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Established in 2003 by the Faculty of Agronomy, Estonian Agricultural University

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Acknowledgement to Referees:

The Editors of *Agronomy Research* would like to thank the many scientists who gave so generously of their time and expertise to referee papers submitted to the Journal.

Abstracted and indexed:

SCOPUS, EBSCO, CABI Full Paper and Thompson Scientific database: (Zoological Records, Biological Abstracts and Biosis Previews, AGRIS, ISPI, CAB Abstracts, AGRICOLA (NAL; USA), VINITI, INIST-PASCAL.)

Subscription information:

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ISSN 1406-894X

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Evaluation of rice by-products, incubation time, and photoperiod for solid state mass multiplication of the biocontrol agents *Beauveria bassiana* and *Metarhizium anisopliae*

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Abstract. The success of biological control of insect pests depends not only on the isolation, characterization, and pathogenicity, but also on the success of the mass production of the microbial agents. The biological control strategy using entomopathogenic fungi like *B. bassiana* and *M. anisopliae* can only be useful if practical and economic methods of mass multiplication are available. Rice by-products like broken rice grains, rice hulls and their combination was evaluated for solid state multiplication of *B. bassiana* and *M. anisopliae*. The influence of photoperiod and incubation time in the production of conidia was also evaluated. This study showed that, broken rice was the most productive substrate for conidial production of both fungal genera, with a yield of 4.62×10^7 and 2.22×10^6 conidia g^{-1} respectively. Also, under the evaluated solid state multiplication conditions, the best conidia production was achieved with a photoperiod of 24 h of light for *B. bassiana* (with 4.43×10^7 conidia g^{-1}) and *M. anisopliae* (with 1.35×10^6 conidia g^{-1}). The results here demonstrated that these two fungal species could viably be multiplied with good yields of conidia on agro-industrial by-products using solid-state culture and regulating some culture conditions.

Key words: entomopathogenic fungi, solid substrates, light, incubation time, propagule, production.

INTRODUCTION

The scenario of pests' treatment in developed agricultures has been changed to an integrated management especially after the development of resistance in pests, the resurgence of pest outbreaks, and different environmental issues with pesticides. The integrated management includes the use of cultural, biological, biotechnical, mechanical and physical methods, and more innovative microbial pesticides (Blanco-Metzler, 2004). In this scenario, entomopathogenic fungi are frequently employed as biocontrol agents reducing insect pest populations in different agro-ecosystems (Bradley et al., 1992; Inglis et al., 2001).

The entomopathogenic fungi have unique mechanisms of invasion, persistence, and propagation that characterize them as excellent agents of biological control against

different insect pests (Charnley, 1997; Shah & Pell, 2003; Santos et al., 2007; Hajek & Delalibera, 2010). Entomopathogenic fungi that are being studied most for the biological control of insect pests are *Metarhizium anisopliae*, *Beauveria bassiana*, *Lecanicillium lecanii*, among others (Lecuona, 1996; Wraight et al., 2000; Butt et al., 2001; Faria & Wraight, 2007).

The success of biological control of insect pests depends not only on the isolation, characterization, and pathogenicity but also on the successful mass production of the microbial agents (Sahayaraj & Namasivayam, 2008).

Similar, for the development and use of a biological pesticide based on fungi, large amounts of inoculum of the biocontrol agent are required for field application (Ibrahim et al., 2002; Babu et al., 2008; Pham et al., 2009; Gao, 2011). Hyphae (biomass) and conidia of fungi are the main infective fungal structures used in biocontrol strategies (James, 2001; Jaronski, 2014; Mascarin & Jaronski, 2016; Jaronski & Mascarin, 2017).

The biological control strategy using entomopathogenic fungi could only be useful if practical and economic methods of mass multiplication are available (Kleespies & Zimmermann, 1992; Pham et al., 2009). However, only a limited number of methods of mass production for some fungi are being studied, developed and updated. Commercially, the most used method for mass production of biocontrol fungi is the fermentation in standard media (Thakre et al., 2011). The fermentation in solid substrates like low-cost agriculture by-products is a prominent method, especially in emerging countries (Prakash et al., 2008; Jaronski, 2014). Currently, solid substrate fermentation of fungi with agriculture by-products and conditions like incubation time and photoperiod remains mainly studied independently.

The multiplication in solid substrates has generated great interest due to advantages of economic and ecological importance that it offers in comparison with the liquid culture, among which we can mention: the use of solid support for microorganisms, low demand of water, simulation of the natural environment, lower sterility requirements, easy aeration using small batches, high productivity, among other features (Chahal, 1985; Deschamps & Huet, 1985; Acuña et al., 1995; Polizeli et al., 2005; Rodríguez & Sanromán, 2005; Ruiz-Leza et al., 2007; Prakash et al., 2008). In addition, this type of multiplication offers the possibility of using substrates that are abundant and cheap as waste and by-products of food or forest industries (Hölker & Höfer, 2004).

In the evaluation of solid substrates for the mass production of fungi, several authors have studied a variety of plant materials like rice grains, broken rice, rice bran, rice husk, barley, cassava chips, sugarcane bagasse, wheat, wheat bran, among others, with different results (Dorta et al., 1996; Taylor et al., 2013; Jaronski, 2014). Also, mass production of entomopathogenic fungi is dependent on different factors, such as the isolates selected, inoculum density and diverse environmental conditions like photoperiod and incubation time (Taylor et al., 2013).

The present study was undertaken to evaluate combinations of different rice milling by-products for mass production of three different strains of *B. bassiana* and two strains of *M. anisopliae*. It was also evaluated the effect of light and incubation time in the conidial production of strains of those entomopathogenic fungi.

MATERIALS AND METHODS

Three strains of *B. bassiana* sensu lato (accession numbers LBM216, LBM211, and LBM192) and two strains of *M. anisopliae* sensu lato (LBM218, and LBM217) were used in the evaluation of solid mass production of entomopathogenic fungi. These fungal strains are deposited in the culture collection of the Universidad Nacional de Misiones.

Three different treatments with locally available substrates were evaluated in the solid state multiplication of entomopathogenic fungi in small scale evaluations. The evaluated treatments comprised 15 x 30 cm polypropylene bags containing either 100 g of broken rice grains, 100 g of rice hulls or a combination of 50 g of broken rice grains and 50 g of rice hulls. Each bag opening was arranged with cotton plugs for better inoculation, aeration, and sampling under aseptic conditions.

After soaking the substrate with 30 mL of distilled water, the bags were autoclaved at a 15-psi pressure at 121 °C for 30 min (Prakash et al., 2008; Pham et al., 2010; Jaronski, 2014). After cooling, the clumps of the substrates were broken and 1 mL of a conidial solution with a concentration of 10^7 conidia mL⁻¹ was added. Each bag was inoculated with a single strain of entomopathogenic fungi. This procedure was carried out under aseptic conditions. Each bag (treatment) was thoroughly agitated for proper distribution of the conidia. Three replicates were maintained for each treatment.

The polypropylene bags were incubated at 28 ± 1 °C and high humidity level (> 80%) for 28 days after inoculation with the entomopathogenic fungi. The samples were taken every seven days for determination of the number of conidia produced.

Also, the influence of light (photoperiod) in the production of conidia was evaluated and three types of photoperiods were considered: 24 h of light, 12 h of light followed by 12 h of dark, and 24 h of dark. The supplementary light was provided by a white light tube at 20 cm (6500 K, 18 w) and the light/dark periods were regulated by a Zurich XTIM03205 digital timer.

To determine the conidia produced by each treatment, the conidia were harvested by suspending under aseptic conditions one gram of each substrate in 10 mL of sterile distilled water containing 0.1% Tween 80 (v v⁻¹) as surfactant agent (Gandarilla et al., 2013; Ibrahim et al., 2015). The number of conidia produced was determined microscopically from each replicate with a Neubauer hemocytometer at 400 x magnification (Alves & Faria, 2010).

The analysis of variance (ANOVA) was carried out using the Statgraphics Centurion XV program (Statpoint). In addition to the tests of overall significance with ANOVA, the Tukey's HSD test was used to check significant differences between the variables with a confidence level of 95%. All figures were generated using the Statgraphics Centurion XV program (Statpoint) by analyzing the data of two factor at time (interaction plots).

RESULTS AND DISCUSSION

Various fermentation containers such as conical flasks, Petri's plates, tubes, trays, and plastic bags can be used for the mass production of entomopathogenic fungi (Wraight et al., 2001; Jaronski, 2014). One of the advantages of solid multiplication using plastic bags is the possibility of breaking the substrate clumps formed and in some cases the use of light for optimal sporulation (Jaronski, 2014).

In our study, mass production potential of *B. bassiana* and *M. anisopliae* were assessed (Fig. 1). Conidial production among different strains of the same species of entomopathogenic fungi (*B. bassiana* or *M. anisopliae*) showed only small, statistically insignificant differences ($F = 2.14$, $df = 2$, $p = 0.12$; and, $F = 2.75$, $df = 1$, $p = 0.1$; respectively).

However, strains of *Beauveria* produced higher amounts of conidia per gram of substrate than strains of *Metharizium*. The results indicated that the sporulation of these fungi differed significantly among different substrates ($F = 133.8$, $df = 2$, $p = 0$, for *Beauveria*; and $F = 141.6$, $df = 2$, $p = 0$, for *Metarhizium*). Highest sporulation was recorded after four weeks of incubation on broken rice for both fungi, with a mean value of 4.62×10^7 ($\pm 0.2 \times 10^7$) conidia g^{-1} for *B. bassiana* and 2.22×10^6 ($\pm 0.09 \times 10^6$) conidia g^{-1} for *M. anisopliae* (Fig. 2).



Figure 1. Small-scale plastic bag-based mass production of *B. bassiana* and *M. anisopliae*.

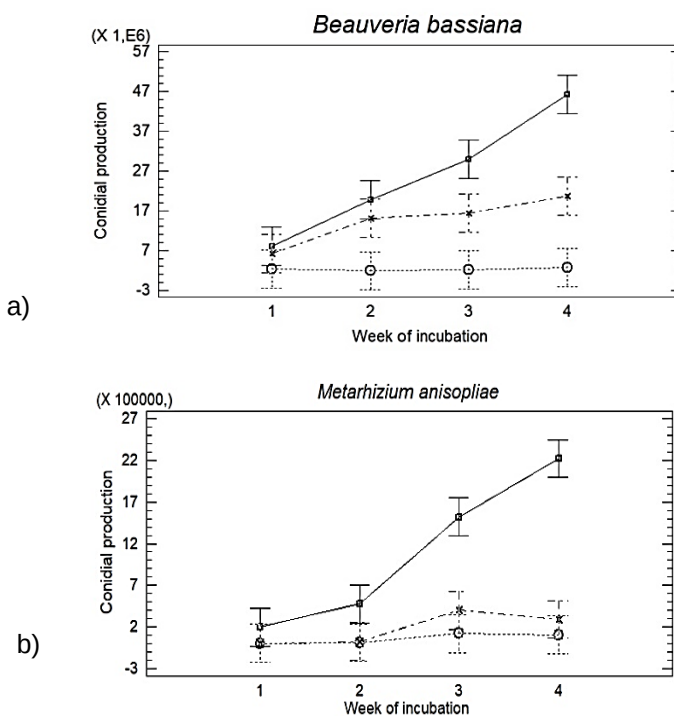


Figure 2. Solid multiplication of the entomopathogenic fungi *B. bassiana* (a) and *M. anisopliae* (b) on different solid substrates. Treatments: —■— 100% Broken rice, -×- 50% Broken rice: 50% Rice hulls. ···○·· 100% Rice hulls.

The evaluated treatments (substrates combinations) differed significantly with respect to sporulation for both entomopathogenic fungal genera. In all treatments broken rice obtained the highest amounts of sporulation for *B. bassiana* and *M. anisopliae* strains. Always the lowest sporulation was recorded on rice hulls (both for *B. bassiana* and for *M. anisopliae* strains) followed by an intermediate sporulation of the combination of 50% of broken rice and 50% of rice hulls treatments.

Values comparable to the present study were reported by Sahayaraj & Namasivayam (2008) with a production close to 1.1×10^7 conidia g^{-1} of substrate, and in their work they proposed rice grains as the most suitable substrate for the mass multiplication of *B. bassiana*. Latifian et al. (2013) evaluated the solid state multiplication of *B. bassiana* on different plant materials, including sugarcane, corn, barley, rice, millet, and sorghum. They found that a selected strain of *B. bassiana* (IRAN441c) recorded a maximum of conidia production of 6.24×10^4 conidia g^{-1} on rice.

Nonetheless, better spore production has been reviewed and commented by Bradley et al. (1992) and Bradley et al. (2002) on different substrates, e.g., barley, where selected *Beauveria* strains produced in the order of 2.6×10^{10} conidia g^{-1} on culture reactors.

Babu et al. (2008) reported that conidial production of the fungus *M. anisopliae* on rice (amended with yeast extract) was significantly greater than on other solid plant substrates, with a mean value of 1.1×10^9 conidia g^{-1} of substrate. When multiplying *M. anisopliae* on rice in conical flasks Latifian et al. (2014) recorded a maximum of conidial production of 2.8×10^6 conidia g^{-1} . Loera et al. (2016) using rice grains as the only substrate for the production of conidia with a selected strain of *M. anisopliae* in plastic bags managed to obtain about 1×10^9 conidia g^{-1} of substrate.

Some authors maintain that the structure of the substrate is as important as the availability of nutrients and that an ideal substrate should provide a large surface area to favor aeration and formation of conidia (Lomer & Lomer, 2008; Machado et al., 2010; Mascarín et al., 2010). Rice hull is a by-product of the rice industry, which has more surface area per gram than the rice grain. However, in the present work any of the rice hulls combinations as a solid mass multiplication substrate produced fewer conidia per gram of substrate than the rice grain for the fungal strains evaluated. This could be due to the fact that rice hulls have few nutrients or little availability of the same for the fungal strains. So, even if rice hull is a by-product of rice milling cheaper than the broken rice, the proportion of nutrient in broken rice is higher, making the last a better option for mass multiplication of biocontrol fungi.

Also, different published protocols of mass multiplication use additives such as *Torula* yeast extract or sugarcane molasses to bypass the need of nutrients of some agricultural substrates and increase the production of conidia (Prakash et al., 2008; Sene et al., 2010; Jaronski, 2014; Mishra et al., 2016). Thus the use of additives could be one possible option to optimize the production the conidia of these entomopathogenic fungal strains in further studies.

We also observed that the incorporation of light has a significant positive effect in the production of conidia by *B. bassiana* ($F = 159$, $df = 2$, $p = 0$) and *M. anisopliae* ($F = 29.1$, $df = 2$, $p = 0$) (Fig. 3). Also, 24 h of light incubation showed higher production of conidia than the treatments with a photoperiod of 12 h of light followed by 12 h of dark, and 24 h of dark.

With respect to the influence of the photoperiod, Kuźniar (2011) and Zhang et al. (2009) observed that exposure to light increased the growth and sporulation of

B. bassiana. Similarly, Onofre et al. (2001) proposed that continuous illumination gave them 2.5 to 5-fold more conidia production of *M. flavoviride*. Oliveira et al. (2017) found that *M. robertsii* grown under blue light produce more conidia than the fungus grown in the dark. Also, they found that white light induced the production of conidia in *Metarhizium* that germinated faster and were more virulent to insects, which is a key factor when the aim is to produce high amounts of fungal propagules (Ibrahim et al., 2002).

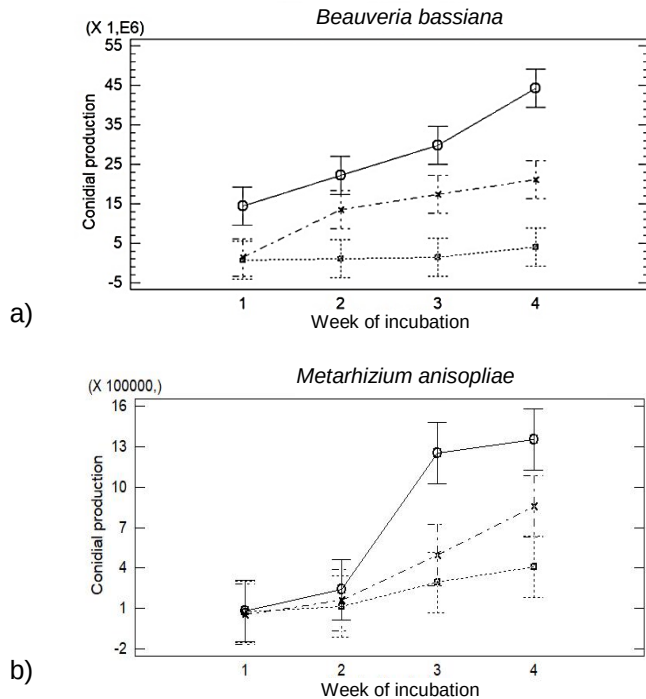


Figure 3. Conidial production by the entomopathogenic fungi *B. bassiana* (a) and *M. anisopliae* (b) with different photoperiods. Treatments: —■— 24 h of light. - × - 12 h of light / 12 h of dark. --○-- 24 h of dark.

However, Bradley et al. (1992) suggested high conidial production of various strains of *B. bassiana* in a completely dark fermentation environment; or Rangel et al. (2011) who evaluated the growth and sporulation of a strain of *M. robertsii*, and observed that the sporulation of the fungus was equivalent under conditions of continuous light or darkness. Therefore, the requirement of a parameter such as light may be a requirement of each fungal strain rather than a general rule.

Similar to the results above, in the simultaneous evaluation of the influence of the factors solid substrates and photoperiod on mass production of the entomopathogenic fungi *B. bassiana* and *M. anisopliae* the best combination was broken rice and 24 h of light ($F = 39$, $df = 4$, $p = 0$; and, $F = 9.09$, $df = 4$, $p = 0$; respectively) (Fig. 4).

Small and medium-scale conidia production varies according to different key parameters like substrate used, pH, temperature, moisture, light, aeration (structure of the substrate), different additives, among others, and optimal conditions must be evaluated for each entomopathogenic fungal species, and even each particular strain (Mascarin et al., 2010; Mar & Lumyong, 2012; Taylor et al., 2013; Muñíz-Paredes et al.,

2017). Further studies with our fungal strains could be deepened in the assessment of mass production on different rice structures or conformations like the grain size or parboiled rice.

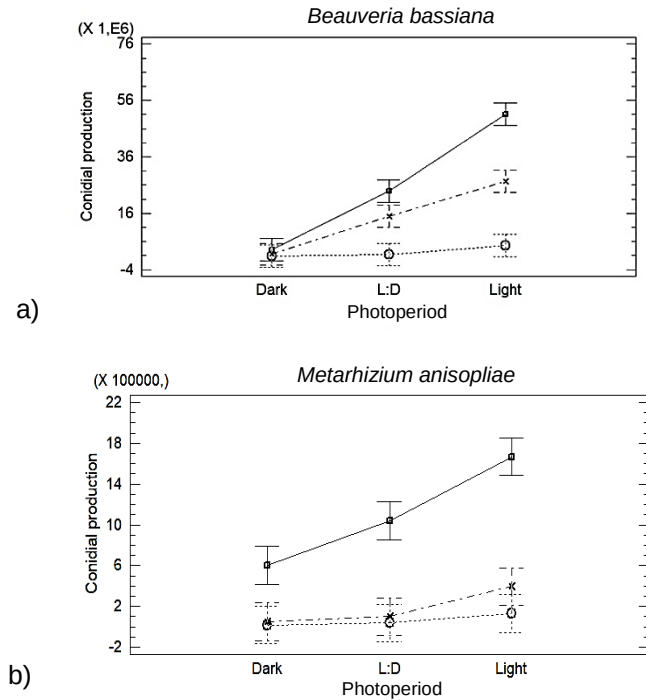


Figure 4. Influence of solid substrates and photoperiod evaluated simultaneously on mass production of the entomopathogenic fungi *B. bassiana* (a) and *M. anisopliae* (b). Treatments: —■— 100% Broken rice. - × - 50% Broken rice: 50% Rice hulls. ···○··· 100% Rice hulls.

CONCLUSIONS

The data of this study showed that broken rice substrate and incubation with 24 h of light were better conditions for mass production of aerial conidia of different strains of *B. bassiana* and *M. anisopliae*. The substrates and parameters evaluated in this study will be a promising strategy even for medium-scale production of conidia for mycoinsecticides with low costs.

For all the above, the results of the present work confirm that each fungal strain has optimal conditions for mass multiplication. In addition the results obtained provide information for a better understanding of key nutritional requirements and culture conditions that can improve the mass production of *Beauveria* and *Metarhizium*. This information can be useful even to small-scale farmers with basic infrastructure to culture these biocontrol fungi easily.

ACKNOWLEDGEMENTS. The authors are sincerely thankful to the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) Argentina for postdoctoral fellowships of Castrillo and Bich.

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The theory of cleaning the crowns of standing beet roots with the use of elastic blades

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Abstract. A standing beet root crown cleaner has been designed. The design comprises the vertical drive shaft that carries two flat elastic cleaning blades installed on axes and connected through the articulated connection. The aim of the study was to develop the new theory of cleaning the crowns of standing roots with the use of an elastic blade installed on the vertical drive shaft in order to determine its optimal design and kinematic parameters. The first step was to design an equivalent schematic model of the interaction between the elastic cleaning blade installed on the vertical drive shaft and the spherical surface of the beet root fixed in the soil. The interaction between the blade and the root's crown took place at the point, where all the forces that can arise during such interaction are applied. A three-dimensional coordinate system was set and the design and kinematic parameters of the considered interaction were designated. Using the original differential equations projected on the set coordinate axes, the system of four nonlinear differential equations of the three-dimensional motion of the elastic cleaning blade on the spherical surface of the root crown was set up, then it was transformed into the system of two differential equations in the normal form. Further, to determine the force that strips off the remaining haulm, which is part of the obtained system of differential equations, the problem of its analytical determination was solved separately. Also, the additional equivalent schematic model of the interaction between the elastic blade as a cantilever beam and the root's crown was designed, the differential equation of the beam's deflection curve (taking into account the beam's simultaneous bending and twisting) was set up and, on the basis of it, the projections of the stripping force on the coordinate axes were found. The values of the force were substituted in the earlier obtained system of differential equations.

Key words: cleaner, elastic blade, harvesting, haulm, sugar beet root.

INTRODUCTION

The state of the art of the implements for mechanized sugar beet harvesting suggests cutting the bulk of herbage from the roots' crowns immediately prior to extracting the roots from the soil, normally with the use of rotary haulm cutting tools set at a higher cutting height, which is followed by final cleaning (after-cleaning) of the crowns from

the remaining tops, while the roots remain standing in the ground throughout the procedure (Pogorely & Tatyanko, 2004; Gruber, 2005; Sarec et al., 2009; Schulze Lammers, 2011). There is quite a number of engineering developments, theoretical and experimental studies (Pogorely et al., 1983; Zhang et al., 2013, Gu et al., 2014; Bulgakov et al., 2016a; Bulgakov et al., 2016b; Bulgakov et al., 2017) aimed at solving the problem of cleaning the crowns of standing roots from the remaining haulm. A considerable number of various standing root crown cleaners exists in practice, they can be bladed, drum-type, annular, sectoral, paraboloidal etc (Eichorn, 1999; Wang & Zhang et al., 2013; Wu et al., 2013). But, looking at their design features, virtually all standing root crown cleaners can be divided into the two groups: the ones with horizontal drive shafts and those with vertical drive shafts rotating their cleaning parts. The widely used types of cleaning parts are elastic blades (from rubber or other elastic materials), brushes from wire, loops (flexible and rigid), metal rings, chains, specially shaped drums, discs with serrated or otherwise profiled surface etc. Also, the cleaners with horizontal drive shafts rotating their cleaning parts can move progressively along the row of roots, in case their rotating shafts are positioned along the row direction, or perpendicularly to the row of beet roots. During this movement, the complete cleaning is performed over the implement's working width and the sweeping-out of all plant residues to the harvested part of the beet field is done.

The primary requirement to the design and kinematic parameters of the implements cleaning root crowns from haulm residues is that they must ensure, during the cleaner's translational movement along the planted rows of sugar beet, its secure contact with a greater area of the spherical (or near-spherical) surface of the root crown (Bentini et al., 2005; Bulgakov et al., 2016a). At the same time, the cleaning parts, which perform by some means the removal of haulm residues from the said surface, must provide for the discharge of the residues, mainly into the plantation inter-row spaces. Also, the beet root crown surface after its final cleaning has to be free from haulm residues with equal degree of quality on all sides of the crown. However, this is not always achieved: for example, the rear side of the beet root, looking along the cleaner's translational movement, is often left totally uncleaned.

It is to be pointed out that, depending on the type of cleaning parts utilised in various standing root crown cleaners and the kinematic parameters of the movements they perform, the haulm residues can be removed by hitting, stripping, scraping, direct cutting (scalping) or by some combination of these methods. Moreover, the cleaners of root crowns from haulm residues shall meet a certain number of established agrotechnical requirements: they must not dislodge the roots from the soil, have to damage possibly little the root crown surface itself, provide for the transfer of the separated mass of residues out of the row zone etc., as well as the requirements of the engineering state of the art: they must have a simple design, the minimum energy and metal intensity, the considerable durability of the cleaning parts etc. However, under the condition of ensuring high cleaning process productivity and relatively simple movements of the cleaners' cleaning parts coupled with the varied properties and states of the beet plantations, it proves not always possible to meet the said requirements in full, as we already mentioned earlier.

It is to be noted that the majority of the beet harvesters produced throughout the world do not provide for the cleaning of standing root crowns from haulm residues at all, – on the contrary, they perform the direct cutting (and, undoubtedly, loss) of the

whole crown together with the haulm residues. Meanwhile, the root crown (which includes the crown itself and the zone of dormant eyes) can make 10 to 20% of the root's total mass and contain up to 10% of its sugar. Thus, it is hardly reasonable to lose knowingly this part of the root.

The aim of our research was to develop the new theory of the cleaning of standing root crowns with the use of an elastic cleaning blade installed on the vertical drive shaft in order to determine the blade's optimal design and kinematic parameters.

MATERIALS AND METHODS

The methods of the mathematics, the theoretical mechanics and the theory of strength of materials were used in the development of the mathematical model of the interaction between an elastic cleaning blade and the crown of a beet root fixed in the soil.

The design and process schematic model of such a cleaner is presented in Fig. 1. The cleaner comprises vertical drive shaft 1 with disc 2 fixed on its end face, arms 3 are pivotally connected to the periphery of the disc, the lower ends of the arms feature axes 4, on which elastic cleaning blades are installed radially and in the cantilever mode. The upper ends of arms 3 are connected to drive shaft 1 through screw mechanisms 6 and articulated joints 7 with mechanisms 8 of their shifting and fixing on shaft 1, which facilitates the pre-setting of blades 5 at various angles in the vertical plane. Cleaning blades 5 are able to rotate on axes 4 and tilt in the radial direction (with regard to the centre line of drive shaft 1).

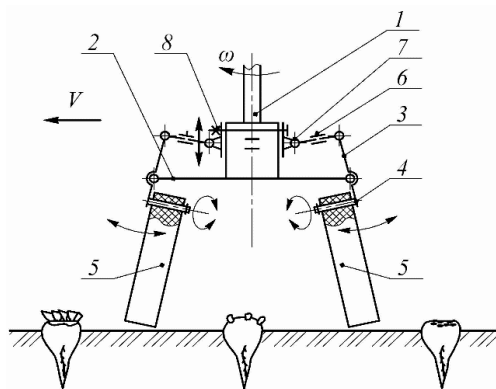


Figure 1. Design and process schematic model of new standing root crown cleaner with vertical drive shaft: 1 – vertical drive shaft; 2 – disc; 3 – double-arm lever; 4 – axis; 5 – cantilevered elastic cleaning blade; 6 – adjustment tensioner; 7 – adjustment slide; 8 – adjustment screw.

The work process of cleaning the standing root crowns from haulm residues with the use of the described type of cleaner proceeds as follows. Cantilevered vertical drive shaft 1 moves progressively along the row of planted beet roots, from which the bulk of haulm was cut earlier. Because of the rotation of shaft 1 with a pre-set angular velocity ω , cleaning blades 5 that are under the action of centrifugal forces tilt from the vertical position by some angle, forming as a result the 'cleaning cone' as it is called (the vertex of which is on drive shaft 1, the base facing down and the generatrices represented by the end faces of cleaning blades 5), which provides for the formation of a sufficiently wide cleaning zone. The cleaner set at a certain height above the soil surface level (in case of a significant quantity of residues on the root crowns and the presence of other vegetable remains in the row the said height must be possibly lower) moves progressively along the row of roots and, as a result of the rotation of drive shaft 1, its cleaning blades 5

hit the front parts of the root crowns, then, bending, move their flat surfaces over the very surfaces of the root crowns and in this phase the work process of cleaning, i.e. the stripping of haulm residues from the root crown surfaces takes place. The use of tilt control screw mechanisms 6, articulated joints 7 and shifting and fixing mechanisms 8 allows to pre-set elastic cleaning blades 5 at different angles to the centre line of cantilevered drive shaft 1. We have carried out the experimental research and field testing of the described cleaner of root crowns from haulm residues and they have produced positive results.

The general view of the cleaner of sugar beet root crowns from haulm residues is presented in Fig. 2.

Fig. 3 shows the view of the cleaning head (a) and one of its eight cleaning blades (b).



Figure 2. Working head for cleaning root crowns from haulm residues mounted on tractor during experimental investigations.

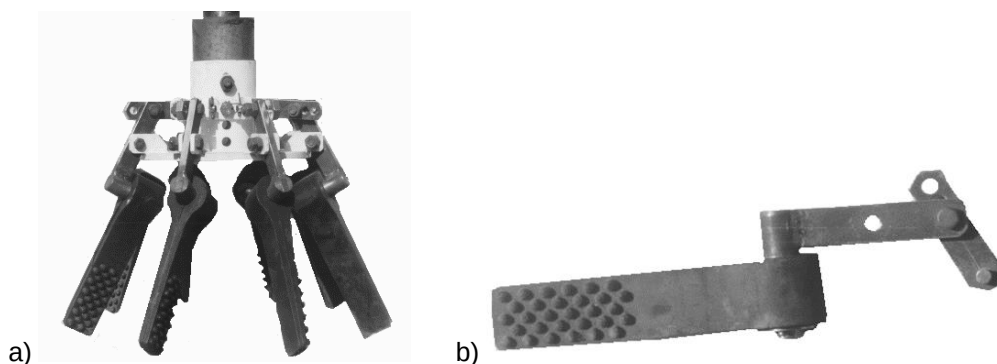


Figure 3. Beet root crown cleaner (a) and cleaning blade (b).

During the cleaner's operation, the vertical drive shaft rotates at a pre-set revolution rate and at the same time moves progressively along the row of beet roots. The cleaning blades hit the beet root crowns, stripping off them haulm residues, and take the latter out of the row zone.

The intensity of cleaning is adjusted by changing the vertex angle of the cone formed by the cleaning blades as well as changing the length and width of the cleaning blades themselves.

In order to determine the optimal design and kinematic parameters of the new standing root crown cleaner designs we proposed the theory of the interaction between the elastic cleaning blade and the root crown in the process of stripping haulm residues from the root's surface, i.e. we developed a model of the movement of the elastic blade, which rotates about the vertical axis and at the same time moves progressively, on the spherical surface of the beet root crown.

In this case, the beet root is modelled as a solid rigidly fixed in the soil, only its crown protruding above the soil surface to a height of h , and that crown is approximated by a spherical surface with a radius of r (Fig. 4). Above it, the cleaner with a vertical axis of rotation, which rotates its cleaning blades with an angular velocity of ω , moves progressively along the row of roots (i.e. in the vertical and longitudinal plane that contains the root's centre line). As it was mentioned above, the cleaning blade is tilted from its vertical position through some angle, therefore, each time when the cleaner meets a root crown, two impacts take place in effect:

the first is from the blade oriented at an obtuse angle to the direction of movement (i.e. from the outer part of the 'cleaning cone', when the cleaner only starts interacting with the root crown) and the second is from the blade oriented already at an acute angle to the direction of movement, i.e. from the inner part of the 'cleaning cone'. During the first impact, the cleaning blade bends toward the inside of the cone, its outer part interacts with the beet root crown and it 'rolls' over the crown's surface (mainly on the small front part of the root crown), stripping the haulm residues, until the end of the contact with the surface of the crown, which is followed by the straightening of the blade. During the second impact, the cleaning blade hits the root crown with its inner part (when the drive shaft axis has already passed the root's centre line, i.e. the root is already inside the cleaning cone), it bends back even more along the direction that is opposite to the cleaner's translational movement and also its inner part rolls over the root crown's spherical surface towards the following end of the contact with it.

In order to develop the analytical model of the interaction between the elastic cleaning blade and the beet root's crown we have to make an equivalent schematic model, in which the movement of the elastic cleaning blade on the spherical surface of the root crown will be examined in the absolute (fixed) three-dimensional Cartesian coordinate system $xOyz$ (Fig. 4).

Under the action of the force generated during the contact between the elastic cleaning blade and the beet root that takes place at point C during the cleaner's

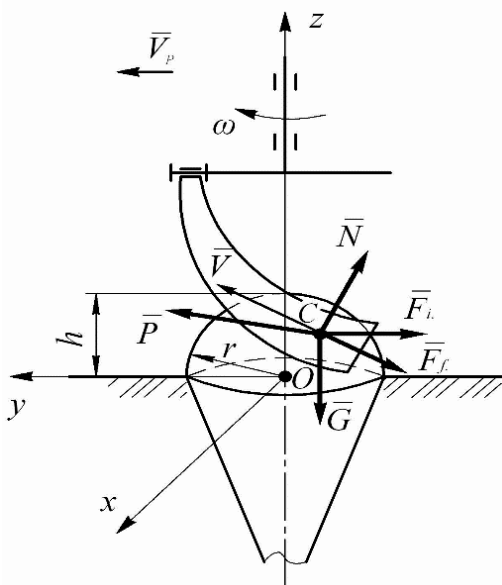


Figure 4. Equivalent schematic model of interaction between elastic cleaning blade and beet root crown.

translational movement (with a velocity of $\bar{V}_p$ as well as the action of the drive's angular momentum the blade will undergo simultaneously the bending and twisting deformations.

Force $\bar{P}$ generated by the mentioned deformations is in effect the working force that strips the haulm residues from the surface of the beet root crown. In addition to the said force $\bar{P}$, frictional force $\bar{F}_f$, centrifugal force of inertia $\bar{F}_i$, normal constraint force $\bar{N}$ and weight $\bar{G}$ of the blade itself, directed as shown in Fig. 4, are also applied to the root crown at contact point C . Hereafter, the movement of the point C of the contact between the blade and the root crown will be considered as the movement of nominal material point C specifically under the action of the mentioned forces: $\bar{P}$, $\bar{N}$, $\bar{F}_f$, $\bar{F}_i$, $\bar{G}$. That said, point C itself moves over the surface of the root crown with translational movement velocity $\bar{V}$.

RESULTS AND DISCUSSION

First of all, the differential equation of the movement of point C on the surface of the beet root crown in the vector form must be generated. It will have the following representation (Dreizler & Lüdde, 2010):

$$m\bar{a} = \bar{P} + \bar{N} + \bar{F}_f + \bar{F}_i + \bar{G}, \quad (1)$$

where m – mass of cleaning blade (kg); α – acceleration in the movement of the cleaning blade on the root crown (m s^{-2}).

The equation (1) in the projections on the axes of the assumed Cartesian coordinate system $xOyz$ will be represented by a system of differential equations and appear as follows:

$$\left. \begin{aligned} m\ddot{x} &= P_x + N_x + F_{f,x} + F_{i,x}, \\ m\ddot{y} &= P_y + N_y + F_{f,y} + F_{i,y}, \\ m\ddot{z} &= P_z + N_z + F_{f,z} + G. \end{aligned} \right\} \quad (2)$$

The right members of the equations in the system (2) represent the sums of the projections of the forces applied at the point C of the contact between the elastic cleaning blade and the beet root crown on the respective coordinate axes.

Further, the analytical expressions of the force of friction F_f , as well as the centrifugal force of inertia F_i and their projections on the axes x and y have to be written down. They will appear as follows, respectively:

– for the force of friction:

$$F_f = f_f N \quad (3)$$

where $\bar{f}_f$ – coefficient of friction of the elastic blade on the beet root crown surface;

– for the centrifugal force of inertia:

$$F_i = m\omega^2 R \quad (4)$$

– for the projections of the centrifugal force $\bar{F}_i$ on the mentioned axes x and y :

$$\begin{aligned} F_{i,x} &= m\omega^2 R \cos \omega t, \\ F_{i,y} &= -m\omega^2 R \sin \omega t, \end{aligned} \quad (5)$$

where m – mass of cleaning blade, (kg), ω – angular speed of rotation of the cleaner's drive shaft (s^{-1}); R – radius of rotation of point C about axis z (m).

Considering the fact that frictional force vector $\vec{F}_f$ has a direction that is opposite to the direction of velocity vector $\vec{V}$ of the movement of contact point C as well as basing on (3) and (5), the system of differential equations (2) can be written as follows:

$$\left. \begin{aligned} m\ddot{x} &= P_x + N \cos(\hat{x}, \vec{\bar{N}}) - f_f N \cos(\hat{\dot{x}}, \vec{\bar{V}}) + m\omega^2 R \cos \omega t, \\ m\ddot{y} &= P_y + N \cos(\hat{y}, \vec{\bar{N}}) - f_f N \cos(\hat{\dot{y}}, \vec{\bar{V}}) - m\omega^2 R \sin \omega t, \\ m\ddot{z} &= P_z + N \cos(\hat{z}, \vec{\bar{N}}) - f_f N \cos(\hat{\dot{z}}, \vec{\bar{V}}) - mg. \end{aligned} \right\} \quad (6)$$

Further, the direction cosines that appear in the right members of the equations (6) have to be determined. According to Vasilenko (1996), they are as follows:

$$\begin{aligned} \cos(\hat{x}, \vec{\bar{N}}) &= \frac{\partial f}{\partial x} \cdot \frac{1}{\Delta f}; \quad \cos(\hat{y}, \vec{\bar{N}}) = \frac{\partial f}{\partial y} \cdot \frac{1}{\Delta f}; \quad \cos(\hat{z}, \vec{\bar{N}}) = \frac{\partial f}{\partial z} \cdot \frac{1}{\Delta f}; \quad \cos(\hat{\dot{x}}, \vec{\bar{V}}) = \frac{\dot{x}}{V}; \\ \cos(\hat{\dot{y}}, \vec{\bar{V}}) &= \frac{\dot{y}}{V}; \quad \cos(\hat{\dot{z}}, \vec{\bar{V}}) = \frac{\dot{z}}{V}. \end{aligned} \quad (7)$$

where $\Delta f = \sqrt{\left(\frac{\partial f}{\partial x}\right)^2 + \left(\frac{\partial f}{\partial y}\right)^2 + \left(\frac{\partial f}{\partial z}\right)^2}$ – modulus of the gradient of function $f(x, y, z)$; $f(x, y, z) = 0$ – constraint equation; $V = \sqrt{\dot{x}^2 + \dot{y}^2 + \dot{z}^2}$ – modulus of velocity vector.

In this case, the constraint is the beet root crown itself, which, as already mentioned earlier, can be approximated by a spherical solid and that can be represented by the following constraint equation:

$$f(x, y, z) = x^2 + y^2 + z^2 - r^2 = 0, \quad (8)$$

where r – radius of sphere of the beet root crown (m).

Thus, taking into account the expressions for the direction cosines of force vectors $\vec{\bar{N}}$ and $\vec{\bar{F}}_t$ and adding the equation of sphere (8) to the system of equations (6), the following system of differential equations of the three-dimensional motion of the elastic cleaning blade on the beet root crown surface is obtained:

$$\left. \begin{aligned} m\ddot{x} &= P_x + \frac{N}{\Delta f} \frac{\partial f}{\partial x} - f_f N \frac{\dot{x}}{V} + m\omega^2 R \cos \omega t, \\ m\ddot{y} &= P_y + \frac{N}{\Delta f} \frac{\partial f}{\partial y} - f_f N \frac{\dot{y}}{V} - m\omega^2 R \sin \omega t, \\ m\ddot{z} &= P_z + \frac{N}{\Delta f} \frac{\partial f}{\partial z} - f_f N \frac{\dot{z}}{V} - mg, \\ x^2 + y^2 + z^2 - r^2 &= 0. \end{aligned} \right\} \quad (9)$$

Hence, this system (9) of differential equations describes the movement of the blade contact point C on the spherical surface of the crown under the action of the forces that are applied by the cleaning blade to the beet root crown.

Further, the partial derivatives of the function $f(x,y,z)$ with respect to the variables x , y and z and the gradient of function Δf , which are parts of the system of equations (9) have to be calculated. The result will be as follows:

$$\begin{aligned}\frac{\partial f}{\partial x} &= 2x, \\ \frac{\partial f}{\partial y} &= 2y, \\ \frac{\partial f}{\partial z} &= 2z,\end{aligned}\tag{10}$$

$$\Delta f = \sqrt{(2x)^2 + (2y)^2 + (2z)^2} = 2\sqrt{x^2 + y^2 + z^2} = 2r.$$

After substituting the obtained expressions (10) in the system of differential equation (9), the following system of equations with respect to the variables x , y , z , N is obtained:

$$\left. \begin{aligned}m\ddot{x} &= P_x + \frac{x}{r}N - f_f \cdot N \frac{\dot{x}}{V} + m\omega^2 R \cos \omega t, \\ m\ddot{y} &= P_y + \frac{y}{r}N - f_f \cdot N \frac{\dot{y}}{V} - m\omega^2 R \sin \omega t, \\ m\ddot{z} &= P_z + \frac{z}{r}N - f_f \cdot N \frac{\dot{z}}{V} - mg, \\ x^2 + y^2 + z^2 - r^2 &= 0.\end{aligned}\right\}\tag{11}$$

The next step is to reduce the obtained system of four equations with the four variables x , y , z , N to a system of two equations with the two variables x , y and a separate formula for determining the normal constraint force N .

