closely–related mould species can be confusing and lead to misidentification (Samson et al., 2007; Silva et al., 2011; Jang et al., 2012). In addition, morphological identification cannot be used for contamination source tracking at strain level. Instead, DNA based methods should be used for it. Information regarding effectiveness of different molecular markers for identification of food contaminating fungi is so far limited (Le Lay et al., 2016; Garnier et al., 2017). Presently, there is no single molecular marker or method advised for fungal identification or genotyping (Vanhee et al., 2010; Araujo, 2014). Sequencing of Internal Transcribed Spacer (ITS) region, D1/D2 region of 28S subunit, β–tubulin, actin, calmodulin and RPB2 gene regions are used for identification (Vanhee et al., 2010; Araujo, 2014).

The aims of our research were to isolate contaminative fungi from rye breads supplied by different Estonian bakeries and to test the effectiveness of different molecular markers in identifying common rye bread contaminative fungi. Discrimination level of random amplified polymorphic DNA (RAPD) analysis using M13 primer followed by sequencing of β–tubulin gene, ITS region and D1/D2 region of 28S subunit for different fungal species was evaluated in order to compose fast and reliable protocol for identification and tracking mould contaminations in baking industry.

MATERIALS AND METHODS

Bread samples and fungi isolation

Ten sliced sourdough rye breads produced by five different Estonian bakeries, designated A, B, C, D and E were purchased. Loaves were made without any preservatives and packed into plastic bags. The presence of fungal colonies on the surface of each loaf was evaluated visually after one, two, three, and four weeks of storage at room temperature. At the end of the fourth week all fungal colonies with different morphology were isolated from the surface of each bread onto Sabouraud Dextrose agar (10.0 g l^{-1} peptone, 12.0 g l^{-1} agar, 40.0 g l^{-1} D–glucose, pH = 5.3). These fungi were then cultivated at 25 °C for 7 days and isolated to obtain monosporal cultures. Pure cultures were cultivated on Yeast Extract Sucrose agar (YES) (yeast extract 20 g l^{-1}, sucrose 150 g l^{-1}, $MgSO_4 \cdot 7H_2O$ 0.5 g l^{-1}, agar 20 g l^{-1}) at 25 °C for 7 days and stored at + 4 °C for further use.

DNA extraction from fungal cultures

The pure fungal cultures were cultivated in Sabouraud Dextrose broth (10.0 g l^{-1} peptone, 40.0 g l^{-1} D–glucose, pH = 5.3) at 25 °C for 5 days. DNA from the resulting fungal biomass was extracted according to Azevedo et al. (2000).

Random amplified polymorphic DNA analysis using M13 primer

A PCR reaction was performed in 25 µl volumes containing 100 ng of fungal DNA, 40 pmol of M13 primer (5'–GAGGGTGGCGGTTCT–3') (Meyer et al., 1999) (Microsynth AG, Switzerland) and 5 µl of 5x HOT FIREPol® Blend Master Mix (Solis BioDyne, Estonia). The PCR was performed as it was described by Meyer et al. (1999) with some modifications. Initial hold at 95 °C for 15 minutes was followed by 35 cycles of 94 °C for 20 seconds, 50 °C for 1 minute, and 72 °C for 20 seconds with a final extension at 72 °C for 6 minutes. The amplified DNA was then analyzed on 15 g kg^{-1} agarose gel at 70 V for 45 minutes and visualized under UV light.

Identification of fungal isolates

The ITS region was amplified using ITS1 (5'–TCCGTCGGTGAACCTGCGG–3') and ITS4 (5'–TCCTCCGCTTATTGATATGC–3') (Kwiatkowski et al., 2012) (Microsynth AG, Switzerland) primer pair. D1/D2 region of the 28S rDNA was amplified using forward primer NL–1 (5′–GCATATCAATAAGCGGAGGAAAAG–3') and reverse primer NL–4 (5′–GGTCCGTGTTTCAAGACGG–3') (Kwiatkowski et al., 2012) (Microsynth AG, Switzerland). For each primer set PCR reactions were performed in 50 µl volumes containing 100 ng of fungal DNA, 40 pmol of each primer, and 10 µl of 5x HOT FIREPol®Blend Master Mix (Solis BioDyne, Estonia) with the remaining volume consisting of ultrapure water. Amplifications of ITS region and D1/D2 region were performed according to Kwiatkowski et al. (2012) with minor modifications. Amplification of ITS region was performed with initial denaturation at 95 °C for 15 minutes followed by 30 cycles of 95 °C for 30 seconds, 55 °C for 1 minute and 72 °C for 1 minute and a final extension at 72 °C for 6 minutes. For the amplification of D1/D2 region after initial denaturation at 95 °C for 15 minutes 30 cycles of 94 °C for 15 seconds, 55 °C for 30 seconds and 68 °C for 2 minutes with a final extension at 68 °C for 5 minutes were performed.

The β–tubulin gene sequences were amplified using Bt2a (5'– GGTAACCAAATCGGTGCTGCTTTC–3') and Bt2b (5'– ACCCTCAGTGTAGTGACCCTTGGC–3') (Zampieri et al., 2009) primer pair (Microsynth AG, Switzerland). PCR protocol was modified from Silva et al. (2011). The 50 µl of PCR mixture contained 20 ng of fungal DNA, 40 pmol of each primer, and 10 µl of 5x HOT FIREPol®Blend Master Mix (Solis BioDyne, Estonia) with the remaining volume consisting of ultrapure water. The mixture was subjected to the following amplification program: initial hold at 95 °C for 15 minutes; followed by 35 cycles of 94 °C for 30 seconds, 50 °C for 30 seconds, and 72 °C for 90 seconds; followed by a final extension at 72 °C for 5 minutes.

PCR products were purified with GeneJET PCR Purification kits (Thermo ScientificTM, Tartu, Estonia) and sequencing of the fragments was carried out at a commercial facility (Estonian Biocenter, Tartu, Estonia). The sequences obtained were compared with the GenBank database using the BLAST algorithm (National Center for Biotechnology Information, USA).

RESULTS AND DISCUSSION

The growth of fungal colonies on sourdough rye breads obtained from bakeries B, C and D was observed during the first week of storage, whereas breads from bakeries A and E got spoiled at the end of third and fourth week, respectively (Table 1). In total, 50 colonies were picked up from studied breads and the monosporal cultures were obtained. Detected fungal morphological biodiversity varied among breads produced by different bakeries. Two fungal morphotypes were detected on A_{bakery} (A1, A111) and C_{bakery} breads (C111, C121) and three morphotypes on breads from B_{bakery} (B111, B123, B213) (Fig. 1). The most morphologically diverse fungal community was observed on D_{bakery} breads (D131, D121, D221, D231, Fig. 1), whereas in samples from E_{bakery} only one type of fungi was detected (E1, Fig. 1).

Table 1. Identified fungal species in bread samples from five Estonian bakeries (A, B, C, D and E) and the duration of mould–free shelf life

Representatives of isolated fungal strains	RAPD pattern	Presence in fungal population of each bakery				
		A	B	C	D	E
Penicillium corylophilum A111, C111	RAPD I	+	–	+	–	–
Wickerhamomyces anomalus A1	RAPD II	+	–	–	–	–
Aspergillus niger B213, D231	RAPD III	–	+	–	+	–
Aspergillus flavus/oryzae B111, D131	RAPD IV	–	+	–	+	–
Aspergillus tubingensis B123, C121	RAPD V	–	+	+	–	–
Aspergillus chevalieri D121	RAPD VI	–	–	–	+	–
Saccharomyces cerevisiae E1	RAPD VII	–	–	–	–	+
Penicillium citrinum D221	RAPD VIII	–	–	–	+	–
Mould–free shelf life, weeks		< 3	< 1	< 1	< 1	> 3

Most colonies isolated from A_{bakery} breads in the end of the storage period were of the white variety and grew to about 4 cm in diameter on YES agar without any clear signs of sporulation (A111, Fig. 1). A minority of isolates produced smaller colonies on YES agar without forming mycelium (A1, Fig. 1). Upon microscopic examination of A1 type isolates we detected the presence of budding yeast–like cells.

Moulds isolated from B_{bakery} breads produced large pale – yellow colonies with yellow and white outlines (B111, Fig. 1) or large ivory colonies with pale– and dark–brown conidia respectively and rich sporulation (B123, B213, Fig. 1). A part of moulds isolated from C_{bakery} breads created colonies similar to A111 isolates (C111, Fig. 1), whereas others formed large ivory colonies with pale–brown spores in the middle and were rather similar to B123 isolates (C121, Fig. 1). Moulds isolated from D_{bakery} breads produced large pale–yellow colonies similar to B111 isolates (D131, Fig. 1), small (about 15 mm in diameter) white colonies with grey and yellow edges (D121, Fig. 1), small white–grey colonies without any signs of sporulation (D221, Fig. 1) and large ivory colonies with dark–brown conidia in the middle similar to B213 isolates (D231, Fig. 1). Finally, fungi isolated from E_{bakery} breads created small ivory colonies with smooth matte surface on YES agar (E1, Fig. 1) and their microscopic examination revealed yeast–like cells.