For this purpose, the following transformations will be carried out. After differentiating the equation of sphere (8) with respect to time t , we obtain:

$$2x\dot{x} + 2y\dot{y} + 2z\dot{z} = 0,$$

or

$$x\dot{x} + y\dot{y} + z\dot{z} = 0.\tag{12}$$

On the second differentiation of the equation of sphere (8) with respect to time t , we obtain:

$$x\ddot{x} + \dot{x}^2 + y\ddot{y} + \dot{y}^2 + z\ddot{z} + \dot{z}^2 = 0$$

and from that we have

$$(x\ddot{x} + y\ddot{y} + z\ddot{z}) + (\dot{x}^2 + \dot{y}^2 + \dot{z}^2) = 0$$

Since $\dot{x}^2 + \dot{y}^2 + \dot{z}^2 = V^2$, the following formula for the squared velocity V of the movement of the point C on the beet root crown is concluded from the last expression:

$$V^2 = -(x\ddot{x} + y\ddot{y} + z\ddot{z}).\tag{13}$$

The obtained formulae (12) and (13) will be applied for the further transformation of the system of differential equations (11). After multiplying the first equation of the system (11) by x , the second equation by y , and the third equation by z , the following is obtained:

$$\left. \begin{aligned} m\ddot{x} \cdot x &= P_x \cdot x + \frac{x^2}{r} N - f_f \cdot N \frac{\dot{x}}{V} \cdot x + m\omega^2 R \cdot x \cdot \cos \omega t, \\ m\ddot{y} \cdot y &= P_y \cdot y + \frac{y^2}{r} N - f_f \cdot N \frac{\dot{y}}{V} \cdot y - m\omega^2 R \cdot y \cdot \sin \omega t, \\ m\ddot{z} \cdot z &= P_z \cdot z + \frac{z^2}{r} N - f_f \cdot N \frac{\dot{z}}{V} \cdot z - mg \cdot z, \end{aligned} \right\} \quad (14)$$

Adding term by term all three equations of the system (14), we come to:

$$\begin{aligned} m(x\ddot{x} + y\ddot{y} + z\ddot{z}) &= (P_x x + P_y y + P_z z) + \frac{N}{r} (x^2 + y^2 + z^2) - \\ &- f_f \cdot \frac{N}{V} (x\dot{x} + y\dot{y} + z\dot{z}) + m\omega^2 R x \cos \omega t - m\omega^2 R y \sin \omega t - mgz. \end{aligned} \quad (15)$$

Since $x^2 + y^2 + z^2 = r^2$, the expression (15) can be represented as follows:

$$\begin{aligned} m(x\ddot{x} + y\ddot{y} + z\ddot{z}) &= (P_x x + P_y y + P_z z) + rN - \\ &- f_f \cdot \frac{N}{V} (x\dot{x} + y\dot{y} + z\dot{z}) + m\omega^2 R (x \cos \omega t - y \sin \omega t) - mgz. \end{aligned} \quad (16)$$

Taking into account the formulae (12) and (13), we obtain:

$$\begin{aligned} -mV^2 &= (P_x x + P_y y + P_z z) + rN + m\omega^2 R (x \cos \omega t - \\ &- y \sin \omega t) - mgz. \end{aligned} \quad (17)$$

From the equation (17) the value of the normal pressure N at the point C of the contact between the cleaning blade and the beet root crown is found. It is equal to:

$$N = -\frac{1}{r} \left[P_x x + P_y y + P_z z + mV^2 + m\omega^2 R (x \cos \omega t - y \sin \omega t) - mgz \right]. \quad (18)$$

Further, from the equation (12) the following can be obtained:

$$\dot{z} = -\frac{x\dot{x} + y\dot{y}}{z},$$

then

$$\dot{z}^2 = \frac{(x\dot{x} + y\dot{y})^2}{z^2},$$

or

$$\dot{z}^2 = \frac{(x\dot{x} + y\dot{y})^2}{r^2 - (x^2 + y^2)}. \quad (19)$$

Taking into consideration (19), we obtain the expression for the squared velocity V of the movement of the cleaning blade on the beet root crown surface, which does not contain the variable z , but only contains the variables x , y and their first-order derivatives, that is:

$$V^2 = \dot{x}^2 + \dot{y}^2 + \dot{z}^2 = \dot{x}^2 + \dot{y}^2 + \frac{(x\dot{x} + y\dot{y})^2}{r^2 - (x^2 + y^2)} \quad (20)$$

Substituting the formula (18) in the first two equations of the system (11), we obtain:

$$\left. \begin{aligned} m\ddot{x} &= P_x - \left(\frac{x}{r} - f_f \cdot \frac{\dot{x}}{V} \right) \left[P_x x + P_y y + mV^2 + m\omega^2 R (x \cos \omega t - y \sin \omega t) - mgz \right] \frac{1}{r} + m\omega^2 R \cos \omega t, \\ m\ddot{y} &= P_y - \left(\frac{y}{r} - f_f \cdot \frac{\dot{y}}{V} \right) \left[P_x x + P_y y + mV^2 + m\omega^2 R (x \cos \omega t - y \sin \omega t) - mgz \right] \frac{1}{r} - m\omega^2 R \sin \omega t, \\ x^2 + y^2 + z^2 - r^2 &= 0. \end{aligned} \right\} \quad (21)$$

Finally, taking into account the formula (20) and the last equation of the system (21), a system of two differential equations of motion with the two variables x and y is obtained:

$$\left. \begin{aligned} m\ddot{x} &= P_x - \left[\frac{x}{r} - f_f \cdot \frac{\sqrt{r^2 - x^2 - y^2} \cdot \dot{x}}{\sqrt{(\dot{x}^2 + \dot{y}^2)(r^2 - x^2 - y^2) + (x\dot{x} + y\dot{y})^2}} \right] \times \\ &\times \left[P_x x + P_y y + m\dot{x}^2 + m\dot{y}^2 + \frac{m(x\dot{x} + y\dot{y})^2}{(r^2 - x^2 - y^2)} + \right. \\ &\left. + m\omega^2 R (x \cos \omega t - y \sin \omega t) - mg\sqrt{r^2 - x^2 - y^2} \right] \frac{1}{r} + m\omega^2 R \cos \omega t, \\ m\ddot{y} &= P_y - \left[\frac{y}{r} - f_f \cdot \frac{\sqrt{r^2 - x^2 - y^2} \cdot \dot{y}}{\sqrt{(\dot{x}^2 + \dot{y}^2)(r^2 - x^2 - y^2) + (x\dot{x} + y\dot{y})^2}} \right] \times \\ &\times \left[P_x x + P_y y + m\dot{x}^2 + m\dot{y}^2 + \frac{m(x\dot{x} + y\dot{y})^2}{(r^2 - x^2 - y^2)} + \right. \\ &\left. + m\omega^2 R (x \cos \omega t - y \sin \omega t) - mg\sqrt{r^2 - x^2 - y^2} \right] \frac{1}{r} - m\omega^2 R \sin \omega t. \end{aligned} \right\} \quad (22)$$

The obtained system of equations (22) is a system of nonlinear differential equations with respect to the unknown functions $x(t)$ and $y(t)$ in what is known as the normal form, when the derivatives of higher order are expressed in terms of unknown functions and lower-order derivatives. Since the system of differential equations under consideration is nonlinear, it can be solved only with the use of approximate numerical techniques on a PC under the specified initial conditions.

After determining the unknown functions $x(t)$ and $y(t)$, it is possible to find the unknown function $z(t)$ from the constraint equation (8):

$$z = \sqrt{r^2 - x^2 - y^2}. \quad (23)$$

Then it becomes possible to calculate the normal constraint force N with the use of the expression (18) and perform the comparative assessment of the obtained value with respect to the force acceptable for beet roots under the condition of their remaining undamaged. The said condition appears as follows:

$$N \leq [N], \quad (24)$$

where $[N]$ – acceptable normal force.

Thus, the system of differential equations (22) of the three-dimensional motion of the elastic cleaning blade on the surface of the beet root crown has been obtained. Nevertheless, this system of differential equations contains the force $\bar{P}$, which causes the separation of haulm residues from the beet root crown during its cleaning in the standing condition. Without determining the said force it is impossible to solve the obtained system of differential equations (22).

The force in question $\bar{P}$ can be determined on the basis of the conditions of bending and twisting of the elastic cleaning blade during its movement on the root crown surface. According to the principle of superposition, it is possible to analyse the deformations of bending and twisting of the elastic cleaning blade separately. The bending deformation arises in consequence of the contact between the cleaning blade and the beet root crown during the cleaner's translational movement along the planted row and also the action of the centrifugal force $\bar{F}_i$ of the cleaning tool rotation.

In order to solve the set problem analytically, the cleaning blade will be analysed separately as cantilever beam AB with a fixed end (Fig. 5), to which the force $\bar{P}_b$ is applied at a distance of d , which is the distance from the contact point C to the free end of the blade (point B). The Cartesian coordinate system xAy , the axis Ax of which coincides with the centre line of the blade, is assumed.

The force P_b can be determined from the differential equation of the beam deflection curve (Grote, K.-H. & Antonsson, E.K., 2008). The moment of deflection in a random beam section at a distance of X from the origin of coordinates (point A) is:

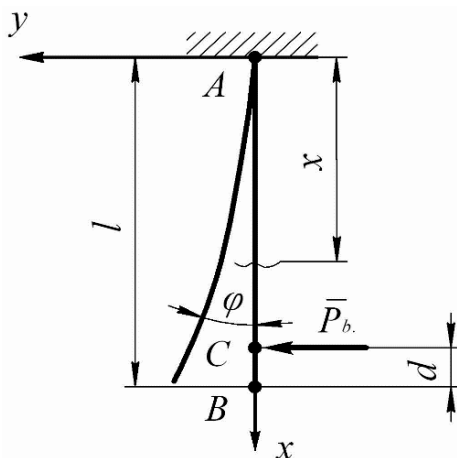


Figure 5. Deformation of elastic cleaning blade under the action of force $\bar{P}_b$.

$$M_x = -P_b (l - d - x). \quad (25)$$

The differential equation of the beam deflection curve will appear as follows:

$$EJ \frac{d^2 y}{dx^2} = M(x), \quad (26)$$

where EJ – beam stiffness; y – beam deflection.

Then, taking into account the formula (25), the following is obtained:

$$EJ \frac{d^2 y}{dx^2} = -P_b (l - d - x). \quad (27)$$

In order to determine the amount of deflection $y(x)$, it is necessary to integrate the obtained differential equation (27) two times. The first integration will produce:

$$EJ \frac{dy}{dx} = -\int P_b (l - d - x) dx,$$

or:

$$EJ \frac{dy}{dx} = -P_b (l - d)x + P_b \frac{x^2}{2} + C_1. \quad (28)$$

The second integration will give:

$$EJ y(x) = -\int P_b (l - d)x dx + \int P_b \frac{x^2}{2} dx + C_1 x + C_2,$$

or:

$$EJ y(x) = -P_b (l - d) \frac{x^2}{2} + P_b \frac{x^3}{6} + C_1 x + C_2. \quad (29)$$

where C_1, C_2 – arbitrary constants of integration.

In order to determine the arbitrary constants of integration C_1 and C_2 , the following initial conditions will be set: at $x = 0$:

$$\begin{aligned} \frac{dy}{dx} &= 0, \\ y(x) &= y(0) = 0. \end{aligned}$$

It can be found from the equation (28), after substituting in it the initial conditions, that $C_1 = 0$, and it is found from the equation (29) that $C_2 = 0$. Then the expressions (28) and (29) will finally appear as follows:

$$EJ \varphi(x) = EJ \frac{dy}{dx} = -P_b \left[(l - d)x - \frac{x^2}{2} \right], \quad (30)$$

$$EJ y(x) = -P_b \left[(l - d) \frac{x^2}{2} - \frac{x^3}{6} \right], \quad (31)$$

or

$$\varphi(x) = -\frac{P_b}{EJ} \left[(l - d)x - \frac{x^2}{2} \right], \quad (32)$$

$$y(x) = -\frac{P_b}{EJ} \left[(l-d) \frac{x^2}{2} - \frac{x^3}{6} \right]. \quad (33)$$

In the obtained equations (30) – (33): $\varphi(x) = \frac{dy}{dx}$ – angular displacement of the cleaning blade section at the arbitrary point x ; $y(x)$ – amount of deflection of the cleaning blade at the arbitrary point x . Further, the angular displacement of section φ and amount of deflection y at the point C of contact between the cleaning blade and the beet root crown, i.e. at the point $x = l - d$, will be determined:

– angular displacement:

$$\varphi(l-d) = -\frac{P_b}{EJ} \left[(l-d)^2 - \frac{(l-d)^2}{2} \right],$$

after transformation:

$$\varphi(l-d) = -\frac{P_b}{2EJ} (l-d)^2. \quad (34)$$

– deflection:

$$y(l-d) = -\frac{P_b}{EJ} \left[\frac{(l-d)^3}{2} - \frac{(l-d)^3}{6} \right],$$

after transformation:

$$y(l-d) = -\frac{P_b}{3EJ} (l-d)^3. \quad (35)$$

The expressions (34) and (35) can be regarded as the functions of the angular displacement of section and deflection of the elastic cleaning blade with respect to the value d , because the value d is variable during the movement of the cleaning blade on the root crown surface.

For the case, when $d = 0$, i.e. when the contact point C is at the free end of the cleaning blade, the following is obtained:

$$\varphi(l) = -\frac{P_b l^2}{2EJ}, \quad (36)$$

$$y(l) = -\frac{P_b l^3}{3EJ}. \quad (37)$$

If $\varphi(l)$ or $y(l)$ are known values, then it can be determined from the equations (36) and (37) that:

$$P_b = -\frac{2EJ \varphi(l)}{l^2}, \quad (38)$$

$$P_b = -\frac{3EJ y(l)}{l^3}. \quad (39)$$

The force $\bar{P}_{pr.}$ that has the same modulus as the force $\bar{P}_b$, but has the opposite direction, is the force pressing the elastic cleaning blade to the beet root crown. This force is a useful force, which performs the stripping of haulm residues as a consequence of the bending deformation. It can be assumed approximately that the said force is in line with the course of the cleaner's movement over the row of beet roots.

Hence, the pressing force $\bar{P}_{pr.}$ is equal to $P_{pr.} = -P_b$, i.e.:

$$P_{pr.} = \frac{2E J \varphi(l)}{l^2}, \quad (40)$$

or

$$P_{pr.} = \frac{3E J y(l)}{l^3}. \quad (41)$$

Further, the twisting deformation of the cleaner's cleaning blade due to the rotary motion of the tool about its axis is to be analysed. The twisting deformation of the blade occurs because of the action of the moment of rotation imparted by the drive of the cleaning tool. It can be conventionally assumed that the blade itself is a shaft with a rectangular cross-section of $h \times b$, where h – blade thickness and b – blade width.

It is known that the angle of torsion Θ for rectangular cross-section shafts is determined by the formula (Grote & Antonsson, 2008):

$$\Theta = \frac{M_{tw.} l}{G J_{tw.}}, \quad (42)$$

where $M_{tw.}$ – moment of rotation causing the twisting; l – length of shaft; G – shear modulus of the material; $J_{tw.}$ – moment of inertia under torsion.

The moment of inertia $J_{tw.}$ is determined by the following formula:

$$J_{tw.} = \alpha h^4. \quad (43)$$

The coefficient α depends on the ratio $b : h$ and is selected from the tables (Grote & Antonsson, 2008). Further, knowing the blade's torsion angle Θ , it is possible to determine from the formula (42) the moment of rotation that causes the blade's twisting. It will be:

$$M_{tw.} = \frac{G J_{tw.} \Theta}{l}. \quad (44)$$

Then the force $P_{tw.}$ that twists the blade can be expressed as follows:

$$P_{tw.} = \frac{M_{tw.}}{b},$$

where b – blade width,

or, taking into account the expression (44):

$$P_{tw.} = \frac{G J_{tw.} \Theta}{l b}. \quad (45)$$

The cleaning force $\bar{P}_p$, which has a modulus equal to that of the force $\bar{P}_{tw.}$ and the opposite direction, is a useful force, which performs the stripping of haulm residues due to the blades elasticity in its twisting, because $P_p = P_{tw.}$

It can be assumed that this force has a direction along the tangent to the trajectory of the rotary motion of the cleaning blade on the beet root crown. Then the resulting haulm residue stripping force P will be as follows:

$$\bar{P} = \bar{P}_{pr.} + \bar{P}_p. \quad (46)$$

The vector equation (46) in terms of its projections on the axes of the system of coordinates $xOyz$ (46) can be written as follows:

$$\begin{aligned} P_x &= P_{pr.x} + P_{p.x}, \\ P_y &= P_{pr.y} + P_{p.y}, \\ P_z &= P_{pr.z} + P_{p.z}. \end{aligned} \quad (47)$$

Since:

$$\begin{aligned} P_{pr.x} &= 0, \quad P_{pr.y} = P_{pr.}, \quad P_{pr.z} = 0, \\ P_{p.x} &= P_p \sin \omega t, \quad P_{p.y} = P_p \cos \omega t, \quad P_{p.z} = 0, \end{aligned}$$

then the equations (47) will appear as follows:

$$\begin{aligned} P_x &= P_{p.x}, \\ P_y &= P_{pr.} + P_{p.y}, \\ P_z &= 0, \end{aligned}$$

or, taking into account the expressions of the projections:

$$\begin{aligned} P_x &= P_p \sin \omega t, \\ P_y &= P_{pr.} + P_p \cos \omega t, \\ P_z &= 0. \end{aligned}$$

After substituting in the latter equations the expressions of the forces $P_{pr.}$ and P_p in accordance with (41) and (45), we obtain, respectively:

$$P_x = \frac{GJ \Theta}{lb} \sin \omega t, \quad (48)$$

$$P_y = \frac{3EJ y(l)}{l^3} + \frac{GJ_{tw} \Theta}{lb} \cos \omega t. \quad (49)$$

The obtained expressions (48) and (49) for the stripping force projections are substituted in the system of differential equations (22). The final result will be as follows:

$$\begin{aligned}
& m\ddot{x} = \frac{GJ_{tw.}\Theta}{lb}\sin\omega t - \left[\frac{x}{r} - f_f \cdot \frac{\sqrt{r^2 - x^2 - y^2} \cdot \dot{x}}{\sqrt{(\dot{x}^2 + \dot{y}^2)(r^2 - x^2 - y^2) + (x\dot{x} + y\dot{y})^2}} \right] \times \\
& \times \left[\frac{GJ_{tw.}\Theta}{lb}\sin\omega t \cdot x + \left(\frac{3EJ y(l)}{l^3} + \frac{GJ_{tw.}\Theta}{lb}\cos\omega t \right) y + m\dot{x}^2 + m\dot{y}^2 + \right. \\
& \left. + \frac{m(x\dot{x} + y\dot{y})^2}{r^2 - x^2 - y^2} + m\omega^2 R(x\cos\omega t - y\sin\omega t) - mg\sqrt{r^2 - x^2 - y^2} \right] \times \\
& \times \frac{1}{r} + m\omega^2 R\cos\omega t, \\
& m\ddot{y} = \frac{3EJ y(l)}{l^3} + \frac{GJ_{tw.}\Theta}{lb}\cos\omega t - \\
& - \left[\frac{y}{r} - f_f \cdot \frac{\sqrt{r^2 - x^2 - y^2} \cdot \dot{y}}{\sqrt{(\dot{x}^2 + \dot{y}^2)(r^2 - x^2 - y^2) + (x\dot{x} + y\dot{y})^2}} \right] \times \\
& \times \left[\frac{GJ_{tw.}\Theta}{lb}\sin\omega t \cdot x + \left(\frac{3EJ y(l)}{l^3} + \frac{GJ_{tw.}\Theta}{lb}\cos\omega t \right) y + m\dot{x}^2 + m\dot{y}^2 + \right. \\
& \left. + \frac{m(x\dot{x} + y\dot{y})^2}{r^2 - x^2 - y^2} + m\omega^2 R(x\cos\omega t - y\sin\omega t) - mg\sqrt{r^2 - x^2 - y^2} \right] \times \\
& \times \frac{1}{r} - m\omega^2 R\sin\omega t.
\end{aligned} \tag{50}$$

Thus, we have obtained the system of two differential equations (50) of the motion of the elastic cleaning blade on the spherical root crown surface, which models the process of haulm residue stripping and contains two unknown variables (x and y) as well as kinematic and design parameters of the cleaner with a vertical axis of rotation.

The obtained system of differential equations (50) was solved numerically with the use of the Runge-Kutta method on the PC, resulting in the plotting of the diagrams shown in Figs 6–11. It becomes obvious from the graphs in Fig. 6 that the use of reinforced rubber, which has the highest elasticity modulus E , as the material of the cleaning blade ensures the generation of the sufficiently strong stripping force P (graph 2). Any smaller values of the elasticity modulus E will not always facilitate the high quality cleaning of sugar beet root crowns from short, green and sufficiently strong haulm residues, while its higher values will result in the considerable damage to the root crowns.

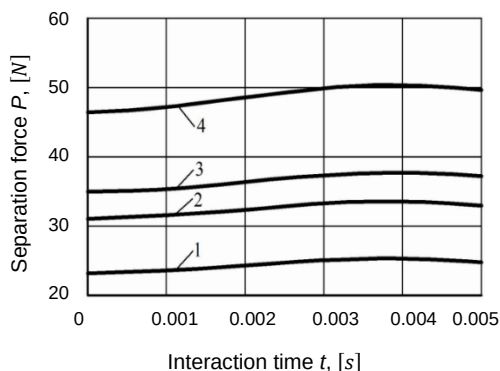


Figure 6. Relation between cleaning blade stripping force P and time t at different values of elasticity modulus E : 1) 1.80; MPa; 2) 2.42 MPa; 3) 3.17 MPa; 4) 4.3 MPa.

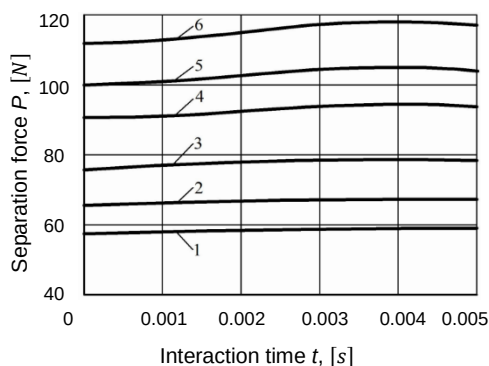


Figure 7. Relation between cleaning blade stripping force P and time t at different values of blade length: 1) 32 cm; 2) 28 cm; 3) 24 cm; 4) 20 cm; 5) 18 cm; 6) 16 cm (blade width 2 cm).

It can be concluded from the graphs in Figs 7–11 that the magnitude of the stripping force P to a great extent depends on the length of the blade and its width. On the basis of the results obtained from the numerical calculations on a PC a conclusion can be drawn that the optimal dimensions of the blade are its length equal to 20–25 cm and its width equal to 4–5 cm, in which case the total stripping force P will not exceed its maximum permitted value of 30.0 N (Pogorely et al., 1983).

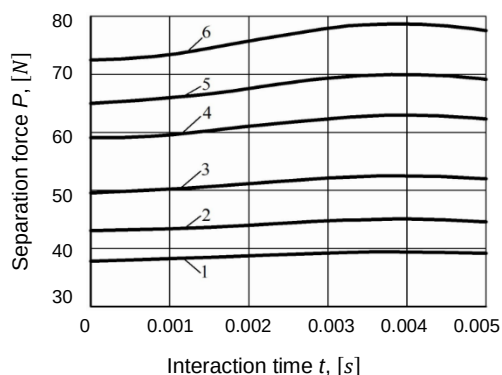


Figure 8. Relation between cleaning blade stripping force P and time t at different values of blade length: 1) 32 cm, 2) 28 cm, 3) 24 cm, 4) 20 cm, 5) 18 cm, 6) 16 cm (blade width 3 cm).

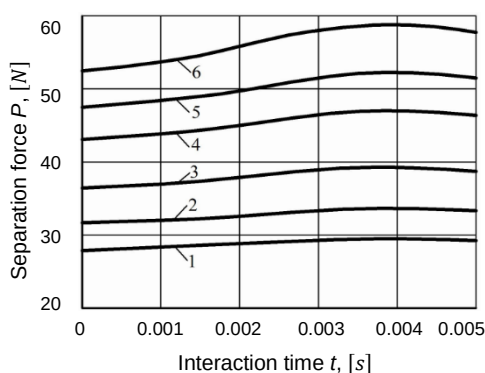


Figure 9. Relation between cleaning blade stripping force P and time t at different values of blade length: 1) 32 cm, 2) 28 cm, 3) 24 cm, 4) 20 cm, 5) 18 cm, 6) 16 cm (blade width 4 cm).

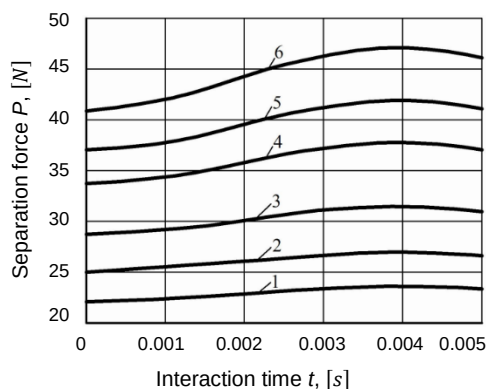


Figure 10. Relation between cleaning blade stripping force P and time t at different values of blade length: 1) 32 cm, 2) 28 cm, 3) 24 cm, 4) 20 cm, 5) 18 cm, 6) 16 cm (blade width 5 cm).

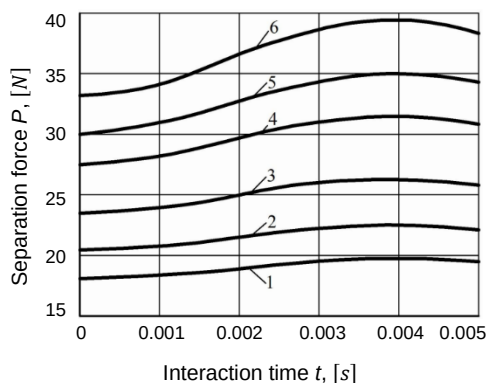


Figure 11. Relation between cleaning blade stripping force P and time t at different values of blade length: 1) 32 cm, 2) 28 cm, 3) 24 cm, 4) 20 cm, 5) 18 cm, 6) 16 cm (blade width 6 cm).

CONCLUSIONS

1. A new design of the standing root crown cleaner has been developed. The cleaner consists of the vertical drive shaft, on which two elastic cleaning blades installed as cantilevers with the use of articulated joints and axes. The experimental studies and field tests of the cleaner have produced positive results.

2. The theory of the interaction between an elastic cleaning blade that is installed as a cantilever on the vertical drive shaft and the crown of a beet root fixed in the soil has been developed. For that purpose initially the equivalent schematic model of the interaction between the elastic cleaning blade and the spherical surface of the beet root crown was formulated. It was assumed that the interaction between the blade and the root crown took place at the point, where all the forces acting in such interaction were applied. The three-dimensional coordinate system was set and the design and kinematic parameters of the interaction were designated.

3. Basing on the use of the original differential equations in the form of their projections on the assumed coordinate axes, the system of four nonlinear differential equations of the three-dimensional motion of the elastic cleaning blade on the spherical surface of the root crown was set up. Thereafter, the system of differential equations was transformed into a system of two differential equations in the normal form.

4. In order to determine the haulm residue stripping force, the additional equivalent schematic model of the interaction between the cleaning blade as a cantilever-fixed beam and the root crown was composed, then the differential equation of the beam deflection curve was set up (taking into account the simultaneous bending and twisting of the beam) and on its basis the projections of the stripping force on the assumed coordinate axes were obtained.

5. The finally obtained system of differential equations of the movement of the elastic cleaning blade on the spherical surface of the beet root crown with the specified haulm residue stripping force taken into account can be solved with the use of numerical methods on a PC after compiling the respective software. Substituting then various

design and kinematic parameters of the cleaner, it is possible to select their optimal values.

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Experimental investigation of the work of a ploughing aggregate, operating according to the system ‘push-pull’

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Abstract. The use of ploughing machine-and-tractor aggregates, operating according to the principle of ‘push-pull’, finds increasing application in the world since it allows ensuring the stability of the movement and the depth of ploughing, reduction of the energy indicators. The aim of this experimental study is to determine under field conditions the dynamic and operational technological parameters of the ploughing machine-and-tractor aggregate, operating according to the ‘push-pull’ system. This aggregate was an integral ploughing-tillage wheeled HTZ 16131 tractor, in front of which there was mounted a two-bottom plough, and at the rear – four-bottom ploughs. This aggregate has satisfactory path parameters of the movement during the execution of the technological process of ploughing. The oscillations of the furrow path for this aggregate are of a low-frequency nature and are concentrated in a rather narrow frequency range (0–50 m⁻¹). At the operating speed of 2.0 m s⁻¹ it is 0–0.16 Hz. The maximum value of the mutual correlation function between the input impact (the turning angle of the driven wheels of the aggregating tractor) and the output value – its relative bearing is positive and reaches a value of 0.88. Stability of the ploughing depth for the aggregate according to the ‘2 + 4’ scheme is ± 1.65 cm², which is normally the smaller value of the same indicator for a serial ploughing aggregate (± 1.98 cm²).

Key words: ploughing, aggregate, system ‘push-pull’, indicators.

INTRODUCTION

One of the most important tasks of agricultural production is to reduce the energy costs of ploughing (Barwicki et al., 2012; Valainis et al., 2014). An important point in solving this problem is to increase the tractive-coupling properties of the aggregating tractor by increasing its coupling weight (Bulgakov et al., 2016).

As part of a ploughing machine-and-tractor aggregate, this can be achieved by using ploughs attached to the aggregating tractor according to the scheme 'push-pull' (Rucins & Vilde, 2005). As investigations show, due to the vertical component of the draught resistance of the frontal plough, additional load of the frontal wheels increases, and, consequently, the coupling weight of the tractor increases. As a result, this leads to a certain reduction in slipping and lower fuel consumption by the ploughing aggregate (Nadykto et al., 2017).

However, if the frontal plough is not correctly connected to the source of power, not additional loading of the frontal wheels of the aggregating tractor may occur, but vice versa – their insufficient loading with inevitable loss of the steering ability and stability of the movement of the entire ploughing machine-and-tractor aggregate (Bulgakov et al., 2017).

In order to meet agrotechnical requirements for the quality of ploughing during the study of the operation of the frontal plough as part of the ploughing aggregate, operating according to the push-pull scheme on the basis of a modular source of power tool MEZ-200 (Bulgakov, 2008, Bulgakov et al., 2015), it was suggested to locate the support wheel of the frontal plough outside the furrow, i. e., on the untilled field at the same time, the removal of the said wheel from the furrow generates a stability problem of the movement of the frontally mounted ploughing tool in a horizontal plane when it is pivotally attached to the source of power. Without a support the frontal plough may take the extreme (left or right) deviated position, and not leave it afterwards. It is quite clear that further work of such a ploughing machine-and-tractor aggregate will be practically impossible.

An important point in assembling a ploughing machine-and-tractor aggregate, operating according to the 'push-pull' scheme, is to determine the ratio between the number of bodies of the frontal and the rear-mounted ploughs. The least common option is ratio 1:1. It has been established by investigations of the previous years (Köller, 1983) that the front-mounted plough should have fewer bodies than the rear plough. Usually the smallest number of bodies of a frontal ploughing tool is two. No less common variant in the world, operating according to the 'push-pull' scheme, is application of a three-furrow ploughing frontal tool.

In selecting a ratio between the number of the frontal and the rear plough bodies, a fact is taken into account that stability in a horizontal plane of the movement of an aggregate operating according to the '2 + 4' scheme can be higher than that of the aggregates assembled according to the schemes '2 + 3' or '3 + 4' (Kasymov, 1988). The authors of this same work believe that for better steering ability of the movement, the draught resistance of the front-mounted plough should not exceed 40% of the total draught resistance of the entire ploughing machine-and-tractor aggregate.

At the same time, according to various publications, the highest increase in the efficiency of ploughing is achieved by ploughing aggregates, assembled according to the '3 + 4' and '3 + 5' schemes. The increase in this indicator reaches 58–60% (Köller, 1983). In this case somewhat doubtful is the 57% reduction in the specific fuel consumption by the aggregate, assembled according to '2 + 3' scheme.

Our theoretical studies have established that, in order to avoid insufficient loading (underloading), and, vice versa, – unloading of the frontal wheels of the tractor with a nominal tractive effort of 30–32 kN, the frontal plough should have two bodies, and the rear plough should have 4 bodies (the '2 + 4' assembling scheme) (Kistechok, 2016). The aggregating wheeled tractor moving with the right-side wheels along the furrow with the

frontal plough being rigidly attached to it in a horizontal plane and the supporting wheel of this plough located outside the furrow.

The aim of the research is to carry out under field conditions experimental evaluation of the draught-and-power indicators and agrotechnical indicators of the operation of a ploughing aggregate, operating according to the ‘push-pull’ scheme with the number of plough bodies ‘2 + 4’.

MATERIALS AND METHODS

The ploughing machine-and-tractor aggregate, studied by us, was assembled according to the ‘push-pull’ scheme and consisted of a HTZ 16131 tractor with engine power 132 kW (category III of the tractor power in accordance with ISO 730/1 and 730/3-82), a frontal two-furrow plough (of our design), and a rear-mounted tensometric plough (Fig. 1). As it was mentioned above, in order to avoid a case of underloading, and, vice versa, – unloading the frontal wheels of the said wheeled aggregating tractor, the rear plough must have 4 bodies. For this purpose, in the process of experimental field investigations with a five-furrow tensometric plough, one body was removed. In this way an experimental ploughing aggregate was assembled according to the ‘2 + 4’ scheme, which had a frontal two-furrow and a rear-mounted four-furrow plough.

The following parameters were recorded during the tests: humidity and density of the soil, the longitudinal-vertical profile of the field, the draught resistance and the working width of the ploughs, the speed of the movement, skidding of the wheels and the hourly fuel consumption by the tractor, as well as the depth of ploughing.



Figure 1. A ploughing aggregate, assembled according to the ‘push-pull’ scheme.

The physical characteristics of soil were determined by the methodology described in (Arshad et al., 1996; Standard 5180-2015, 2016). The moisture content of soil, according to this methodology, was determined using a drying chamber.

The density of soil was determined by a specially made densitometer in the form of a metallic cylindrical beaker. In this case we were guided by the following procedure (Dospehov, 1985; Tamm et al., 2016).

The depth of ploughing by the aggregate was measured during the research process by a developed depth gauge. The number of measurements was 50, and the measurement pitch (interval) was 0.5 m.

For the characteristics of the agrotechnical background, the required number of sampling replications (n) was determined from the known expression (Nalimov & Chernova, 1975):

$$n = \text{Intg} \left(\frac{tV}{\rho} \right)^2 \quad (1)$$

where t – a normalised value of Student's t -criterion at a confidence level of 95% ($t = 1.96$); V , ρ , – the coefficient of variation and the allowed deviation (the accuracy factor) of the measured parameter.

It is known that for most technical problems it is not necessary to determine the measurement error with accuracy greater than 10% (Venikov, 1986). Proceeding from this, we assumed in the calculations that $\rho = 0.1$.

It has been established by many years' experimnts that the variation coefficient of the soil humidity and density usually does not exceed 10%, that is, the variability of these parameters is low. In this connection we assumed that $V = 0.1$. Uneven soil contamination is usually higher, therefore, taking into account the results of previous measurements, the variation coefficient for this process, was assumed to be equal to $V = 0.2$. As a result, we obtained the following replication rates for the experiments: when measuring the soil humidity and density, $n = 4$; when determining the field contamination, $n = 16$.

The experimental studies were conducted on a field with the following characteristics: the type of the soil – dark chestnut; the relief – flat; the microrelief – levelled; the agrotechnical background – a disked sunflower stubble; the soil humidity in a layer of 0–30 cm – 13–14%; the soil bulk density in a layer of 0–30 cm – 1.26–1.29 g cm⁻³; the humus content – 4.7%, the soil weediness – 95 pcs m⁻².

Taking into account the design features of the investigated ploughing machine-and-tractor aggregate to carry out its experimental studies, we developed a complex of measuring and recording equipment, which allowed obtaining unbiased evaluation of the investigated parameters (Fig. 2).

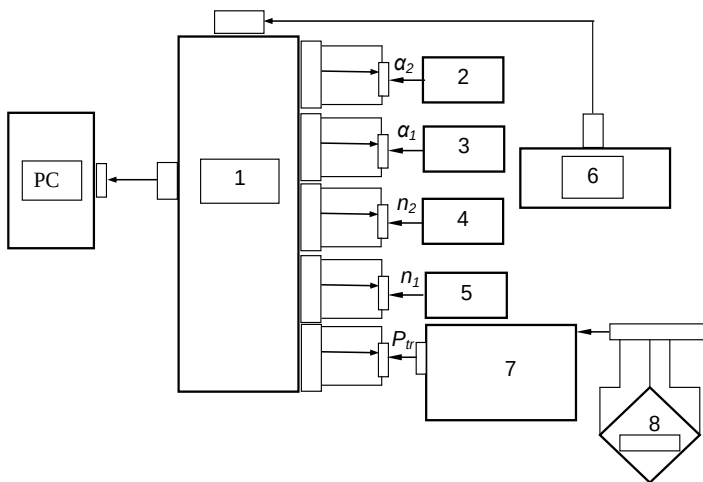


Figure 2. S block diagram of the measuring and recording complex: PC – a computer; 1 – an analogue-to-digital converter; 2, 4, 5 – current collectors; 3 – variable resistance; 6 – a power supply unit; 7 – a kb-8 balancing box; 8 – a tensometric bridge; α_1 – the turning angle of the lower links of the tractor rear linkage; α_2 – the relative bearing of the tractor; n_1 – revolutions of the right-side frontal wheel of the tractor; n_2 – revolutions of the track measuring wheel; p_{tr} – the draught resistance of the rear-mounted plough.

During the experimental field studies of the ploughing aggregate the following data were synchronically transferred to the analogue-to-digital convert and recorded (Fig. 2): the draught resistance of the rear-mounted plough; revolutions of the tractor wheels; revolutions of the track measuring wheel; the relative bearing of the tractor; the turning angle of the lower links of the HTZ 16131 tractor rear linkage in a horizontal plane; the tractor.

The moisture content of the soil was determined by the conventional hot drying method. To measure the agrotechnical background density, a method and instrument specially developed by the authors were used (Nadykto, 2017). The amplitude fluctuations and the frequency of the field profile irregularities in a longitudinal direction were measured with the help of a special profilograph (Fig. 3) according to the procedure described in (Kuvachov, 2008).

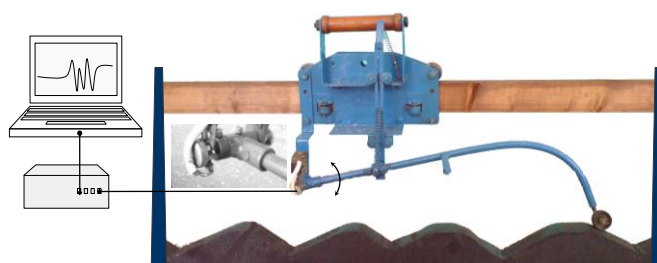


Figure 3. A scheme of the measuring equipment for profiling the irregularities of the agricultural background (agrophone).

The draught resistance of the ploughs was measured and then recorded using a tensometric link designed for a tractive effort up to 40 kN. In order to measure the draught resistance of the rear-mounted plough, the latter was equipped with a frame pivotally attached to it. With its front part this frame was connected to the rear linkage attachment of the tractor HTZ 16131, but with its rear part – through the measuring element – to the frame of the plough. The speed of the working movement of the ploughing aggregate was fixed by means of a track measuring wheel installed on the tractor. On the hubs of its frontal and rear axles, counters of revolutions were installed the electric signals of which were taken by means of current collectors.

Two impulse-type flowmeters IP-151 were used to measure the hourly fuel consumption of the investigated aggregating tractor. One of them fixed the supply of fuel to the fuel pump of the tractor, and the other – the amount of fuel returned to the fuel tank. Electrical signals processed by a profilograph, the tensometric unit, the track measuring wheel, the revolution counters and the fuel meter were recorded on the PC, passing them through an analogue-to-digital converter. Repeatability of the measurements of all the parameters was 5.

The frontal plough and the rear-mounted plough of this ploughing machine-and-tractor aggregate were adjusted for a 25 cm depth of ploughing. The operational and technological parameters of the explored ploughing machine-and-tractor aggregate, assembled according to the ‘push-pull’ scheme, were calculated using a methodology laid out in standard (Standard 24055-2016, 2016) and specially developed software.

RESULTS AND DISCUSSION

As a result of investigations, it was found out that fluctuation in the field profile irregularities were of a high-frequency nature. This fact is unambiguously shown by the length of the correlation link of the ordinates of this process, which was less than 0.3 m. The normalised correlation function of the field surface profile fluctuations included a certain periodic component with a period of approximately 0.75 m. The dispersion of the fluctuations was also small (1.21 cm^2) and was concentrated in the frequency range of $0\text{--}12 \text{ m}^{-1}$.

At a speed of the ploughing aggregate 1.98 m s^{-1} this is $0\text{--}24 \text{ s}^{-1}$ or $0\text{--}4 \text{ Hz}$ (Fig. 4). It follows from this that a relatively high frequency and a small dispersion of the irregularities of the longitudinal-vertical field profile can be generators of more or less significant fluctuations of the draught resistance of the frontal and the rear-mounted plough. The basic trend of this parameter (i.e., the draught resistance) should be formed by the internal structure of the soil environment with which the operating surfaces of the ploughing tools are in contact.

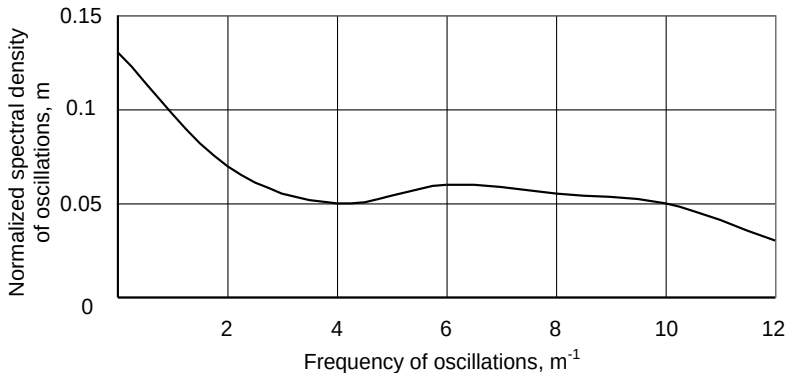


Figure 4. A normalised spectral density of the field profile fluctuations.

For the rear-mounted plough the draught resistance varied within the limits of 21.0–23.1 kN. The draught resistance of a double-furrow frontal implement was 10.5–11.6 kN. The total resistance of both ploughs was 31.5–34.7 kN. With this in mind, we can say that with an average quadratic deviation of $\pm 5.0 \text{ kN}$, the variability of the draught resistance of the entire ploughing aggregate according to the ‘push-pull’ scheme was average since the coefficient of variation of this process was within the range of 14–16%. Analysis of the normalised correlation functions of the draught resistance of ploughs showed that the time of the correlation link was within 0.24–0.26 s. Such duration (in time) of the correlation link characterises the process as a relatively high-frequency one. The real proof of this is the dispersion spectrum of the fluctuations in the draught resistance of the investigated ploughing aggregate, concentrated within the frequency range of $0\text{--}25 \text{ s}^{-1}$ or $0\text{--}4 \text{ Hz}$ (Fig. 5).

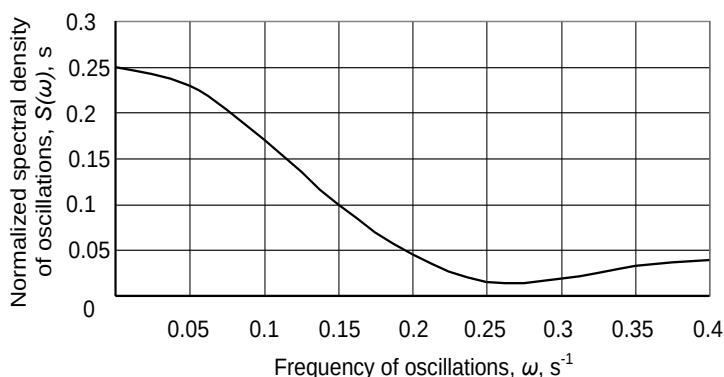


Figure 5. Normalised spectral densities of fluctuations in the draught resistance of a rear-mounted plough within the ploughing machine-and-tractor aggregate assembled according to the scheme ‘push-pull’.

The results of the experimental field research of the ploughing machine-and-tractor aggregate, operating according to the ‘push-pull’ scheme, are presented in Table 1. Because of the frontal plough, additional loading of the frontal wheels of the investigated tractor, and, consequently, its coupling weight increased, which allowed it to aggregate a total of 6 bodies of the plough, in contrast to the basic version of the ploughing aggregate, based on it, aggregated only with the rear-mounted five-furrow plough. This provided a possibility to increase the actual working width of the ploughing aggregate, assembled according to the ‘push-pull’ scheme, by 20.9% (Table 1). As a result, the efficiency of the investigated aggregate per hour of the basic time turned out to be by 19.5% higher than that of the ploughing aggregate with a rear-mounted 5-furrow plough.

Table 1. The results of the experimental research of the ploughing aggregate, operating according to the scheme ‘push-pull’

Composition of the ploughing aggregate, assembled according to the ‘push-pull’ scheme	Operating speed, m s^{-1}	Operating width, m	Efficiency per 1 hr of the basic time, ha hr^{-1}	Depth of ploughing, cm	Skid-ding of the wheels of the tractor, %	Draught resistance of the plough, kN	Hourly fuel consumption, kg h^{-1}	Specific fuel consumption, kg ha^{-1}
HTZ 16131 + frontal two-furrow plough + a rear-mounted four-furrow plough	1.98	2.14	1.53	25.1 ± 0.1	14.4	33.1	22.3	14.6

It is known that one of the main agrotechnical indicators of the operation of a ploughing aggregate is the uniformity of the tillage depth. According to the experimental data, the mean square deviation of the ploughing depth and working with a ‘push-pull’ aggregate did not exceed agrotechnical requirements ($\pm 2 \text{ cm}$) and amounted to $\pm 1.52 \text{ cm}$. In addition, higher stability in the tillage depth was observed. One of the

reasons for the implementation of the ploughing process with a higher stability of the tillage depth may be a circumstance that the front axle of the tractor HTZ 16131, having a frontal implement, produces lesser vertical vibrations when moving along the furrow. In general, this is positively reflected in the smoothness of the movement of both the frontal and the rear-mounted ploughs.

Analysis of the normalised correlation function and the spectral density of the fluctuations of the ploughing depth of the ploughing aggregate assembled according to the 'push-pull' scheme showed that the length of the correlation link is at least 21 m. The main share of dispersion of the ploughing depth of the tested ploughing aggregate is concentrated within a rather narrow frequency range: 0–0.45 m⁻¹. When the speed of the forward movement of the ploughing aggregate is at the level of 2.0 m s⁻¹, this is 0–0.90 s⁻¹, or only 0–0.14 Hz. The fluctuations in the ploughing depth themselves do not contain any latent periodic component. And this, despite the fact that the correlation function of the field profile fluctuations includes such a component with a period of approximately 0.75 m. The manifestation of the latter phenomenon can be explained by the fact that the basic soil tillage was carried out across the rows of the harvested sunflower, sown with a row spacing of 0.7 m. In the process of its growing hilling of the sprouts was carried out, which caused the appearance of periodic hillocks on the field at a distance of about 0.7 m. The results of the operational and technological evaluation of a ploughing aggregate, operating according to the 'push-pull' scheme on the basis of the HTZ 16131 tractor, are presented in Table 2.

Table 2. The operational and technological evaluation of a ploughing aggregate, operating according to the 'push-pull' scheme on the basis of the HTZ 16131 tractor

Indicator	Value
Operating conditions:	
– working width, m	2.15
– operation travel speed, km h ⁻¹	7.2
– installed depth of ploughing, cm	25
– length of the furrow, m	1,150
Amount of the performed work, ha	40
Efficiency of the work, ha h ⁻¹ :	
– basic time	1.55
– shift time	1.33
– operating time	1.30
Specific fuel consumption, kg ha ⁻¹	14.4
Operational and technological coefficients:	
– the use of the shift time	0.86
– the use of the operating time	0.84
– reliability of the technological process	0.99
– the use of the operation travels	0.90
The average duration value of one 'pear-shaped' turn, s	53
Width of the turning strip, m	32.1
Agrotechnical indicators:	
– average value of the ploughing depth, cm	25.7
– uniformity of the ploughing depth, ±cm	1.65
– uniformity of the working width, ± cm	6.8
– gaps (stoppages)	none

Analysis of the data, obtained from testing the experimental ploughing aggregate (Table 2), in contrast to the serial ploughing aggregate, showed a 14.7% decrease in labour costs, direct costs by 17.0%, specific capital investments by 10.8%, and reduced costs by 15.8%, indicating undoubted advantages of the aggregates of the 'push-pull' system.

CONCLUSIONS

1. The conducted experimental field investigations show that the advantage of frontal aggregation of agricultural implements with a wheeled HTZ 16131 tractor, makes it possible to create on its basis highly efficient ploughing-machine-and-tractor aggregates operating according to the 'push-pull' scheme. The results of the experimental study provided a possibility to establish that the discrepancy between the theoretical and experimental amplitude-frequency characteristics of the movement is not more than 8%.

2. The ploughing aggregate of such a scheme, composed from the HTZ 16131 aggregating wheeled tractor, a double-furrow frontal and a four-furrow rear plough ('2 + 4' scheme), allows to increase the actual working width by 20.9%. As a result, the efficiency per hour of the basic time of the ploughing aggregate, combined according to the 'push-pull' scheme, was by 19.5% higher than the base machine-and-tractor aggregate with one rear-mounted 5-furrow plough. Due to the achieved higher efficiency of work, the specific metal content of the investigated ploughing aggregate was by 10.7% less.

3. The fluctuations of the furrow path for this aggregate are of a low-frequency nature and are concentrated in a rather narrow frequency range (0–50 m⁻¹). The operating speed of the movement being 2.0 m s⁻¹, this is 0–0.16 Hz. With an operating speed of 2.0 m s⁻¹ this is 0–0.16 Hz. The maximum value of the mutual correlation function between the input impact (the turning angle of the driven wheels of the aggregating tractor) and the output value – its relative bearing, is positive and reaches a value of 0.88.

4. The machine-and-tractor ploughing aggregate, working according to the 'push-pull' scheme, carries out the technological process of ploughing with a higher depth stability of processing. The average square deviation of the ploughing depth of the investigated aggregate is ± 1.52 cm and it is within the allowed agrotechnical limits. The ploughing depth stability of the aggregate, working according to the '2 + 4' scheme, is ± 1.65 cm², which is naturally a smaller value of the same index for the serial ploughing aggregate (± 1.98 cm²).

5. The comparative tests of this ploughing aggregate with the serial one allow reducing: labour costs by 14.7%, direct costs by 17.0%, specific capital investments by 10.8%, reduced costs by 15.8%.

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Experimental study of an improved root crop cleaner from admixtures

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Abstract. One of the ways to raise the quality of sugar beet harvesting is the use of improved digging tools that are able to dig out root crops from the soil without any loss and considerable damage, as well as cleaners of the heap from admixtures. Perspective are the root crop harvesting machines, built according to the modular principle, where, depending on the state of the beet plantation, the composition of the cleaning tools, and the kinematic and technological modes of their operation are determined. To carry out experimental studies, experimental equipment was made which, under laboratory and field conditions, made it possible to obtain qualitative separation indicators of the heaps of sugar beet roots with wide variation in the range of kinematic and design parameters of the improved cleaner. As the results of the laboratory and field experimental studies showed, in each of the two stages of cleaning the beet heap, a sufficiently high degree of removal of the soil admixtures and plant residues is ensured. Thus, at the first, preliminary stage of cleaning, the removal of admixtures amounted to 65.5–75.8%. After the second, basic stage of cleaning, the transported heap contained no more than 1.9% of admixtures. The results of the laboratory and field tests indicate that the proposed design of an improved sugar beet root cleaner from admixtures is prospective.

Key words: sugar beet, harvesting, cleaning, admixtures.

INTRODUCTION

One of the ways to raise the quality of sugar beet harvesting is the development of new trailed (or semi-trailed) root harvesting machines that would be equipped with digging tools suitable for different soil and climatic conditions, equipped with such improved cleaning implements that provide high-quality cleaning of root crops under any conditions of the beet plantation and that are designed according to the so-called modular principle (Halemendik, 2001; Pogorely & Tatyanko, 2004).

Separators of the beet heap should provide steady and qualitative technological process under heavy harvesting conditions, and at various characteristics of the material to be processed. The systems of the separating tools, often used in the sugar beet harvesters and root harvesting machines, do not always ensure a sufficient level of separation of soil and the plant residues from the beet raw materials (Ivančan et al., 2002; Lilleboe, 2014; Bulgakov et al., 2015). This is due to clogging or sticking of the surfaces of the cleaning tools with moist soil and green plant residues (Lammers et al., 2010; Bulgakov et al., 2014).

Many researchers and designers have worked in order to solve the problem of creating reliable and efficient machines for cleaning beet heap (Smith, 1991; Gevko, 1999; Linnik, 2014). However, in spite of a large number of separate works on the improvement of some technological processes of cleaning the beet and potato heaps (Bulgakov et al., 2017) during harvesting – there are no practical studies on the development and operational testing of the separators of a modular design. Consequently, investigations are necessary in this area for the beet harvesting industry and for solving the problem of cleaning the heap of root crops, in general.

The aim of the study is to determine the quality indicators of cleaning heaps of sugar beet root crops after passing through stages 1 and 2 of the improved cleaner of a modular type, and to evaluate the impact of the rotation speed of the screw upon the soil separation quality from the root crop.

MATERIALS AND METHODS

A new design of improved cleaning and transportation implements was developed, which were installed on the trailed root-harvesting machine MKP-6 (the test samples of which were made according to our drawings at the Ternopil Combine Plant (Zykov, 2010). New cleaning and transportation implements were installed (according to the modular principle) in the middle of the self-propelled root harvesting machine.

As can be seen from the presented diagram of the root harvesting machine, the basic cleaning of the sugar beet roots from the soil admixtures and plant residues takes place inside the cleaning drum formed by the driven auger-type or bar rolls, inside of which there is installed with clearance either a transverse screw conveyor, or a drum with arc-shaped cells.

In order to estimate the cleaning efficiency of the sugar beet roots from admixtures during digging, field equipment was designed and manufactured under factory conditions (see Fig. 1). The main components of this experimental equipment are disk diggers 1 with beaters 2, which are installed on serial root crop harvesters, on the improved cleaning device 3–5 and the loading elevator 6 of a new design. The cleaning device consists of 80 mm diameter screw rollers which form an S-shaped cleaning surface, in the cavity of which there is a transverse screw with a diameter of 650–850 mm conveying the beet roots in the axial direction to the discharge conveyor.

After the experimental installation was made, it was tested under field conditions. The economic tests showed positive results (Bulgakov et al., 2014), which made it possible to make equipment in the future with improved cleaning and transportation implements for carrying out experimental tests in order to find out the quality indicators of the cleaning and transportation devices under various harvesting conditions.

To conduct laboratory and field experimental tests, a methodology was developed for conducting field experiments, including the following:

1. The cleaner was conditionally divided into the first and the second stage of cleaning. The first stage of cleaning consists of auger-type rolls 3 (Fig. 1) that rotate in one direction, the second stage consists of screw rolls 4 that have counter-rotational movement and are located inside an S-shaped (in the longitudinally vertical plane) surface of the large-diameter auger 5.

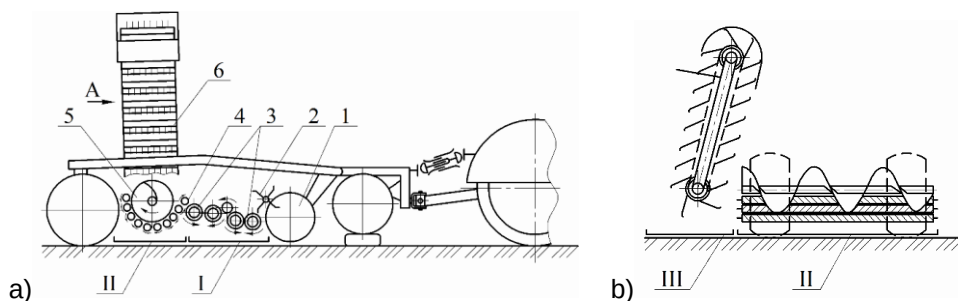


Figure 1. The technological scheme of the laboratory and field experimental equipment (a) and its view A according to arrow (b).

2. The experimental equipment was installed in a stationary manner so that the first stage of cleaning I occupied a horizontal position (see Fig. 1). This was because the root crop of sugar beet, fed as a single specimen, will have a chaotic nature of its movement along the surface of the cleaner (if there is no support for the entire heap), but it needs to be directed along the cleaner to the loading elevator, as it occurs at volume feeding of a heap of root crops to be cleaned. The drive of the operating tools of the experimental equipment was from the power take-off shaft of the tractor which provided conditions for its stable operation.

3. For step-by-step investigation of the cleaning and transportation devices three storage bins (I, II and III) were made (for the first stage of cleaning, for the second stage, as well as for the common discharge of the beet roots from the cleaner) and placed under the cleaning unit. During the laboratory tests, the loading elevator was disconnected from the root-harvesting machine.

4. Massive amounts of soil, together with the root crops, were excavated manually and weighed.

5. When the drive of the root harvester was switched on, the soil masses with the root crops were fed to the first stage of cleaning, opposite the first digger. When the root crops on the cleaner reached the loading elevator, the drive of the operating tools of the machine was switched off. From the storage bins I, II and III, soil and other residues were removed and divided into fractions, each of them being carefully weighed with accuracy to 1 gram, and the measurement data were recorded in a table for their further statistical processing. Further, measurement was made of the cleaning degree of the root crops, fed from the other five diggers.

For laboratory studies the massifs of soil, together with the root crops, were dug out manually, and preliminarily weighed. During these studies, the amount of the screened soil and its remains in the heap were determined.

6. Further investigations were carried out changing the rotation speed of the transverse screw conveyor, which was achieved by means of replacement sprockets of the chain drive.

Experimental field tests of the improved root-harvesting machine were carried out in autumn 2016 at the Ukrainian Scientific Research Institute for Prognostication and Testing of Machinery and Technologies (Kiev Region, Ukraine). During the field tests only the amount of unseparated soil and its quantity on the surface of the root crops were determined.

The necessary number of replications of experiments (N) was determined from the following dependence (Nalimov & Chernova, 1975):

$$N = \text{Integer} \left(\frac{t \cdot v}{\rho} \right)^2 \quad (1)$$

where t – the normalised value of Student's t-test. With a confidence level of 95% ($t = 1.96$); v , ρ – the coefficients of variation and the allowed deviation (the accuracy factor) of the measured parameter.

It is known that for most technical problems it is not necessary to determine the measurement error with accuracy greater than 10% (Venikov, 1986). Proceeding from this, we assumed in the calculations that $\rho = 0.1$.

Previous studies (Halemendik, 2001) have shown that the variation coefficient of the soil screened off during its separation usually does not exceed 12%, that is, the variability of the process is low. Therefore we assumed that $v = 0.12$. As a result, the obtained the following value of the necessary amount of replications of experiments $N = 6$. Thus the investigations were conducted in with a 6-fold replication. At the same time, for each of the six diggers, a pre-weighed massif of the soil with the sugar beet roots was supplied.

For the data obtained during the laboratory-field experimental studies the following statistical characteristics were calculated: the mean value H , g; the mean square deviation σ , g; the coefficient of variation K , %. In this paper approximation of the obtained data by a polynomial of the second degree was carried out, and the Pearson correlation coefficient (R) was calculated (Benjamin & Cornell, 1970; Dospehov, 1985; Tamm et al., 2016).

The data obtained during the laboratory and field experimental tests were statistically processed and presented in the form of graphs.

RESULTS AND DISCUSSION

The results of the efficiency determination of the beetroot cleaner from admixtures are presented in the form of graphs (Fig. 2 and Fig. 3), where the angular speed of the screw rotation is plotted along one axis, the screened and the unscreened soil – along the second axis, which is expressed as percentage of the total soil quantity fed for cleaning and transport devices.

As we can see from the graphs (Fig. 2), the largest mass of residues (65–75%) is separated in the first stage of cleaning. In this case, there is no doubt that the angular speed of rotation of the transverse screw does not significantly affect the cleaning process.

Fig. 3 shows the impact of the angular speed of the screw rotation upon the final quality of cleaning of the sugar beet roots by improved cleaning and transportation implements. These graphs are constructed by measuring the weight of the soil residues that have remained on the heads and bodies of the sugar beet roots at the output of the cleaner.

Figs 2 and 3 showed that, maximum weight of the soil and plant residues after cleaning did not exceed 1.9%. The amount of soil firmly bound with the surface of the sugar beet root did not exceed 2.2% of its total mass. Increasing the angular speed of rotation of the transverse screw reduced the amount of the free soil at the outlet the cleaner. However, increase in the angular speed of the screw, did not lead to improved cleaning of the lateral surfaces of the sugar beet root from the bound soil. Thus, at an angular speed of 22.3 s^{-1} , the amount of the bound soil is only 1.7%, and, increasing the angular speed of rotation to 28.7 s^{-1} , the amount of soil is 2.2%.

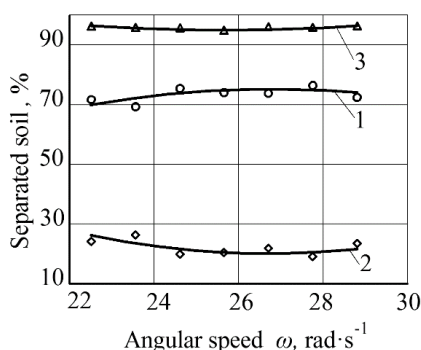


Figure 2. Dependence of the soil sifted off during its separation on the angular speed of rotation of the screw: 1 – the first stage of cleaning ($R = 0.64$); 2 – the second stage of cleaning ($R = 0.68$); 3 – total amount of the separated soil ($R = 0.75$).

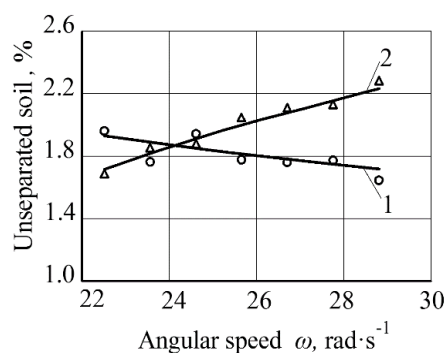


Figure 3. Impact of screw rotation speed upon the separation quality of the soil from the root crop: 1 – the free soil ($R = 0.71$); 2 – the soil bound with the root crop ($R = 0.67$).

The conducted analysis and comparison of the research results under similar conditions of work (Gevko, 1999; Zykov, 2010; Nikitin, 2018) of the root harvesting machines KSP-6; MKP-6, etc. (made in Russia) and the West European firms Holmer, Franz Klein, Agrifak, etc. showed that the quality indicators of their work often do not meet the agrotechnical requirements. For instance, in heavy waterlogged soils (humidity more than 22%), the content of the soil admixtures in the gathered heap reaches 20%. Besides, more than 70% of the soil is in a cohesive state.

Consequently, the improved cleaning and transportation operating tools of the MKP-6 root crop harvesting machine point to a significant advantage over the existing cleaning devices of serially produced root crop harvesting machines because the total number of residues in the heap of the collected root crops did not exceed 3.9%.

Such a combination of the cleaning and transportation tools made it possible to efficiently perform cleaning of the heap of root crops, both from the soil and plant admixtures. So, the counterrotating rolls effectively seize and remove from the heads of root crops the residues of the haulm. They also grab and separate other plant residues. A large-diameter auger, which conveys the root crops in an axial direction, provides

effective cleaning of the lateral surfaces of the root crops from the stuck soil. Together with the small-diameter screw conveyors it creates significant relative movements for sugar beet roots, which essentially affects the duration of their contact with the surfaces of the cleaning operating tools.

CONCLUSIONS

Operational tests and field studies of the experimental sugar beet root cleaner from admixtures (according to the developed program and methodology) showed a higher efficiency of the machine in contrast to the existing commercial machines. The majority of the residues (65–75%) was separated in the first stage of cleaning while the total amount of residues in the heap of the collected root crops did not exceed 3.9%.

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Particle size distribution analysis of pine sawdust: comparison of traditional oscillating screen method and photo-optical analysis

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Abstract. Particle size and particle size distribution (PSD) are crucial parameters which affect properties of particulate and agglomerated materials, and have an impact on a quality and utilization of a final product. The aim of this paper was to determine PSD as well as to assess dimensional features of pine sawdust fractions via mechanical sieve analysis and photo-optical analysis. The first one is a traditional and standard method taking into account only one parameter of particle shape and the second one is a modern method based on a digital image processing that considers also irregular shapes of biomass particles. Pine sawdust was grinded into three fractions: 4, 8 and 12 mm and analysed using two mentioned methods. A horizontal vibrating sieve shaker comprising 11 sieves and a bottom pan was used, and the obtained data of retained particles on each sieve were evaluated. For comparison, a computerized photo-optical particle analyser was applied with max Feret's diameter as a measurement algorithm for a particle length, and PSD was analyzed by grouping the particles according to their distinct lengths adjusted to the sieves' sizes used in the screening method. Moreover, additional results in dimensions and parameters of PSD were obtained and evaluated through the photo-optical method. Pine sawdust particles can be described as non-uniform, mainly prolonged, finer particles dominated in all fraction samples. The study showed differences in the results, inaccuracy and other drawbacks of the conventional sieving method such as clogging and falling-through phenomena as well as the limitations of the machine vision. Strong sides of both methods were discussed, too. Overall, the results contributed to a better knowledge of the material properties and different methods of PSD analysis.

Key words: computerized particle analyzer, image analysis, mechanical sieving, machine vision, particle size classification.

INTRODUCTION

Obligations to comply stated norms of EU directives on green energy production and the increasing demand for biofuels from various organic waste materials including forest biomass go hand in hand with a necessity of a better knowledge about an input material's properties (Gendek et al., 2018). Particle size and particle size distribution (PSD) are listed among the main physical factors influencing different properties of particulate and agglomerated materials, and provide important information about overall material's/product's quality and performance. They significantly affect flow ability and

handling, compaction, compressibility, bulk density, strength and durability of densified products (Pietsch, 2008; Tumuluru et al., 2011; Guo et al., 2012; Shanthi et al., 2014; Zhang & Guo, 2014; Febbie et al., 2015; Muntean et al., 2017; Chaloupková et al., 2018). Due to the fact that biomass is comprised of diversely shaped and sized particles (Guo et al., 2012; Febbi et al., 2015) it is essential to control and measure the PSD precisely and rapidly to secure the high-quality final products (Igathinathane et al., 2009a).

PSD analysis is a procedure assessing dimensional and morphological characteristics of particulate materials (Igathinathane et al., 2009a; Vaezi et al., 2013). Commonly, the results of PSD analysis indicate percentage of particles retained on the sieves, cumulative undersize distribution, geometric and arithmetic mean value, and the related standard deviation as well as other parameters depending on an applied method (Igathinathane et al., 2009a). PSD of biomass material is standardly determined by the mechanical sieving/screening procedure (UNE-EN ISO 17827-1:2016, 2016), where the material is separated by sieves of different sized apertures/openings. A number of studies have reported the PSD results of different biomass materials, e.g. wheat straw, switchgrass, corn stover (Bitra et al., 2009), barley straw (Mani et al., 2006), *Cynara cardunculus* L. (Igathinathane et al., 2009b), miscanthus, pine sawdust (Igathinathane et al., 2009b; Chaloupková et al., 2016), wood sawdust and shavings mixture (Vítěz & Trávníček, 2010), industrial wood particles (Li et al., 2014) and hemp (Dinh, 2014; Chaloupková et al., 2016). The conventional method considers only one parameter: general particle shape. This is given by the aperture of a sieve (no detailed individual results of particles' lengths, width or shapes could be obtained), thus it is solely suitable for spherical particles (Igathinathane et al., 2009a). Although biomass particles are characterized by highly irregular sizes and shapes (Guo et al., 2012; Febbi et al., 2015), these irregularities increase errors in the PSD estimation (Shanthi et al., 2014). Therefore, many authors propounded that more precise results could be acquired by machine vision and image analysis (Igathinathane et al., 2009a; Igathinathane et al., 2009b; Souza & Menegalli, 2011; Kumara et al., 2012; Vaezi et al., 2013; Gil et al., 2014; Febbi et al., 2015). However, the results of the conventional sieving method and the advanced photo-optical analysis were not found to be confronted in one study before, and selected machine vision method was not applied for pine sawdust.

The aim of the present study was to compare two PSD analysis methods: conventional screening vs. photo-optical measuring. While photo-optical method is efficient and time saving, it is not yet to be standardized employing different biomass types. Three fractions of pine sawdust were tested for the purpose of this study.

MATERIALS AND METHODS

Pine sawdust (*Pinus* L.), a traditional wooden feedstock material for production of densified biofuels (Deac et al., 2015) was used in the present study. The material was obtained from the Czech Republic and for comparison purposes it was grinded by a hammer mill (model 9FQ-40C, Pest Control Corporation, Vlčnov, Czech Republic) into three different initial fraction sizes of 4, 8 and 12 mm. Shape of obtained particles was irregular and prolonged/elongated particles were predominated, as it was identified by the analysis of sphericity (the overall particle shape and similarity to a sphere) and roundness (the description of the particles' corners) within a photo-optical analysis

method. Moisture content (w.b.) of 4 mm, 8 mm and 12 mm samples was 9.91%, 8.82%, and 10.35%, respectively.

PSD of the pine sawdust fractions was determined by the sieve analysis according to a valid standard (UNE-EN ISO 17827-1:2016, 2016). A horizontal vibrating sieve shaker Cisa (model RP 08, Mervilab, S.A., Madrid, Spain) comprising 11 standard calibrated sieves with the diameter of 20 cm and opening sizes of 16.0, 8.0, 3.15, 2.8, 2.0, 1.4, 1.0, 0.5, 0.25, 0.125, 0.063 mm, and the bottom pan was used. During the sieve analysis, a representative weighed sample was poured into the top sieve with the largest screen opening size, and 30-minute sieve shaking time and amplitude 3.0 mm g⁻¹ were applied. After the sieving process the material retained on each sieve was analysed. The percentage of the material retained on any sieve was found by the Eq. 1 below. Three repetitions were performed for each fraction (with sieving loss error approx. 0.3%) and the average value was considered as the final result.

$$\% \text{ Retained} = \frac{W \text{ Sieve}}{W \text{ Total}} \times 100\% \quad (1)$$

where W Sieve – weight of the material in the sieve (g); W Total – total weight of the material (g).

For comparison, a computerized photo-optical particle analyser Haver (model CPA 4–2, Haver & Boecker OHG, Oelde, Germany) was used to analyse PSD together with the other particle characteristics (number of analysed particles, maximum and minimum particle length, average particle size and amount of variation). The analyzer worked under the particle measuring range from 0.091 up to 90 mm, which was selected with respect to the material character (according to the manual another measuring possibility is a range of 0.035–15 mm, but it is suitable mainly for fine materials like ash). The analyser consisted of a feeding unit with the high of 6 mm being set for the regular particle spread on a vibration channel, a vibratory channel itself, a CCD-line digital scan camera with the high-resolution (4,096 pixels line resolution), which scanned all free-falling particles of the studied samples against the background of a LED lighting array module with a high recording frequency (up to 28,000 line scans per second) (Haver & Boecker, 2015). Amplitude of the vibrating feeder was automatically regulated by the analyzer. All individual particles were measured, and their profile parameters processed via Haver CpaServ software (Haver & Boecker OHG, Oelde, Germany). The suitable shape of analysed sample was assigned as an elongated. The data were transferred into spreadsheet for further analyses. The maximum Feret's diameter was set as a measurement algorithm (Fig. 1) for particle length. This parameter gives the value of the minimum sieve size through which the particle can pass through without any obstacle (Shanthi et al., 2014). To compare PSD results with the conventional sieve method, the data were grouped into the groups in accordance with the particles' distinct lengths corresponding to the sieve sizes used in the screening method. PSD analyses

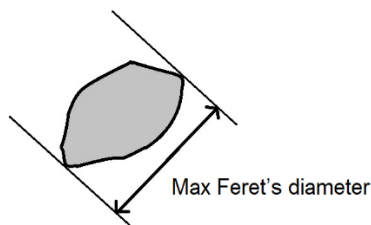


Figure 1. Max Feret's diameter as a measurement algorithm of a particle size (length).

were confronted as the percentages of particles' weights retained on the sieves and the percentages of particles' numbers retained on virtual sieves.

The data from both analyses were processed using MS Excel (version 2007, Microsoft, Redmond, WA, USA) and Statistica software (version 13.3, TIBCO Software Inc., Palo Alto, CA, USA); afterwards the obtained results were tabulated, graphically plotted and discussed.

RESULTS AND DISCUSSION

Oscillating screen analysis

The weight values and the percentage weight values of different particle sizes of sawdust fractions obtained by sieving analysis are presented in the Table 1, together with the cumulative percentage of the material's weight. Fig. 2 presents the plotted cumulative percentage values.

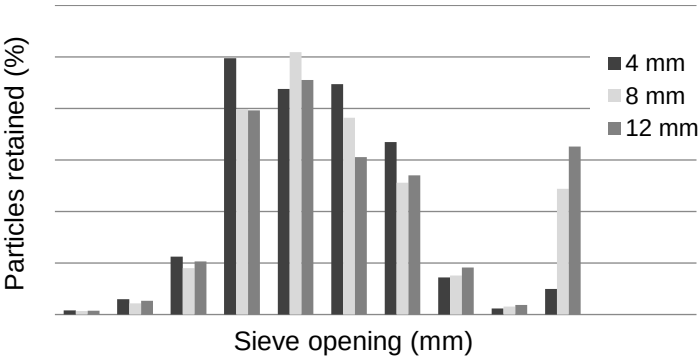


Figure 2. Plotted comparison of PSD of examined fractions via the sieve analysis.

Table 1. Tabulated PSD of the examined material's fractions via the sieve method

Sieve opening size (mm)	4 mm fraction			8 mm fraction			12 mm fraction		
	g	%	Cum.%	g	%	Cum.%	g	%	Cum.%
16.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3.15	0.99	2.48	2.48	4.90	12.20	12.20	6.56	16.29	16.29
2.80	0.23	0.58	3.06	0.31	0.77	12.97	0.38	0.94	17.23
2.00	1.44	3.61	6.67	1.52	3.79	16.76	1.84	4.57	21.80
1.40	6.68	16.73	23.40	5.14	12.80	29.56	5.44	13.51	35.31
1.00	8.93	22.36	45.76	7.67	19.10	48.66	6.15	15.27	50.58
0.50	8.74	21.89	67.65	10.22	25.45	74.11	9.17	22.77	73.35
0.25	9.93	24.87	92.52	8.00	19.93	94.04	7.98	19.82	93.17
0.125	2.24	5.61	98.13	1.81	4.51	98.55	2.07	5.14	98.31
0.063	0.59	1.48	99.61	0.44	1.10	99.65	0.53	1.32	99.63
<0.063	0.16	0.40	100.00	0.14	0.35	100.00	0.15	0.37	100.00

And as it can be seen from the results (Table 1, Fig. 2), all fraction samples had very fine particles. Majority of the material comprised of the particles with a size between 0.25 and 1.4 mm; for 4 mm fraction it was 86% of the material, for 8 and 12 mm

it was 71% and 77%, respectively. Privious study of Chaloupková et al. (2016) also determined that the pine sawdust fraction of 12 mm consists mainly of the particles smaller than 1.5 mm.

Sieves 8.0 and 16.00 mm did not catch any material. The minimum of the material was captured by the sieve 2.8 mm as well as by the smallest sieve 0.063 mm and the bottom pan. Besides, decreased screens' opening size resulted in decreased particle sizes partly.

Photo-optical analysis

Number of particles determined by the photo-optical procedure and grouped based on sieve sizes used in the screening method, together with the percentage values and cumulative percentage values are presented in the Table 2 and Fig. 3.

Table 2. Tabulated comparison of PSD of the examined fractions via the photo-optical analysis

Sieve opening size (mm)	4 mm fraction			8 mm fraction			12 mm fraction		
	N	%	Cum.%	N	%	Cum.%	N	%	Cum.%
16.00	24	0.00	0.00	22	0.00	0.00	39	0.01	0.01
8.00	747	0.10	0.10	638	0.12	0.12	830	0.15	0.16
3.15	29,648	4.03	4.13	18,887	3.47	3.59	20,253	3.78	3.94
2.80	12,419	1.69	5.82	8,173	1.50	5.09	8,196	1.53	5.47
2.00	62,227	8.46	14.28	41,271	7.58	12.67	42,031	7.84	13.31
1.40	124,805	16.97	31.25	88,805	16.31	28.98	89,291	16.65	29.96
1.00	167,081	22.72	53.97	127,499	23.42	52.40	124,463	23.21	53.17
0.50	263,131	35.78	89.75	204,136	37.49	89.89	193,457	36.08	89.25
0.25	49,767	6.77	96.52	36,670	6.74	96.63	38,071	7.10	96.35
0.125	25,599	3.48	100.00	18,349	3.37	100.00	19,527	3.64	99.99
0.063	0.00	0.00	100.00	0.00	0.00	100.00	0.00	0.00	99.99
<0.063	0.00	0.00	100.00	0.00	0.00	100.00	0.00	0.00	99.99

Table 2 and Fig. 3 showed that the results of all fractions are very similar/more precise. PSD can be described as non-uniform with the finer particles dominated. In the case of photo-optical analysis, the majority of the material composed of the particles with a size between 0.5 and 1.4 mm, where more than one third of the material had the length of 0.5 mm.

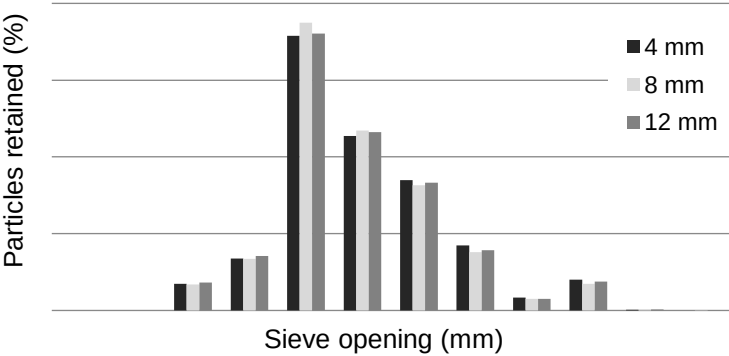


Figure 3. Plotted comparison of PSD of examined fractions via the photo-optical analysis.

According to Shanthi et al. (2014) max Feret’s diameter used in image analysis as a measurement algorithm for the particle length which gives a high level of accuracy in case of irregular shapes, thus it can replace the sieve analysis method very precisely (Fernlund, 1998; Al-Thyabat & Miles, 2006, Hamilton et al., 2013).

Additional to photo-optical analysis, Table 3 provides detailed statistics about the particles, i.e. total number of analysed particles, arithmetic mean of a particle length, together with the maximum and minimum length values. Average particle length was 1.26 mm. Minimum measured length for all fractions was 0.1257 mm which can be explained and limited by the given minimum measuring range of the photo-optical analyser (0.091 mm). Maximum length of the particles was over 20 mm. Although just the minimum amount of the material had the length over 8 and 16 mm, these particles were not measured by the sieve analysis at all, most probably due to a ‘falling-through’ effect of prolonged particles through the smaller sieve apertures (Igathinathane et al., 2009b; Chaloupková et al., 2016).

Table 3. Descriptive statistics of the particle size

Fraction size	N of particles	Mean	Std. Dv.	Min. length	Max. length
4 mm	735,448	1.2812	0.9183	0.1257	20.0739
8 mm	544,450	1.2433	0.8936	0.1257	21.2136
12 mm	536,158	1.2638	0.9421	0.1257	20.5417

Comparison between mechanical sieving and machine vision analysis

PSD analysis from the both procedures expressed as the percentages of particles retained on sieves and cumulative particles retained is presented in the Figs 4–6, for each fraction separately. The comparison of both methods was made with respect to the weight percent and the number percent.

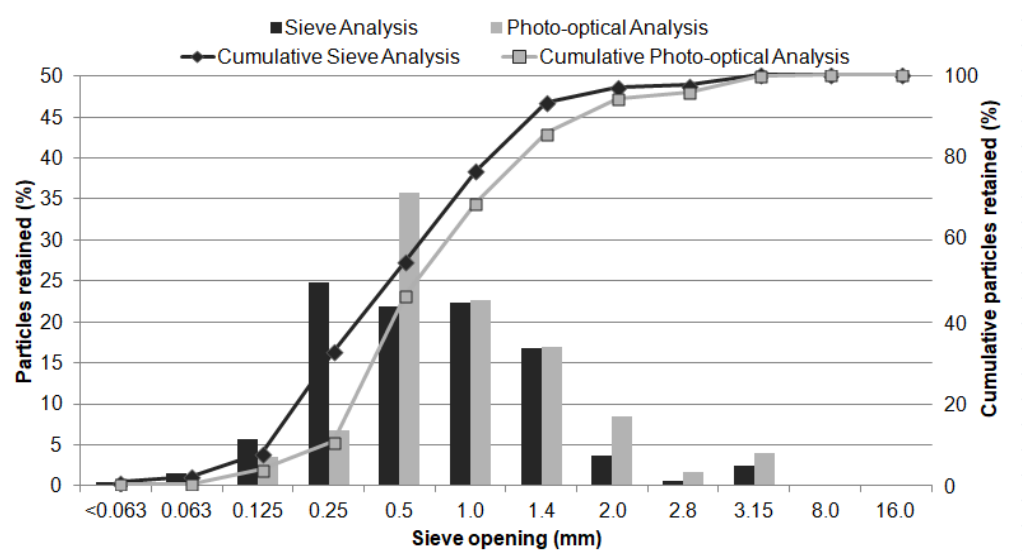


Figure 4. PSD analyses of pine sawdust fraction 4 mm.

The analyses did not show fully comparable results. And, they also confirmed a higher precision of photo-optical method and more possibilities in measurements. In accordance with Igathinathane et al. (2009a) mechanical sieving is an effective method in case of uniform spherical particles, what for the results are not so reliable in our case of prolongly shaped particles. From the presented comparisons, a consistent less number of particles retained for all fractions in the bottom pan with mechanical scening compared to machine vision method was not caused by sieve clogging phenomenon as it is reported by Igathinathane et al. (2009a) and Glé at al. (2013). Clogging phenomenon could be observed for 3.15 mm sieve in case of 8 and 12 mm fraction (Figs 5 and 6).

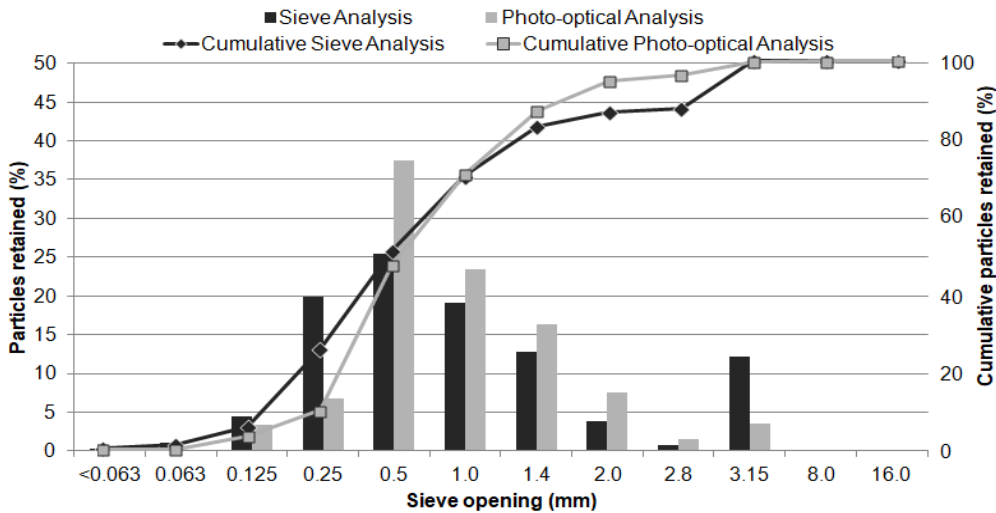


Figure 5. PSD analyses of pine sawdust fraction 8 mm.

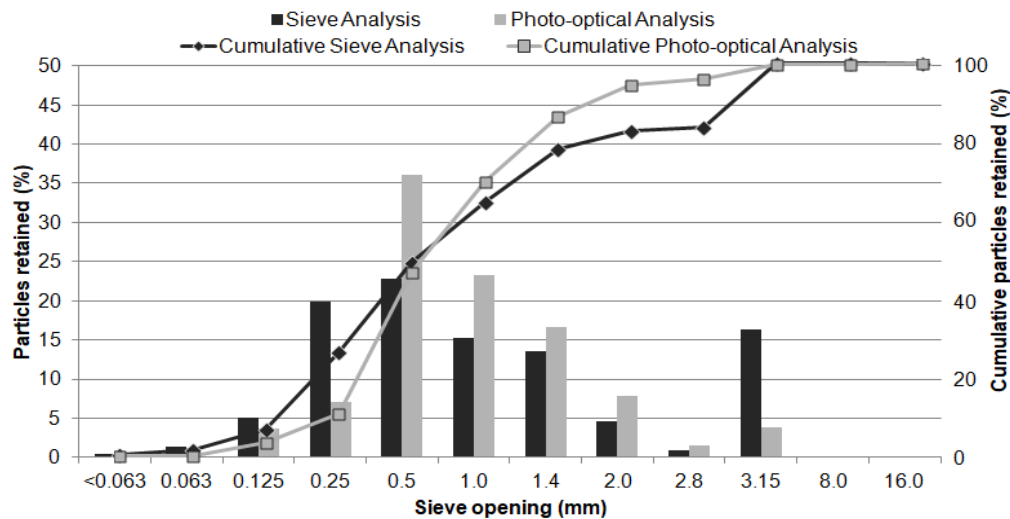


Figure 6. PSD analyses of pine sawdust fraction 12 mm.

The Figs 4–6 also indicated the mentioned ‘falling-through’ effect of sieve analysis, which was previously detected by Igathinathane et al. (2009b) and Chaloupková et al. (2016). As it can be seen, starting from the sieve 2.8 mm (more markedly from the sieve 2.0 mm) physically longer particles passed through the sieves with the smaller apertures and passed until the sieve 0.125 mm, where the sieve 0.125 and especially the sieve 0.25 mm captured significantly more material than in a reality should retain. In case of the last sieve (0.063 mm) and the bottom pan it was not possible to compare the results of two methods due to the limited measuring range of the photo-optical analyzer.

Besides the clogging phenomenon and the falling-through effect, sieve analysis is a time-consuming process, particles are not measured individually and their shape highly affects the final result (Fernlund, 1998; Febbi et al., 2015). It also has limited set of available standard sieves and limited number of sieves held in the shaker. On the other hand, classical sieve analysis is an easy, simple, standardized and inexpensive tool (Al-Thyabat & Miles, 2006; UNE-EN ISO 17827-1:2016, 2016) giving a possibility to physically separate the particle size fractions. In comparison, photo-optical analysis based on a machine vision and an image processing provides more accurate and precise PSD analysis results, time savings, particles are examined individually, and it gives an additional information relating to shapes and the number of particles. On the contrary, photo-optical analysis is associated with higher investment costs, only two dimensional projection of the particles is captured and measured, and the method does not provide the possibility of separation of the particle size fractions (Fernlund, 1998; Igathinathane et al., 2009a). Also, as it was observed in this study, the analysis was limited by the minimum measuring range.

CONCLUSIONS

Particle size and PSD are both important factors that influence the final product’s quality. In this study PSD analysis of pine sawdust fractions (4, 8 and 12 mm) was conducted using the photo-optical analyzer based on digital image processing and the conventional method based on sieving.

In case of sieve analysis, the material was spread and caught mostly on the sieves with the opening sizes between 0.25 mm and 1.4 mm; the photo-optical analysis showed that the material was comprised of the particle with the size between 0.5 mm and 1.4 mm, and the particle length of 0.5 mm was greatly predominant. Inequalities of used methods were caused by the clogging phenomenon and ‘falling-through’ effect of longer particles through smaller sieve apertures observed within the sieve analysis that was influenced by the prolonged shape of analysed particles.

During application of both methods their merits and drawbacks were reported. The procedure of sieve analysis is easy and standardized; on the contrary the results were less accurate and consistent owing to the non-spherical particle shape. The photo-optical analysis is fast, and it provided extensive and more precise results, however, the possibility of separation of particle size fractions is missing and the measuring range is limited.

ACKNOWLEDGEMENTS. This research was supported by the Internal Grant Agency of the Faculty of Tropical AgriSciences (FTA), Czech University of Life Sciences, Prague [grant number 20175011 and 20185011] and the mobility training grant of FTA provided through the International Relation Office. Acknowledgement also goes to Miguel Fernández Llorente from the laboratory of CEDER-CIEMAT for his assistance and valuable advice during the photo-optical analysis.