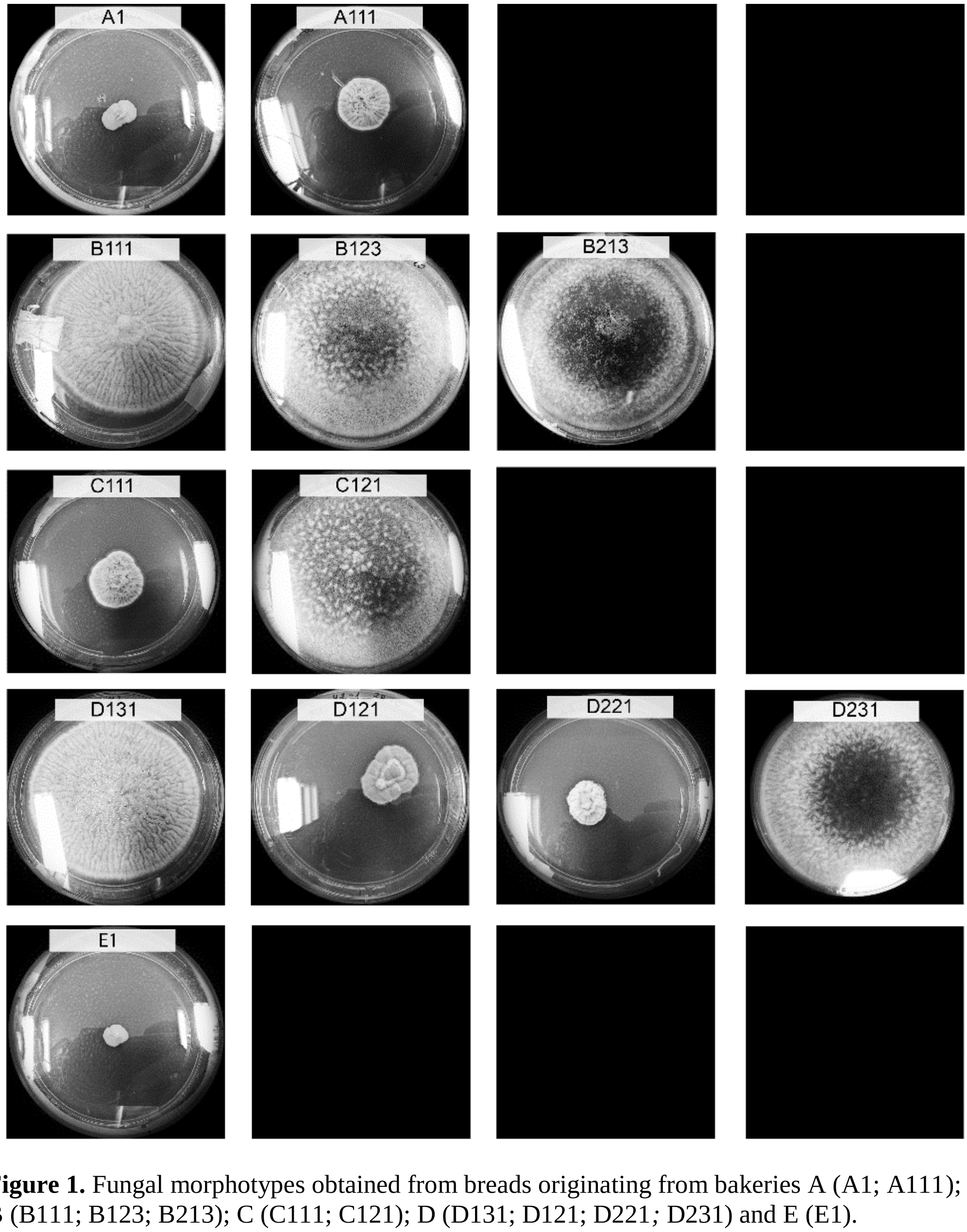

Figure 1. Fungal morphotypes obtained from breads originating from bakeries A (A1; A111); B (B111; B123; B213); C (C111; C121); D (D131; D121; D221; D231) and E (E1).

All 50 fungal isolates were genotyped by RAPD–PCR using M13 primer. Eight reproducible RAPD patterns with clear banding profiles were obtained (Fig. 2). Several RAPD patterns were bakery–specific (RAPD II, RAPD VI–VIII, Table 1), whereas representatives of other RAPD patterns were found on breads produced by different bakeries (Table 1). It is considered that biodiversity of contaminating fungi is related to the frequency of fungal infections in bakeries (Lund et al., 1996). Indeed, in our study the direct correlation between the numbers of moulds with different RAPD patterns and duration of shelf–life of rye bread was observed. Samples from A_{bakery} and E_{bakery}, where only yeasts or one mould species were detected, had the longest shelf–life among all bread samples evaluated in this study (Table 1).

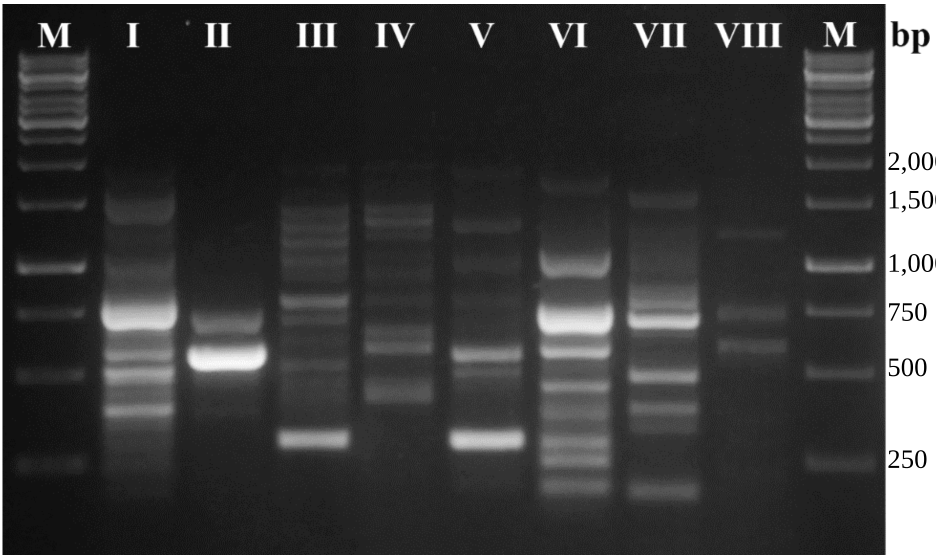

Figure 2. Eight RAPD patterns of fungal isolates obtained by RAPD–fingerprinting with M13 praimer (see the Table 1 for more detailed information). M: 1 kb DNA ladder.