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Comparative characteristics of antioxidant capacity of some forage plants of the Baltic Sea Region (a case study of the Kaliningrad Region and Estonia)

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Abstract. In this paper, we investigate changes in the antioxidant levels (anthocyanins, leucoanthocyanins, catechins) and the total water-soluble antioxidants capacity in forage plants in relation to their geography, i.e. proximity to northern or coastal areas. We demonstrate that the antioxidant content increases in unfavorable conditions, being higher in plants growing closer to the sea and in northernmost plants. Thus, since the total water-soluble antioxidants capacity is influenced by ecological factors, it may be used as one of the indicators in complex environmental assessment.

Key words: water-soluble antioxidants, anthocyanins, leucoanthocyanins, catechins.

INTRODUCTION

The Baltic Sea Region countries: Poland, Lithuania, Latvia and Estonia are EU members geographically closest to Russia. Recently, they have been largely contributing to the implementation of European standards and regulations in industry and agriculture, especially in the border regions. For example, Russia is now developing its own system of field certification and precise farming, not unlike the one actively implemented by the Baltic EU member states. At the same time, the criteria for assessing quality of cultivated farmland crops are constantly being updated. The total water-soluble antioxidants capacity in agricultural crops can be used as one of such criteria (Noormets et al., 2010).

Antioxidants are sensitive components of plants, whose levels in a given plant is directly related to the state of environment (pollution of the soil and/or air with xenobiotics) and ecological conditions that determine the growth and development of plants (temperature, light, mineral nutrition) (Chupakhina, 2009). This topic is especially relevant for coastal areas, due to climatic features and specific vegetation period of plants (Chupakhina et al., 2014).

In addition, plant antioxidants are valuable biologically active compounds, with diverse biological effects. We distinguish between antioxidant enzymes and non-enzymatic (low-molecular-weight) antioxidants. The latter can be hydrophilic and hydrophobic. The hydrophilic antioxidants include ascorbic acid, reduced glutathione, and bioflavonoids. There are also fat-soluble antioxidants, such as ubiquinone, carotenoids, tocopherols. The protective effect of antioxidants is related to their ability to utilize free radicals in cells thus preventing premature aging and development of various diseases: atherosclerosis, different types of cancer, neurodegenerative and cardiovascular diseases (Maslennikov et al., 2014).

Thus, identifying plants that are high in antioxidants becomes an important task. Understanding antioxidant value of different plants is necessary not only for characterizing the quality of plant food, but also for identifying plants that can be used as nutritional supplements, functional food or genetic engineering material.

The purpose of this research was to investigate the effect of environmental factors on the total water-soluble antioxidants capacity, as well as the individual antioxidant levels of plants in meadow plant communities in the of the Baltic Sea coast regions and countries (Kaliningrad region, Russian Federation) and the Puhja municipality (Estonia).

RESEARCH SUBJECT AND METHOD

Our research concentrated on forage plants: timothygrass (*Phleum pratense* L.), red fescue (*Festuca rubra* L.), cock's-foot (*Dactylis glomerata* L.), white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), bush vetch (*Vicia sepium* L.), cow vetch (*Vicia cracca* L.), large-leaved lupine (*Lupinus polyphyllus* Lindl.); and on the plants with medicinal properties: dandelion (*Taraxacum officinale* Webb.), yarrow (*Achillea millefolium* L.), nettle (*Urtica dioica* L.) and curly dock (*Rumex crispus* L.). Plant samples from the Kaliningrad region were harvested in the Zelenogradsky urban district (near the village of Roshchino) located 2 to 4 km from the sea (coastal zone), and in the Gusevsky urban district (near the city of Gusev), located 160 to 170 km inland (continental zone). In Estonia, plant samples were collected in the Puhja municipality (aproximately 25 km from Tartu), 140 to 150 km inland.

To assess the antioxidant status of plants we determined the total water-soluble antioxidants capacity and the levels of anthocyanins, leucoanthocyanins, and catechins. The analysis was carried out on freshly harvested leaves.

Total contents of antioxidants have been estimated by an amperometric method using a TsvetYauza-01-AA (NPO Khimavtomatika Inc., Moscow, Russia) according to Yashin (Yashin, 2008). Plant extract preparation: 0.2–0.5 g of plant material was homogenized with 50 mL of eluent (solution of phosphoric acid with the molar concentration of 2.2 mM). The mixture was then filtered and used for analyse in day of preparation. Amperometric method is based on measuring an electric current in the detector cell which occurs during oxidation of the extract on the working electrode surface when certain potentials are applied. The signal was recorded as differential output curves. The areas or peak heights (of the differential curves) were calculated for the extract and for the reference substance. The average value of three to five consecutive measurements was used for the analysis. The calibration curve was made by preparing quercetin solutions at different concentrations.

The total anthocyanins content was determined spectrophotometric after their extraction from plant samples. Plant extract preparation: 0.3–0.5 g plant material was homogenized with 10 mL of extraction solvent (1% HCl water solution). The extracts were centrifuged for 30 min at 4,500 rpm. The optical density of the above supernatant was determined at 510 nm (UV-3600, Shimadzu, Japan). To correct for the content of green pigments P.V. Maslennikov suggested to consider the optical density of the extracts obtained at 657 nm. As a standard the solution of cyanidin-3-glucoside was used (Chupakhina et al., 2016).

To determine the leucoanthocyanins and catechins, the weighed portion of the plant material was homogenized in the presence of an acidified 96% ethanol (20:1). The homogenate was centrifuged at 4,500 g for 30 minutes. The content of leucoanthocyanins was measured by Butanol-HCl assay. 1 mL of the supernatant was placed into test tubes with 19 mL of a 5% solution of HCl in n-butanol. The resulting solution was vortexed. The tubes were placed in a boiling water bath for 50 minutes, after thermostating the tubes were cooled. The absorbance at 520 nm was recorded using Shimadzu UV3600 (Japan). Cyanidin-3-glucoside was used as a standard (Feduraev et al., 2011).

The vanillin method based on the reaction of vanillin with condensed tannins which lead to formation of colored complexes was used for determination of catechins. In pre-prepared tubes with 4 mL of vanillin reagent and hydrochloric acid (2.5 mL of a 5% alcohol solution of vanillin + 47.5 mL of concentrated HCl) was poured 1 mL of supernatant – starting with blank solution. The contents of each tube were mixed and transferred to the cuvettes. The absorbance was measured 5 minutes later after adding the extract to the vanillin reagent, the reference solution was used as a control. The measurements were carried out at a wavelength of 520 nm. (+)- Catechin was used as a standard (Feduraev et al., 2011).

The content of the tested antioxidants in plant samples was shown in mg g⁻¹ of dry weight (for the total water-soluble antioxidant capacity and catechins in curly dock), and in mg 100 g of dry weight (for anthocyanins, catechins and leucoanthocyanins).

All measurements were performed in Natural Antioxidants Laboratory of the Immanuel Kant Baltic Federal University.

One-way analysis (ANOVA) was performed using the SigmaPlot 12.3 (Systat Software GmbH, Erkrath, Germany). Before ANOVA data were checked for normality and the homogeneity of variance. To identify the difference between species in different region the 1-factorial ANOVA was conducted for each zone separately. And then the reliability of differences in means of antioxidants content between different regions for each plant species was calculated. Difference among means were determined by Tukey's test at a significance level of $p < 0.05$. The results were reported as mean $\pm$ standard deviation (SD).

RESULTS AND DISCUSSION

The total water-soluble antioxidants capacity in forage plants of meadow plant communities in the Kaliningrad Region, located at different distances (2–4 km and 160–170 km) from the sea are shown in Fig. 1.

The total water-soluble antioxidant capacity was significantly higher in cock's-foot, timothy-grass, red clover and large-leaved lupine growing in close proximity to the Baltic coast. We found no major differences in water-soluble antioxidant capacity in white clover and cow vetch. Red clover ranked the highest in total water-soluble antioxidant capacity: with more than 2.5 mg g it had up to 5 times the capacity of other plant species.

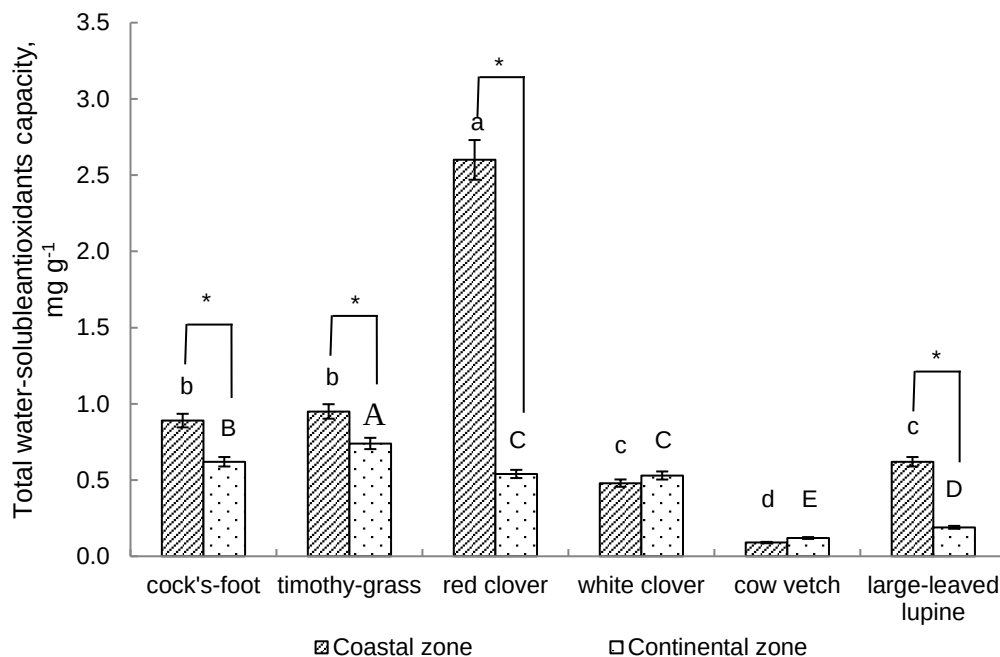


Figure 1. Total water-soluble antioxidant capacity in plants of the Kaliningrad region at different sites. Different lower case letters indicate significant differences among plant species in coastal zone, upper case letters indicate significant differences among plant species in continental zone ($p < 0.05$), asterisk * indicate significant differences among coastal and continental zone ($p < 0.05$) based on post hoc Tukey's tests.

Anthocyanins were higher in timothy-grass, cock's-foot and curly dock from meadow plant communities of the coastal part of the region compared to those growing in the continental zone (Fig. 2). In general, we found the greatest amount of anthocyanins in the leaves of curly dock in coastal zone (more than 5 mg per 100 g).

We also looked at catechins content in plants of meadow plant communities at different distances from the Baltic Sea. It was established that the most coastal zone samples had higher catechins values (Fig. 3). However, timothy-grass plants from continental zone were characterized by higher content of catechins. It is necessary to note there was no significant difference between catechins contents in curly dock of coastal and continental zones. Curly dock had the highest accumulated catechins content - up to 400 mg 100 g, almost 50 times higher than in the other plants.

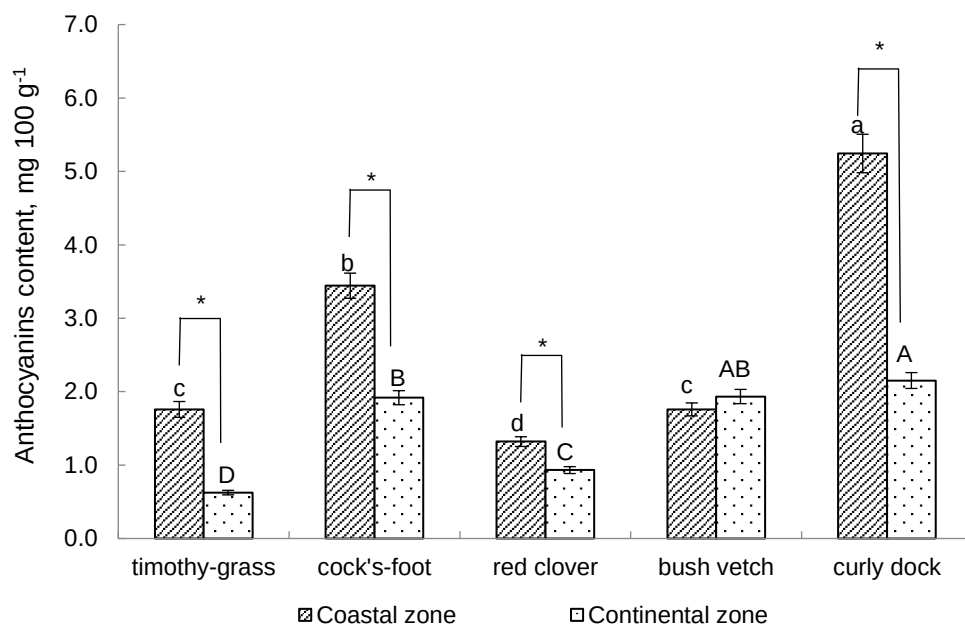


Figure 2. Anthocyanins content in plants of the Kaliningrad region at different sites. Different lower case letters indicate significant differences among plant species in coastal zone, upper case letters indicate significant differences among plant species in continental zone ($p < 0.05$), asterisk * indicate significant differences among coastal and continental zone ($p < 0.05$) based on post hoc Tukey's tests.

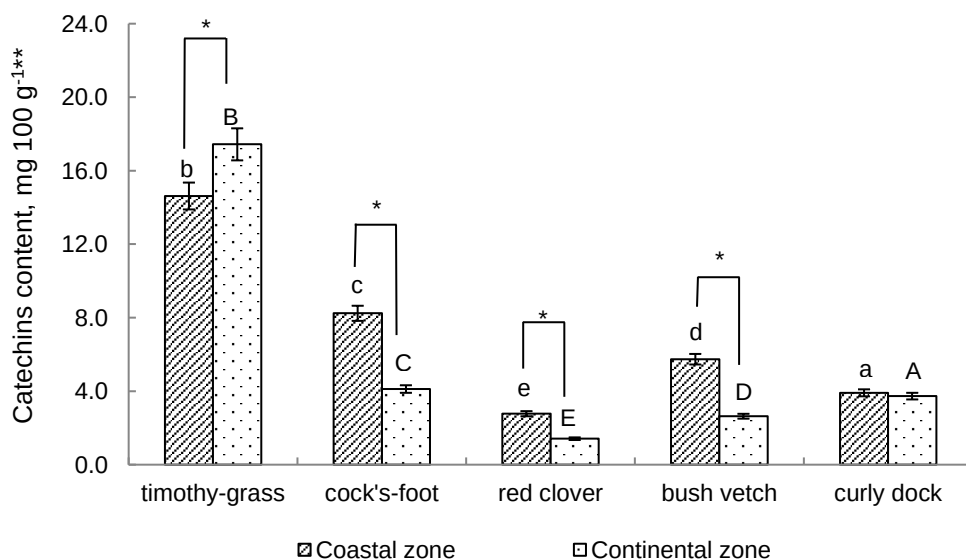


Figure 3. Catechins content in plants of the Kaliningrad region at different sites (** – catechins content in curly dock are shown in mg g⁻¹). Different lower case letters indicate significant differences among plant species in coastal zone, upper case letters indicate significant differences among plant species in continental zone ($p < 0.05$), asterisk * indicate significant differences among coastal and continental zone ($p < 0.05$) based on post hoc Tukey's tests.

A comparative study of leucoanthocyanins showed that their levels were higher in curly dock, cock's-foot, timothy-grass and bush vetch harvested in the coastal area (Fig. 4). Curly dock showed the highest content of these compounds, more than 35 mg 100 g⁻¹.

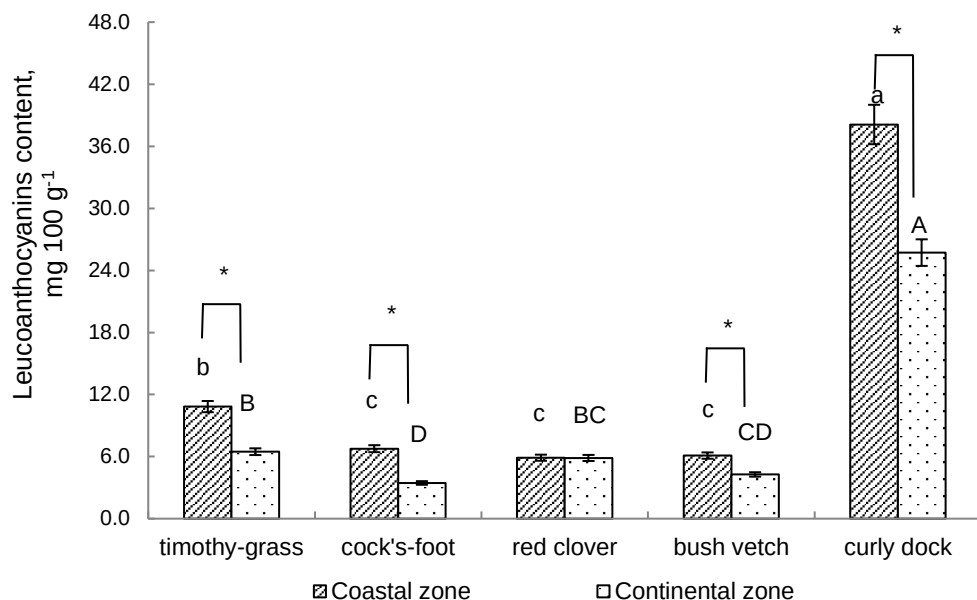


Figure 4. Leucoanthocyanins content in plants of the Kaliningrad region at different sites. Different lower case letters indicate significant differences among plant species in coastal zone, upper case letters indicate significant differences among plant species in continental zone ($p < 0.05$), asterisk * indicate significant differences among coastal and continental zone ($p < 0.05$) based on post hoc Tukey's tests.

The results of the studies of total water-soluble antioxidants capacity in fodder plants of meadow plant communities in the territory of the Puhja municipality, Estonia, as well as a comparison of the collected data with the results for the samples of the same plant species harvested near the city of Gusev in the Kaliningrad region, also located more than 150 km inland are shown in Fig. 5.

The data presented in Fig. 5 shows that plants growing in Estonia generally display higher total water-soluble antioxidants capacity. Reliably higher values were established for all plant species, except dandelion and nettle, where the difference in the antioxidant content between the plants of the Kaliningrad region and Estonia was statistically inconsistent. The same species of plants were characterized by a generally lower content of water-soluble antioxidants, compared to other plant species both in Estonia and in the Kaliningrad region.

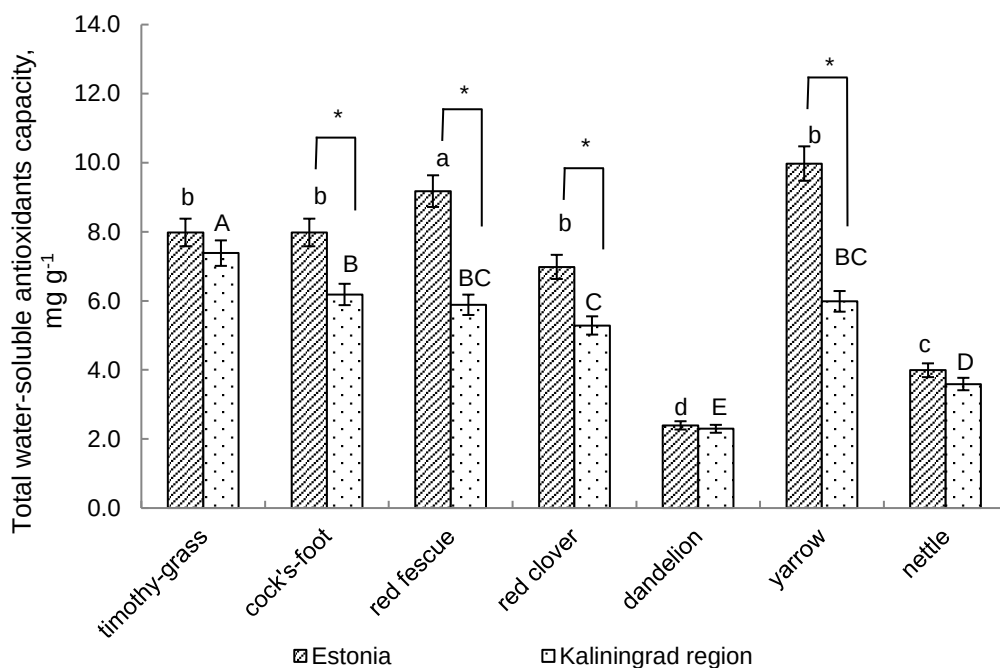


Figure 5. Comparative characteristics of the total water-soluble antioxidants capacity in the meadow plant species of the Puhja municipality (Estonia) and the vicinity of Gusev (Kaliningrad region). Different lower case letters indicate significant differences among plant species in Estonia, upper case letters indicate significant differences among plant species in Kaliningrad region ($p < 0.05$), asterisk * indicate significant differences among Estonia and Kaliningrad region ($p < 0.05$) based on post hoc Tukey's tests.

A study of the accumulation of antioxidants in plants of the Kaliningrad region at different sites showed that almost all plant species of the coastal zone (cock's-foot, timothy-grass, red clover, large-leaved lupine) had higher antioxidant content. In these plants, the total water-soluble antioxidants capacity (Fig. 1) was higher (or significantly, up to 5 times higher, as in the case of red clover), and the level of individual antioxidants – anthocyanins (Fig. 2), catechins (Fig. 3), leucoanthocyanins (Fig. 4) – was also higher. The climate features are illustrated by the Table 1. The Baltic Sea creates special microclimatic conditions in this part of the region: the wind is constant and is generally stronger, the summer temperatures are relatively low and the humidity is high (Dedkov & Fedorov, 2015). Thus, increased levels of antioxidants in the coastal plants may probably be explained by the antioxidants' protective functions. It is known that under the influence of stress created, for example, by low temperatures, the content of ascorbic acid (Golovina et al., 2008), anthocyanins (Chon et al., 2012), polyphenols (Ramakrishna & Ravishankar, 2011) increases in plants. The results obtained in this study are consistent with the findings of a previous study of the total water-soluble antioxidants capacity in forage plants at different sites of the Kaliningrad region, which also revealed a direct correlation between the distance from the coast and the level of antioxidants in plants (Chupakhina et al., 2013). The protective function of antioxidants can explain a higher level of water-soluble antioxidants in plants growing in Estonia

(near Tartu), located further north from the Kaliningrad region (58° 22'00"N and 54° 35'00" N, respectively).

Table 1. Climatic parameters in different zones (coastal and continental) of the Kaliningrad Region and Estonia for the summer period (April – October)

	Zone	Average temperature, °C	Precipitation, mm	Average air humidity, %	Average wind speed, m s ⁻¹
Kaliningrad	Coastal	18.7	525	76	5–6
	Continental	21.4	475	70	3.5–4
Estonia*	Coastal, Vilsandi	11.9	358	81	5.6
	Continental Tartu-Tõravere	11.8	457	75	2.9

Source of data: *The characteristics of Estonian climate are based on the database of the Estonian Weather service (Riigi Ilmateenistus, 2018) of the Kaliningrad region the climate characteristics are based on the information from the Hydrometeorology and Environmental Monitoring Center of Kaliningrad (Maslennikov et al., 2018).

Moreover, the results obtained during the study give the opportunity to consider the level of antioxidants as an indicator of the state of the environment. For this purpose, either total water-soluble antioxidant capacity or concentrations of individual antioxidants could be used. The greatest change under the influence of environmental factors is established for the content of anthocyanin pigments. It must be noted, however, that such change is not specific to this cause. A number of studies have shown an increase in the number of anthocyanins stimulated by a deficiency of mineral components in the soil (Henry et al., 2012; Msilini et al., 2014), by various pollutants (Chupakhina & Maslennikov, 2004; Maslennikov et al., 2013), high light intensity (Zhang et al., 2010), or by UV irradiation (Gao & Yang, 2016). Therefore, an integral indicator (total water-soluble antioxidant capacity) is better suited for judging the state of the environment.

Among the studied so far plants, legumes were the most sensitive to environmental changes. The pool of water-soluble antioxidants in legumes was less stable than in grasses (Poaceae), where the level of antioxidants was less dependent on whether the plant community was coastal or not. Since accumulation of antioxidants is species-specific (Maslennikov et al., 2014), comparative studies of plants in different climatic and/or environmental conditions should focus on a single species to eliminate inter-species variations of indicator values. For the region in this study the species of choice can be red clover.

CONCLUSION

We have demonstrated that the antioxidant status of plants species dominant in the meadow plant communities of the Kaliningrad region is, among other factors, determined by the proximity to the sea: the level of antioxidants was higher in plants growing closer to the Baltic Sea than in those growing further inland. Geographical zoning, and, more specifically, latitude, also plays a role: in more northern locations the antioxidants capacity of plants was higher than that in the southern ones. These conditions affect the content of anthocyanins, leucoanthocyanins, catechins, and the total water-soluble antioxidants capacity of plants. The latter can be used as a marker in assessing the state of the environment. Legumes are well-suited to serve as indicator

species, since their pool of water-soluble antioxidants is not as stable as that of grasses (Poaceae). For the studied region, we recommend using red clover as an indicator species.

Red clover is native to Europe, Western Asia and North Africa and is naturalised in many other parts of the world (Edwards et al., 2015) that can also be explained by an active system of antioxidant protection (Kaurinovic et al., 2012), which makes it possible to adapt the physiology of this plant to various environmental conditions.

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Can sustainability match quality citrus fruit growing production? An energy and economic balance of agricultural management models for ‘PGI Clementine of Calabria’

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Abstract. This paper analyses energy and economic balances for different growing methods (conventional, integrated and organic cultivation) for Protected Geographical Indications (PGI) Clementine of Calabria, a quality-oriented citrus species in South Italy. Through a double methodological approach, the economic and energy sustainability of each production system was assessed by accounting for the farm net value (FNV) of farms. The energy employment in terms of direct (D) and indirect (I) sources and in terms of renewable (R) and non-renewable (NR) energy sources was also analysed. Regarding FNV, the results show that in the presence of European subsidies, organic farming (with 6.06 k€ ha⁻¹) is more profitable than other systems (4.33 k€ ha⁻¹ for integrated farming and 4.99 k€ ha⁻¹ for conventional farming) due to the higher sales price of organic PGI clementines, which allow producers to obtain the highest remuneration for their capital (1.65 B/C organic, 1.48 B/C integrated, 1.61 B/C conventional). In addition, from an energy perspective, the organic farming systems showed better performances than conventional and integrated systems because they required the lowest average energy employment (49.5 GJ ha⁻¹ year⁻¹) compared with the integrated (57.2 GJ ha⁻¹ year⁻¹) and conventional scenarios (59.1 GJ ha⁻¹ year⁻¹).

Key words: agricultural sustainability, citrus growing, economic analysis, energy balance, PGI.

INTRODUCTION

The sustainability of agricultural production is one of the most interesting fields of discussion among current research frontiers (Finco et al., 2007; Zanolì et al., 2012; De Luca et al., 2015a and 2015b; Mariani & Vastola 2015). There are many analytical and methodological approaches to establishing criteria to measure the impact of agricultural crops on the surrounding environment (Rigby & Càceres 2001). To that end, since the early 1990s, many scientists have tried to establish objective standards based on the use of specific indicators (Rigby et al., 2001), providing specific guidelines to measure the impacts of agricultural practices both per unit surface and per unit product (Van der Werf & Petit 2002). According to De Olde et al. (2016), even if new indicator-

based tools for the assessment of agricultural sustainability are rapidly increasing, a lack of consensus on how to choose sustainability indicators remains.

The ever-growing dependence of modern agriculture on synthetic chemicals, such as fertilizers and pesticides, has certainly caused serious repercussions on public health and on the environment (Pimentel 2005a). Therefore, the need to ensure a more rational balance in the conservation of soil, water, energy, and biological resources has led to the growth of organic farming. Its benefits are well established in terms of conserving the organic matter of soil and using less fossil energy, with similar production yields as conventional systems. The increased organic efficiency in retaining soil wetness and water resources is also highlighted because this is particularly beneficial in drought conditions (Pimentel et al., 2005b). In addition, more sustainable practices should have positive effects for biodiversity and consumers. In fact, products obtained through organic agriculture are healthier and have a lower environmental impact because they contain a lower amount of pesticides than conventional systems (Finco et al., 2007; D'Amico et al., 2016).

Several analyses have deepened the main features of sustainable entrepreneurship linked to specific agricultural sectors, such as olive oil (Di Vita et al., 2015; Bernardi et al., 2016; Stillitano et al., 2016 and 2017; Bernardi et al., 2018; De Luca et al., 2018) and wine grape growing or the wine industry (Pellicanò & De Luca 2016; Schimmenti et al., 2016). Other studies have assessed the environmental impact of different cultivation practices (Falcone et al., 2015; Sgroi et al., 2015a; Falcone et al., 2016; Sgroi et al., 2015b; Nicolò et al., 2017; Strano et al., 2017). Furthermore, a large strand of literature has evaluated the impact of organic versus conventional cultivation of citrus. Among them, particular relevance has been found for energy and economic analyses (Banaeian et al., 2011; Pergola et al., 2013).

Concerning the economic analysis, several methodological approaches have been developed to evaluate economic sustainability in terms of the profitability of grain production (Hanson et al., 1997), current Mediterranean orchards (De Gennaro et al., 2012; De Luca et al., 2014; Lontakis & Tzouramani 2016) and other agro-food productions (Strano et al., 2015). In addition, energy analysis has taken on increased importance in the existing literature, being widely debated in several economic studies (Ozkan et al., 2004; Wood et al., 2006; Pergola et al., 2013) and focusing on energy use efficiency (Banaeian et al., 2011; Mohammadi et al., 2014).

However, certain aspects still deserve further attention, especially with regard to the specificity of cultivation environments related to the quality of orchards, e.g., geographical indications or others certified labels identifying specific characteristics of agricultural products. Another important aspect is measuring the sustainability levels of the organic cultivation method versus conventional farming. With an energy, environmental and production cost analysis, Pergola et al. (2013) evaluated the impact of every citrus fruit product on the environment, observing that the overall production cycles of lemons and oranges on organic farms can be considered more sustainable than those of conventional farms. In the context of citrus, a joint application of life cycle methodologies was performed by De Luca et al. (2014) to simultaneously assess the environmental and economic sustainability of clementine crops by confirming the advantages of organic orchards. Several studies comparing the energy consumption between organic and conventional farms can be found in the literature (Ozkan et al., 2004; Astier et al., 2014; Aguilera et al., 2015a and 2015b; Lee et al., 2015; Taxidis et

al., 2015; Lin et al., 2016). Among these, Ozkan et al., 2004 found that the direct use of energy as well as the emissions of greenhouse gases are higher for organic farms compared to conventional farms. However, the contribution of indirect factors, which exercise greater pressure on the environment, appears to be negatively correlated with conventional farms, causing a substantially higher overall impact.

As already mentioned, because most of the relevant research has aimed to evaluate different environmental impacts of organic and conventional cultivation of citrus fruit (Chinnici et al., 2013; De Luca et al., 2014; Ribal et al., 2016) typical agro-food productions have received very little attention. Further studies are needed with respect to the specific features of growing cultivation areas, such as Protected Geographical Indications (PGI) and Protected Designation of Origin (PDO). In fact, yields and inputs can be strongly influenced by the production specifications of each producer association. In this direction, we believe it would be informative to compare the results of clementine producers derived from the three different growing methods (conventional, integrated and organic cultivation) in a homogenous citrus growing area, i.e., the PGI Clementines of Calabria, assessing energy and economic performances in terms of average total costs and average net values. Clementines are a typical citrus fruit with specific characteristics cultivated in a specific area of Calabria, a region of southern Italy. The authenticity of this fruit has been recently demonstrated by a multi-element fingerprint (Benabdelkamel et al., 2012), and it was also recently awarded with EU PGI designation.

This study aims to evaluate the environmental and economic effects of different agricultural management models for quality citrus fruit production. The remainder of the paper consists of five different sections. The next section briefly describes the specificity of three different farming models: organic, conventional and integrated cultivation. The third section describes the methodological approach used in the study and the data sampling method. The fourth section presents the main economic and energy results, whereas the fifth part discusses the main outcomes and implications in terms of farms profitability and environmental and socio-economic sustainability. The last section provides some conclusions and directions of future work.

MATERIALS AND METHODS

The EU agricultural management models analysed

The three different farming systems identified in this study, conventional, integrated and organic practices, are characterized by specific regulations related to the use of fertilizers, pesticides, fungicides, herbicides and fito-regulators.

The conventional farming system represents the freest alternative, allowing the use of all chemical products authorized by European and national regulations. In particular, the use of fertilizers is constrained in Europe by Council Regulation (EC) no. 2003/2003 (EC 2003), whereas the use of phytoiatric compounds is constrained by Council Regulation (EC) no. 1107/2009 (EC 2009). Excluding other specific limitations related to specific areas susceptible to fertilizers and chemicals leaching, synthetic agricultural products can be used following the technical guidelines provided by fertilizer manufacturers.

In addition, organic farming systems are specifically regulated by Council Regulation (EC) no. 834/2007 (EC 2007) on organic production and the labelling of organic products, which limits the typology of products allowed and in some cases the

quantity (e.g., for copper compounds, the norm limits the quantity to $6 \text{ kg ha}^{-1} \text{ year}^{-1}$ of copper metal). National audit bodies, which monitor for fraud and allow companies to use the organic labels for verified products, guarantee compliance with the rules. Organic productions are characterized by the substitution of chemical fertilizers with organic compounds (e.g., manure, horn meal, poultry manure etc.) and chemical phytoiatric compounds with organic compounds, the biological control of pests and mechanical operations (e.g., mechanical weeding). Generally, organic systems have lower yields than conventional systems due to both the low use of inputs and the higher amount of rejected products due to damage.

Integrated production, compared to conventional production, attempts to move the goal from yield maximization to cost reduction and the quality of the product (Tamis & Van Den Brink, 1999) by implementing management strategies to limit as much as possible the use of synthetic compounds and the release of hazardous slag. In particular, this type of farming system is normed at the local level by specific procedural guidelines of regional authorities, which describe the most appropriate cultivation techniques for single species and fix the typology and the quantity of inputs allowed. All products in organic production are also allowed in the integrated production. In particular, for citrus cultivation, and especially for clementines in the Calabria region, the production rules fix the active ingredients allowed for each disease, the period of treatments, and the maximum amount allowed (Regione Calabria, 2016). For fertilizers, specific limits are fixed for nitrogen (120 kg ha^{-1}), for phosphorus pentoxide (60 kg ha^{-1}) and for potassium oxide (100 kg ha^{-1}). These limits are referred to as normal conditions, but incremental values are allowed in specific contexts (e.g., for a high yield and/or for low soil fertility). In particular, for nitrogen, the quantity can be increased up to 75 kg ha^{-1} . For phosphorus pentoxide, it can be increased up to 80 kg ha^{-1} . For potassium oxide, it can be increased up to 45 kg ha^{-1} . Specific recommendations are also made for tillage, with preference for soft operations, low energy consumption and conservative ploughings in terms of soil fertility and soil biodiversity.

Theory and modelling

This paper presents a double methodological approach to evaluate economic and energy sustainability. The first part of the analyses was addressed to evaluate the profitability among organic, integrated and conventional cultivations. According to previous research (Di Vita et al., 2013), this first analysis was mainly oriented towards evaluating the economic results of the sampled farms by comparing the farm net value (FNV) of each of the production systems. The farm net value was calculated as a mean for each homogeneous area by subtracting from total output (TO) the production costs (PC), which include total specific costs, farming overheads and depreciation. TO includes total crops saleable (production expressed in tons per average price). With the aim of reducing the biases arising from changes in the level of inputs, prices and seasonal productive trends (De Luca et al., 2014; Di Vita et al., 2014), the values of the TO and FNV were determined using their average values for at least four years (2012–2015).

Concerning the PC, the analysis identified three main classes of costs: materials, labour and services, and quotas and other duties (Gresta et al., 2014; Stillitano et al., 2016). The materials item includes the costs of all non-capital inputs (fertilizers, pesticides, herbicides, fuel, water and other crop specifics) and was calculated taking into consideration both the amount effectively used by the farm during the accounting

years and the current market prices. The labour and services item identifies all expenditures for the remuneration of labour, considering all workers directly employed in the production process as well as the external farming services. Costs for farmworkers were evaluated in terms of opportunity cost and were equal to the employment of temporary workers for manual and mechanical operations, assuming current hourly wages. The expenditure for services and specialized labour provided by external agencies was considered as rental costs of mechanical means. Furthermore, in this typology of costs, all expenses for insurance, product sale mediation and transport were accounted for. The quotas and other duties item include depreciation costs for machinery, equipment, land and buildings, circulating and current capital, taxes and fees. Direct subsidies were also included in the analysis by calculating the support of the Common Agricultural Policy (CAP) for the citrus fruit sector provided per hectare, according to Council Regulation (EC) no. 1307/2013 (EC 2013).

The second methodology applied in this paper focuses on an energy analysis approach. In particular, an input-output energy analysis was applied to deepen all energy requirements connected to agricultural production, including the indirect contribution made by the manufacturing of agricultural inputs. With the aim to assess the energy demand of different citrus farming techniques, an approach ‘from gate to gate’ was chosen (Fig. 1).

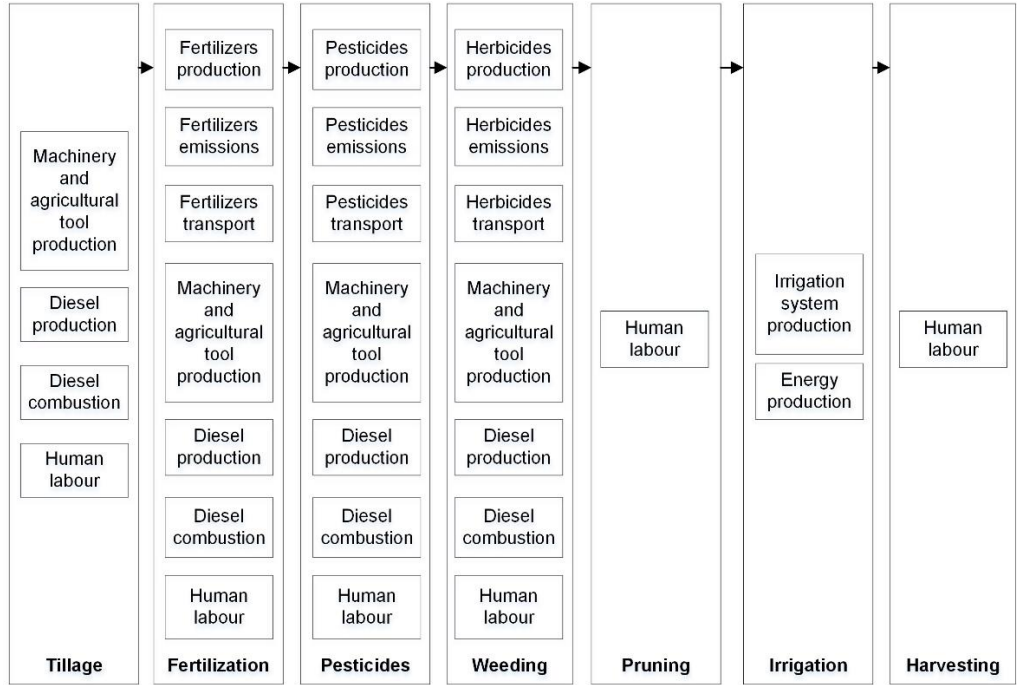


Figure 1. Flow chart of citrus farming system.

According to Ribal et al. (2016), the energy balance assessment was carried out taking into account the full production phase of orchards, which represents the most representative phase in terms of practices, material inputs and environmental impacts.

A reference unit equal to 1 ha year⁻¹ was adopted. In terms of comparing different land utilizations, it appears to be more appropriate than a mass unit (e.g., kg of product), especially for practical implications pertinent to managerial strategies for farmers and policy makers (Cerutti et al., 2015). Data on the input quantities were directly measured from primary sources, whereas the energy equivalent requirement for each input (Table 1) was estimated according to Namdari et al. (2011). To note the different typologies of employed energy, inputs connected to clementine production were classified in direct (D) and indirect (I) sources and in renewable (R) and non-renewable (NR) energies (Yilmaz et al., 2005).

Table 1. Energy equivalent requirement for each input and output considered

Input	Sources typology	Energy typology	Measurement unit	Characterization factors (MJ unit ⁻¹)	Reference
Diesel Fuel	(D)	(NR)	L ha ⁻¹	56.31	Mohammadi et al., 2008
Human Labor	(D)	(R)	h ha ⁻¹	1.96	Ozkan et al., 2004
Water	(D)	(R)	m ³ ha ⁻¹	1.02	Mohammadi et al., 2008
Electricity	(D)	(NR)	kWh ha ⁻¹	11.93	Ozkan et al., 2004
Machinery	(I)	(NR)	h ha ⁻¹	62.70	Ozkan et al., 2004
Manure	(I)	(R)	kg ha ⁻¹	0.30	Canakci et al., 2005
N	(I)	(NR)	kg ha ⁻¹	66.14	Mohammadi et al., 2008
P ₂ O ₅	(I)	(NR)	kg ha ⁻¹	12.44	Mohammadi et al., 2008
K ₂ O	(I)	(NR)	kg ha ⁻¹	11.15	Mohammadi et al., 2008
Pesticides	(I)	(NR)	kg ha ⁻¹	199.00	Ozkan et al., 2004
Fungicides	(I)	(NR)	kg ha ⁻¹	92.00	Ozkan et al., 2004
Herbicides	(I)	(NR)	kg ha ⁻¹	238.00	Ozkan et al., 2004

The investigation covered the areas of clementine of Calabria PGI production. Representative farms were identified in each of three most representative areas, Cosenza, Reggio Calabria and Catanzaro, taking into account the characteristics of the territories and the ordinariness of the production units. Because of the diversity of citrus cultivation and to ensure that the sample adequately reflected this heterogeneity, we stratified the universe of farms using the following criteria: average production, specialized farms, age of cultivation (constant production stage) and plant density. As a consequence, 27 representative farms, equally distributed in three different areas, were totally identified. Data were collected during face-to-face interviews with each producer using a custom-fitted survey questionnaire. The final organization of the questionnaire was derived using outcomes, items and information obtained in a previous focus group. The questionnaire consisted of two main parts; the first one took into account the structural and entrepreneurial characteristics of farms, and the second section was aimed at gathering data on economic aspects and energy use.

Synthetically, the data gathering concerned farm production (yield), farm inputs (types and quantities of agricultural inputs), machinery use for farm management (e.g., fertilizer application, tillage, pruning, weed mowing, etc.), outsourced cost items (e.g., expert consultancies, transport and outsourced cultivation operations), wages, and all cost items not directly attributable to specific growing operations, represented by quotas (depreciation, maintenance and insurance), levies, and interests (remuneration of working capital) and rent (remuneration of land). Table 2 reports the main features characterizing the sample of analysed farms.

Table 2. Main features of sampled farms (means)

Production systems	Cultivated ha area	Diesel Fuel (L ha ⁻¹)	Human Labor	Machinery (h ha ⁻¹)	Water (m ³ ha ⁻¹)	Manure (kg ha ⁻¹)	N (kg ha ⁻¹)	P ₂ O ₅ (kg ha ⁻¹)	K ₂ O (kg ha ⁻¹)	Pesticides (kg ha ⁻¹)	Herbicides (kg ha ⁻¹)	Fungicides (kg ha ⁻¹)	Energy (kW ha ⁻¹)	Yield (ton ha ⁻¹)
Organic	5.6	302.7	432.1	66.2	6,552.0	1,698.9	0.0	0.0	0.0	65.9	0.0	30.0	931.1	29.0
Conventional	6.1	231.9	473.7	50.4	6,685.0	0.0	217.8	142.7	168.8	19.1	5.4	16.1	966.7	36.05
Integrated	6.4	232.6	461.6	53.6	6,776.0	0.0	165.6	120.0	124.4	18.0	5.2	14.9	966.7	34.16

RESULTS AND DISCUSSION

The results are organized in two different subsections. The first analysed the economic results in terms of FNV obtained both including and excluding CAP aids, whereas the second part reported the energy analysis carried out according to the current existing literature (Gündoğmuş 2006; Namdari et al., 2011; Pergola et al., 2013).