The representative isolates of different RAPD types were identified based on sequences of the ITS region, the β–tubulin gene, or the D1/D2 region of the large subunit of the 28S rDNA as *Aspergillus chevalieri, Aspergillus flavus/oryzae, Aspergillus niger, Aspergillus tubingensis, Penicillium citrinum, Penicillium corylophilum, Saccharomyces cerevisiae* and *Wickerhamomyces anomalus* species (Annex 1). None of the molecular markers used were suitable for identification of all fungal isolates either due to low discriminative capacity towards a certain species or a continuous failure of amplification/low quality of sequences (Annex 1). In case of isolates belonged to RAPD pattern I sequencing of the β–tubulin gene and ITS region yielded 100% identical sequence data for *Penicillium corylophilum*/*Penicillium obscurum* or *Penicillium corylophilum/Penicillium obscurum/Penicillium chloroleucon* respectively (Annex 1). All identified species belonged to *Penicillium* section *Exilicaulis*. However, as the result of the recent revision of this section by Visagie et al. (2016), both *P. obscurum* and *P. chloroleucon* were considered as synonyms for *P. corylophilum* species. Thus, RAPD I isolates can be identified as *P. corylophilum* based on sequences of β–tubulin gene and

ITS region. This mould species is widely found in cereals and damp buildings (Magan, Arroyo & Alfred, 2003; McMullin, Nsiama & Miller, 2014) and has been previously reported as dominant species in contaminated rye breads made without chemical preservatives (Lund et al., 1996).

Another species of genera *Penicillium* was represented by isolates belonging to RAPD VIII pattern and was identified as *Penicillium citrinum* by all three molecular markers used (Annex 1). There is no data concerning the contamination of rye bread by *P. citrinum* species. However, *P. citrinum* was found in wheat flour and bread in the USA (Bullerman & Hartung, 1973). This species is widely found in soil and plants (Houbraken & Samson, 2011), and thus it may contaminate the bakery environment via flour particles that spread through the air and also by landing on equipment used for slicing and packaging.

Representatives of RAPD III, RAPD IV, RAPD V and RAPD VI patterns belong to the *Aspergillus* genera and were identified at species level only based on β–tubulin gene sequence (Annex 1) including closely–related black–spore *Aspergilli* species *Aspergillus tubingensis* (RAPD V) and *Aspergillus niger* (RAPD III). The latter are the most difficult groups to identify using morphology–based methods (Varga et al., 2000; Varga, Frisvad & Samson, 2011; Jang et al., 2012). Whereas *A. niger* is frequently reported as bread contaminant (Legan, 1993; Lund et al., 1996; Saranraj & Geetha, 2012), there is no data regarding the contamination of rye bread by *A. tubingensis*. RAPD VI pattern was composed by *Aspergillus chevalieri*, a xerophilic mould growing on food with water activity down to 0.65 like rolled oats, chocolate, some dried fruits and nuts (Pomeranz, 1991). Its telemorph, *Eurotium chevalier*, has been detected as a contaminant of milk bread rolls (Le Lay et al., 2016). RAPD IV group also belonged to *Aspergillus* genera but it was not identified at species level. Sequences of both β–tubulin gene and D1/D2 region have the similar identity to the sequences of both *Aspergillus flavus* and *Aspergillus oryzae* species (Annex 1). These are very closely–related species, which are genetically almost identical (Chang & Ehrlich, 2010; Amaike & Keller, 2011) but have different economic impact. While most *A. flavus* strains are aflatoxigenic and infect preharvest and postharvest seed crops, representatives of *A. oryzae* species have been widely used for preparation of traditional fermented foods and beverages. Genome sequence data supports the view that *A. flavus* and *A. oryzae* are the same species with the latter representing a domesticated clade of *A. flavus* (Amaike & Keller, 2011). Although Nikkuni et al. (1998) showed that these two species could be distinguished based on ITS region sequence, Jang et al. (2012) reported that sequences of all targeted regions (ITS, D1/D2 region and β–tubulin gene) were not variable enough to distinguish *A. flavus* from *A. oryzae*.

Isolated morphotypes A1 and E1, which were microscopically identified as yeasts, clustered into RAPD II and RAPD VII patterns, respectively (Table 1). Representatives of RAPD II pattern were identified as *Wickerhamomyces anomalus* based on sequences of ITS and D1/D2 regions while amplification of β–tubulin gene repeatedly failed. All three selected primer pairs performed equally well for the identification of RAPD VII as *Saccharomyces cerevisiae* (Annex 1). In the study performed by Lund et al. (1996) significant part of fungi isolated from spoiled rye breads belonged to yeast species causing surface spoilage of baked products known as ‘chalk moulds’. In our trial yeasts were isolated only from samples obtained from bakeries A and E. Whereas only single cases of contamination by *S. cerevisiae* have been previously described (Spicher, 1985), *Wickerhamomyces anomalus* together with *Endomyces fibuliger* and *Hyphopichia burtonii* yeast species belongs to the most frequently reported cause of 'chalk mould' bread defect (Lund et al., 1996; Deschuyffeleer et al., 2011).

Although the ITS region is considered as the universal barcode for fungal identification (Schoch et al., 2012) in our study its discriminative capacity was insufficient for identification of most fungal isolates belonging to *Aspergillus* genera. Garnier with co–authors (2017) noticed its limited taxonomic resolution for *Penicillium* and *Cladosporium* species. Thus, the β–tubulin gene should be recommended as a primary molecular marker for identification of fungi associated with rye breads. Preliminary clustering of fungal isolates with RAPD–PCR appeared to be an efficient way to reduce the sequencing expenses. Additional studies should be performed to evaluate the efficiency of RAPD–PCR fingerprinting with M13 primer to track fungi contamination source at strain level.

CONCLUSIONS

1. Fungi isolated from mouldy rye breads mainly belonged to *Aspergillus* and *Penicillium* species.
2. The β–tubulin gene sequence has higher taxonomic resolution for identification of mould isolates belonging to *Aspergillus* genera comparing to ITS region and D1/D2 region.
3. Clustering of fungal isolates with RAPD–PCR using M13 primer followed by identification based on the β–tubulin gene sequence can be recommended as a protocol for identification and tracking mould contamination in baking industry.

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Effects of compaction pressure on silage fermentation in bunker silo

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Abstract. The aim of this research was to determine the effects of compaction pressure on maize silage fermentation under field conditions. The CAT 955 L type work machine was used for the compaction of the material. In this research, a pressure measurement system was developed to measure the compaction pressure in bunker silos. In bunker silos, 24 points for pressure and temperature measurement were identified. Chemical and microbiological analyzes were made by taking samples from each measurement point. The lowest temperature is measured in the back wall of the silo. There is a significant relationship between pressure and temperature. Pressure had a significant effect ($P < 0.05$) on silage fermentation. There was a significant correlation between regions in bunker silo and pressure ($R^2 = 0.914$, $P < 0.01$).

Key words: Silage, pressure, compaction, bunker silo, pressure measure method.

INTRODUCTION

Ensiling is a common preservation method for moist forage crops. It is based on lactic acid bacteria (LAB), convert water-soluble carbohydrates (WSCs) into organic acids, mainly lactic acid (LA), under anaerobic conditions. As a result, pH decreases and the forage is preserved from spoilage microorganisms (McDonald et al., 1991; Filya, 2004).

Chopped whole crop maize is the major crop ensiled in Turkey. Silage making in bunker silos and stack type silos are generally more common than other types of silo. For this reason, the compaction process is an important process for silage. High losses and low quality can occur if the compaction is too low. Toruk et al. (2010) reported that fermentation characteristics of the silage were affected positively by increasing compaction. Darby & Jofriet (1993) found that the density of silage in bunker silos increased by increasing compaction equipment mass. For this reason, work machine or heavy tractors of 20 t and more are often used in large bunker silos. Roy et al. (2001) indicated that more compacting time will be needed to achieve an enough density. Compaction equipment mass, compaction time, packing time and layer thickness were important factors on the silage density and silage quality (Ruppel et al., 1995; Muck & Holmes, 2000; Roy et al., 2001). In previous studies, the pressure on the bunker silo material was generally measured under laboratory conditions. In a laboratory study, Savoie et al. (2004) found that the average dry matter (DM) density of maize silage was significantly affected by layer thickness, crop processing, and pressure but not by time of compaction or moisture content.

The effect of pressure on the ensiling properties of maize was studied under field conditions. The pressure measurement system (PMS) was developed and used in this study. This system is based on the compaction pressure determination. The pressure sensing rubber globes were used to detect the pressure coming from each direction. Turner & Raper (2001) used similar a method for determination of soil compaction. Pressure sensing rubber globes and temperature sensors retrieved after opening the silo.

The aim of this study was to determine the effect of pressure on the fermentation of maize silage under field conditions.

MATERIALS AND METHODS

Whole crop maize (*Zea mays* L.) was harvested at early dent maturity stage (25% DM) on 18 October 2014. The effect of pressure on the ensiling properties of maize was studied under field conditions. The pressure measurement system (PMS), which was developed by the researchers, was used to measure the pressure applied on the silage.

Compaction equipment

The work machine was used as the compaction equipment (Fig. 1). The technical specifications of the machine are given in Table 1.

Figure 1. Compaction equipment.

Table 1. The technical specifications of compaction equipment

Specification		
Power	130/96.9	HP per kW
Weight	13,700	kg
Track shoe width	432	mm
Length of track on ground	2,355	mm
Ground contact area	2.03	m^2

Bunker silo and measurement points

The size of the bunker silo was 7,500 mm wide by 27,000 mm long by 2,250 mm high (Fig. 2). The location of pressure and temperature sensors in bunker silos are given in Table 2.

To characterize the silage profile, three location factors were chosen (D'Amours & Savoie, 2005).

The silo was divided into three regions (A, B, C) (Fig. 2).

Three locations were defined in each region (right, center and left).

Three layers of thickness from the floor were taken (0.5 m, 1 m and 1.5 m). The distance between sensors was equal.