Economic results (comparison between organic and conventional growing)

Observing data reported in Tables 3, 4 and 5, the more profitable results were found for organic cultivation in terms of both total output (TO) and farm net value (FNV) in the absence of CAP aids. Obviously regarding FNV in the presence of incentives (which include those for Mediterranean cultivation plus those for organic cultivation), the outcome is even more favourable for organic farming. In fact, despite the total costs of production being the highest in organic farms, the higher sale price of organic PGI clementines allowed producers to obtain the highest remuneration of their capital. Organic cultivation allows producers to obtain specific aid provided for the organic method in addition to the agricultural incentives provided by the EU for each Italian citrus fruit farm.

Differences were also observed for the three different samples with respect to materials and quotas and other duties, whereas statistically relevant differences were observed for the costs linked to labour and services. In organic cultivation, the expenditures for materials, especially for fertilizers and pesticides, are lower than those in conventional and integrated systems. Our results confirm those reported in other studies (Padel & Lampkin, 1994). As expected in the organic cultivation, the expenditure for quotas and other duties is the highest. This result is due to the fees for control required by the inspection body for the certification of organic process.

Concerning the second management model based on integrated agricultural practices, it registers the lowest economic performance compared to the others methods. The lower profitability of the integrated management model is due mainly to the presence of higher average costs, despite the total output of production being on average slightly higher than conventional systems, causing a more favourable price in the final markets. This result is strictly coherent with other studies carried out on organic farming of perennial crops of the Mediterranean Basin (Sgroi et al., 2015a).

Table 3. Economic results of the organic farming systems (expressed in k€ ha⁻¹)

Farm no.	Materials	Labour and services	Quotas and other duties	Production Cost (PC)	Total Output (TO)	Farm Net Value (FNV)	Farm Net Value (FNV) + CAP aids	B/C	(B+CAP aids) /C
Org 1	1.07	3.27	3.89	8.23	12.80	4.57	6.38	1.56	1.77
Org 2	0.99	3.87	3.86	8.71	12.18	3.47	5.27	1.40	1.61
Org 3	0.98	3.72	3.84	8.54	11.20	2.66	4.46	1.31	1.52
Org 4	1.53	4.59	3.37	9.49	14.00	4.51	6.32	1.48	1.66
Org 5	1.46	4.56	3.37	9.40	15.00	5.60	7.41	1.60	1.79
Org 6	1.44	4.61	3.80	9.85	13.75	3.90	5.71	1.40	1.58
Org 7	1.52	3.70	4.22	9.45	14.46	5.00	6.81	1.53	1.72
Org 8	1.43	3.97	4.66	10.06	14.80	4.74	6.54	1.47	1.65
Org 9	1.55	4.51	4.30	10.36	14.21	3.85	5.65	1.37	1.55
Min	0.98	3.27	3.37	8.23	11.20	2.66	4.46	1.31	1.52
Max	1.55	4.61	4.66	10.36	15.00	5.60	7.41	1.60	1.79
Mean	1.33	4.09	3.92	9.34	13.60	4.26	6.06	1.46	1.65
Sd	0.24	0.49	0.42	0.72	1.28	0.88	0.88	0.09	0.10

Table 4. Economic results of the integrated farming systems (expressed in k€ ha⁻¹)

Farm no.	Materials	Labour and services	Quotas and other duties	Production Cost (PC)	Total Output (TO)	Farm Net Value (FNV)	Farm Net Value (FNV) + CAP aids	B/C	(B+CAP aids)/C
Int 1	1.63	4.66	2.95	9.27	11.55	2.28	3.48	1.25	1.38
Int 2	1.64	3.95	2.80	8.39	11.88	3.49	4.69	1.42	1.56
Int 3	1.50	4.09	2.77	8.35	10.85	2.50	3.70	1.30	1.44
Int 4	1.55	3.59	3.70	8.84	12.96	4.12	5.32	1.47	1.60
Int 5	1.70	4.26	3.49	9.45	12.25	2.80	4.01	1.30	1.42
Int 6	1.66	3.36	3.83	8.86	13.30	4.44	5.65	1.50	1.64
Int 7	1.53	4.04	3.48	9.04	12.16	3.12	4.32	1.35	1.48
Int 8	1.37	4.40	3.39	9.16	11.78	2.62	3.83	1.29	1.42
Int 9	1.65	4.63	3.27	9.55	12.35	2.80	4.01	1.29	1.42
Min	1.37	3.36	2.77	8.35	10.85	2.28	3.48	1.25	1.38
Max	1.70	4.66	3.83	9.55	13.30	4.44	5.65	1.50	1.64
Mean	1.58	4.11	3.30	8.99	12.12	3.13	4.34	1.35	1.48
Sd	0.10	0.44	0.38	0.42	0.73	0.74	0.74	0.09	0.09

The conventional farming system shows the lowest cost of all three samples. It is less rentable than organic agricultural method, but it registers a higher profitability than the integrated system. The average total costs of conventional farms amount to 8.13 k€ ha⁻¹, with a minimum of 6.99 and a maximum of 8.95 k€ ha⁻¹. Quotas and other duties constitute the most significant proportion of total costs, which differs from that

detected for the other two systems, whereas Labour and services constitute the major cost. These last results were consistent with earlier findings reported by other authors arguing that organic management systems are more economically sustainable than conventional systems (Pergola et al., 2013; Sgroi et al., 2015a).

Table 5. Economic results of the conventional farming systems (expressed in k€ ha⁻¹)

Farm no.	Materials	Labour and services	Quotas and other duties	Production Cost (PC)	Total Output (TO)	Farm Net Value (FNV)	Farm Net Value (FNV) + CAP aids	B/C	(B+CAP aids)/C
Conv 1	1.53	2.06	3.71	7.30	9.50	2.20	3.41	1.30	1.47
Conv 2	1.41	1.95	3.63	7.00	9.24	2.24	3.45	1.32	1.49
Conv 3	1.43	2.18	3.85	7.46	10.50	3.04	4.25	1.41	1.57
Conv 4	1.64	2.93	3.80	8.37	11.44	3.07	4.28	1.37	1.51
Conv 5	1.58	2.86	3.71	8.15	11.75	3.60	4.81	1.44	1.59
Conv 6	1.56	2.98	3.89	8.44	12.16	3.73	4.93	1.44	1.58
Conv 7	1.54	4.44	2.91	8.89	14.00	5.11	6.32	1.57	1.71
Conv 8	1.68	4.28	3.00	8.95	15.00	6.05	7.25	1.68	1.81
Conv 9	1.76	4.02	2.92	8.70	13.75	5.05	6.25	1.58	1.72
Min	1.41	1.95	2.91	7.00	9.24	2.20	3.41	1.30	1.47
Max	1.76	4.44	3.89	8.95	15.00	6.05	7.25	1.68	1.81
Mean	1.57	3.08	3.49	8.14	11.93	3.79	4.99	1.46	1.61
Sd	0.11	0.96	0.42	0.72	2.02	1.34	1.34	0.13	0.12

Energy analysis: comparison among organic, integrated and conventional farming

As previously observed in the economic analysis, from an energetic point of view, the organic farming system (Table 6) shows better performances than the conventional and integrated systems (Tables 7 and 8). Organic clementines require the lowest average energy employment (49.55 GJ ha⁻¹ year⁻¹) compared with the integrated (57.21 GJ ha⁻¹ year⁻¹) and conventional scenarios (59.09 GJ ha⁻¹ year⁻¹). The larger amount of energy consumption in the organic farming systems is related to the depletion of fossil fuels due to machinery use (on average 34.5% of total), followed by the use of electricity and irrigation (22.3%). Fertilization represents only 1.03% whereas the use of plant protection products accounts for 18.6%. Analysing the standard deviation, a higher value was reached for the 'Diesel Fuel' category (2.6%) followed by the 'Pesticides' (2.1%) and 'Electricity' (1.8%) categories. The other categories show, on average, values approximately 0.5%, indicating low dispersion of the distribution of results.

As mentioned above, the integrated farming system represents the second less impactful scenario in terms of energy consumption, with a higher energy requirement in terms of 'fossil energy'. The ratio of this energy category consumption to the total requirement is less than that for the organic scenario and is, on average, 24%. Fertilization represents the most impactful operation overall, particularly nitrogen fertilizer (20.1%), together with phosphorus pentoxide and potassium oxide, which brings the share of total energy required for fertilizers to 25.3%.

Table 6. Energy results of the organic farming systems

Farm no.	Diesel Fuel (%)	Human Labor (%)	Water (%)	Electricity (%)	Machinery (%)	Manure (%)	Pesticides (%)	Fungicides (%)	TOTAL (GJ ha ⁻¹ year ⁻¹)
Org 1	36.25	1.98	12.72	20.70	8.80	1.00	12.00	6.55	44.95
Org 2	37.57	1.97	12.23	19.62	9.09	1.04	12.21	6.27	42.57
Org 3	37.92	1.88	12.78	20.36	9.21	0.94	10.64	6.28	43.95
Org 4	30.41	1.57	14.60	23.88	7.35	1.03	15.55	5.61	52.45
Org 5	31.97	1.64	13.98	24.96	7.77	1.03	13.76	4.90	52.59
Org 6	32.54	1.64	13.98	21.61	7.90	0.94	16.02	5.37	49.68
Org 7	33.08	1.56	13.28	23.71	8.08	1.08	14.38	4.82	55.35
Org 8	35.43	1.61	13.83	23.90	8.73	1.14	9.92	5.44	52.42
Org 9	35.80	1.65	13.54	21.79	8.74	1.04	12.14	5.31	52.02
Min	30.41	1.56	12.23	19.62	7.35	0.94	9.92	4.82	42.57
Max	37.92	1.98	14.60	24.96	9.21	1.14	16.02	6.55	55.35
Mean	34.55	1.72	13.44	22.28	8.41	1.03	12.96	5.62	49.55
Sd	2.64	0.17	0.75	1.88	0.65	0.07	2.11	0.62	4.57

Irrigation was the third most energy expensive operation, accounting for 21% of the total energy requirement. The standard deviation was higher for electricity (2.9%) and nitrogen fertilizer (2.5%), whereas for the other inputs, it was, on average, 0.5%.

Table 7. Energy results of the integrated farming systems

Farm no.	Diesel Fuel (%)	Human Labor (%)	Water (%)	Electricity (%)	Machinery (%)	N (%)	P ₂ O ₅ (%)	K ₂ O (%)	Pesticides (%)	Fungicides (%)	Herbicides (%)	TOTAL (GJ ha ⁻¹ year ⁻¹)
Int 1	24.44	1.72	10.93	17.93	6.06	24.23	2.80	2.62	4.16	2.42	2.68	53.22
Int 2	26.11	1.80	10.76	17.14	6.62	22.81	2.74	2.46	4.46	2.82	2.28	52.19
Int 3	25.25	1.85	10.98	16.95	6.30	21.48	3.28	2.83	5.87	2.80	2.41	49.28
Int 4	24.04	1.71	14.07	23.02	5.96	17.02	2.63	2.36	4.25	2.54	2.41	54.41
Int 5	22.16	1.62	13.71	25.30	5.98	18.70	2.31	2.07	3.76	2.28	2.10	56.58
Int 6	23.53	1.67	13.28	22.64	6.26	17.93	2.47	2.22	5.08	2.33	2.58	55.32
Int 7	22.46	1.47	13.14	22.22	5.53	21.28	2.74	2.74	4.09	2.34	2.02	59.05
Int 8	23.83	1.52	13.14	22.09	6.36	18.66	2.74	2.75	4.42	2.60	1.89	56.70
Int 9	23.98	1.58	13.24	21.35	6.26	18.34	2.89	2.79	5.18	2.47	1.92	55.89
Min	22.16	1.47	10.76	16.95	5.53	17.02	2.31	2.07	3.76	2.28	1.89	49.28
Max	26.11	1.85	14.07	25.30	6.62	24.23	3.28	2.83	5.87	2.82	2.68	59.05
Mean	23.98	1.66	12.58	20.96	6.15	20.05	2.73	2.54	4.59	2.51	2.25	54.74
Sd	1.24	0.12	1.31	2.93	0.31	2.47	0.27	0.27	0.66	0.20	0.29	2.88

The conventional farming system has the worst results; however, the mean value is close to that of the integrated farming system. In terms of the incidence of a single input to total energy requirements, fertilization represents the most wasteful operation. In

particular, the use of nitrogen share is on average 24.45% of the total, representing overall the most impactful input. Considering phosphorus pentoxide and potassium oxide, the use of fertilizers constitutes approximately 30.5% of the energy required. As seen above for the integrated farming system, diesel fuel (22.1%) and electricity (19.44%) represent the second and the third most influential inputs, respectively. The standard deviations have higher values in the nitrogen category (3.1%) and in the electricity category (2.55%) but generally have values comparable with the other farming systems.

Table 8. Energy results of the conventional farming systems

Farm no.	Diesel Fuel (%)	Human Labor (%)	Water (%)	Electricity (%)	Machinery (%)	N (%)	P ₂ O ₅ (%)	K ₂ O (%)	Pesticides (%)	Fungicides (%)	Herbicides (%)	TOTAL (GJ ha ⁻¹ year ⁻¹)
Conv 1	21.56	1.78	10.56	16.83	5.23	27.87	2.81	2.52	5.50	3.12	2.24	53.16
Conv 2	24.80	1.54	9.51	15.45	6.03	27.41	2.58	2.31	5.37	2.54	2.47	57.92
Conv 3	24.31	1.57	9.84	16.29	5.89	27.09	2.55	2.28	5.31	2.83	2.03	58.59
Conv 4	20.79	1.50	12.39	22.22	4.96	25.71	2.42	2.17	3.67	2.24	1.93	61.73
Conv 5	21.86	1.47	12.21	20.40	5.23	24.43	2.45	2.19	4.95	2.36	2.44	58.47
Conv 6	19.73	1.57	12.64	21.68	4.77	25.79	2.57	2.30	4.45	2.13	2.36	60.52
Conv 7	21.71	1.58	12.08	20.17	5.31	21.47	4.04	5.13	4.17	2.33	2.01	59.15
Conv 8	21.92	1.61	12.07	20.58	5.33	20.86	3.92	4.98	4.45	2.12	2.15	60.87
Conv 9	22.37	1.54	12.29	21.36	5.40	19.38	3.65	4.63	4.25	3.00	2.13	61.43
Min	19.73	1.47	9.51	15.45	4.77	19.38	2.42	2.17	3.67	2.12	1.93	53.16
Max	24.80	1.78	12.64	22.22	6.03	27.87	4.04	5.13	5.50	3.12	2.47	61.73
Mean	22.12	1.57	11.51	19.44	5.35	24.45	3.00	3.17	4.68	2.52	2.20	59.09
Sd	1.59	0.09	1.20	2.55	0.40	3.13	0.67	1.32	0.63	0.38	0.19	2.62

In terms of the type of energy used in the different farming systems, the share of non-renewable energy is higher than that of renewable. For the conventional and integrated scenarios, renewable energy represents only 13%, whereas for the organic scenario, the share increases up to 16% (Fig. 2).

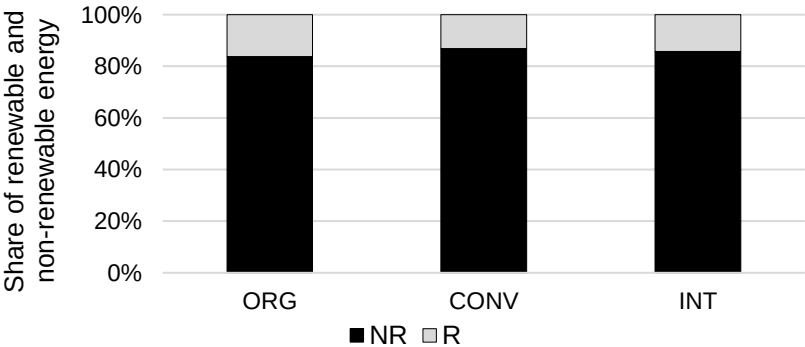


Figure 2. Average share of renewable and non-renewable energy for different farming systems.

In terms of direct and indirect energy, the conventional and integrated systems show a similar trend, using 55% and 60% of direct energy and 45% and 40% of indirect energy, respectively. Conversely, the organic farming systems use 72% of direct energy and only 28% of indirect energy (Table 9).

Table 9. Average share of direct and indirect energy for different farming systems

	(D)			(I)								
	Diesel Fuel	Human Labor	Water	Electricity	Machinery	Manure	N	P	K	Pesticides	Fungicides	Herbicides
ORG	34.39%	1.71%	13.49%	22.42%	8.37%	1.03%	0.00%	0.00%	0.00%	13.02%	5.57%	0.00%
CONV	22.10%	1.57%	11.54%	19.52%	5.35%	0.00%	24.37%	3.00%	3.18%	4.66%	2.51%	2.19%
INT	23.93%	1.65%	12.63%	21.07%	6.14%	0.00%	20.00%	2.73%	2.53%	4.57%	2.50%	2.25%

Discussion

The results confirm the differences in the energy and economic performances among three different farming systems, and the outcomes seem to be in line with previous research concerning these issues. The analyses showed the best economic performance for organic farming, unlike the findings obtained in an analogous study conducted on orange farming that showed the highest profitability for the conventional method (Chinnici et al., 2013). These initial results, confirmed by most of the subsequent studies (Pergola et al., 2013; Patil et al., 2014; Sgroi et al., 2015a), are due to current availability of European Union farm support to help organic growers gain additional value from citrus fruit production. At the same time, this result is justified by the progressive increase in the consumer prices of clementine that benefit from a favourable price due to it being organic and having PGI certification, confirming current trends of modern consumers that show an increasing appreciation for organic products and the origin of fresh fruit production (Lombardi et al., 2013). From a consumer behaviour perspective, further analysis can be directed to evaluating a hedonic price function of the effects of each certification on the final price of clementines. In addition, the analysis noted that organic farming is more labour intensive than the other scenarios. As a consequence, from a macroeconomics approach, it would seem that organic and even integrated farming requires a larger amount of work in relation to the final output. In this sense, our results seem to have interesting implications for rural and local development because the outcomes show that putting more effort into the development of sustainable practices in the agriculture of PDO and PGI areas would require greater use of manpower. As a result, this increasing demand in terms of extra labour could be redirected towards structural employment policies for both skilled and generic agricultural jobs.

Therefore, we can reasonably affirm that organic agriculture produces positive economic effects not only due to the higher prices or to EU additional payments for organic growing, which lead to an increase in farm profitability but also because it generates favourable social effects and benefits on the local system thanks to the major participation of local organic stakeholders. This result appears to be in line with a study arguing the role of organic farming in preventing the abandonment of rural areas (Testa

et al., 2015). Therefore, the increasing demand of employment and more profitable incomes of organic farming can ensure a more favourable impact overall on the territory, with positive economic and social effects within the rural areas (Timpanaro et al., 2013; Spampinato et al., 2013; Zarbà et al., 2013; De Luca et al., 2015a; Frischknecht et al., 2015). Concerning environmental sustainability, by observing the results obtained in the energy analysis, it was found that the organic farming system requires less energy input than the conventional system. This result appears to be consistent with earlier studies that compared these different farming systems using energy input-output analyses (Gündoğmuş et al., 2006; Pergola et al., 2013). Similar results have been obtained in different life cycle assessment (LCA) studies in which the depletion of non-renewable resources was considered (Falcone et al., 2015; Ribal et al., 2016); however, these energy source generally considers only fossil fuel consumption (Frischknecht et al., 2015). Furthermore, the results are comparable with some studies that assessed the energy consumption of citrus orchards (Namdari et al., 2011; Pergola et al., 2013).

In particular, taking into account the study of Ozkan et al. (2004), their results determined an energy requirement for mandarins that is lower than our findings (48.84 GJ ha^{-1}), but they used different cultivation techniques and characterization factors. For the energy requirements of mandarins, the results of Namdari et al. (2011) are very similar to ours (77.50 GJ ha^{-1}), and in this case, the cultivation techniques are different and strongly connected to the area of the survey. Considering only one year of production, the results of Pergola et al. (2013) show a higher energy demand compared to our findings, but their results are relative to the full production phase and a different citrus species. In contrast, our results appear consistent, especially considering that the area analysed by Pergola et al. (2013) is relatively close to that considered for this study.

In terms of energy demand by farming operations, as mentioned above, the fertilization and pesticide distributions constitute approximately 40% of the total energy requirement for the conventional and integrated scenarios, according to Ozkan et al. (2004) and Namdari et al. (2011), whereas for the organic system, it covers approximately 20%. Also in Beccali et al. (2010) fertilization is the most impactful operation, but it is not possible to attribute the share of the cumulative energy demand linked to this operation for the different reference unit used (1 kg of transformed product) and in the absence of an in-depth analysis of the agricultural phase. Tillage and irrigation generally represent the second and the third most energy-expensive operations, due to the use of fossil fuels and electricity, according to Ozkan et al. (2004) and Namdari et al. (2011). On the contrary, Pergola et al. (2013) observed that the most impactful operation is harvesting in lemon cultivation for both the organic and conventional systems. This result is due to the higher planting density of lemon orchards and to the distribution throughout the year of the harvesting of lemon fruits.

Considering one ha of cultivated surface, the organic farming systems exhibited better performances respect than the conventional and integrated systems, but these could be subject to relevant changes considering, as a reference unit, one kilo of product. For example, considering the mean values of energy consumption and the average yield (organic $29,000 \text{ kg ha}^{-1}$; integrated $34,167 \text{ kg ha}^{-1}$; conventional $36,056 \text{ kg ha}^{-1}$), the results of the present study change 1.71 MJ kg^{-1} for organic, 1.64 MJ kg^{-1} for conventional and 1.60 MJ kg^{-1} for integrated farming systems. The alternative results in

terms of the kg of products show that the integrated system performed better compared to the conventional and/or organic systems, apparently, contrasting the results outlined above; however, it is only a perspective question. In fact, the results expressed in terms of mass are strictly connected to the difference in the yield between cultivation techniques (which is lower in the organic system). The use of a mass FU favours the integrated and conventional scenarios, according to Mattsson (1999), Nicoletti et al. (2001) and Cerutti et al. (2015). Therefore, in terms of energy consumption, it might be plausible to assert that organic practices are not always sustainable. A thorough environmental assessment should consider other indicators, for example, the effects on biodiversity at a local scale and the impact on soil quality. Only considering the energy footprint, it would be hazardous to affirm that integrated or conventional agriculture is in anyway better than organic agriculture (Cerutti et al., 2015).

CONCLUSIONS

This study provides empirical research on the economic and energy sustainability of different citrus cultivation practices in Southern Italy to ascertain whether differences exist among different agricultural management models in terms of profitability and energy use in PGI areas. The results allowed us to compare the economic and energy performances of each farming typology, describing the outcomes for three different scenarios: organic, integrated and conventional farming.

Economic analysis found the highest economic and social sustainability performances for organic farming. In terms of product quality, this production method ensures the highest profitability and seems to be more beneficial in terms of rural development and environmental protection. Furthermore, concerning energy analysis, the organic farming system yields better results. The results referring to the cultivated area could be useful for defining energy-oriented development strategies. From a consumer perspective, referring to the assessment of the product, the results revealed that the increase of the yield plays a key role, allowing a greater distribution of energy consumption. Increasing the yield of the organic farming system should be the path to obtaining more sustainable products.

Therefore, the present paper confirms the main outcomes of a large strand of existing literature on organic farming and introduces for the first time new insights linked to the energy balance for crops cultivated in protected geographical indication areas.

Further analysis of sustainability in other PDO and PGI areas is needed to corroborate our results. More in-depth studies could be useful for understanding the different levels of sustainability by investigating additional environmental and economic indicators through life cycle methodologies and financial analysis.

ACKNOWLEDGEMENTS. This research is funded by the Italian Ministry of University and Research (MIUR) within the research project: Distretto ad alta tecnologia agroindustriale della Calabria AGRIFOODTECH (PON03PE_00090_3) ‘Modelli sostenibili e nuove tecnologie per la valorizzazione delle filiere vegetali mediterranee’.

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The characteristics of wheat collection samples created by *Triticum aestivum* L/*Triticum spelta* L hybridisation

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Abstract. The aim of our research was to create, analyzes, and systematise wheat collection samples for the selection of valuable initial forms, to involve them in breeding process, and to create new productive cultivars. For this purpose the hybridisation of soft and spelt wheat was carried out, along with an evaluation of the hybrids that were obtained, between 2006 and 2018 (F₅–F₁₀). A collection of wheat samples, containing more than a thousand numbered items, was formed from the obtained diversity of samples. The economically-valuable and morphological characteristics of newly-developed materials were analysed. According to the results of our research, we selected forms of spelt, soft wheat, and speltoid samples which have high levels of productivity and high gluten and protein contents in grains.

Spelt wheat sample 1817 contains 45.2% gluten, 22.3% protein, and has a yield capacity of 6.55 t ha⁻¹. Soft wheat sample 1689 has 32.4% gluten, 15.8% protein, and a yield capacity of 7.19 t ha⁻¹. These samples were submitted for state scientific and technical expert evaluation in 2018. The created varieties of European spelt wheat and Artemisia soft wheat were included in the ‘State Register of Plant Varieties Suitable for Distribution in Ukraine’.

The varieties, Artaniia and Artaplot, were submitted for state scientific and technical expert evaluation.

Key words: initial material, hexaploid species, speltoid hybrids, protein content, gluten content, ear density.

INTRODUCTION

The main task of wheat breeding consists of the creation of highly-productive varieties with excellent grain quality (Guzman et al., 2016).

However, in recent years there has been a tendency to increase yield capacity along with a noticeable deterioration in the quality of grain (Nazarova & Zhdanova, 2017).

Therefore a number of scientific institutions are working on developing wheat varieties with high productivity levels, resistance to unfavourable environmental factors, and high grain quality. The department of genetics, plant breeding, and biotechnology at Uman National University of Horticulture (Ukraine) has been conducting research into winter wheat breeding, applying the methods of intraspecific and remote hybridisation.

Worldwide practice has shown that an effective breeding method is the crossing of geographically remote forms, but success significantly depends upon a proper selection of hybridisation components - that is, the initial material (Xie et al., 2015; Longin et al., 2016). It is appropriate to use genetically remote forms to create new wheat varieties that would meet the demands of contemporary agricultural production (Polyanetska, 2012). In this respect wild, semi-wild, and now-forgotten forms act as donors of high protein content, gluten, lysine, and resistance to diseases and pests. It is reasonable to use spelt wheat as a donor of economically valuable characteristics.

It is a hexaploid species with genomic structure, A^uBD; therefore its hybridisation with a soft wheat of the same genomic structure is easy to carry out, although there are certain problems which are related to the morphological structure of plants and flowering time (spelt wheat is high-growing, while varieties that are used in hybridisation are mainly low-growing or semi-dwarf). At present this wheat species is used in breeding programmes because it is a donor of high protein content, and contains almost all of the nutrients in a balanced state that are necessary for the human body (Ikanović et al., 2016; Rapp et al., 2017).

Research by Ukrainian and foreign breeders has shown a positive effect in crossing soft wheat with spelt, in particular a substantial expansion of existing genetic diversity in the wheat and creating new forms that combine a high protein content and gluten from spelt and high productivity levels from soft wheat (Guzman et al., 2012; Polyanetska, 2012). However, according to the opinion of Rybalko (2011), these crossovers are undesirable because they lead to a deterioration in the grain quality of spelt and the inheritance of complicated grain threshing problems and soft wheat's 'fragile ear'.

Breeders from many countries deal with the improvement of the quality parameters of wheat grain by means of hybridisation with spelt. In this field some achievements were achieved in Switzerland, Austria, and Serbia, where the following spelt varieties were created: Bauländer, Schwabenkorn, Frankenkorn (Austria), Nirvana (Serbia), Altgold Rotkorn (Sweden) (Dvorak et al., 2012). In Ukraine a profound area of research in this area is being conducted at Uman National University of Horticulture, the All-Ukrainian Scientific Institute of Breeding, the plant production institute which was named in memory of V. Ya. Yuryev.

A number of research on the hybridisation of soft wheat and spelt have been conducted at the Uman National University of Horticulture, which allows a collection of samples to be created from obtained varieties of breeding materials which is unique in terms of morphological, biological, and biochemical characteristics. This is the source of valuable genetic plasma which can be used to improve existing varieties of wheat and to create new ones.

The objective of our research was to create new materials through the hybridisation of *Triticum aestivum* L./*Triticum spelta* L., and to systemise the collection wheat samples with the aim of selecting valuable initial forms with high levels of grain quality, using them in the breeding of highly-productive varieties.

MATERIALS AND METHODS

Specialists at Uman National University of Horticulture (Ukraine) carried out a number of studies on soft wheat and spelt hybridisation. Wheat variety samples were created by using the methods of intraspecific and remote hybridisation with multiple

individual selection. The creation of a collection of wheat samples began in 2006 under the guidance of F M Parii, doctor of biology. Zoned soft winter wheat varieties, Favorytka, Smuglianka, Podolianka, Zolotokolosa, Harus, Bilotserkivska semi-dwarf, Murhad, Kruzhunka, Farandol, Ermak, Selyanka, Panna, Olesya, Olvia, Povernna, Slavna, Krasnodarska 99, Panna, and samples of spelt wheat from local breeding in the foothills of the Carpathians were involved as initial material in species crossovers. When the research began (in 2006) there were no spelt wheat varieties in Ukraine. Hybridisation was carried out by the manual castration of female flowers and the subsequent forced pollination of the male parent.

The F_{2-5} hybrid progenies were analysed according to the manifestation of morphobiological traits and economically valuable parameters (such as plant height, length, colour, and the density of the ear, the threshability of the grain, the weight of grain from the main ear, the weight of a thousand grains, the content of protein and gluten in the grain, gluten quality, yield capacity, and so on). In the fifth generation (F_5), when splitting was no longer observed, and when considering plant habitus and the morphological structure of the ear, all of the materials created were divided into soft wheat, spelt wheat, and intermediate (speltoïd) forms. The best samples which had economically valuable parameters were selected from each group for further testing. The testing of the selected samples was carried out during 2012–2017 (F_5 – F_{10}).

All analysis and observations were conducted in accordance with the ‘Ukraine state methodology for the testing of agricultural crops’ (2011). The gluten content was detected by using the methodology of the state scientific and technical expert evaluation of plant varieties (2011). The height of the plants was measured in the field prior to harvesting. The grouping of wheat samples was carried out according to the height of the plants, using the procedure which had been drawn up by Dorofeyev et al. (1987). Harvesting and the recording of grain yields was carried out during the firm ripening stage. The soft winter wheat variety, Podolianka, was used as a standard for the soft wheat group, while the winter spelt wheat, Zoria Ukrainy, was used as a standard for the spelt wheat group, and for the intermediary group both of the aforementioned varieties were used as standards.

A method involving the systematic placement of plots with an accounting area of 10 m^2 was used in experiment. Numbered plants were placed in blocks with a plant density of 400,000 units for each hectare. Experiments were conducted in repetitions of five. Plant biometrics were determined for fifty plants, each of which were selected from each plot in two nonadjacent repetitions. The threshing of grain was carried out and yield capacity was defined after all measurements had been taken. The credibility of the research, the degree of variation of the characteristics, and the significance of differences from the parameters of productivity in the experiments were all evaluated by making use of the methodology which had been developed by Ermantraut et al. (2000), using MS Excel.

RESULTS AND DISCUSSION

Hybridisation between highly productive zoned winter soft wheat varieties and spelt wheat samples of our own selection was carried out during the process of our research. The descendant samples that were obtained were self-pollinated or re-crossed with parental forms. Individual family selection amongst descendants was used to select

the samples, with these being characterised by a significant diversity according to economically-valuable characteristics, and morphological and biological properties. Today the wheat collection includes more than a thousand samples. The collection consists of sample varieties of soft wheat, spelt wheat, and speltoid hybrids with a set of valuable properties, such as early ripeness, dwarfness, and high winter resistance. Some materials surpassed the initial varieties in terms of yield capacity, and the protein and gluten content.

All of the materials created beginning from the fifth generation (F_5), while considering plant habitus and the morphological structure of the ear, were divided into soft wheat, spelt wheat, and intermediary (speltoid) forms. The soft wheat group includes samples with a medium-dense or dense ear (between 16–28 spikelets for each 10 cm of ear), with normal glume levels and easy grain threshing. The spelt wheat group comprises forms with a long, loose ear (< 16 spikelets for each 10 cm of ear), plus rough glume, and a complicated grain threshing. The samples which, according to their ear morphological structure, had an intermediary position amongst the parental forms, were classified in the speltoid wheat group.

The collection includes a wide range of forms which are categorised according to their height. The height variability of the plants ranges between 52–129 cm. The samples created were grouped according to the Dorofeev classification (1987) into tall-growing (> 120 cm), medium-growing (105–119 cm), low-growing (85–104 cm), semi-dwarfs (60–84 cm) and dwarfs (< 60 cm). The most numerous and productive examples were low-growing and semi-dwarf samples.

Spelt wheat is a tall-growing species. This is why reducing plant height with a resultant saving of high protein and gluten content is an important task for plant breeding. The height of spelt wheat plants varies within the range of 75–127 cm, the variation coefficient exceeding 20% ($V = 36\%$), which indicates a significant variation in the samples created according to this parameter. A significant decrease in plant height when compared to the standard height was recorded in ten samples (Table 1).

The semi-dwarf 1559 and dwarf 1817 samples of spelt wheat, which are characterised by high yields for this type of wheat (6.36 and 6.55 t ha⁻¹ respectively), both were selected.

The main purpose behind the hybridisation of soft wheat with spelt wheat was to create new wheat forms which produced high levels of protein and gluten content. The gluten content in soft wheat grain varies between 26–30%, and protein levels vary between 12–14%. For spelt wheat grain these indicators are significantly higher: the gluten content reaches 45–50% and the protein content is over 20%.

In those spelt wheat samples which have been created, the protein content varied between 16.4–24.0%, while the gluten content fell within 35.1–48.8% depending upon the genotype. The high values of variation coefficient indicate a significant range of variability in terms of the content of protein and gluten. Samples of spelt wheat surpassed the forms of soft wheat and speltoid hybrids in this parameter. The highest content of protein and gluten were in varieties of spelt wheat, 1721 and 1691. Their protein content consisted of 24.0% and 22.8% respectively, while their gluten content was at 47.8% and 48.8%, which slightly exceeded the standard.

The negative features of spelt wheat are its low yield and difficult grain threshing. It was expected that its hybridisation with soft wheat would allow new forms of spelt wheat to be created with improved threshing qualities and higher productivity levels. As the result of this research, those forms were selected which surpassed the standard in yield capacity (samples 1695, 1691, 1755, 1559, 1674, 1817 and 1786). At the same time samples 1559 and 1817 showed high parameters of grain quality – in particular their protein content which reached 21.2% and 22.3% respectively, and a gluten content of 44.5% and 45.2%.

Table 1. Economically valuable parameters for collection samples of spelt wheat, average for 2012–2017

Breeding material	Origin	Plant height, cm	Grain weight of the head ear, g	Ear length, cm	1,000 grains weight, g	Gluten content, %	Protein content, %	Yield capacity, t ha ⁻¹
Zorya	ASIB*	116	1.82	15.8	50.5	48.2	23.7	5.52
Ukrainy (st)								
1730	Favoritka × spelt	127	1.74	15.7	45.5	37.7	15.8	4.81
1695	Farandol × spelt	129	2.72	16.1	50.8	40.8	19.2	6.52
1691	Krasnodarska 99 × spelt	120	2.08	15.8	55.1	47.8	22.8	5.81
1719	Panna × spelt	109	1.87	16.8	52.1	42.2	20.1	5.74
1721	Panna × spelt	106	1.62	17.6	43.8	48.8	24.0	4.83
1725	Kopylivchanka × spelt	110	1.36	17.2	44.2	40.4	18.7	4.42
1755	Panna × spelt	98	2.33	17.5	51.2	39.2	18.1	6.04
1731	Favoritka × spelt	100	1.66	17.6	42.8	40.2	19.2	4.93
1559	Kryzhynka × spelt	87	2.45	18.3	65.0	44.5	21.2	6.36
1694	Farandol × spelt	98	1.78	18.1	43.4	41.2	19.4	5.15
1674	Farandol × spelt	89	2.06	15.0	55.5	35.1	16.4	5.86
1817	Kharys × spelt	75	2.67	18.3	50.2	45.2	22.3	6.55
1786	Favoritka × spelt	82	2.05	15.1	51.7	42.4	20.7	5.84
LSD ₀₅		3	0.07	0.4	1.67	1.4	0.7	0.19
$\bar{x} \pm S_x$		102.0 ± 10.1	2.03 ± 0.25	16.9 ± 0.7	50.1 ± 3.8	42.0 ± 2.3	19.8 ± 1.4	5.60 ± 0.42
Min		75.0	1.36	15.0	42.8	35.1	15.8	4.42
Max		129.0	2.72	18.3	65.0	48.8	24.0	6.55
V, %		36	8.55	8.4	79.6	35.4	28.7	8.88
S _{xy} , %		4.6	0.06	2.0	3.5	2.5	3.3	0.03

* ASIB – All-Ukrainian Scientific Institute of Breeding - the originator of each variety.

The problem of reducing plant height for speltoid forms is also relevant, since this feature can manifest itself in intermediary forms which are similar to spelt. According to the height of the plants, significant variation was observed in speltoid forms (V = 28%) (see Table 2).

Table 2. Economically-valuable parameters for spelt-like samples, average for 2012–2017

Breeding material	Origin	Plant height, cm	Grain weight from the head ear, g	Ear length, cm	1,000 grains weight, g	Gluten content, %	Protein content, %	Yield capacity, t ha ⁻¹
Podolyanka IPPG* (st)		85	2.32	9.8	52.4	29.4	13.8	6.78
Zorya Ukrainy (st)	ASIB*	116	1.82	15.8	50.5	48.2	23.7	5.52
1669	Panna × spelt	99	1.45	14.0	45.8	33.6	16.2	4.95
1766	Favoritka × spelt	97	1.88	13.6	42.4	34.9	16.5	5.65
1710	Zolotokolosa × spelt	100	1.99	14.0	59.2	35.8	17.0	5.87
1626	Ermak × spelt	87	1.75	14.1	50.5	30.4	14.3	5.41
1561	Kryzhynka × spelt	102	2.27	14.6	51.4	36.4	17.5	6.45
1694	Selyanka × spelt	80	1.45	12.3	50.2	32.1	15.6	4.87
1809	Kopulivchanka × spelt	78	1.65	14.0	45.7	39.1	18.1	5.98
1800	Kharus × spelt	75	1.48	12.8	50.1	35.0	16.5	4.80
1628	Ermak × spelt	58	1.28	12.5	43.7	44.3	21.4	4.68
1635	Podolyanka × spelt	55	1.49	12.7	45.7	35.1	16.7	5.01
LSD ₀₅		3	0.06	0.4	1.7	1.2	0.6	0.18
$\bar{x} \pm S_x$		83.1 ± 12	1.67 ± 0.21	13.5 ± 0.6	48.5 ± 3.4	35.3 ± 2.6	16.9 ± 1.3	5.27 ± 0.40
Min		55.0	1.28	12.3	42.4	30.4	14.3	4.68
Max		102.0	2.27	14.6	59.2	44.3	21.4	6.45
V, %		28.1	4.14	5.4	49.2	38.2	20.6	5.09
S_{x^2} , %		6.5	5.76	1.9	3.2	3.3	3.4	3.41

*IPPG – Institute of Plant Physiology and Genetics NAS of Ukraine; *ASIB – All-Ukrainian Scientific Institute of Breeding.

Speltoid forms are significantly inferior to the Zoria Ukrainy variety in terms of plant height, and samples 1809, 1800, 1628, and 1635 are significantly inferior to both standards. Significant variation was recorded in the weight of a thousand grains within 42.4–59.2 g. There was a considerable decrease in the weight of a thousand grains in speltoid forms when compared to the standard Podolyanka variety. The only exception was sample 1710 with a weight for a thousand grains at the level of 59.2 g, which is the highest index in the experiment. In speltoid materials, only the low-growing sample 1561 is characterised by a combination of high productivity levels and an increased gluten content in its yield capacity, in which it approached the Podolyanka variety (6.45 t ha⁻¹) and considerably surpassed it in terms of its content of gluten (36.4%) and protein (17.5%).

There were no high-growing samples in the soft wheat group. Two medium-growing samples were selected, although they failed to show high parameters in productivity and grain quality. The height of plants in this group varied between 52–100 cm, and in this case the variation coefficient reached 28%, which indicates a considerable variation in this feature (Table 3).

Table 3. Economically-valuable parameters for the collection of soft wheat samples, average for 2012–2017

Breeding material	Origin	Plant height, cm	Grain weight from the head ear, g	Ear length, cm	1,000 grains weight, g	Gluten content, %	Protein content, %	Yield capacity, t ha ⁻¹
Podolyanka IPPG (st)		8	2.32	9.8	52.4	29.4	13.8	6.78
1692	Krasnodarska 99 × spelt	100	2.45	8.5	55.2	30.1	14.2	7.02
1687	Myrchad × spelt	87	2.12	9.8	53.1	29.7	13.7	6.45
1688	Myrchad × spelt	89	1.55	6.4	48.7	35.4	16.5	5.49
1684	Ermak × spelt	90	1.78	8.8	45.7	38.1	17.8	5.74
1685	Ermak × spelt	95	2.35	9.8	52.0	30.2	14.2	6.87
1682	Selyanka × spelt	90	2.10	9.4	50.8	27.5	12.9	6.36
1694	Smuglianka × spelt	80	2.02	9.5	52.5	34.6	16.1	5.97
1689	Zolotokolosa × spelt	80	2.52	9.0	53.4	32.1	15.8	7.19
1686	Kharus × spelt	77	2.10	8.7	50.1	31.7	15.2	6.40
1681	Kharus × spelt	75	1.58	6.4	46.5	36.4	17.1	5.38
1675	Selyanka × spelt	60	1.98	9.5	48.9	33.4	16.1	5.80
1678	Selyanka × spelt	58	2.22	8.8	46.8	32.2	16.0	6.30
1514	BCNK × spelt	55	2.01	8.0	48.2	28.8	13.5	6.74
1598	Podolyanka × spelt	52	1.85	9.4	47.8	33.8	16.4	5.95
LSD ₀₅		3	0.07	0.3	1.7	1.1	0.5	0.22
$\bar{x} \pm S_x$		77.7 ± 9	2.0 ± 0.17	8.7 ± 0.6	50.0 ± 1.7	32.4 ± 1.8	15.4 ± 0.8	6.26 ± 0.32
min		52.0	1.6	6.4	45.7	27.5	12.9	5.38
max		100.0	2.5	9.8	55.2	38.1	17.8	7.19
V, %		31.1	4.14	13.7	17.1	28.6	14.2	5.09
S _x , %		5.4	3.80	3.4	1.6	2.5	2.5	2.41

*IPPG – Institute of Plant Physiology and Genetics NAS of Ukraine.

In this group of plants there was a significant variation ($V = 28.6\%$) in terms of gluten content. The range of variability fell between 27.5–38.1 g. A significant increase in gluten and protein content when compared to the standard was recorded in all samples, except 1692, 1687, 1682, and 1514, which have these indicators at the level of the control variant.

The forms with a different ear shape were selected for the seed plot. Considering the ear morphological structure, all of the materials obtained were divided into six morphological types: spelt (Fig 1, a), speltoid forms (Fig 1, b), forms with the typical ear for a soft wheat (Fig 1, c), squareheads (Fig 1, d), subcompactoids (Fig 1, e), and compactoids (Fig 1, f).



Figure 1. Wheat morphotypes by ear shape.

The characteristics of each morphotype are given in Table 4.

Table 4. The morphotype characteristics of wheat collection samples by ear shape

Morphotype	Sample	Ear density, pcs. of spikelets/ 10 cm of ear	Ear length, cm	Characteristics
Spelts	1786, 1817, 1674, 1559, 1731, 1755, 1725, 1721, 1719, 1691, 1695, 1730	< 16	> 15	Long, loose ear with tough glume and difficult grain threshing of the ear
Speltoides	1635, 1628, 1800, 1809, 1561, 1626, 1710, 1766, 1669	< 16, 17–22	12–15	Elongated, loose or medium dense ear with difficult grain threshing
Typical soft wheat	1598, 1514, 1675, 1686, 1682, 1685, 1684, 1692	17–22	8–12	Medium dense ear with soft glume and easy grain threshing
Squareheads	1689, 1687, 1678	17–22, 23–28	8–12	Compacted upper part of ear
Subcompactoids	1688, 1675	23–28, > 28	6–8	Shortened ear with compacted upper and middle part
Compactoids	1598, 1693	> 28	< 6	Short very dense ear

From the practical point of view, speltoids with the typical ear of a soft wheat and squareheads are the most valuable, because these forms have a well-grained ear with an easy grain threshing which insures high crop yields. In our studies these forms were the most productive. In particular, the squarehead samples 1689 and 1692 with the typical ear of a soft wheat produced the highest yield in the trial (with 7.19 and 7.02 t ha⁻¹).

Spelt doesn't have a high ear grain content and, as a result, its productivity levels are lower. However, the main obstacle to the large-scale introduction into manufacture of spelt is the difficult threshing of its ear grain (the threshing capacity of grain makes up about 60% of the total), which complicates the process of mechanically gathering their crops.

Among the collection samples, the most productive spelt wheat forms were samples 1695, 1755, 1559 and 1817, which all showed high yield capacity for this wheat species: 6.04–6.55 t ha⁻¹, with these results significantly exceeding the Zorya Ukrayny variety (5,52 t ha⁻¹) at LSD_{0.05} = 0.19. Forms with a long, loose ear have a number of advantages, in particular their rapid ear drying after rain, which helps to lower their susceptibility to disease, plus they are able to form large grains which have better technological qualities.

High fertility levels of the pollen and a better yield capacity were recorded in these forms. Therefore the aforementioned samples which have a long ear and high yields can be used for wheat breeding improvement programmes according to a number of economically valuable properties.

In subcompactoid and compactoid forms the number of grains in the head ear can reach up to 70 pcs, but their grain is shrunken and small, which negatively influences productivity. In our studies two subcompactoid samples were selected, 1688 and 1681, with a yield of 5.38–5.49 t ha⁻¹ and with a short (6.4 cm), well-grained (54 pcs) ear. Eleven compactoid samples were also created, with grain levels of 70 pcs per ear, but their productivity levels remain low (about 5.0 t ha⁻¹).

Grain weight from the head ear is an important parameter. It positively correlates with yield capacity and can be used in selecting high-yielding genotypes at early stages of breeding work. In wheat collection samples, grain weight from the head ear varied between 1.28–2.72 g. Spelt wheat samples 1695, 1755, 1559 and 1817, soft wheat samples 1692, 1685, and 1689, and speltoid sample 1561 were the best varieties according to this indicator and they all exceeded the standard figures. The samples created differed significantly according to the duration of their vegetation period.

Spelt wheat ripens between 7–10 days later than soft wheat. The collection includes spelt wheat samples which have the same ear formation and maturation period as early ripening soft wheat varieties. Samples 1674 and 1719 have a vegetation period of between 280–285 days, and the yield capacity of grain significantly exceeded the standard (5.76–5.84 t ha⁻¹). Early-ripening genotypes were also selected from the soft wheat group and speltoid forms. These are samples 1685 and 1710 with a vegetation period of between 280–285 days, which ripened 7–10 days earlier than the Podolyanka variety.

In certain years of the research (between 2013–2015) there was a significant spreading of brown rust in wheat crops. Up to 80% of plants were damaged by this pathogenic agent. During this period spelt wheat samples 1674 and 1721 and soft wheat samples 1685 and 1692 showed high resistance to this pathogen. The intensity of damage to the material was less than 5% of the leaf surface, which corresponds to scores of 8–9 according to the scale of resistance. These samples can be used in the wheat breeding process as donors of brown rust resistance genes.

Collection samples are constantly tested, and the search has been successfully conducted for new donor forms which exhibit valuable traits. The highest yield capacity and parameters for ear productivity were recorded in soft wheat. Amongst the fourteen samples from this group, two considerably exceeded the standard according to yield capacity and two samples equalled the standard in this parameter. It is worth noting that spelt wheat samples 1695 and 1817 combined a high gluten content (> 40%) and protein (about 20%) with yield capacity.

As a result of the research, the Europe spelt wheat variety and the soft wheat varieties Artemisia, Artaniia, and Artaplot were created, while the Europe and Artemisiia varieties were also included in the State Register of Plant Varieties Suitable for Distribution in Ukraine, while the Artaniia and Artaplot varieties were submitted for state scientific and technical expert evaluation.

The varieties created had the following characteristics during the period in which the state scientific and technical expert evaluation was being carried out (over the course of three years) in various soil and climatic zones across Ukraine:

- Europe spelt winter wheat (breeding sample 1725). An awned form of spelt wheat, with 90% of grain separating from glume during threshing. The height of the plant is 110 cm. Average yield during testing reached 5.8 t ha⁻¹ (2012–2015). Gluten content is 40%, and protein is 18%. The weight of a thousand grains is 45 g. Grain unit is 670 g L⁻¹. This variety is characterised by resistance to brown rust, powdery mildew, and snow mould, and a tolerance to yellow spotting, fusarium head blight, and root rot. Since 2015 this variety has been included in the State Register of Plant Varieties Suitable for Distribution in Ukraine;

- Artemisiia soft winter wheat (breeding sample 1686). The height of the plant is 79 cm. Average yield during testing was 6.5 t ha⁻¹ (2012–2015). Gluten content is 38%, and protein is 16%. The weight of a thousand grains is 43 g. The grain unit is 690 g L⁻¹. This variety has resistance to powdery mildew and snow mould, and a tolerance to root rot, yellow spotting, and fusarium blight. This variety is frost and drought-resistant. Since 2015 it has been included in the State Register of Plant Varieties Suitable for Distribution in Ukraine;

- Artaniia soft winter wheat (breeding sample 1684). The height of the plant is 80 cm. The ear is awnless. The average yield in the experiment was 5.5 t ha⁻¹ (2015–2017). Gluten content is 38%, and protein content is 18%. The weight of a thousand grains is 45 g. The grain unit is 725 g L⁻¹. Resistant to powdery mildew, root rot, and snow mould;

- Artaplot soft winter wheat (breeding sample 1809). The height of the plant is 78 cm. The ear is awned. The average yield in the experiment was 6.4 t ha⁻¹ (2015–2017). Gluten content is 39%, and protein content is 18%. The weight of a thousand grains is 45 g. The grain unit is 690 g L⁻¹. Resistant to fusarium and septoria blight, and also powdery mildew, and is tolerant to root rot and brown rust.

CONCLUSIONS

1. The collection of wheat samples which consisted of a thousand numbered items was created by the remote hybridisation of soft winter wheat and spelt wheat. Samples were analysed according to economically-valuable parameters and suitability for wheat breeding improvement. The collection includes unique recombinant forms which differ in terms of economically-valuable parameters, and in morphological and biochemical traits.

2. Spelt wheat forms, and soft wheat and speltoid samples were selected which combine high productivity levels with high gluten and protein content. These include spelt wheat sample 1817 with a gluten content of 45.2%, a protein content of 22.3%, and a yield capacity of 6.55 t ha⁻¹, and soft wheat sample 1689 which contains 32.4% gluten and 15.8% protein, and shows yields of 7.19 t ha⁻¹. These samples will be submitted to the State Scientific and Technical Expertise body in 2018.

3. The Europe spelt variety and the Artemisia soft wheat variety were created and were included in the State Register of Plant Varieties Suitable for Distribution in Ukraine, and the varieties, Artania and Artaplot, were submitted to the Ukraine State Scientific and Technical Expert Evaluation.

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Impact of faba bean (*Vicia faba* L.) cultivation on soil microbiological activity

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Abstract. Faba bean (*Vicia faba* L.) is widely grown not only as an important protein source for food and feed, but as a component in different cropping systems to improve soil quality. Beans are grown using different soil management practices, moreover, legume seeds often are inoculated before sowing. Microorganisms, introduced in the soil as an inoculum, affect not only inoculated plants, but these microorganisms can remain in the soil for the next growing season and can also affect the subsequent crops. Seed inoculation can stimulate production of root exudates as well as change microbial diversity and structure. The aim of the present study was to estimate the soil microbiological activity in soils where faba beans were cultivated with different rhizobia inoculants obtained from collection of Latvia University of Life Sciences and Technologies. Another trial was established where faba beans were included in different crop rotations under two tillage systems. During both trials, soil microbiological activity was analysed. Soil respiration intensity was measured by changes of carbon dioxide. Soil enzymatic activity was assessed by dehydrogenase activity and fluorescein diacetate (FDA) hydrolysis intensity. The total number of bacteria, fungi and rhizobia was expressed as colony forming units (CFU) g⁻¹ dry soil. Soil microbiological activity depended on the cultivated crop and the crop rotation. Faba bean inoculation method had less impact on the ratio between analysed microorganism groups than on the activity of soil enzymes.

Key words: tillage, crop rotation, Rhizobium, soil enzyme.

INTRODUCTION

Intensification of soil use in agriculture has caused the fear that soil quality and sustainability is decreasing. Soil management practices influence micro- and macro-organisms, so more environmentally friendly farming practices are being introduced in agriculture. That means thinking about improving soil cultivation, crop rotation systems and cultivation techniques. Attention is being paid to the impact of these processes on the quantity and composition of soil organic matter and availability of plant nutrients (Mikanova et al., 2009).

Legumes including faba beans (*Vicia faba* L.) are widely grown not only as an important protein source, but as a component in different cropping systems to improve soil quality. Currently, the use of legumes is expanding and developing also in the areas of processing, to improve the potential for legume production. Faba bean is increasingly investigated from the environmental point of view. Growing legumes not only reduces

the required mineral nitrogen consumption, thereby reduces N₂O emissions from soil caused by microbiological processes and improves the structure of soil (Jensen et al., 2010; Jensen et al., 2012; Schwenke et al., 2015). In addition, faba bean is a good pre-crop for cereals (Mikanova et al., 2009; Köpke & Nemecek, 2010). Diversity of crop species, cropping intensity, and crop rotation affect microbial activity and microorganisms diversity. Legumes effect the following crop by influencing the amount of available mineral elements, and by changing the microbiological processes. Different crops create specific environment in the rhizosphere (Thomas & Kevan, 1993). The biological processes in the rhizosphere are influenced by both the change of nutritional composition and root exudates. Most of the fixed N in legumes is harvested, but experimental data suggest that legumes can deposit significant amounts of N in the soil (Lupwayi & Kennedy, 2007), affecting the soil P dynamics and rhizosphere properties during growth (Yadav & Verma, 2014; Maltais-Landry, 2015). The root exudates can influence the structure and function of soil microorganisms' community and activity. The metabolic characteristics and the diversity of soil microbial communities are known to be sensitive to soil management and may provide information on the status and activity of the microbial community as well as the resilience of the community to stress. Additionally, the size and diversity of specific functional microbial groups, such as rhizobia, nitrifying bacteria or arbuscular mycorrhizal fungi communities also have potential to characterise the effects of management on the sustainability of soil (Bending et al., 2000; Bending et al., 2004).

Often faba bean is grown using seed inoculation with rhizobia that can affect not only symbiotic nitrogen assimilation, but also the rhizosphere processes by altering the composition of the inoculated plant exudates. Seed inoculation stimulates production of phytohormones, siderophores, and release of phenolic compounds and enzymes in the soil (Siczek & Lipiec, 2016). Soil enzyme activity is a significant indicator of soil microorganisms' diversity if they reflect changes in enzymes activity. Activity of fluorescein diacetate (FDA) hydrolase, dehydrogenase, glycosaminidase, and phosphatases characterises microbial activity in soils. These enzymes are involved with decomposition of complex organic compounds and with nitrogen mineralization, and are correlated with fungal and microbial biomass (Kandeler, 2007).

Conventional tillage may negatively affect soil microbial and biochemical properties through a reduction of soil organic matter content that provides a substrate source for soil microorganisms. A decline in water-stable macroaggregates that provide a favourable microhabitat for soil microorganisms and changes in environmental conditions such as moisture and temperature (Balota et al., 2003; Kabiri et al., 2016). Soil biological and biochemical processes may also affect the formation of symbiotic associations and their effectivity. Legume cultivation using symbiotic microorganisms instead of mineral nitrogen fertilisers is of great importance.

Faba beans as an important leguminous crop are studied mostly as feed and food source. But the interaction of faba bean with soil microorganisms is investigated mostly from the symbiotic nitrogen fixation point of view. However, little information is available about the effect of faba bean on microbiological activity in soil under different agricultural management practices. The aim of this study is to compare the number of bacteria and fungi as well as soil enzymatic activity under different soil cultivation methods for faba bean.

MATERIALS AND METHODS

The description of experimental site. A stationary field experiment was carried out at the Study and research farm ‘Peterlauki’ of the Latvia University of Life Sciences and Technologies. The data were obtained during the period 2014–2016. Soil microbiological activity was determined in two sets of experiments: 1) Tillage experiment, where microbiological activity was compared in faba bean trials with different tillage methods, and 2) Rhizobia experiment, where microbiological activity was compared in faba bean trials with seed inoculation. Soil agrochemical properties are characterized in Table 1.

1) *Tillage experiment.* Soil microbiological activity in faba bean sown with conventional (CT) and reduced (RT) tillage was analysed. Conventional tillage consisted of ploughing to a depth of 22–23 cm with a mouldboard plough, in contrast to minimum shallow tillage (RT) to a depth of 10–12 cm with a disc harrow. The experiment had two replications. Each plot size was 24×100 m. The soil according to WRB 2015 was a Cambic Calcisol (Aric, Bathyraptic, Episiltic, Protostagnic). An additional stationary field experiment tested different crop rotation systems. The dataset described in this paper contains only the plots with faba beans sown after grains, and compared with winter wheat plots cultivated without crop rotation.

2) *Rhizobia experiment.* Soil microbiological activity was analysed in the experimental plots where faba bean was grown with and without rhizobia inoculation. The trials were established on a field where the pre-crop was cereals and where trials with rhizobia bacteria have not been carried out for more than 10 years. The soil according to WRB 2015 was an Endocalcaric Endoabruptic Luvisol (Aric, Endoclayic, Cutanic, Hypereutric, Ochric, Endoraptic, Anosiltic, Protostagnic, Epiprotovetric). Nitrogen fertilizer was applied only to the faba bean plots without rhizobia inoculation. The experiment included variants without seed inoculation (K), without seed inoculation but with additional mineral nitrogen fertilizer (KN), and different inoculation variants a) with individual rhizobia strains (RP023 and RV407) or a mixture (R), b) with mycorrhizae inoculum (M) and mycorrhizae with additional mineral nitrogen fertilizer (MN), and c) double inoculation with rhizobia strain and mycorrhizae (RP023M, RV407M or RM).

Table 1. Soil agrochemical properties at the depth of 0–20 cm

Experiment field	pH _{KCl}	Carbonate, %	C _{org} , %	P ₂ O ₅ , mg kg ⁻¹	K ₂ O, mg kg ⁻¹
Tillage experiment	6.8	0.46	1.06	134.5	245.3
Rhizobia experiment	7.2	1.37	1.39	247.4	328.3

Soil sampling. For the *tillage experiment*, the presented results were obtained in the spring (April) and at the end of the crop vegetation period (November). Soil samples were collected after the faba beans were harvested and the next crop (winter wheat) was sown. Soil samples from the *Rhizobia experiment* were collected after the yield was harvested.

For both experimental sets, soil samples were prepared similarly – soil samples were taken from 0–20 cm of soil layer using an auger with a 2-cm diameter. A composite sample of 10–15 drillings was taken from each plot. For the analysis of soil biological activity, field-moist samples were stored in plastic bags at 4 °C.

Biological activity was determined by soil respiration intensity. Soil basal respiration was determined by placing 50 g of field-moist soil and a beaker containing 5 mL of 0.1 M KOH solution into a 500 mL glass jar; the jar was sealed and placed in the dark at 30 °C for 24 hours. Afterwards, the KOH solution was removed and titrated with 0.1 M HCl to determine the amount of CO₂ evolved with the soil microbial respiration (Pell et al., 2005). Results were compared with average soil respiration intensity from all measurements obtained from 2010 until 2014, when the soil intensity assessment was initiated.

Soil moisture was determined by drying the sample for 24 hours at 105 °C.

Soil enzymatic activity. The dehydrogenase activity (DHA) was determined as described by Kaimi et al. (2007) using iodonitrotetrazolium chloride (INT). INT is reduced by the enzyme reactions to a red formazan (INTF), the concentration of which was determined spectrophotometrically at 460 nm.

Soil fluorescein diacetate hydrolysis activity was determined according to Schnürer & Rosswall (1982). Fluorescein diacetate was added to the soil sample and incubated at 24 °C. After incubation, fluoresceine, the product of enzymatic conversion of FDA, was determined spectrophotometrically at 490 nm. All measurements of soil enzymatic activity were done in four replicates.

Soil microbiological analysis was performed in 2016 after the crop was harvested. Total number of cultivable bacteria (Nutrient agar, *Scharlau Chemie*, S.A. Spain), fungi (Czapek agar, *Scharlau Chemie*, S.A. Spain) and number of *Rhizobia* on mannitol media were determined¹.

The experimental data were analysed by two-factor analysis of variance using software ANOVA. The parameters were considered as significant at $P < 0.05$. In the *Rhizobia* experiment, correlation coefficients between number of microorganisms and soil microbiological activity were determined.

RESULTS AND DISCUSSION

Legumes, including faba bean, are grown not only as an important source of protein but also as a component of crop rotation. Therefore, the microbiological activity of the soil in different bean sowings was estimated. In reduced tillage, there was consistently less microbial respiration under continuous cereal than under faba bean (Fig. 1). In conventional tillage, the same trend was apparent except in April 2015 when there was no significant difference between the crops. The values under winter wheat were always below average. In both years, microbiological activity was higher in the autumn, except in the case of faba bean with reduced tillage in 2016.

1 <http://www.lf.llu.lv/sites/lf/files/2017-01/Eurolegume%20D3%201%20-%20Handbook%20of%20protocols.pdf>

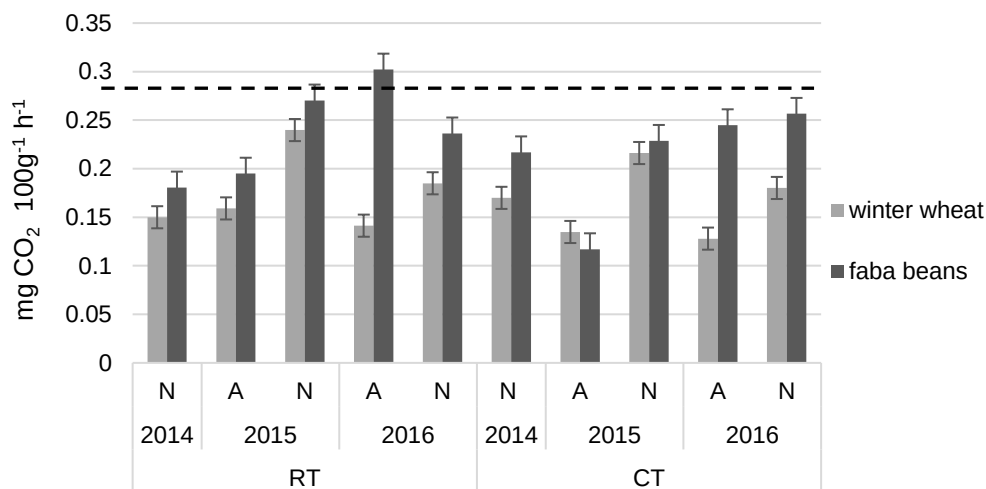


Figure 1. Soil respiration intensity in winter wheat and faba bean trials with reduced and conventional tillage: A – April; N – November; RT – reduced tillage; CT – conventional tillage; - - - - -average soil respiration intensity.

According to Copeck et al. (2015), the soil treatment affects the proportion of small soil particles in the soil, which can affect soil water content, aeration, and temperature. In 2016, from the second decade of July, precipitation was 18 to 33 percent more than in 2015, which could have affected soil properties and microbial activity.

According to Kainiemi et al. (2015), there are no consistent results in the literature about tillage effect on soil microbiological activity. Some authors achieved higher fixed soil CO₂ emission in conventional tillage (La Scala et al., 2006), but Kainiemi et al. (2013) obtained higher soil respiration intensity after reduced tillage. In contrast to the results of Kainiemi et al. (2015), our results show that higher respiration intensity was in the soil collected in November (both tillages). Furthermore, soil respiration intensity is influenced by the crop rotation. Such effect was detected in conventionally tilled plots in 2016. Differences between microorganisms' activity in monoculture and crop rotation has been noticed previously. Lower soil respiration intensity in winter wheat monoculture has been related with decrease in microorganisms' biomass and diversity (Gajda & Martyniuk, 2005). In addition, Köpke & Nemecek (2010) emphasized the positive effect of faba beans on soil microbial diversity.

Activity of dehydrogenases were unstable (Fig. 2). Enzyme activity after faba bean cultivation was similar in the CT and RT soil, but in winter wheat plots significantly higher dehydrogenase activity was observed in conventionally tilled plot in 2016. In two experiment years from three raised dehydrogenase activity in conventionally tilled faba bean fields was observed. Tamm et al. (2016) noticed that more substantial dehydrogenase activity changes between soil layers were found in reduced tillage, but in conventionally tilled soil enzyme activity fluctuated less.

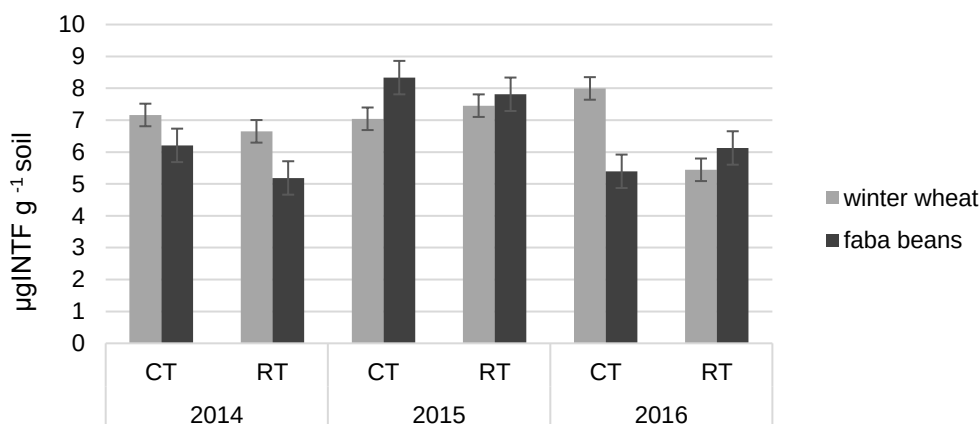


Figure 2. Dehydrogenase activity in winter wheat and faba bean trials at the end of the vegetation period: CT – convention tillage; RT – reduced tillage.

In contrast with dehydrogenase, in most cases activity of hydrolytic enzymes were higher in reduced tillage plot soils. Two thirds of the results indicated that the inclusion of faba beans in crop rotation after grains reduced the activity of hydrolytic enzymes at the end of the vegetation period. (Fig. 3).

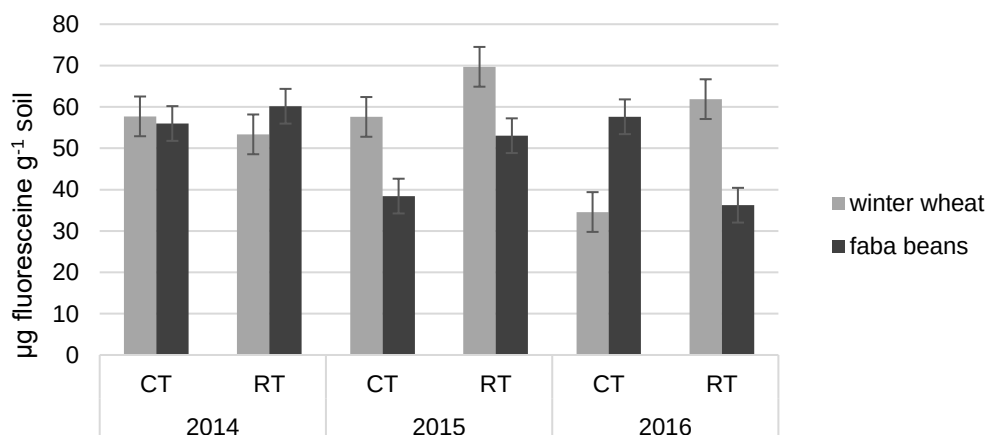


Figure 3. FDA hydrolysis intensity in winter wheat and faba bean trials at the end of vegetation period: CT – convention tillage; RT – reduced tillage.

Microbiological activity in the faba beans root zone was significantly higher at the end of vegetation period (Fig. 4). During flowering, faba bean is intensively fixing symbiotic nitrogen, and energetic processes are occurring more intensively in nodules (Hirsch, 1992; Kiers & Denison, 2008). At the end of the vegetation period, the progressive death of roots increases the carbon source for microorganisms, and a considerable amount of *Rhizobium* bacteria leave senescing nodules. The combination of these processes can also lead to higher soil respiration during this period.

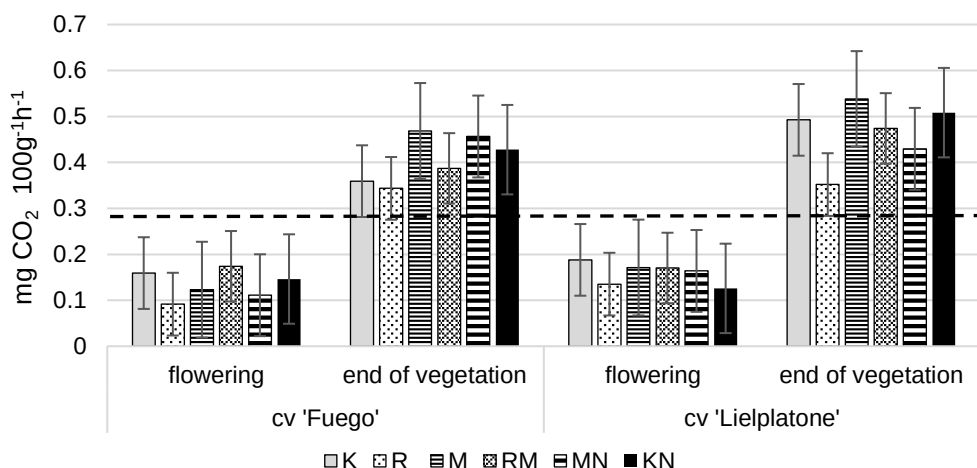


Figure 4. Soil respiration intensity in faba bean trials with different seed inoculation variants: K – control without seeds inoculation; R – inoculation with mixture of rhizobia strains RV407 and RP023; M – inoculation with mycorrhizae fungi; RM – inoculation with rhizobia bacteria and mycorrhizae fungi; MN – inoculation with mycorrhizae fungi and with additional mineral nitrogen fertilizer; KN - control without seeds inoculation, but with additional mineral nitrogen fertilizer; - - - - : average soil respiration intensity.

Dehydrogenase activity at the end of vegetation period varied between seed inoculation variants, but not significantly (Fig. 5). Values were lower in cv 'Fuego' than in cv 'Lielplatone'. Highest dehydrogenase activity in cv 'Fuego' was obtained with rhizobia strain RV407 and with mycorrhizae fungi, whereas the highest value in cv 'Lielplatone' was observed with rhizobia strain RP023 followed by the double inoculation RP023M.

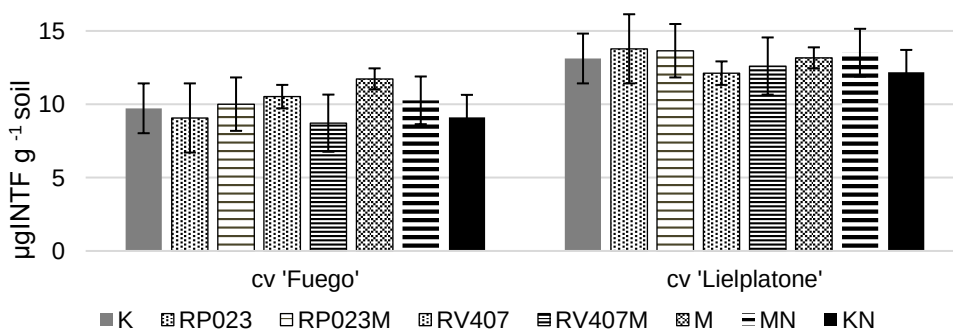


Figure 5. Comparison of DH activity in faba bean trials with different seed inoculation variants: K – control without seeds inoculation; RP023 – inoculation with rhizobia strains RP023; RV407 – inoculation with rhizobia strains RV407; M – inoculation with mycorrhizae fungi; RP023M – inoculation with rhizobia strains RP023 and mycorrhizae fungi; RV407M – inoculation with mixture of rhizobia strains RV407 and mycorrhizae fungi; MN – inoculation with mycorrhizae fungi and with additional mineral nitrogen fertilizer; KN – control without seeds inoculation, but with additional mineral nitrogen fertilizer.

Fluorescein diacetate (FDA) results differed between inoculation variants and between cultivars (Fig. 6), with the lowest values in both cultivars from the RV407 rhizobial inoculant. For cv 'Fuego', the highest FDA hydrolysis intensity was observed in the treatment with both mycorrhiza and nitrogen fertilizer, but for cv 'Lielplatone' it was in the treatment with rhizobia strain RP023.

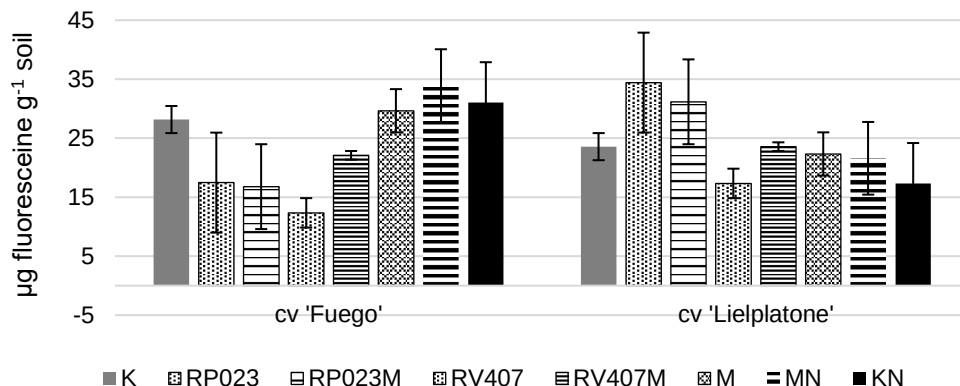


Figure 6. FDA hydrolysis intensity in faba bean trials with different seed inoculation variants. Abbreviations are clarified in Fig. 5

Soil microbiological analysis showed that the number of microorganisms in the root zone was influenced more by the crop rotation and legume seed inoculation than it was by soil tillage method. Number of bacteria and fungi in the root zone of RT faba beans were about 71% and 55% higher respectively than in root zone of winter wheat, but in the CT plots 38% and 40% respectively. Differences between the number of microorganisms in RT and CT root zone were 21% and 30%, respectively.

In the rhizobia experiment, soil microbiological analysis at the end of the vegetation period showed differences between inoculation treatments. During the vegetation period, the number of rhizobia bacteria in the root zone increased by 19–32% for both faba bean cultivars. The highest increase was observed for treatments with double inoculation – for cv 'Fuego' 32% increase, but cv 'Lielplatone' 28%. A 2% decrease in rhizobia population was observed in mycorrhizae variant with additional nitrogen fertilizer for cv 'Lielplatone'. The total number of bacteria did not increase significantly. The highest increase was observed in variants with mycorrhizae: in the root zone of cv 'Fuego' by 16%, and in cv 'Lielplatone' by 49%. In the rhizobia experiment a significant correlation ($r = 0.43$) between total number of bacteria and dehydrogenase activity was detected.

CONCLUSIONS

Including faba bean in a crop rotation increased the number of soil microorganisms, regardless of tillage treatment.

Inoculation of bean seed influenced microorganism activity and interaction near plant roots.

Additional nitrogen fertilizer decreased the number of fungal colony forming units in the variant with mycorrhiza fungi inoculant.

Seed inoculation effectively increased rhizobia numbers.

The soil enzymatic activities were not similarly affected by different *Rhizobium* inoculation variants.

ACKNOWLEDGEMENTS. The experiments with faba bean inoculation and assessment of microbiological activity was supported by the 7th Research Framework Programme of the European Union project 613781, EUROLEGUME (Enhancing of legumes growing in Europe through sustainable cropping for protein supply for food and feed).

The faba beans field trials with different soil management and assessment of microbiological activity was supported by the State research programme 'Agricultural Resources for Sustainable Production of Qualitative and Healthy Foods in Latvia' Project No. 1 Sustainable use of soil resources and abatement of fertilisation risks (SOIL).

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Effects of Dormex (Hydrogen Cyanamide) on the performance of three seedless table grape cultivars grown under greenhouse or open-field conditions

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Abstract. Greenhouse cultivation of table grapes is still limited to some experimental trials at Lebanese coast. One major constraint facing this type of cultivation is the lack of enough chilling hours causing irregular bud-break and yield reductions. Dormex, with Hydrogen Cyanamide as active ingredient, is an effective mean for dormancy release adopted in warm winter regions. The work investigated separate and combined effects of two factors: greenhouse cultivation and Dormex application on vine buds (following winter pruning) on three-year old seedless cultivars (ARRA15, ARRA18, and ARRA19). Control consisted of non-treated plants grown in open-field. Results showed that Dormex application under greenhouse induced budburst uniformity, increased budburst percent (by 60%), number of flowers and fruits per shoot (by 83%) and vine productivity (by 90%) in all cultivars compared to control. Bud formation was increased under greenhouse and was reduced by Dormex treatment. Under greenhouse, elongation of current season shoots was delayed and shoot length was reduced in treated plants, harvest was earlier by 7, 14, and 30 days in non-treated plants of ARRA18, ARRA19 and ARRA15 respectively and full fruit set (100%) occurred for all plants. Dry weight of shoots was improved by Dormex application in both cultivation systems. All ARRA cultivars responded similarly to experimental factors except ARRA 19 under greenhouse where shoot length was enhanced in all plants while bud formation only in treated plants. Finally, treating vine by Dormex under greenhouse was found as efficient tool to improve bud break and advance harvest under the specific Lebanese coastal conditions.

Key words: bud break, greenhouse, Hydrogen Cyanamide, seedless cultivars.

INTRODUCTION

The diversity of micro-climates in Lebanon is an asset for new introduced table grape cultivars which are characterized by a higher productivity compared to local ones. Lebanese farmers are becoming increasingly interested in diversifying into popular seedless cultivars targeting high value local and export markets. However, excess production of such cultivars could negatively affect their prices at local market especially when the quality of products intended for export is affected by inadequate storage

facilities (DAI, 2014). Consequently, it is of great practical significance to change the grape growth period and promote the grape to go on the market ahead of time through greenhouse cultivation pattern. According to Qin (2013) under greenhouse yield is higher, quicker and easier to manage compared to open-field cultivation. Also, the fruit is of good quality, and fruit maturity and harvest period can be easily controlled.

Recently, greenhouse cultivation has been rising at Lebanese coast; however it is still limited to some experimental trials and has not yet reached commercial volumes. One major constraint facing this cultivation technique in coastal regions is the lack of enough chilling hours that cause irregular bud-break. When grapes do not receive sufficient winter chilling to release buds from dormancy, a delayed and erratic bud-break may result causing reductions in shoots and clusters number per vine and irregular ripening (Lavee et al., 1984; Hashim-Maguire, 2015). Inadequate winter chilling could also reduce fruit yield and fruit quality (Dokoozlian & Williams, 1995).

There are three successive phases of bud dormancy in grapevines; paradormancy that is regulated by physiological factors within the plant but outside the dormant structure, endodormancy that is regulated by physiological factors within the bud itself and ecodormancy that is imposed by environmental factors after endodormancy release ending when warm temperatures cause ecodormant buds to burst (Balandier et al., 1993; Egea et al., 2003). Shoot growth begins with budburst and initially the growth is slow, but soon it enters a phase of rapid growth which typically continues until just after fruit set (Goldammer, 2015).

On the other hand, early studies have pointed out the efficient role of Hydrogen Cyanamide (HC) as a plant growth regulator that supplements chilling and causes earlier and more uniform bud-break (George et al., 1992; Cline, 2003) improves yield (Carreno et al., 1999; Abdalla, 2007; Hussein, 2009) and ameliorates growth uniformity (Hashim-Maguire, 2015; Silvestre et al., 2017). Under experimental conditions, applying 1.25% HC, 2.50% HC (Dokoozlian & Williams, 1995) and 1.5% HC (Botelho et al., 2007) has improved bud sprouting in vine cuttings. Similar trend was reported by Pérez & Lira (2005) and Mohammed & Gouda (2017) in open-field conditions where the application of 5% HC has maximized and advanced bud-break by 26–40 days. Improvement and advances in bud-break were also found by Ben Mohamed et al. (2010), Ben Mohamed et al. (2012) and Khalil-Ur-Rehman et al. (2017) following Dormex (HC as active ingredient) treatment. Additionally, HC improved main shoot length (Ahmed et al., 2014), advanced harvest dates and ameliorated fruiting buds percentage, berry set, clusters number per vine, cluster weight and cluster dimensions (Ahmed et al., 2014; Mohamed & Gouda, 2017).

Moreover, de Almeida et al. (2017) reported positive effects of HC application on grape vines covered by plastic films, however previous trials regarding its potential effects under greenhouse are lacking from literature. Consequently, the experiment was as a first trial conducted in Lebanon to evaluate the effects of Dormex (3.5% HC) application under greenhouse as an attempt to attain more uniform and earlier production, consequently to provide newly introduced seedless ARRA cultivars off-season at the Lebanese market.

MATERIALS AND METHODS

The study was carried out in an experimental field situated at the Lebanese coast (140 m above sea level) where three years old seedless cultivars (ARRA15: white variety, ARRA18: black variety and ARRA19: red variety) were grown in open-field conditions or under greenhouse spaced at 3 x 3 meters apart, trained on pergola system and drip irrigated (30 L per vine per 2 weeks). Vines of all cultivars were grafted on the same rootstock: 1103P and were pruned in mid-November leaving 6 buds per cane. Vines received around 400 chilling hours in open-field, and 90 chilling hours under greenhouse during the period of bud dormancy.

Dormex solution containing 3.5% HC was applied 12 days after pruning (Aly et al., 2015) and 12 days later by wiping the buds with cotton immersed in Dormex solution. Experimental treatments were: greenhouse/without Dormex, greenhouse/with Dormex, open-field/with Dormex and open-field/without Dormex (control).

Date of budburst, fruiting, flowering and harvest were determined through daily visual monitoring on-site. Budburst percent was evaluated on the main cane. Buds on the main cane were assigned as a, b, c, d, e and f. The bud position 'a' being the position of the first bud emerging at the base of the main cane. Measurements carried out were: length of current season's shoot (cm) (evaluated weekly as soon as shoots have emerged), number of buds (formed on current season's shoots), bud burst uniformity (distribution pattern of bursted out and non-bursted out buds on current season's shoots), number of flowers and number of clusters per shoot, fruit set (%), average weight of individual cluster (g) and fruit yield (Kg per vine). Additionally, shoots were collected and oven-dried at 105 °C until constant weight for determine their dry weight (g). A full factorial design was adopted with 4 treatments and 9 replicates per treatment (9 vines). Statistical analysis was done using STATISTICA program. Factorial ANOVA and Chi-square test were applied considering a $P_{value} < 0.05$.

RESULTS

Dormex application increased budburst percent by around 74% under greenhouse and around 31% in open-field with no significant differences among cultivars Fig. 1. Dormex induced significantly lower average length of current season's shoots of all cultivars grown in open-field, and under greenhouse, with the lowest values obtained in ARRA15 and ARRA18 grown under greenhouse Fig. 2. In non-treated plants, shoot elongation in time Fig. 3 was not affected by cultivation system, while in treated plants it was lower under greenhouse except for ARRA19. In addition, average dry weight of shoots Fig. 4 was significantly enhanced by Dormex application mainly in ARRA15 (by 59%) and ARRA18 (by 49%) in open-field and in all cultivars under greenhouse (by around 68%). The highest dry weight of shoots was recorded for ARRA19 in the treatment greenhouse/with Dormex (2,141.6 g).

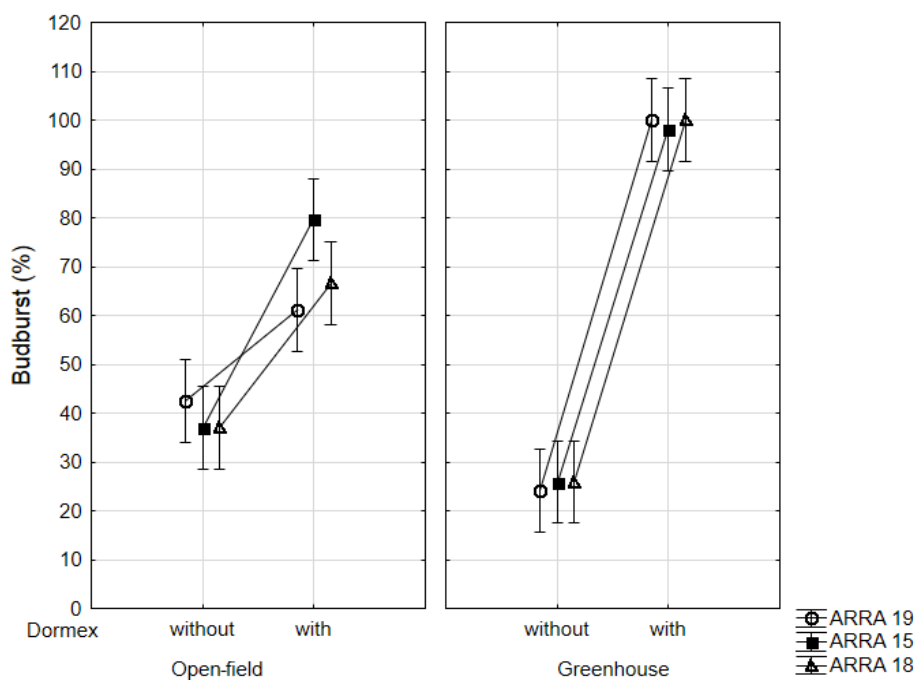


Figure 1. Averages of budburst in percentage (middle markers).