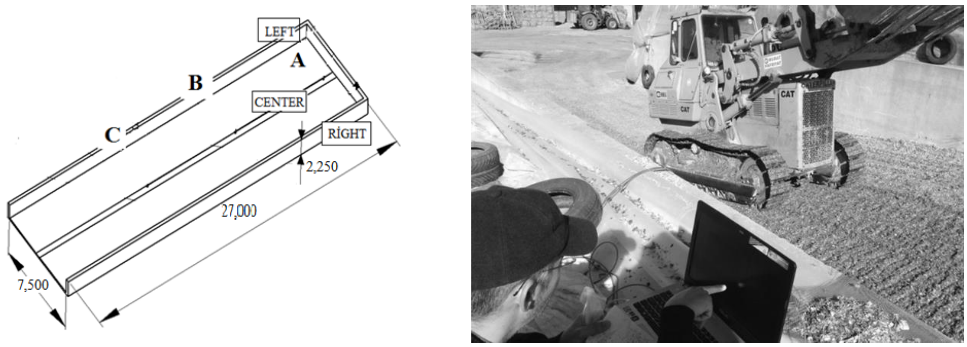

Figure 2. Bunker silo and trials.

Table 2. The location of pressure and temperature sensors in bunker silo

Region		A			B			C		
		Locations								
Heights	Sensor	Left	Center	Right	Left	Center	Right	Left	Center	Right
1.5	P*	AL15	AC15	AR15	BL15	BC15	BR15			
1	P	AL1	AC1	AR1	BL1	BC1	BR1	CL1	CC1	CR1
1	T**	AL1	AC1	AR1	BL1	BC1	BR1	CL1	CC1	CR1
0.5	P	AL05	AC05	AR05	BL05	BC05	BR05	CL05	CC05	CR05

*P: Pressure sensor; **T: Temperature sensor.

The temperature sensors (9 units) are only located in the middle layer (layer thickness 1 m). Pressure sensors (24 units) are located in all layers. For the temperature measurement, the E-348-UA-002-08 model temperature sensors were used.

Measurement system

A pressure measurement system (PMS) was used to determine the compaction pressure. PMS has mainly five units (Fig. 3). These are:

- Pressure sensing rubber globes;
- Hydraulic hose connections;
- Pressure sensors;
- Data collection, recording and storage and
- Portable computer.

Pressure sensing rubber globes were connected to pressure sensors via the hydraulic hoses. The pressure sensing rubber globes can detect the pressure coming from each direction. The sensor outputs were connected to NI DAQ measurement and storage system.

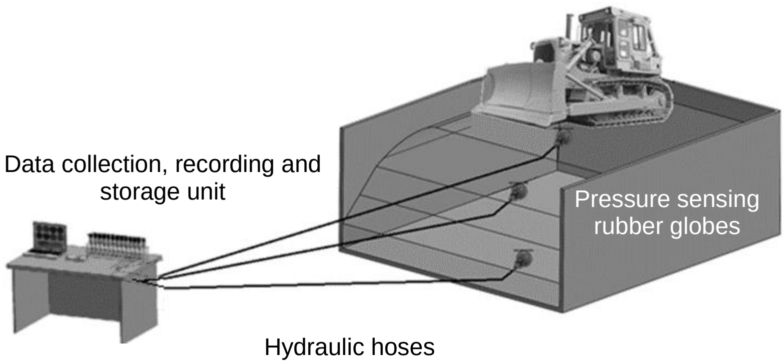

Figure 3. Pressure measurement system (PMS).

Mesens 500 series 4–20 mA of the 4 bar capacity pressure sensors were used. Sensor capacity was determined according to the work machine's weight. Pressure and temperature measurement sensors were installed at these measuring points (Fig. 3). In hydraulically operated system, water was used for pressure transmission (Turner & Raper, 2001). The portable computer and data collection, recording and storage unit of the PMS was placed at the outer surface of the silo. The data acquisition system is based on a graphical programming language NI LabVIEW software and NI CompactDAQ hardware modules. The data is stored in an MS Excel.

Analytical procedures

Chemical and microbial analyses were in triplicate. The DM content of the silages was determined by oven drying for 48 h at 60 °C (Akyıldız, 1984). The pH in fresh material and silage samples was measured according to the British Standard method (Anonymous, 1986). The ammonia nitrogen (NH_3-N) content of silages was determined according to Anonymous (1986). The water soluble carbohydrates (WSC) content of silages was determined by spectrophotometer (Shimadzu UV-1201, Kyoto, Japan) after reaction with antron reagent (Thomas, 1977). LA was determined by the spectrophotometric method (Koc & Coskuntuna, 2003).

Microbiological evaluation included enumeration of lactobacilli on pour-plate Rogosa agar (Oxoid CM627, Oxoid, Basingstoke, UK). Yeast and moulds were determined by pour plating in malt extract agar (Oxoid CM59) that had been acidified, after autoclaving, by the addition of 85% lactic acid at a concentration of 0.5% vol/vol. Plates were incubated aerobically at 32 °C for 48 to 72 h (Seale et. al., 1990).

Statistical analysis

To evaluate statistical significance between the pressure and temperature in bunker silo, the data was analyzed using the ANOVA procedure, and significant differences among means were determined by Tukey test and correlation test was performed between all parameters (SPSS v.15.0).

RESULTS AND DISCUSSION

The fresh maize contained 228 and 150 g kg^{-1} DM, WSC, respectively, and the pH value was 4.48. The log numbers of colony forming unit (cfu g^{-1}) of yeasts and moulds in the fresh material were 1.81 and 1.51 respectively.

The effects of pressure on the chemical composition of the silages according to regions (A, B, C) in bunker silo are given in Table 3. The effects of pressure on the chemical composition of the silages according to locations (right, center, left) in bunker silo are given in Table 4.