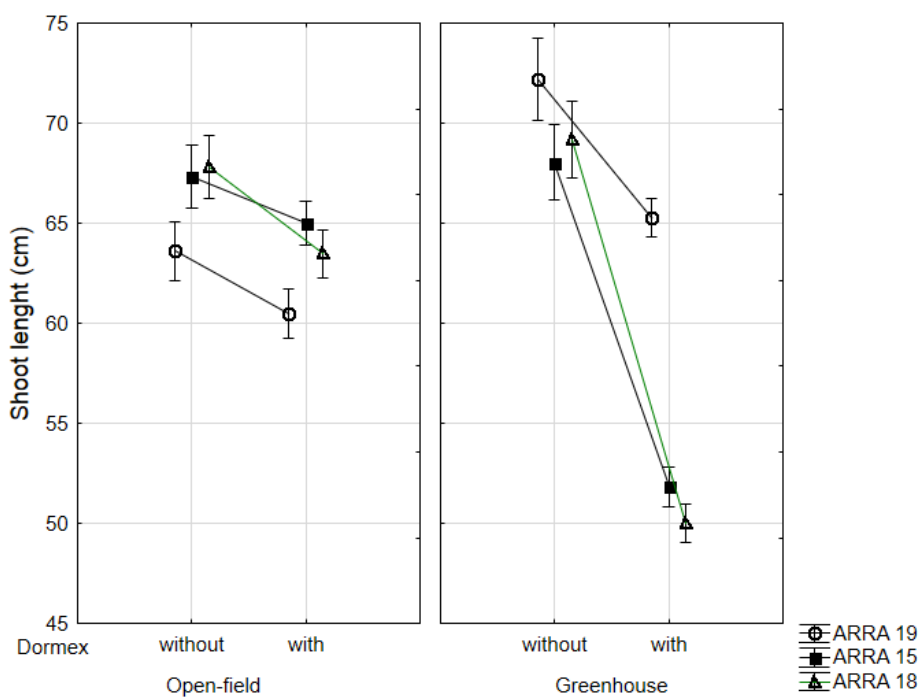


Figure 2. Averages of shoot length in cm (middle markers).

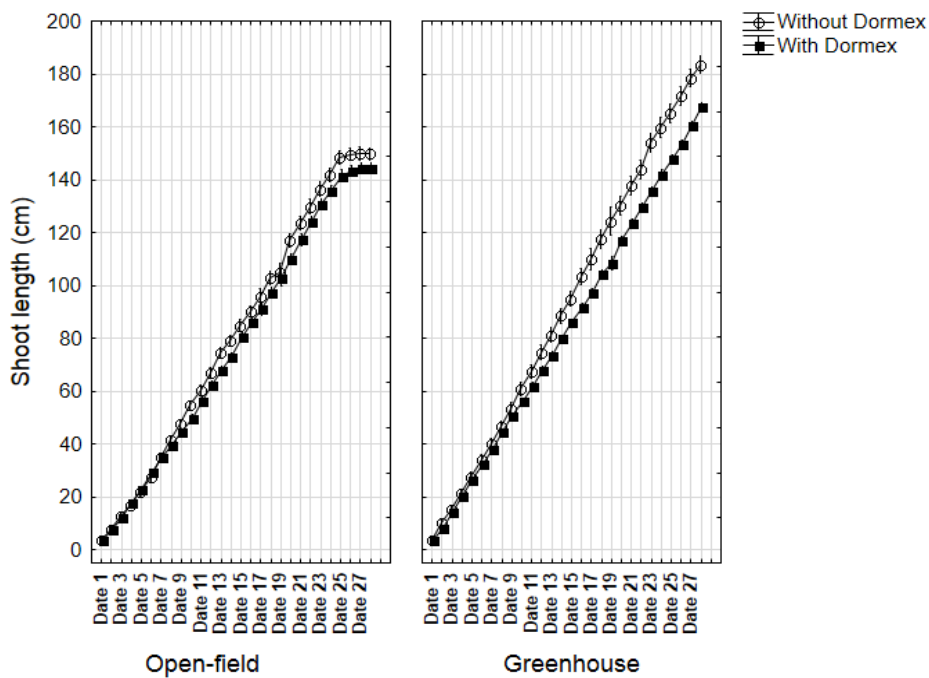


Figure 3. Averages of shoot length in cm (middle markers).

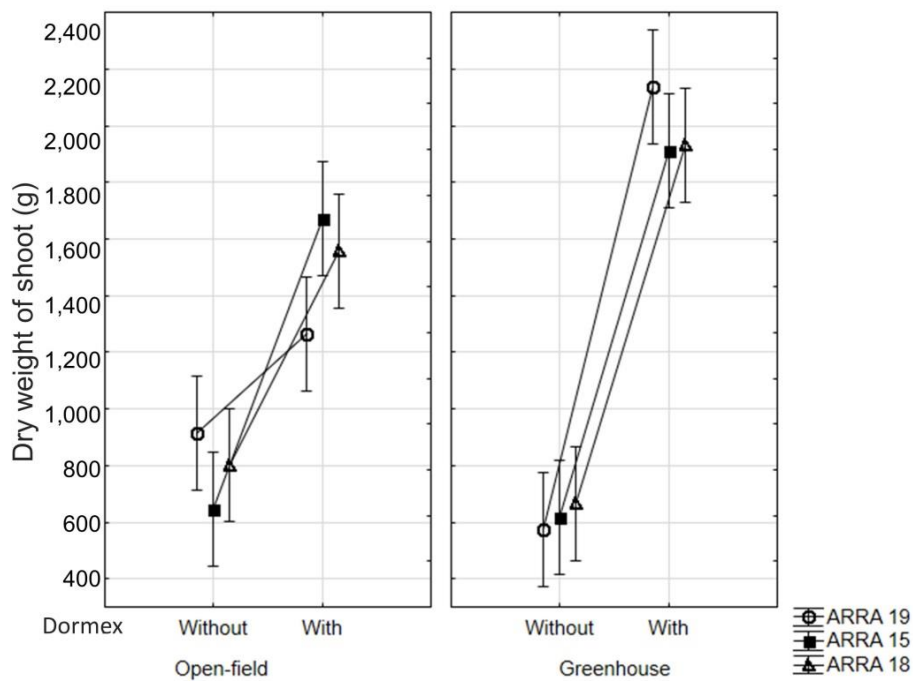


Figure 4. Averages of dry weight of shoots in grams (middle markers).

Average number of buds on current season's shoots was higher under greenhouse compared to open-field in treated (greenhouse/with Dormex: 31, 31 and 39 buds compared to outdoor/with Dormex: 13, 13, and 13 in ARRA15, ARRA18 and ARRA19 respectively) and non-treated plants of all cultivars (greenhouse/without Dormex: 34, 34 and 33 buds compared to open-field/without Dormex: 20, 30 and 17 in ARRA15, ARRA18 and ARRA19 respectively). However, Dormex reduced bud formation in both systems for all cultivars except for ARRA19 under greenhouse (32 and 39 buds in greenhouse/without Dormex and greenhouse/with Dormex respectively).

When comparing the effect of cultivation systems, it was observed that budburst was higher in open-field compared to greenhouse in non-treated plants, while it was the opposite case following Dormex application Fig. 5. Moreover, Dormex induced a more uniform and full budburst (100%) under greenhouse (around 27 buds on the 6 bud positions), while it only improved buds formation in open-field (on all positions with maximization only on the first two positions) with no significant effect on budburst uniformity.

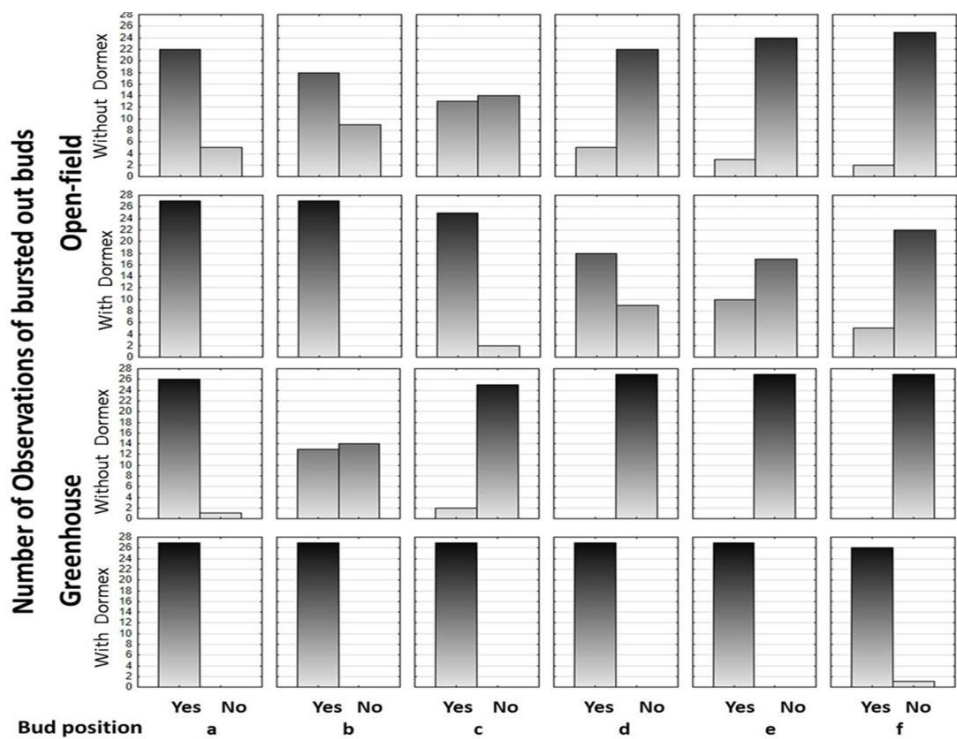


Figure 5. Observed bursted out buds frequencies for the different levels of the experimental factors(a, b, c, d, e and f: position of buds on main shoot).

In general, the dates of flowering and fruit set were advanced under greenhouse compared to open-field. Also, budburst and harvest dates were advanced under greenhouse by one week in treated plants of all cultivars. On the other hand, in non-treated plants budburst was advanced by one week for all cultivars and harvest was advanced by one, three and four weeks for ARRA18, ARRA19 and ARRA15

respectively. Under greenhouse, there was no significant effect of Dormex on various phenological dates while in open-field conditions it induced earlier harvest by 2 and 3 weeks in ARRA19 and ARRA15 respectively Fig. 6.

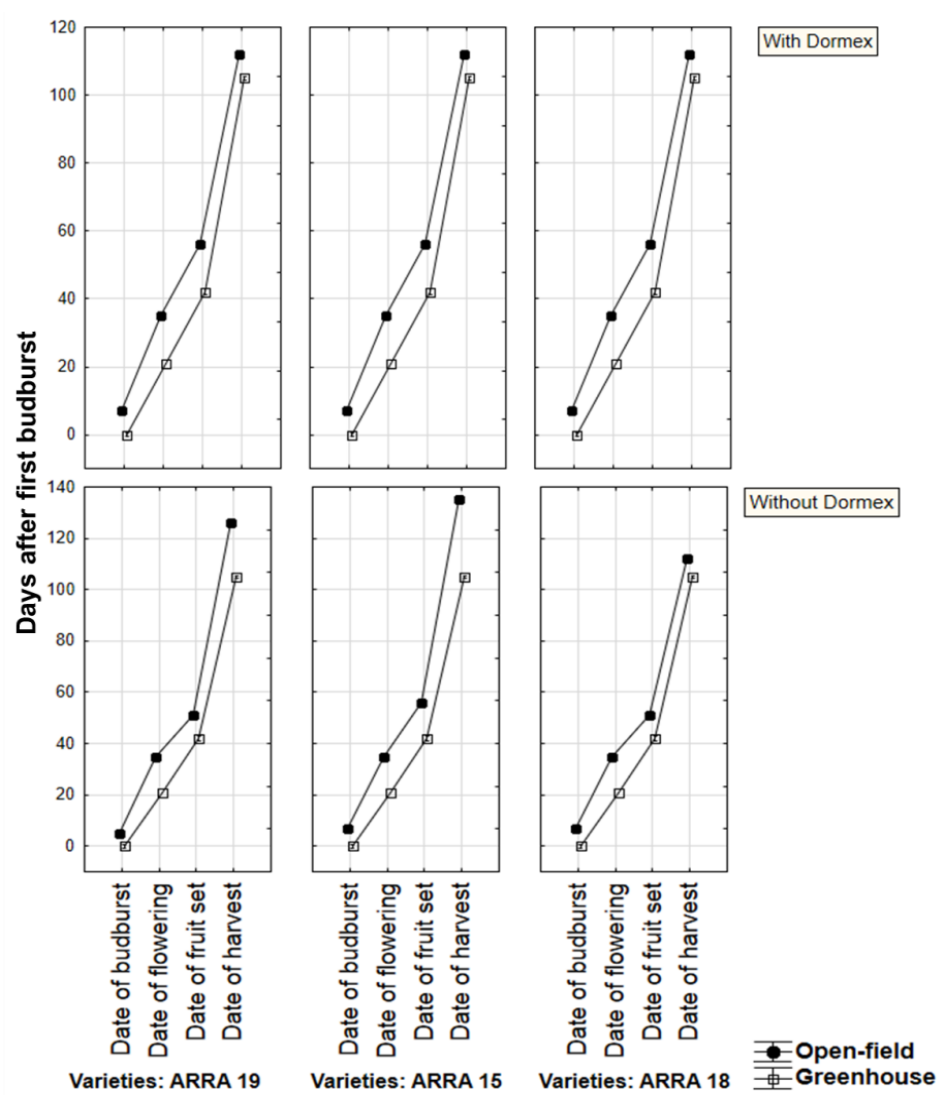


Figure 6. Average days (middle markers) to budburst, flowering, fruit set, harvest dates (days after first budburst).

Fruit set was significantly improved under greenhouse in non-treated plants of ARRA 19 (by 22%) and in treated ones of ARRA 15 (by 46%) and ARRA 19 (by 35%) compared to open-field. Dormex treatment did not positively influence fruit set despite the cultivation systems, in fact it had a negative effect on fruit set of ARRA15 in open-field (decrease by 46%). Moreover, it has a significant positive effect on flowers and fruits number per shoot only under greenhouse where averages of both indicators were both increased (1 flower and 1 fruit in greenhouse/without Dormex compared to

6 flowers and 6 fruits in greenhouse/with Dormex). The highest average cluster weight and average yield per vine were obtained in the treatment greenhouse/with Dormex (708 g, 671 g, 743 g and 26 kg per vine, 25 kg per vine, 27 kg per vine for ARRA19, ARRA15, and ARRA18 respectively). In the remaining treatments, average cluster weight did not exceed 237 g and yields were negligible. As a result, application of Dormex under greenhouse improved plant productivity by 99% for all cultivars.

DISCUSSION

Advanced harvest under greenhouse was related to the earlier budburst and fruit maturity on vines confirming the findings of Novello et al. (2000) and Kamiloğlu et al. (2011). Under greenhouse, the air temperature increases and induces a faster accumulation of growing degree days, which in turn, stimulate an earlier vine budbreak (Novello & de Palma, 2008). The greater shoot development in non-treated plants of ARRA 19 under greenhouse were similarly observed by Novello et al. (2000) on protected 'Matilde' table grapes.

Improvement in budburst percent as a result of Dormex application that was reached in this study (4 times higher) was superior to the one obtained on "Superior Seedless" (2 times higher) in the study of Ben Mohamed et al. (2012a). In fact, hydrogen cyanamide favors the decarboxylation process (Slocum & Flores, 1991) and causes a strong inhibition of the enzyme catalase (Amberger, 1961). Catalase activity is at maximum in dormant buds and decreases with low winter temperature (Nir et al., 1986; Or et al., 2001). Additionally, Dormex counteracted the impact of lacking chilling hours on plants by favoring the allocation of assimilates towards plant shoots and inducing a normal metabolic activity (Ben Mohamed et al., 2012a) in shoot cells. It also might have lowered down and reduced shoot growth at the expense of budburst. However, it did not affect the date of budburst which contradicted earlier findings regarding this indicator (Pérez & Lira, 2005; Ben Mohamed et al., 2012; Hashim-Maguire, 2015; Khalil-Ur-Rehman et al., 2017).

Chilling exposure is a critical factor influencing the response of grapevines to HC which could not play any significant role on bud-break and fruit maturity under conditions where grapevines receive sufficient chilling 800 h at 7 °C (Jensen & Bettiga, 1984; Williams, 1987). In the current work the efficiency of Dormex (3.5% HC) on bud break was high under greenhouse where vines received 90 chilling hours compared to a lower efficiency in open-field with 400 chilling hours. In addition, a high efficiency of Dormex (4% HC) in breaking dormancy and promoting yield was found earlier by Ahmed et al. (2014) when vines had received 200 or 210 chilling hours.

The lowest shoot elongation, thus the more equilibrated distribution of assimilates in shoots caused complete and uniform budburst and improved flower and fruit number in treated vines under greenhouse while in open-field the growing stems have diverted sugars away from axillary buds (Kebrom, 2017). The exceptional low reduction in shoot length and the improvement in budburst in treated ARRA19 plants under greenhouse compared to ARRA 15 and ARRA 18 could reflect a variety-dependent response to Dormex (Lavee et al., 1984).

At the level of each axillary bud and at the plant level, many endogenous and developmental signals have to be integrated to determine bud fate and to establish the number and position of the growing new shoots on the plant. Such regulation is also

strongly dependent on environmental factors (Khayat & Zieslin, 1982; Moulia et al., 1999; Battey, 2000; Cameron et al., 2006; Kim et al., 2010; Huché-Thélier et al., 2011; Demotes-Mainard et al., 2013; Djennane et al., 2014; Pierik & Testerink, 2014).

CONCLUSION

Greenhouse cultivation has shortened the phenological cycle of plants reaching an earlier maturity of fruit clusters and consequently earlier yields. In parallel, improvement in bud-break and the more equilibrated allocation of assimilates in new shoots following Dormex application has resulted in a maximization of yields on shoots of current season. Consequently, there was complementarity in the effects of Dormex and greenhouse and this combination of cultural practices was an efficient way to reach the objectives of the study that were basically focused on resolving the problem of insufficient chilling hours' requirements on the Lebanese coast and would help in spreading table grapes cultivation in warm winter regions and to produce off-season table grapes. Finally, Dormex is prohibited in some countries because of its hazardous effects. Therefore, it is better to use it at recommended concentration and to take all safety measure when applying it.

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Determination of the tension limit forces of a barley malt and a malt crush in correlation with a load size

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Abstract. This article deals with determination of selected parameters of barley malt (whole grain and crushed grain). The barley malt is besides water, hops and brewer's yeast, one of the basic ingredients necessary for the production of traditional Czech pilsner type of beer. The aim of this research is to determine limit force and internal friction angle with depending on the size of the load. The assessed malt crush was produced using a 2-roller malt mill. The 2-roller malt mill is based on the principle of grinding the material in a milling gap between two milling rollers, which is a very commonly used production of the malt crush. By determining the tension limits of the barley malt and the malt crush in correlation with the load, we can obtain very important parameters that inform us of the bulk material behavior, particularly with respect to the storage method (storage shape, height of the stored material layer, and the removal method from the storage, etc.), and to the transport (route gradient, transport speed, etc.). The determination of the tension limits has a direct link with cohesion of the bulk material and thereby contributes to establishing of the basic parameters of the bulk material, such as - the friction angle. The measurement for the angle of internal friction determination were performed on a prototype device. The principle of measurement on a mobile prototype devices is, the upper square chamber slides down the lower square chamber. Barley malt (whole grain and crushed grain) were loaded from 100 g to 5,000 g. The results of measuring were statistically analyzed with software Statistica 12.

Key words: food industry, barley malt, malt crush, bulk material, friction angle.

INTRODUCTION

These basic ingredients are used when manufacturing a traditional Czech pilsener type beer: water, hops, brewer's yeast and barley malt. The light malt is a product made from barley, after four- to five- week ripening in containers. At the beginning of the malt manufacturing technology, there is a phase of pre-cleaning of barley, which is then followed by soaking of barley in special containers so called steeping tanks. A germination of barley was in the past conducted in so called floor malting houses, however nowadays it is being realised by using pneumatic germination drums, or

germination boxes, Saladin or Lausmann boxes, or circular germination towers arranged vertically (germination towers), then it is followed by so called kilning which is drying of the green malt in the drying kiln. Such germinated but still green malt is in the first stage pre-dried by dry air at the temperature of 60 °C and then finished at the temperature 80 °C up to 105 °C.

The moisture content definition of the processed materials, respectively of the actual measured sample, was the basic step of the particular experimental measurements. A knowledge of the initial moisture content of the sample (%) is necessary for securing optimal conditions for the additional experiments and possible detection of correlation between measured quantities and entry material.

For ensuring the optimal progress of the next technological steps, the value of moisture content of the processed (stored) materials is the basic requirement. With regard to relevant economical indicators and ingredient quality, the moisture content optimalization is fundamental because higher moisture content causes i.e. a fast development of mould which contributes to a significant deterioration of the ingredient quality. Therefore the moisture content control in individual food companies is not underestimated. The deterioration of ingredients' quality is not the only factor to determine the initial moisture content because losses (especially energetical) are also caused during grinding, as the amount of moisture content affects the amount of energy needed for malt grinding, thus the malt crush production (Dendy & Dobraszczyk, 2001; Kunze, 2010).

The type of the produced malt depends on the temperature of the air used for drying because lower temperatures of the drying air produce light malts and high temperatures produce dark malts. The dried malt is after kilning cleaned from damaged grains, dust and roots and it is further transported to a container where it has to stay for a certain period followed by another processing, the so called air resting. Light malts in Czechia are generally pilsner types (the temperature of drying air up to 85 °C), or Vienna and Dortmund malt type (Kunze, 2010; Chládek et al., 2013).

Before the actual brewing, the malt is mechanically grinded using malt mills (grinders) that work on the principle of grinding between two counter-rotating rollers (the so-called roller mills respectively Malt grist mills), grinding using rotating hammers (so called hammer mills) and grinding between two discs (so called steel disc mills, respectively dispersants). The product of malt grinding is called a malt crush, which is the basic material for the actual beer brewing (Vaculík et al., 2013; Smejtková et al., 2016).

The traditional beer brewing is bringing together subjects from a wide range of fields that include i.e. fermentation chemistry, microbiology, but also subject of grain treatment, transport and storage. These very subjects of grain treatment, the transport, and the storage are demanding a knowledge of the basic parameters of the processed materials, thus the bulk materials. Among basic properties of the bulk materials, where barley malt and the malt crush undoubtedly belong, can be included: density, bulk density, friction angle (Chotěborský & Linda, 2014).

The paper is aimed at determining the tension limits, depending on the load size of the barley malt and the malt crush. In the case of incoherent (ideally) bulk materials, an external friction coefficient is defined, which is characterizing the frictional properties of the mass on the surfaces of containers and mats (external surfaces) and an internal friction coefficient is characterizing the frictional properties of the internal section area

(Afzalnia & Roberge, 2007; Ibrahim, 2008; Boac et al., 2010; Gil et al., 2013, Liu et al., 2015; Zheng et al., 2017).

For cohesive bulk materials, the interactions between particles are also affected by cohesiveness. An ideally loose substance can be mostly compared to a dry matter with particles larger than 0.25 mm (ČSN ISO TS 17892-10). Finer milled substances are showing a cohesiveness. Cohesive forces impact the contact points of the particles. Therefore, the finer is the substance, the greater is the cohesiveness because in the unit of volume there is a greater number of contact points. Cohesion forces are of different physical nature (Jacobson et al., 2004; Afzalnia & Roberge, 2007; Kaliniewicz, 2013; Sologubik et al., 2013; Füll & Hoffmann, 2015; Kibar, 2016).

The main objective of this paper is a determination of selected mathematical and physical parameters of colored and light malt, i.e. size of the grain, density, bulk density, specific weight of the malt, repose angle and determination of tension limits.

MATERIAL AND METHODS

Before the actual determination of tension limits of the barley malt and the malt crush, depending on the load size, the moisture content of the processed materials was defined.

During grinding of the rated barley malt varieties was used a double roll crusher KVM 130/150 (Fig. 1), manufactured by KVM Uničov, Czech Republic, with a maximum output of 250 kg h⁻¹ and two electric motors, each with a power of 2.05 kW. The distance between the grinding rollers, i.e. the gap between the rollers, was set to 0.4 mm.

Determination of the mathematical and physical parameters

Determining the moisture content of the assessed barley malt was implemented using a moisture analyzer OHAUS MB25 (Fig. 2).



Figure 1. The double roll crusher KVM 130/150.



Figure 2. Moisture analyzer OHAUS MB 25.

Firstly, representative samples of malt were taken, we chose a drying temperature of 105 °C and an automatic drying mode, which is automatically terminated once the mass (weight) constant is reached. The principle of the moisture analyzer OHAUS MB25 is based on a weight reduction of the measured sample due to its heating that is caused using the heat source of the moisture analyzer, which is a halogen emitter. The drying temperature for this analyzer can be set ranging from 50 °C up to 160 °C.

For determining the mean statistical particle size which is a constant for a specific set $\bar{x}$, we applied so called RRSB distribution. For fine-grained materials that are products of grinding different substances, an exponential relationship was found by Mr. Rosen, Mr. Rammler and Mr. Sperling that quite accurately characterizes the distribution of particle sizes in certain grain materials. With respect to the percentage expression of the relative residual on the sieves R , that is determined by the network analysis, this relationship can be expressed in the latter Bennett's adaptation (hence the RRSB distribution) as follows:

$$R = 100 \exp \left[- \left(\frac{x}{\bar{x}} \right)^n \right] \quad (1)$$

where R – aggregate relative residual on the sieves (%); x – dimension of specific particle (limited by two consecutive sieves) (mm); $\bar{x}$ – mean statistical particle size (mm); n – material constant (–).

Setting the tension limits

The measurement is aimed at determining the tension limits, depending on the load size of the barley malt and the malt crush.

Measurement of shear cohesion and friction properties of bulk materials, respectively the determination of the limit tensions as a relation of the barley malt load and malt crush load was conducted on the apparatus (Figs 3, 4), which was adapted to measure the properties of bulk materials. The principle of the measurement on the mobile prototype device is based on the current readings of normal and tangential force during the bulk material slipping or shifting on the mat.

Overall, the friction is generally dependent on dilatation and contraction, consistency, displacement acceleration, lugs' resistance to prevent breaking, and size of normal load.

When determining of limit tensions in relation to a load size of the barley malt and the malt crush on the prototype device, which was adapted to measure the properties of bulk materials. The measurement procedure is as follows:

- using a electric motor 1, the upper square chamber 2 slides down the lower square chamber 3;
- in the dividing gap between the chambers 4 is the bulk material stressed by tangential force T (N);

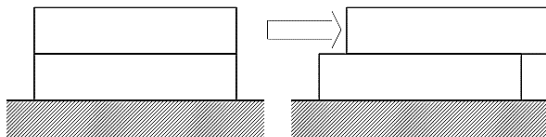


Figure 3. The principle of apparatus operation for measuring shear cohesion and friction properties of bulk materials.

- normal force N (N), exerted by the weight 5 acting on the malt through the loading plate 6;
- depending on the size of the deformation, the force can be determined T (N) (the floor plan of the body in the chamber is known).

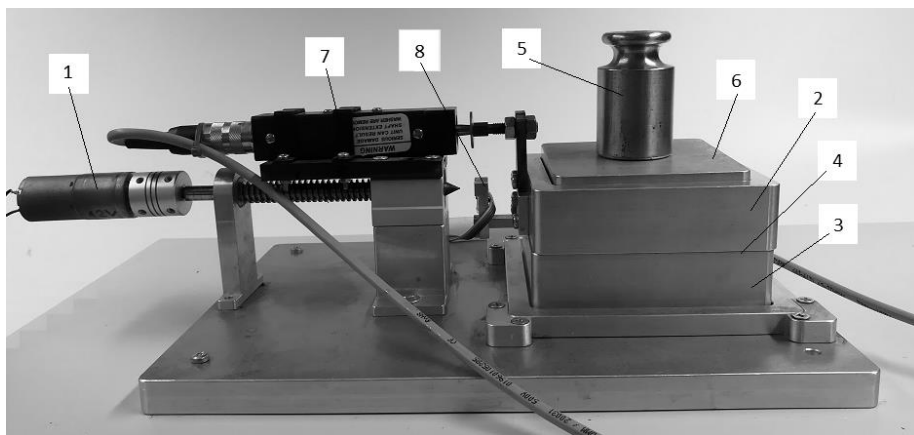


Figure 4. Prototype device for measuring shear cohesion and friction properties of bulk materials. Annotations: 1 – electric motor; 2 – the upper square chamber – movable; 3 – the lower square chamber – fixed; 4 – the dividing gap between the chambers; 5 – weight; 6 – loading plate; 7 – shift sensor; 8 – deformational component with strain gauges for measurement of the force. The device is complemented with measuring electronics and evaluation software.

The height of the upper and lower chamber is 30 mm, the floor area of the chamber 8,100 mm². Load weights were used for loading 100, 500, 1,000, 2,000, 3,000, 4,000 and 5,000 g. The weight of the load plate was 219.68 g.

For the incoherent bulk materials, the maximum tangential stress is dependent. $\tau_{\max}$ in areas of normal tension σ described by:

$$\tau_{\max} = f \cdot \sigma \quad (2)$$

where $\tau_{\max}$ – maximum tangential stress (N); σ – normal tension (N); f – coefficient of internal friction (Pa) (Feynman et al., 2011).

For the cohesive bulk materials, the ratios are more complex and can be characterized as follows:

a) dependence of the maximum tangential stress $\tau_{\max}$ to normal tension σ in area of its impact, which is a characteristic of the yield strength or the yield curve, varies with the degree of material consolidation. (The consolidation means a compression of material by known force for a known period of time prior to the actual measurement);

b) the previous dependence for a given consolidation level only applies to a certain range of normal tensions, and a limited tension from above σ_e . The consolidation level can be characterised by maximum main tension σ_{le} associated with Mohr's circle, which is in contact with the yield strength characteristics at their endpoint (Fig. 5);

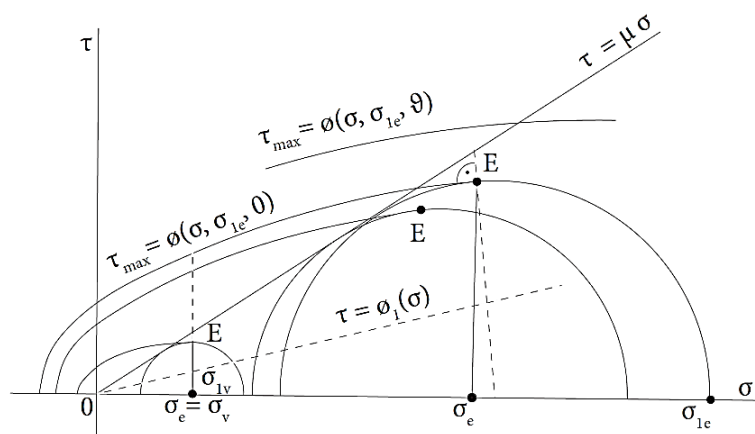


Figure 5. The Mohr's circle (Maloun, 2001).

c) once the yield strength is reached τ_{max} , when tension is $\sigma_v < \sigma_e$, it leads to a 'plastic' deformation (materials movement) that will transform into a new, lower consolidation level $\sigma_{1c} = \sigma_{lv}$, that means into a yield strength characteristics with a endpoint $\sigma_e = \sigma_v$;

d) the envelope of Mohr circles passing through the endpoints of the yield strength characteristics can be called a characteristic of the effective yield strength. This describes the behavior of a cohesive bulk material during a continuous change of the consolidation level;

e) if the compressed material remains at rest, its cohesion is increased;

f) the dependence of tangential stress during external friction to the normal tension is approximately linear, similar to that of cohesive materials. (Maloun, 2001; Feynman et al., 2011).

RESULTS AND DISCUSSION

Determination of the mathematical and physical parameters

The results of the moisture content establishing of the individual samples of processed raw materials are shown in Table 1.

Table 1. The results of the moisture content establishing (drying temperature 105 °C)

Physical parameters	Light barley malt		Colored barley malt	
	Whole grain	Crushed grain	Whole grain	Crushed grain
Humidity, %	1.904 ± 0.01	1.997 ± 0.13	1.877 ± 0.07	1.983 ± 0.1

Furthermore were determined another selected mechanical and physical parameters of the colored and light barley malt and the malt crush (see Table 2). Grain size was determined by network analysis. The Program Statistica 12 (StatSoft 2014) was used for statistical evaluation.

Table 2. Mechanical and physical parameters of the colored and light barley malt and the malt crush

Physical parameters	Light barley malt		Colored barley malt	
Density, kg m ⁻³	1,110		1,180	
Volume weight, kg m ⁻³	570		535	
Friction angle, °	32		34.5	
	Whole grain	Crushed grain	Whole grain	Crushed grain
Size, mm	3.6 ± 0.11	0.91 ± 0.15	3.48 ± 0.13	1.05 ± 0.13

Setting the tension limits

In the following figures (Fig. 6 to 9) are shown the courses of the limit tensions for the barley malt and malt crush in relation to the load size from 100 to 5,000 g. The graphs show the force course on the deformation member, which shows a gradual increase in pressure until the upper cell is slid off, while the chamber moves with an almost constant force course. The size of the tangential force is determined by the grain consolidation and the magnitude of the normal force.

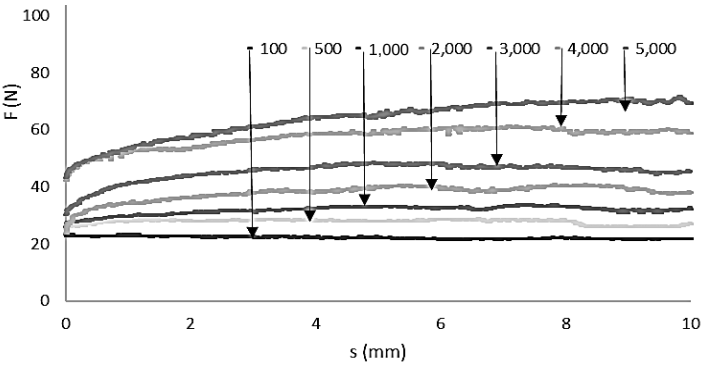


Figure 6. The graph of force dependence on length - light barley malt – whole grain.

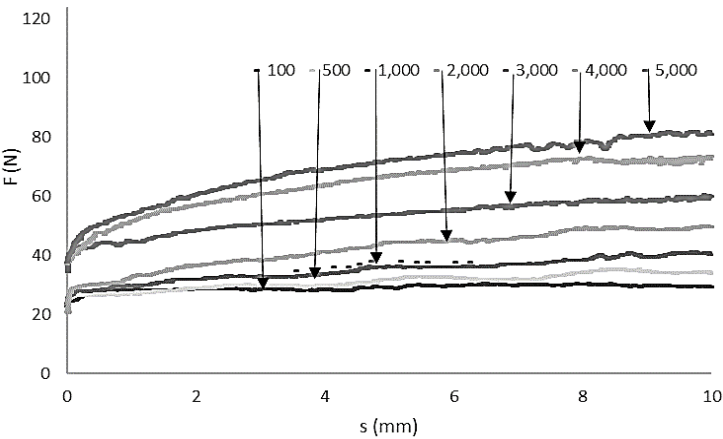


Figure 7. The graph of force dependence on length - light barley malt – crushed grain.

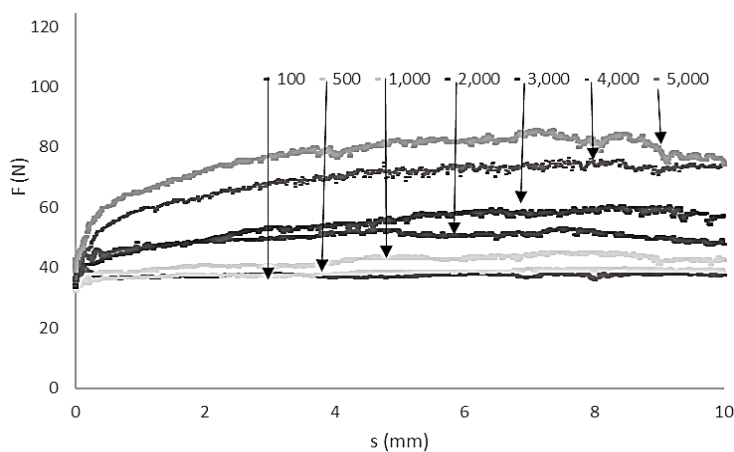


Figure 8. The graph of force dependence on length - colored barley malt – whole grain.

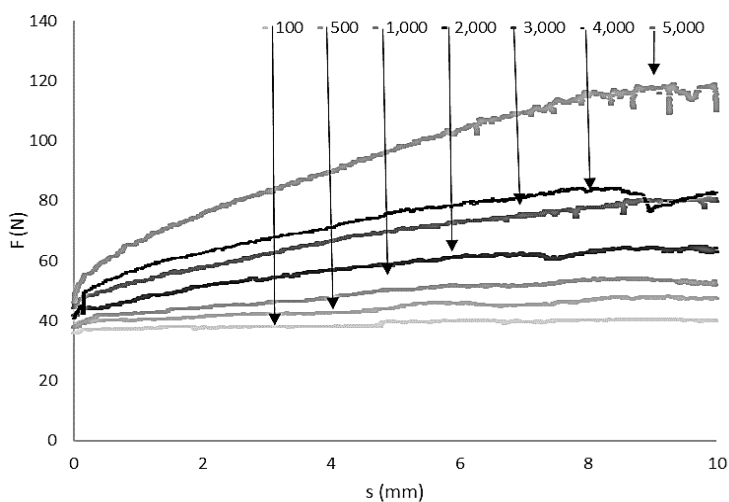


Figure 9. The graph of force dependence on length - colored barley malt – crushed grain.

From the Mohr's circles (Fig. 10 to 13) the internal friction angle and the consolidation stress (Table 3) were determined.

Table 3. The internal friction angle and the consolidation stress

Physical parameters	Light barley malt		Colored barley malt	
	Whole grain	Crushed grain	Whole grain	Crushed grain
Consolidation tension, Pa	2,602.3	3,158.3	3,775.3	4,339.5
Internal friction angle, °	43.95	56.38	44.44	57.52

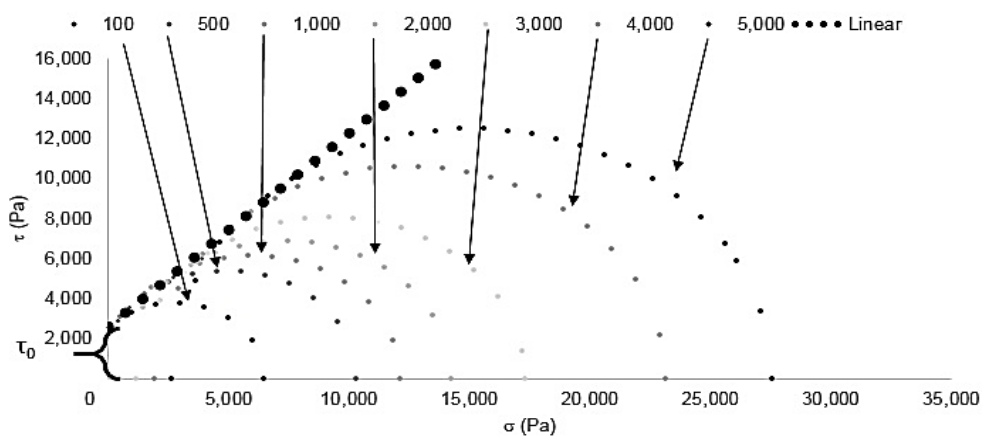


Figure 10. Mohr's circle for the light barley malt – whole grain.

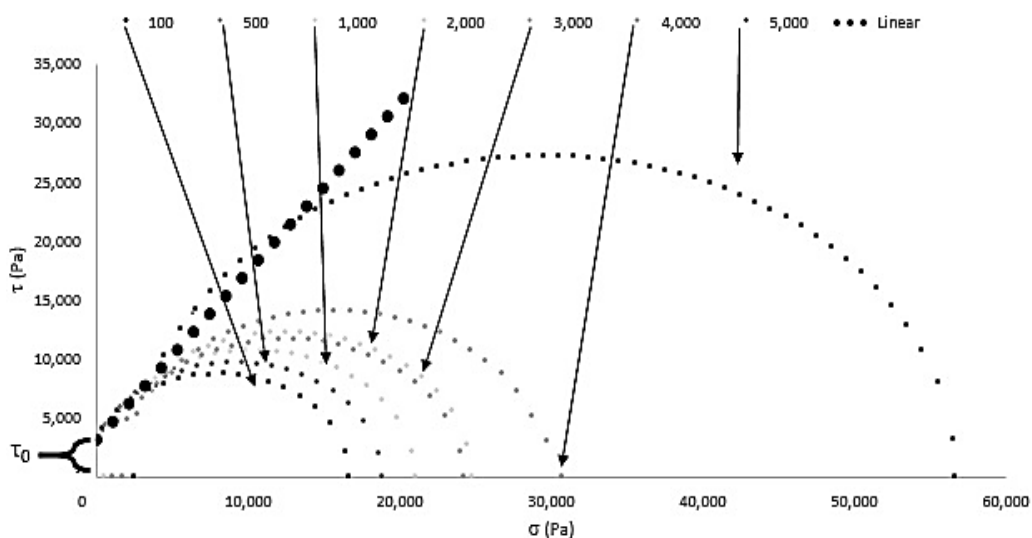


Figure 11. Mohr's circle for the light barley malt – crushed grain.

It was verified by measuring that the whole grain of pale malt has a lower consolidation tension by 556 Pa than the crushed grain. The whole grain of colored malt has a lower consolidation tension by 564 Pa than the crushed grain. The difference in the consolidation tension between the whole grain and the crushed grain of pale and colored malt is the same.

The friction angle of the whole grain pale malt is less by 0.49° than the colored malt, the crushed pale malt is lower by 1.14° than the colored malt. The friction angle of the barley is in according to (Horabik & Housinek, 2002) $27.8^\circ \pm 0.4^\circ$ when humidity at 10% and $33.2 \pm 0.5^\circ$ when at 20%, in according to (Öztürk & Esen, 2008) 19.5° when at 10% and 22.5° when at 14%, and in according to (Moya et. al., 2013) 24.8° when at 8.83%. The friction angle of malting barley is 43.95° for the whole grain, this increase is due to its germination and the grain drying. This modification of the barley's

mechanical parameters must be taken into consideration when designing a transport system where the external friction angle changes on average by 7°, which influences a design of malt silos.

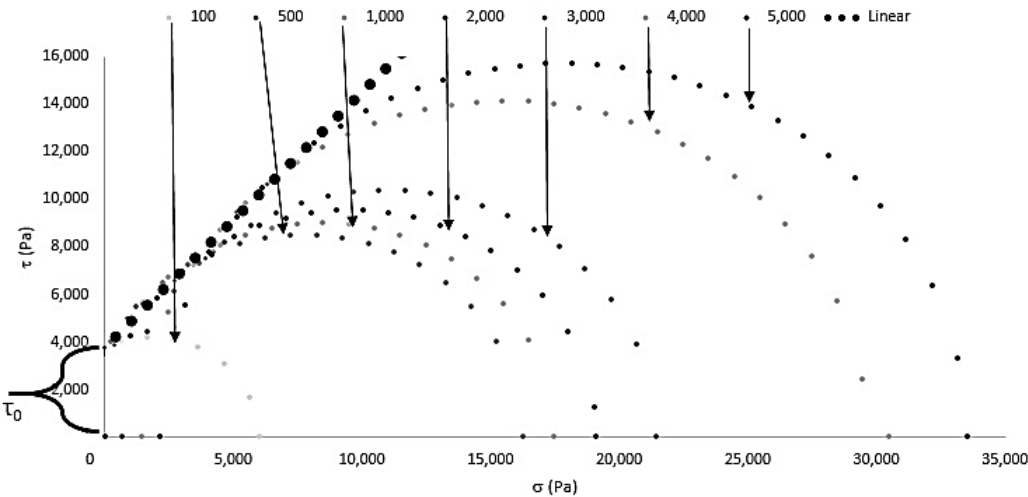


Figure 12. Mohr's circle for the colored barley malt – whole grain.