The effects of compaction pressure on DM content was found statistically significant in regions and insignificant in locations. DM content increased by the increasing pressure. Roy et al. (2001) also reported significant relationship between DM of chopped corn and pressure in experimental range of 120 and 480 kpa. DM content and pH values of the silages were similar to trends found by Yıldız et al. (2010). The lowest DM content was measured in the A region (22.15%), the highest DM content was measured in the C region (26.03%). There was a significant correlation between DM content and pressure (R^2 = -0.624, $P < 0.05$).

The maize silage was well-preserved, as would be expected with carbohydrate rich crops. The pH value of maize silage was lower than the fresh maize. In the experiment, the WSCs in silage decreased by the decrease in pH. The effects of compaction pressure on pH values were found statistically significant in regions and insignificant in locations. pH values were decreased by the increasing pressure.

The lowest pH value was measured in the C region (3.23), which is a result of the fact that the LA content was the highest in the C region, and the highest pH value was measured in the A region (3.73). Peterson (1988) stated that for a good quality silage, pH should be under 4.3. The pH values determined in all regions were found in the appropriate range for fermentation. There was a significant correlation between pH values and pressure (R^2 = 0.910, $P < 0.01$). The pH values of the silage in bunker silo were affected positively by the increasing compaction pressure. One of the main factors affecting silage quality is the rate of decline in ambient pH at the early stage of fermentation. It is desirable that the pH value should be reduced to below 4.2, 4.0 rapidly. The rate of decrease in pH is related to LA production. The changes that can be observed in the silage in terms of these properties depend on the WSC content and composition of the material, the concentration of the epiphytic microorganism and the density of the applied bacteria. In many circumstances, materials with high WSC content have the advantage that suitable fermentation development can be achieved (Davies et al., 1998).

The effects of compaction pressure on NH_3-N was found statistically significant in regions and insignificant in locations. The pressure is generally high at the right side, and low at the left side. The operator was able to compact in regions C and B; however, couldn't compact enough in region A, which is closest to the back wall of the silo. Region A caused the overall average to be lower than expected. NH_3-N value increased by the increasing pressure. The lowest NH_3-N was measured in the C region (0.18%), the highest NH_3-N was measured in the A region (0.45%).

Table 3. Results of chemical and microbiological analysis values according to regions

Region	Pressure (Bar)	Temperature (ºC)	DM (%)	pH	NH_3-N (g kg^{-1})	LA (%)	WSC (g kg^{-1})	Yeast (cfu g^{-1})	Mould (cfu g^{-1})
A	0.25 ± 0.09 **a***	18.30 ± 1.47 **a**	22.74 ± 1.56 **a**	3.80 ± 0.12 **b**	0.45 ± 0.2 **b**	11.36 ± 2.8 **a**	93.92 ± 31.7	1.81 ± 0.1 **b**	0.0 ± 0.0
B	0.35 ± 0.01 **b**	22.46 ± 3.50 **b**	25.34 ± 1.00 **b**	3.76 ± 0.9 **b**	0.22 ± 0.1 **a**	13.36 ± 0.3 **b**	108.8 ± 4.9	1.84 ± 0.1 **a**	0.6 ± 0.9
C	0.38 ± 0.02 **c**	29.2 6 ± 3.51 **c**	26.09 ± 0.50 **b**	3.23 ± 0.5 **a**	0.18 ± 0.2 **a**	14.30 ± 0.3 **b**	111.7 ± 8.1	1.66 ± 0.0 **ab**	0.0 ± 0.0
Mean	0.32 ± 0.05	23.34 ± 5.42	24.72 ± 1.81	3.60 ± 0.2	0.29 ± 0.2	13.1 ± 2.03	104.82 ± 20.8	1.66 ± 0.1	0.2 ± 0.6
Min.	0.21	16.63	20.07	3.16	0.05	7.00	48.80	1.40	0.0
Max.	0.39	33.54	26.93	4.01	0.66	14.80	122.50	4.15	1.81
F	*114.48*	*30.82*	22.52	96.75	6.89	7.43	2.22	0.32	2.28
P	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	*ns*	$P < 0.05$	*ns*

*Mean values on the same column with the same superscript do not differ significantly at $P < 0.05$; *ns*: not significant $P < 0.05$; DM dry matter; NH_3-N concentration of ammonia-nitrogen; LA lactic acid; WSC water soluble carbohydrates.

Table 4. Results of chemical and microbiological analysis values according to locations

Location	Pressure (Bar)	Temperature (ºC)	DM (%)	pH	NH_3-N (g kg^{-1})	LA (%)	WSC (g kg^{-1})	Yeast (cfu g^{-1})	Mould (cfu g^{-1})
Right	0.34.± 0.04	25.99 ± 6.61	24.73 ± 1.96	3.62 ± 0.3	0.22 ± 0.2	13.66 ± 0.5 **b**	93.04 ± 31.4 **a**	1.69 ± 0.0	0.0 ± 0.0
Center	0.32 ± 0.06	22.48 ± 5.75	24.53 ± 2.27	3.57 ± 0.3	0.24 ± 0.1	13.66 ± 0.9 **b**	115.4 ± 5.8 **b**	1.96 ± 0.8	0.0 ± 0.0
Left	0.31 ± 0.06	21.56 ± 2.55	24.91 ± 1.24	3.60 ± 0.2	0.40 ± 0.2	11.70 ± 3.1 **a**	105.9 ± 1.4 **ab**	1.66 ± 0.1	0.4 ± 0.79
Mean	0.32 ± 0.06	23.34±5.42	24.72 ± 1.81	3.60 ± 0.2	0.29 ± 0.2	13.1 ± 2.03	104.82 ± 20.8	1.77 ± 0.4	0.2 ± 0.4
Min.	0.21	16.63	20.07	3.16	0.05	7.00	48.80	1.40	0.0
Max.	0.39	33.54	26.93	4.01	0.66	14.80	122.50	4.15	1.81
F	*0.44*	*1.77*	0.09	0.074	2.30	3.28	3.31	1.01	2.28
P	*ns*	*ns*	*ns*	*ns*	*ns*	$P < 0.05$	$P < 0.05$	*ns*	*ns*

*Mean values on the same column with the same superscript do not differ significantly at $P < 0.05$; *ns*: not significant $P < 0.05$; DM dry matter; NH_3-N concentration of ammonia-nitrogen; LA lactic acid; WSC water soluble carbohydrates.

Table 5. Correlations between all parameters

	DM	pH	NH_3-N	LA	WSC	Yeast	Mould	Pressure	Temperature
Regions	0.770**	-.840**	-.562**	0.606*	*ns*	*ns*	*ns*	-.914**	-.840**
A	0.723**	*ns*	*ns*	-.925**	0.738*	*ns*	*ns*	*ns*	0.986**
B	-.810**	*ns*	0.915**	*ns*	*ns*	*ns*	*ns*	*ns*	0.900**
C	*ns*	*ns*	*ns*	*ns*	-.963**	*ns*	*ns*	0.878**	-.668**
Locations	*ns*	*ns*	*ns*	-0.401*	*ns*	*ns*	*ns*	*ns*	*ns*
Right	-.742*	0.836**	0.860**	*ns*	-.893**	0.710*	*ns*	0.940**	0.846**
Center	-.714*	0.873**	*ns*	-.980**	0.933**	*ns*	*ns*	0.916**	0.911**
Left	*ns*	0.874**	0.938**	-.895**	0.783*	*ns*	0.724*	0.927**	0.907**
Pressure	-0.624*	0.910**	0.461*	-.542**	*ns*	*ns*	*ns*	1	0.747**
Temperature	-0.701**	0.640**	0.635**	-.780**	*ns*	0.749*	0.733*	0.747**	1
DM	1	-.458*	-.413*	*ns*	*ns*	*ns*	*ns*	-.624**	-.701**
pH	-.458*	1	0.390*	-.491**	*ns*	*ns*	*ns*	0.910**	0.640**
NH_3-N	-.413*	0.390*	1	-.586**	-.457*	*ns*	*ns*	0.461*	0.635**
LA	*ns*	-.491**	-.586**	1	*ns*	*ns*	*ns*	-.542**	-.780**
WSC	*ns*	*ns*	-.457*	*ns*	1	*ns*	*ns*	*ns*	*ns*
Yeast	*ns*	*ns*	*ns*	*ns*	*ns*	1	0.830**	*ns*	*ns*
Mould	*ns*	*ns*	*ns*	*ns*	*ns*	0.830**	1	*ns*	0.733*

**. Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed); *ns*: not significant $P < 0.05$; DM dry matter; NH_3-N concentration of ammonia-nitrogen; LA lactic acid; WSC water soluble carbohydrates; A region closest to the back wall of the silo; B region in the centre of the silo, C region in front of the silo; Right 1.0 m from both the right side wall; Center in the middle of the silo; Left 1.0 m from both the left side wall.

There was a significant correlation between NH_3-N and pressure ($R^2 = 0.461$, $P < 0.05$). The lowest NH_3-N was measured on the right while the highest NH_3-N was measured on the left. The low NH_3-N concentration may be attributed to the sharp decline in pH, which made aerobic microorganism and plant enzymes inhibit rapidly, resulting in protein degradation during fermentation process.

The effects of compaction pressure on LA were found statistically significant both in regions ($R^2 = 0.606$, $P < 0.01$) and in locations ($R^2 = -0.401$, $P < 0.01$). LA content increased by the increasing pressure. The lowest LA content was measured in the A region (11.59%), and the highest LA content was measured in the C region (14.3%). Alcicek & Ozkan (1997) stated that the value of LA should be over 2.0% in high-quality silages. The values of this study about LA were sufficient. Toruk et al. (2010) reported that fermentation characteristics of the silage were affected positively by the increasing compaction. There was a significant correlation between LA content and pressure ($R^2 = -0.542$, $P < 0.01$).

There was no statistically significant correlation between WSC, yeast, mould and pressure in both regions and locations.

Correlations between all parameters and pressure were shown in Table 5. There was a significant correlation between regions in bunker silo and pressure ($R^2 = -0.914$, $P < 0.01$).

CONCLUSION

The data of this study indicated that fermentation characteristics of the maize silage were positively affected by the increasing compaction pressure. The pressure showed positive correlation with pH, NH_3-N and temperature, whereas it was negatively correlated with DM and LA. The effects of compaction pressure was found statistically significant in regions, while insignificant in locations. The pressure was the lowest in the back wall of the silo and the highest in the front side of the silo. This may be due to the application of less compression time in the back wall of the silo than in the front of the silo.

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