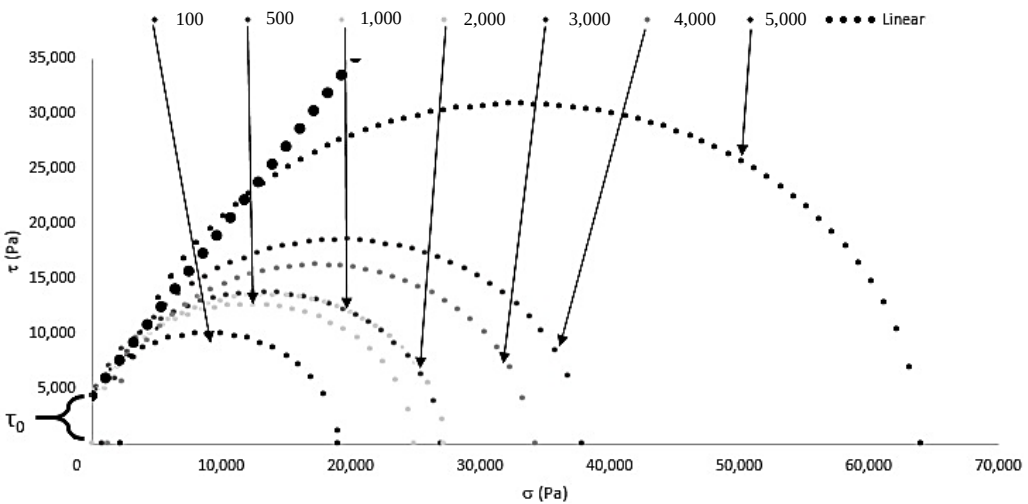


Figure 13. Mohr's circle for the colored barley malt – crushed grain.

CONCLUSION

The importance of determining the tension limits lies in the fact that based on their knowledge we can make calculations of storage and manipulation devices with loose bulk feed and consider a possibility of buckling arches formation that interfere with the function of the device and subsequently use it as a source data for DEM modeling in warehouse management.

ACKNOWLEDGEMENTS. The measurements were made on the device 'Mobile device for shear soil testing' utility model number 29836, authors CHOTĚBORSKÝ, R., LINDA, M., NÝČ, M. <http://www.upv.cz/cs/>. 29836. 04.10.2016.

Thank doc. Ing. Rostislav Chotěborský, Ph.D. and Department of Material Science and Manufacturing Technology for borrowing the device.

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Identification of wet areas in forest using remote sensing data

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Abstract. Aim of this study is to evaluate different remote sensing indices to detect spatial distribution of wet soils using GIS based algorithms. Area of this study represents different soil types on various quaternary deposits as well as different forest types. We analyzed 25 sites with the area of 1 km² each in central and western part of Latvia. Data about soil characteristics like thickness of peat layer and presence of reductimorphic colors in soil was collected during field surveys in 228 random points within study sites. ANOVA test for comparing means of different soil wetness classes and binary logistic regression analysis for evaluating the accuracy of different remote sensing indices to model spatial distribution of wet areas are used for analysis. Main conclusion of this study is that for different quaternary deposits and soil texture classes different algorithms for soil wetness prediction should be used. Data layers for predicting soil wetness in this study are various modifications and resolutions of digital elevation model like depressions, slope and SAGA wetness index as well as Sentinel-2 multispectral satellite imagery. Accuracy of soil wetness classification of soils on moraine, fluvial and eolian sediments exceeds 94%, whereas on the clayey sediments it is close to 80%.

Key words: DEM, satellite imagery, quaternary deposits.

INTRODUCTION

Surface topography and potential energy of gravity of the Earth are main aspects that determines water flow direction and accumulation (Zinko et al., 2005). Infiltration rate on different soil types and underlying sediments may vary because of different hydraulic parameters and therefore surface water and groundwater may infiltrate or accumulate in depressions (Wang et al., 2015). Poorly drained and wet soils are important for biodiversity, water exchange, chemical and other processes, but may be a challenge in forestry, agriculture and similar fields (Detenbeck et al., 1999; McNabb et al., 2001).

Soil disturbance, like rutting and soil compaction is a consequence of timber harvesting operations, but its impact is variable and can be reduced through improved planning of forest management operations (Ares et al., 2005). The level of soil damage resulting from forestry operations depends on factors like machine-applied pressure, soil texture, soil organic matter and water content (Ampoorter et al., 2010). Degraded soil leads to reduced soil bearing capacity, release of sediments and pollutants to surface water, damages aesthetics, unsafe working conditions and increasingly negative public opinion (Campbell et al., 2013). Soil wetness maps can be combined with other data,

such as soil type or soil bearing capacity, in order to contribute to decision making tools in forest operation planning (Mohtashami et al., 2017), therefore associated environmental and financial costs can be limited (Christensen et al., 1996).

Large scale LiDAR (*Light Detecting and Ranging*) surveys have provided scientists with precise topographical data which can be used in soil moisture predicting. Various topographical indices can be used in order to predict spatial distribution of wet soils such as topographical wetness index (TWI) and depth to water (DTW) mapping (Ågren et al., 2014). Topographical information in wet areas can be combined with multispectral satellite imagery, because distribution of plant species and timing and progression of plant development may provide information about plants and their environment like soil moisture, soil temperature, illumination and other aspects (Reed et al., 1994).

The focus of the article is to evaluate different remote sensing indices in modeling of spatial distribution of wet soils. The indices evaluated in the article are based on local depression detecting, slope analysis, Saga wetness index analysis and Sentinel-2 multispectral imagery analysis (Böhner et al., 2002; Wang & Liu, 2006).

MATERIALS AND METHODS

Study area

Study area consists of 25 objects, which are made to represent various forest types on dry mineral soils and drained mineral soils (Fig. 1) and different quaternary sediment types (moraine, clayey, fluvial and eolian sediments). The size of each site is 1 x 1 km² and consists from up to 10 randomly generated point sample plots. Because of relatively large area of each study object, various forest types can be represented in each of them. In total 228 sample plots was generated and surveyed during field measurements to collect data which is relevant for study but can't be measured by remote sensing methods. Collected data consists of soil texture for depth up to 1 m, depth of peat layer and depth, thickness and severity of reductimorphic horizon.

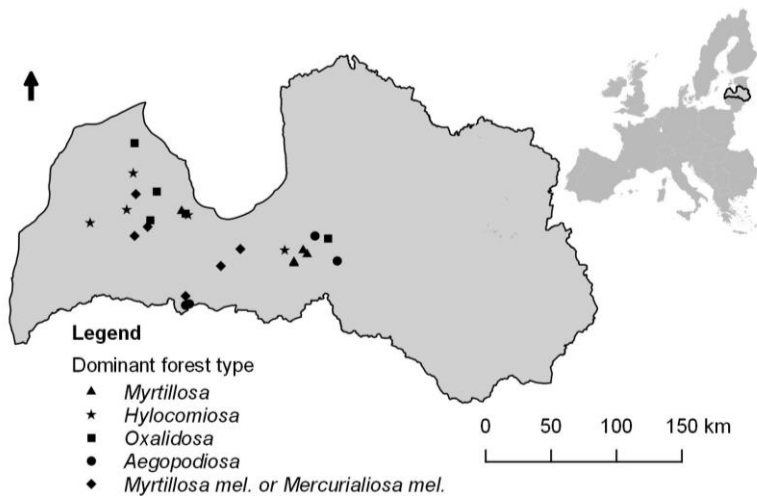


Figure 1. Study area.

Remote sensing data

Remote sensing data for this study is obtained from Latvian Geospatial Information Agency (LiDAR) and European Space Agency (Multispectral satellite imagery). Since 2013, Latvian Geospatial Information Agency is gathering high resolution elevation and vegetation cover scans using LiDAR technology for all of Latvia with a point density of at least 1.5 points per m², an average horizontal point error of 0.36 m and vertical accuracy of 0.12 m.

Bare ground digital elevation models (DEM) for all 25 study sites were created. The area of each DEM is 9 km² (3 x 3 km) and contains study site together with 8 neighboring 1 km² cells. Neighboring cells are added for hydrological runoff modeling for central cell. DEM's are created in various resolutions e.g. 1, 2 and 5 m. This was done in Global Mapper v.15 software through triangulated irregular network (TIN). Resulting DEM's are hydrographically corrected by automatically branching road artifacts at stream crossings. All further DEM processing was carried out with Grass GIS 7.2 and QGIS 2.18.15 modeling tools. DEM's were used to derive following raster datasets in various resolutions: depressions, slope and Saga wetness index. Depressions raster map is generated by extracting original DEM from filled DEM, slope and Saga wetness index raster maps are generate by using designated GIS tools.

Satellite imagery is used to obtain data about forest canopy light reflection spectrum within the study objects. There is significant difference between coniferous and deciduous trees in near infrared spectrum, so red and near infrared spectral bands with 10 m resolution are used in this study. Normalized Difference Vegetation Index (NDVI) is calculated mathematically,

$$NDVI = \frac{(NIR - VIS)}{(NIR + VIS)} \quad (1)$$

where NIR – near infrared spectral band; VIS – visible red spectral band.

Field measurements

Soil wetness was determined and divided into four classes during field surveys according to soil properties and water regime in sample plots. The criteria of separating various wetness classes are occurrence and depth of peat layer and occurrence and intensity of reductimorphic colors in upper soil horizons (0–1 m). The division of soil wetness is as follows:

1 – dry mineral soil with no peat layer and upper soil layer is having no reductimorphic colors; 2 – No peat and upper soil layer is having < 20% of reductimorphic colors; 3 – peat layer is < 30 cm thick and upper soil layer is having 20–50% reductimorphic color; 4 – Peat layer is thicker than 30 cm and/or reductimorphic color is dominant in upper soil layer.

Soil samples from diagnostic horizons were analyzed by feel (Brady & Weil, 2002) and as Vos et al. (2016) points out, it is sufficient to estimate soil particle size distribution using this method instead of conducting expensive and time-consuming laboratory analysis. Soil texture was used to determine sediment type. Soil texture is important parameter which determines the drainage level of soil and rate of infiltration into soil and can be used to better understand distribution of soil wetness. Field works were carried out from July till October and synchronization with satellite imagery were not carried out.

Data analysis

To combine field data with information from raster layers, previously generated random point layer was converted to polygon layer with radius of 10 m. QGIS tool *Raster statistics for polygons* was used to add mean values of remote sensing indices to newly generated polygon layer.

Example of data sampling is shown in Fig. 2. Study plots that represent wet soils are shown as points and dry soils as triangles. There is visible trend that wet soils tends to be in depressions, in areas with low slope gradient and in areas with high Saga wetness index.

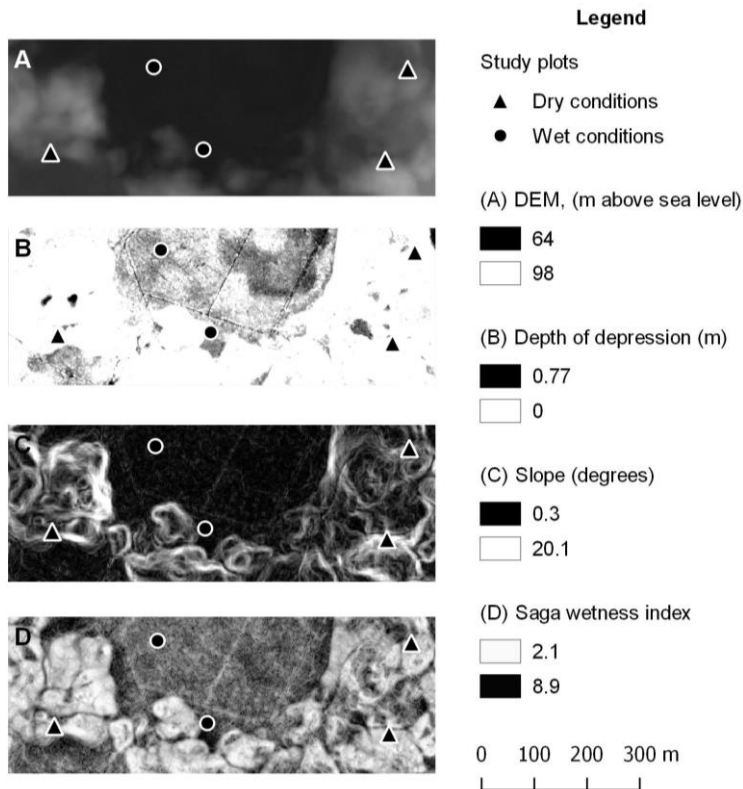


Figure 2. Example of data sampling from various raster layers.

Data statistical analysis

Mean values of various indices derived from remote sensing data were compared using One-Way ANOVA test in SPSS. This analysis was conducted to see which remote sensing indices shows statistically significant differences between soil wetness classes.

Soil wetness classes were reduced to 2 classes to do binary logistic regression analysis. Binary logistic regression analysis in SPSS is used for binary data, when there are only 2 possible outcomes – true or false. In this case all the study plots with dominant reductimorphic horizon were assumed to be true, and all the study plots with reductimorphic color dominance < 50% were assumed to be false. Results from this analysis were used to generate formulae for soil wetness prediction in other areas.

RESULTS AND DISCUSSION

One-Way ANOVA test shows, that there is statistically significant difference ($\alpha < 0.05$) for some of used parameters between different soil wetness classes and significantly different parameters vary depending on settings of quaternary deposits.

Binary logistic regression analysis, similarly to One-Way ANOVA test, shows that various wetness prediction algorithms for different quaternary deposits gives results with various precision, therefore they should be analyzed separately. Results of binary logistic regression analysis are used to get coefficients for soil wetness probability prediction. Depression detection algorithm gives best results for wetness prediction for soils on moraine sediments. Resultant formula gives result from 0 to 1, where 0 means dry conditions and 1 – wet conditions. Field data classification gives 94.6% accuracy when cut value is set to 0.15.

$$\frac{\exp(-3.645 + 48.749x)}{\exp(-3.645 + 48.749x) + 1} \quad (2)$$

where x – average depth of depression from raster with resolution 2 m in 10 m radius.

There are similar trends for soils on clayey sediments, however drainage systems are widely used in these areas and natural water flow is disturbed, so wetness indices are predicting soil wetness conditions with lesser accuracy. Field data classification gives 79,8% accuracy when cut value is set at 0.35. To improve quality of soil wetness prediction slope and saga wetness index values are added to formula:

$$\frac{\exp(5.260 - 2.205x + 4.749y - 0.966z)}{\exp(5.260 - 2.205x + 4.749y - 0.966z) + 1} \quad (3)$$

where x – average slope from raster with resolution 5 m; y – depth of depression from raster with resolution 2 m; z – value of saga wetness index from raster with resolution 1 m. All values are taken as averages from area of 10 m radius.

Hydraulic conductivity for soils on fluvial sediments is higher, so local depressions are less important for determination of soil wetness. Field data classification gives 94.1% accuracy when cut value is set to 0.5. Saga wetness index and slope values are used in soil wetness prediction:

$$\frac{\exp(-11.305 + 1.905x - 0.05y + 2.232z - 2.505m)}{\exp(-11.305 + 1.905x - 0.05y + 2.232z - 2.505m) + 1} \quad (4)$$

where x – saga wetness index value from raster with resolution 1 m; y – saga wetness index value from raster with resolution 2 m; z – average slope from raster with resolution 1 m; m – average slope from raster with resolution 5 m. All values are taken as averages from area of 10 m radius.

Sentinel-2 multispectral imagery together with saga wetness index and slope values is used in classification and predicting of soil wetness on eolian sediments. Field data classification gives 100% accuracy when cut value is set to 0.5. Resulting formula is:

$$\frac{\exp(-9,313.517 + 8,427.14x + 0.388y + 197.023z + 32.007m)}{\exp(-9,313.517 + 8,427.14x + 0.388y + 197.023z + 32.007m) + 1} \quad (5)$$

where x – NDVI value (Sentinel-2 scene from 30.08.2017); y – value of infrared band (Sentinel-2 scene from 30.08.2017); z – saga wetness index value from raster with

resolution 2 m; m – average slope from raster with resolution 5 m. All values are taken as averages from area of 10 m radius.

Spatial distribution of various quaternary sediment types in Latvia is fragmented and topography has largely been formed as a result of last Weichselian event of Pleistocene glaciation. Main processes that affected sedimentation in Latvia were transgressive and regressive processes of glacial accumulation as well as proglacial meltwater activity (Zelcs & Markots, 2004).

Proposed methodology for wet areas detection in forest are based on various indices derived from LiDAR based DEM and multispectral satellite imagery. Similar data sources are used in other studies (Case et al., 2004; Ågren et al., 2014), however they used ready to use models and accuracy of those were analyzed. Accuracy of wet areas detection of *depth-to-water* model proposed by Murphy et al. (2011) in Swedish case study was 85% (Ågren et al., 2014), which is similar to proposed methodology, but it doesn't consider variation in quaternary deposits.

CONCLUSIONS

Predicting of wet soils spatial distribution using LiDAR data and multispectral satellite imagery is a perspective method and can be used in practice for planning of forestry operations. The results of statistical analysis show that using the data obtained in field works, the accuracy of soil wetness classification of soils on moraine, fluvial and eolian sediments exceeds 94%, whereas on the clayey sediments it is close to 80%.

This study shows that different geological deposits have various effect on the spatial distribution of soil moisture. This means that different geological settings must be considered when designing a soil moisture map.

An essential disadvantage of introducing this method in practice is the lack of precise geospatial data on roads and watercourses in Latvia. These data are needed to make DEM corrections to do accurate modeling of water runoff.

ACKNOWLEDGEMENTS. The study is implemented within the scope of the Forest Sector Competence Center project No. 1.2.1.1/16/A/009

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Environmental risk assessment studies on new plant protection products which have been elaborated from coniferous tree bark

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Abstract. Nowadays there are still various chemical pesticides being applied in the course of ensuring plant protection. Since 2010, we have been working on the development of new, environmentally-friendly plant protection products which will provide an effective tool against pathogenic fungi and bacteria which cause disease in crop plants. The specific aim of this study was to evaluate a risk assessment for new plant protection products that have been elaborated on the basis of coniferous tree bark. Various products were tested which are extracted during the processing of wood bark from pine (*Pinus sylvestris* L.) and spruce (*Picea abies* (L.) Karst.). Ethanol extracts were formulated and applied during these experiments. Two formulations, which showed anti-fungal activity *in vitro* and in field trials on fruit crops (involving strawberries and raspberries) were selected for the risk assessment studies. The impact was studied of formulation treatment on crop plants and soil biological activity, and the accumulation of residues of active substances in crop plants and soil. The application of new formulations did not show any negative effect on the chlorophyll content and the chlorophyll fluorescence of plant leaves. The results showed that pine and spruce bark extract formulations contain active compounds (coumaric acid, quercetin, epicatechin, and ferulic acid) within the range of 5.1–5.9 mg kg⁻¹ and 11.1–443.9 mg kg⁻¹ respectively. The amount of active substances which were determined in most cases was higher in the spruce bark extract formulation when compared to the pine bark extract formulation. Our results confirmed the presence of active compounds – epicatechin, quercetin, and coumaric acid – in strawberry fruits which remained untreated and in those that were treated with spruce ethanol extract formulation. Untreated raspberry fruits contained all four active substances within the range of 81–5,300 µg kg⁻¹. We observed a significant increase of coumaric acid and quercetin in raspberries after their having been treated with spruce bark extract formulation in a 2% concentration, $P < 0.05$, and did not find any negative impact for spruce bark extract formulations when used on soil microbial biomass.

Key words: coumaric acid, epicatechin, ferulic acid, pine bark ethanol extract, raspberry, residues in soil and plants, spruce bark ethanol extract, strawberry, quercetin.

INTRODUCTION

Plant pathogens induce considerable economic losses in the agricultural production industry; therefore, it is felt that more attention should be paid to the development and implementation of environmentally-friendly techniques. Pest management is one of the major tasks to be associated with growing berries, one which occasionally involves 70% of total growing expenses (Prits & Handley, 1998). Fungal diseases occur in flowers, fruits, leaves, crowns, and roots, reducing yields and the quality of fruit (Paulus, 1990). Grey mould (caused by the fungal pathogen *Botrytis cinerea* Pers.), phytophthora crown rot (caused by *Phytophthora cactorum* (Lebert and Cohn) J. Schrot.), anthracnose fruit rot (caused by *Colletotrichum acutatum* J H Simmonds), strawberry leaf spot (caused by *Mycosphaerella fragariae* (Tul)), verticillium wilt (caused by *Verticillium dahliae* Kleb.), and fruit rot (caused by *Rhizopus* sp.) are amongst the most widespread diseases which can affect raspberries and strawberries (Paulus, 1990). Most of the fungicides that have been developed for fungal diseases control are site-specific inhibitors with a high risk of resistance development. These problems result in the necessity for alternative methods to be developed, methods which must be safe and are able to replace fungicide treatments. Several studies have been carried out which focus on the investigation of the antifungal effect of different components from coniferous trees (Micales et al., 1994; Zarins & Daugavietis, 1998; Hong et al., 2004; Laugale & Daugavietis, 2009; Zarins et al., 2009; Co et al., 2012; Gabaston et al., 2017). For the most part, the effectiveness of products that have been obtained from resin and tree needles have been evaluated.

Coniferous bark is one of the by-products of forest exploitation which can also be used in the production of plant protection products. Various active substances have been isolated from coniferous bark. For instance, Pan & Lundgren (1995) isolated 28 phenolic compounds from spruce root bark. It is known that coniferous needles and bark contain a wide variety of phenolic compounds with antibacterial, antifungal, antioxidant, and metabolic activities (Richter & Wild, 1992; Co et al., 2012; Salem et al., 2016). Studies which have been carried out on the chemical composition of coniferous bark taken from trees which have been grown in Latvia had been carried out by Verovkins et al. (2008). The major phenolic compounds which are contained in spruce needles and bark are catechin, epicatechin, vanillic acid, *p*-coumaric acid, *o*-coumaric acid, and ferulic acid, with possible others (Iravani & Zolfaghari, 2014; Sadeghi et al., 2014).

In recent years, several plant protection products which have been produced from the biomass of coniferous tree were produced in cooperation between the Latvian State Forest Research Institute 'Silava' and the Institute of Biology, University of Latvia. Various products were tested which are extracted during the processing of wood from pine (*Pinus sylvestris* L.) and spruce (*Picea abies* L.). Different solvents (ethanol, butanol, sodium carbonate, sodium hydroxide, and water) were used for extraction purposes (Jankevica et al., 2013; Laugale et al., 2013). Between 2011 and 2017 several laboratory and field investigations were carried out in order to be able to test the effectiveness of extracts and formulations against important diseases which can infect berry crops.

The laboratory experiments (involving radial growth tests) showed that ethanol extract formulations from coniferous bark are the most effective, and these significantly inhibited the mycelial growth of phytopathogenic fungi: *Botrytis cinerea*, *Colletotrichum acutatum*, *Phytophthora cactorum*, and *Mycosphaerella fragariae*, at the highest dosage level of 20 g L⁻¹ resulted in the complete and total mycelial growth

inhibition of fungi. *B. cinerea*, *C. acutatum*, and *P. cactorum* did not differ from the conventional fungicide, Signum® (Minova et al., 2015). In field trials on strawberries and raspberries the extract formulations showed significantly lower levels of effectiveness than fungicide Signum®. The application of spruce bark extract in concentrations of 1% significantly reduced the development of leaf spots in 2012, and in both 1% and 2% concentrations in 2013, compared to the untreated control (Volkova et al., 2014). A risk assessment of new plant protection products and active substances needs to be tested to see how they match up to Regulation (EC) No 1107/2009.

The aim of the research was to develop a new, environmentally-friendly plant protection product, one which is usable in organic farming and integrated pest management, by carrying out an environmental risk assessment of new plant protection products which have been developed on the basis of coniferous tree bark.

MATERIALS AND METHODS

Bark extracts and formulations

Spruce bark extract (containing dry matter at an amount of 30%) and pine bark extract (containing dry matter at an amount of 26%) were prepared at the Latvian State Forest Research Institute 'Silava'. Bark was crushed with an M-1 extrusion-type grinder. The resulting mass was fractionated using sieves, and a fraction with particles size of between 0.5–1.0 mm was used for further production. The extraction was carried out using a 'Büchi' B-811 Universal Extraction System and the Soxhlet regime. Extraction was carried out in three and-a-half hours, which is a sufficient amount of time (according to our previous experience) for complete extraction. Ethanol at 96% (volume) was used as a solvent (Table 1). A determination of phenols was based on an optical density measurement of coloured oxidation products, which was obtained using a Folin-Ciocalteu reagent (tungstic acid in an alkaline medium results in a blue colour). Gallic acid was used as a reference substance (Pasqualini et al., 2003; Mechnikova et al., 2007). The density of the blue-coloured substances and reference substance (gallic acid) was measured at 765 nm. The concentration of the total flavonoids was measured using a differential spectroscopy method. Optical densities of the coloured substances after their reaction with aluminium chloride were measured at 410 nm. A Genesys 10 UV scanning spectrophotometer was used for optical density measurements.

Table 1. The characteristics of those plant extracts being used for the development of plant protection product formulations

Source	Solvent for extraction	Plant extraction method	pH	Dry matter (%)	Content of flavonoids in dry matter (%)	Content of phenols in dry matter (%)	Properties
Spruce bark	96% (vol) ethanol	in Soxhlet apparatus	3.8	30	1.2	32.3	thick dark product
Pine bark	96% (vol) ethanol	in Soxhlet apparatus	3.6	26	1.2	20.9	thick brownish product

Extracts were formulated to improve adhesion to the plant. Formulations of bark extracts were developed at the University of Latvia's Institute of Biology. The formulations consisted of: bark ethanol extract 67.0% (dry matter 26.0% or 30.0%); water 26.78%; a binding agent, Trifolio S – Forte (Trifolio-M GmbH, Germany) 3.2%; an emulsifier, Tween-80 (Scharlau, Spain) 2.5%; KOH 0.4%; stabiliser 0.1%; and a preservative at 0.02%. When forming the preparations, KOH was added in order to normalise the pH content. The pH value for the formulations that were developed was at 7.5 ± 0.2 . Before use dilutions of 1%, 2%, and 4% of the extract formulation were prepared using warm, clean tap water.

Measurements of chlorophyll content and chlorophyll α fluorescence

The influence was evaluated of two coniferous extracts on the plant photosynthetic parameters on strawberry plants, cv. 'Senga Sengana', propagated *in vitro*. Plantlets were removed from the medium and were planted into plastic pots which contained commercial peat with mineral nutrients. The plants were kept in a growth chamber at 25 °C, photoperiod 18hrs, relative humidity $60 \pm 5\%$. The light was provided by fluorescent lamps with $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ of light intensity. Two month old plants were used for the experiments. A working solution was made up with 1% and 2% concentrations of each coniferous extract's formulations and this was spread over ten plants. The control plants were sprayed with tap water. A visual inspection of the treated plants was carried out. The chlorophyll content and chlorophyll fluorescence levels were measured on ten leaflets at 48, 72, and 168 hours after treatment.

Chlorophyll α fluorescence was measured on the abaxial side of the leaves by using the fast fluorometer PEA (Hansatech, England). The leaves were placed into clips, being darkened for twenty minutes, and then illuminated for five seconds using red diodes (peak 650 nm, the maximum PPFD on the leaf surface was $3,000 \mu\text{mol m}^{-2} \text{s}^{-1}$). The samples were characterised by the parameter Fv/Fm. Chlorophyll content was measured by a SPAD-502 chlorophyll meter (Konica- Minolta, Osaka, Japan). Ten consecutive readings were taken across the surface of each leaf. The SPAD-502 determines the relative amount of chlorophyll by measuring the absorbance of the leaf in two wavelength regions – blue (400–500 nm) and red (600–700 nm). Using these absorbance levels, the meter calculates a numerical SPAD value which is proportional to the amount of chlorophyll present in the leaf. The mean value was calculated using the internal function of the chlorophyll meter. The data was statistically analysed using the Student's *t*-test (level of significance: $P \leq 0.05$).

Field trials

Field research was carried out in cooperation with scientists from the Pure Horticultural Research Centre and the Latvian Plant Protection Research Centre. The trials were conducted in 2012 and 2013 within the grounds of Ķekavas Dārzs Ltd (Ķekava, Latvia) on one-year-old strawberry plantings (*Fragaria* $\times$ *ananassa* Duch.) using cv. 'Induka' (2012) and cv. 'Rubin' (2013) and on the grounds of the Pure Horticultural Research Centre (Pure, Latvia) on six year-old primocane raspberry plantings (*Rubus idaeus*) cv. 'Gerakl' (2013), according to the European and Mediterranean Plant Protection Organisation guidelines (EPPO 1996; EPPO 2012). Before making a start on preparing any of the formulation, we tested the impact of all of the additives in a 1% concentration. No significant impact was observed on chlorophyll content, chlorophyll

α fluorescence, or transpiration (Samsone, unpublished). Field experiments were carried out with spruce ethanol extract preparations because the spruce extracts contained more active substances. There were three experimental plots created for the spruce bark ethanol extract formulation (concentrations of 1%, 2%, and 4%) and a control without treatment. We applied the formulation with its respective levels of concentration on strawberries at an interval of seven to eight days, from the beginning of flowering (24 May 2012 and 28 May 2013) until the maximum level was reached for fruit harvesting (29 June 2012 and 1 July 2013). The rate of treatment was 500 L ha⁻¹ of the working solution. On the raspberry plantings we applied solutions at concentrations of 1% or 2% (600 L ha⁻¹) with an interval of seven days, from the beginning of flowering (27 July 2013). We used a randomised block design with four replicates per treatment and untreated plants as a control. A visual inspection of the plants was carried out at each treatment stage. The yield was harvested between two and three times a week. At the end of the 2013 season, four subsamples were taken from each sampling plot. Subsamples of berries were mixed in order to obtain a representative sample and this was delivered to the laboratory for a determination of the residues of active substances.

A determination of active substances in the preparation, plant, and soil

We selected four active compounds – coumaric acid, quercetin, epicatechin, and ferulic acid – which were easy detectable in extracts and which can be used as model substances to show the dispersion and accumulation of the prepared formulations in the production (berries) and in the soil. Berry samples were frozen and stored prior to the start of the analysis. Before the analysis started, the samples were unfrozen, and 50 g of samples were taken and homogenised. All samples were prepared according to the following procedure: 10 mL acetonitrile was added to 5 g of sample and this was shaken using a laboratory shaker for a total of ten minutes; 4 g of anhydrous magnesium sulphate and 0.5 g of sodium chloride were added, and then vigorously shaken for one minute; the mixture was centrifuged for ten minutes at 3,000 rpm; 5 mL of extract was evaporated into a dry mass in a nitrogen flow at a temperature of 40 °C; dry residue was dissolved in 200 μ L water and an acetonitrile mixture of 8:2, v/v with 0.1% formic acid, and this was used for high resolution HPLC-MS/MS detection. HPLC-MS/MS measurements were carried out with the Waters Alliance 2690 system, which was connected to a Quattro LC mass spectrometer (Waters). Chromatography analysis was carried out with a Luna C18 column (100 mm $\times$ 2.0 mm, particle size 5 μ m, 100Å pore size; Phenomenex) at a temperature of 40 °C with an injection volume of 50 μ L. The mobile phase flow rate was 0.3 mL min⁻¹. The mobile phase composition was as follows: ‘A’ – 0.1% of formic acid solution in water; ‘B’ – 0.1% of formic acid solution in acetonitrile. The time taken for chromatography was 25 mins. A Quattro LC mass spectrometer is equipped with an ESI source in negative mode with the following parameters: 2.5 kV capillary voltage, 150 °C source temperature, 350 °C desolvation temperature, 600 L h⁻¹ desolvation gas flow, and 30 L h⁻¹ cone gas flow. Statistical analyses were carried out using the R 2.14.1 software. To be able to determine significant differences, the resultant data was submitted to a one-way ANOVA, followed by Tukey’s honest significant difference test ($p < 0.05$).

The impact of active substances on soil microbial biomass

Soil samples were collected for an analysis of microbial biomass from the field trial location at the end of the 2013 season. The sampling procedure, transportation, and storage were carried out according to ISO 10381-6 (2009). Four soil subsamples from each sampling plot were taken to a depth of 0–10 cm. The subsamples were mixed and analysed as one sample. The substrate-induced respiration method (ISO 14240-1, 1997) was used to determine soil microbial biomass or soil microbial carbon (SMC).

RESULTS AND DISCUSSION

The impact was evaluated of the use of the formulation treatment on crop plants and of the residues of active substances which have accumulated in crop plants and in the soil. The effect of coniferous extracts on a plant's physiological state was characterised by the chlorophyll content and chlorophyll *a* fluorescence. It has been reported that conifers produce many compounds which may influence other plant growth (Wilt et al., 1993; Aliloo et al., 2012; Cádiz-Gurrea et al., 2014); for example, pine needle inhibitory compounds belong to substances that hinder photosynthesis (Nektarios et al., 2005). The Fv/Fm ratio is used as a stress indicator and describes the potential yield of the photochemical reaction. According to our findings, none of the coniferous bark extracts that were used for the treatment of plants showed any negative effect on the Fv/Fm chlorophyll fluorescence ratio. The results showed that the value of chlorophyll fluorescence (parameter Fv/Fm) was in the range of 0.82–0.84 in all treatments, expressing a high potential activity for photosystem II. The application of spruce and pine bark extract formulations of 1%, 2%, and 4% concentrations did not show any negative effect on the chlorophyll fluorescence of plant leaves (Table 2).

Table 2. Chlorophyll concentration (SPAD units) and chlorophyll *a* fluorescence parameter, Fv/Fm, on strawberry leaves at 48, 72, and 168 hours after treatment with 1% and 2% pine and spruce bark extract formulations (mean $\pm$ standard deviation)

Formulation, concentration	Chlorophyll concentration (SPAD units)			Chlorophyll <i>a</i> fluorescence parameter Fv/Fm		
	48 hrs after treatment	72 hrs after treatment	168 hrs after treatment	48 hrs after treatment	72 hrs after treatment	168 hrs after treatment
Spruce bark extract, 1%	40.6 $\pm$ 2.2	40.1 $\pm$ 2.2	40.0 $\pm$ 2.0	0.84 $\pm$ 0.01	0.83 $\pm$ 0.03	0.83 $\pm$ 0.01
Spruce bark extract, 2%	41.4 $\pm$ 2.9	41.2 $\pm$ 2.7	40.9 $\pm$ 2.8	0.84 $\pm$ 0.01	0.83 $\pm$ 0.01	0.83 $\pm$ 0.01
Pine bark extract, 1%	38.6 $\pm$ 3.3	39.9 $\pm$ 1.5	39.4 $\pm$ 2.2	0.84 $\pm$ 0.01	0.83 $\pm$ 0.01	0.83 $\pm$ 0.01
Pine bark extract, 2%	34.0 $\pm$ 2.6*	40.2 $\pm$ 3.6	40.9 $\pm$ 3.2	0.84 $\pm$ 0.01	0.83 $\pm$ 0.01	0.83 $\pm$ 0.01
Control	40.5 $\pm$ 2.8	40.0 $\pm$ 3.3	40.0 $\pm$ 2.8	0.83 $\pm$ 0.01	0.82 $\pm$ 0.01	0.82 $\pm$ 0.01

* test, $P < 0.05$.

We observed, on the first days following treatment with the 2% pine bark ethanol extract formulation, that the chlorophyll content of the leaves decreased ($P < 0.05$), and slightly increased after 72 hrs in comparison to non-treated leaves (Table 2). During the visual plant inspection on the day of spraying, in variants in which spruce and pine park

extracts of a 4% concentration had been applied, brown spots were detected on the plant leaves. Since the chlorophyll fluorescence parameter Fv/Fm shows an optimum value, it cannot be assumed that the physiological state of the plants would have deteriorated due to the treatment.

Volkova et al. (2014) reported that in field trials the treatments with spruce biomass extract had no significant effect on strawberry yield and fruit size; however the highest concentration (4%) of the extract had a negative influence on fruit taste and aroma, and it slightly reduced the average size of the berries, although the reduction was not statistically significant.

Selected major components – coumaric acid, quercetin, epicatechin, and ferulic acid – were determined by using the HPLC-MS/MS method in newly developed formulations (Fig. 1, a and b). The results showed that pine and spruce bark extract formulations contained all four active substances in the range of 5.9–35.1 mg kg⁻¹ and 11.1–443.9 mg kg⁻¹ respectively (Table 3). The amount of determined active substances in most cases was higher in the spruce bark extract formulation than they were in the pine bark extract formulation. The amount of epicatechin in the spruce bark extract formulation was ten times higher than the amount of other substances. Therefore we used the spruce ethanol extract formulation in field trials.

Using the High Performance Liquid Chromatographic (HPLC) method, flavonoids (kaempferol, quercetin, and myricetin) and phenolic acids (p-coumaric, caffeic, ferulic, p-hydroxybenzoic, gallic, and ellagic acids) were detected in nineteen berries, including strawberries and raspberries (Hakkinen et al., 1999). Research by Hakkinen & Törrönen (2000) shows that the phenolic contents in strawberries are between 421–544 mg kg⁻¹, and flavonols (quercetin, myricetin, and kaempferol) and phenolic acids (ellagic, *p*-coumaric, caffeic, and ferulic acids) are major components. Our results confirmed the presence of the active compounds, epicatechin, quercetin, and coumaric acid, in strawberry fruits, both untreated and those which had been treated with spruce bark extract formulations (Table 4).

Table 3. Amount of active substances (mg kg⁻¹) in the developed bark extract formulations as determined by the HPLC-MS/MS method (relative standard deviation 2%)

Active substance	Substance volume (mg kg ⁻¹)	
	Pine bark extract formulation	Spruce bark extract formulation
Epicatechin	35.1	443.9
Ferulic acid	32.9	11.1
Quercetin	9.2	43.1
Coumaric acid	5.9	40.6

Table 4. The amount of active substances (determined by the HPLC-MS/MS method) in strawberry fruits in field trials, both untreated and those treated with spruce bark extract formulations in 1%, 2%, and 4% concentrations

Formulation, concentration	Substance amount (µg kg ⁻¹)			
	Quercetin	Epicatechin	Ferulic acid	Coumaric acid
Control	77 a	5,000 a	< 100	49 a
Spruce bark extract formulation, 1%	47 b	5,400 a	< 100	30 c
Spruce bark extract formulation, 2%	45 b	3,900 b	< 100	51 a
Spruce bark extract formulation, 4%	88 a	2,900 c	< 100	43 b

Those values which have the same suffix letters within the columns are not significantly different at *P* < 0.05.

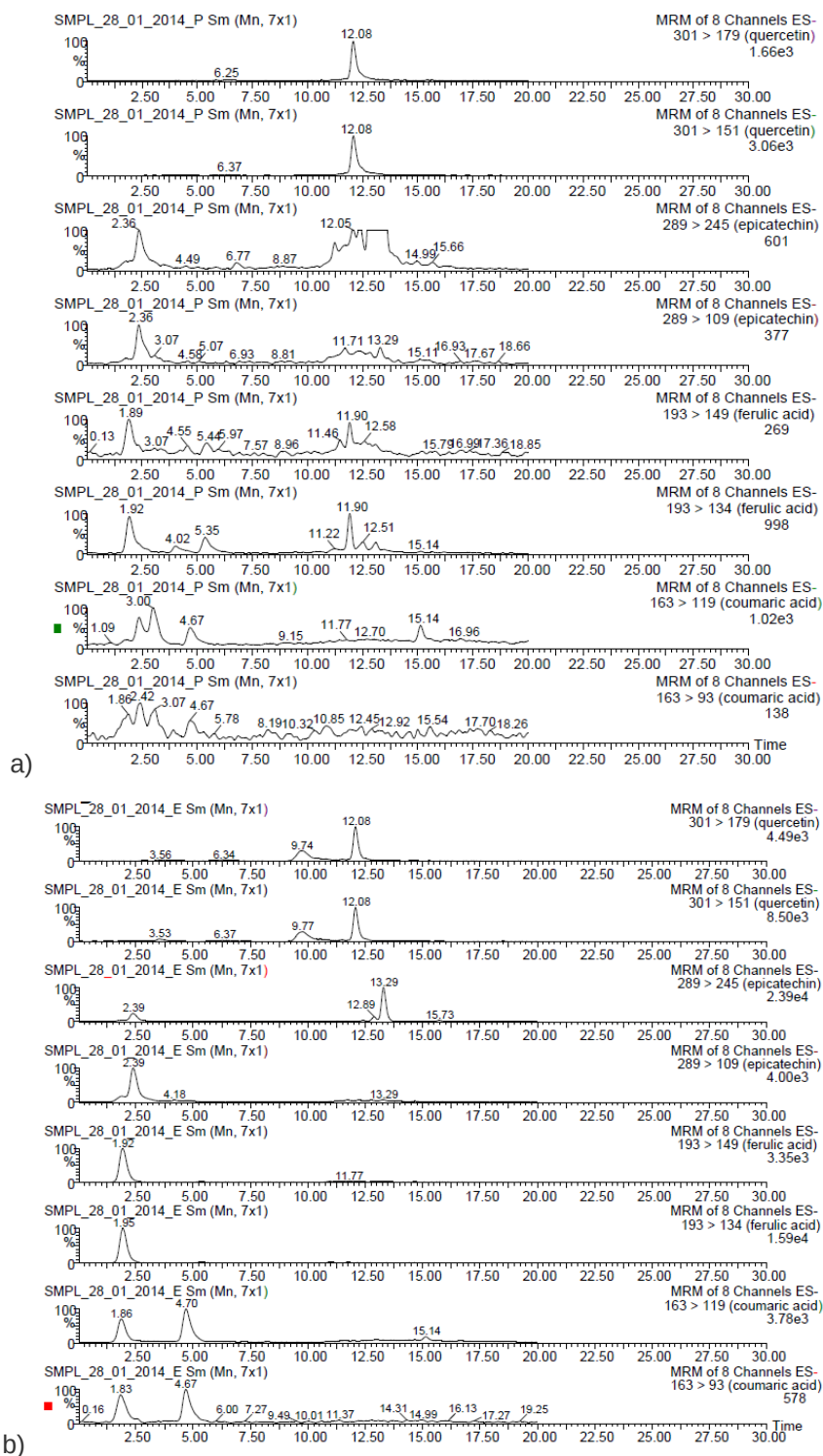


Figure 1. The chromatographic profile of the bark extract formulations: a – the pine bark ethanol extract formulation; b – the spruce bark ethanol extract formulations.

We observed significant differences in the levels of phenolic compounds in strawberry fruits after their treatment with different concentrations of the spruce extract formulation, $P < 0.05$ (Table 4). It is already known that phenolic content in strawberries is slightly affected by cultivation technique, cultivars, the ripening stage, and the growing conditions (Hakkinen & Törrönen, 2000; Huang et al., 2012).

The red raspberry is characterised by higher concentrations of phenolic acids in comparison to flavonols. Untreated red raspberries showed a high concentration of epicatechin, at $5,300 \mu\text{g kg}^{-1}$, and ferulic acid, at $654 \mu\text{g kg}^{-1}$. Quercetin and *p*-coumaric acid were found at lower concentrations (Table 5). These results are in accordance with those reported by Hakkinen et al (1999).

Table 5. The amount of substances (as determined by the HPLC-MS/MS method) in raspberry fruits from the untreated control field and fields which had been treated with working solutions of spruce bark extract formulation at a 1% and 2% concentration

Formulation, concentration	Substance amount ($\mu\text{g kg}^{-1}$)			
	Quercetin	Epicatechin	Ferulic acid	Coumaric acid
Control sprayed with water	111 b	5,300 a	654 b	81 b
Spruce bark extract formulation, 1%	114 b	4,846 c	645 b	84 b
Spruce bark extract formulation, 2%	138 a	5,000 b	675 a	199 a

Those values which have the same suffix letters within the columns are not significantly different at $P < 0.05$.

We observed a significant increase of coumaric acid, ferulic acid, and quercetin in raspberries after their treatment with a spruce bark extract formulation of 2% concentration, $P < 0.05$ (Table 5). Considering the fact that the average samples were tested and knowing that the content of the phenols in berries is affected by environmental factors, the planting technology, and the ripening stage, the scattering results for the amount of active substances cannot be limited to the effect of the extracts.

We did not find quercetin, epicatechin, ferulic acid, or coumaric acid in soil samples from the control fields or those fields which had been treated with bark extract formulations (of 1%, 2%, and 4% concentrations).

It is already known that soil microbial communities can significantly influence the productivity and overall quality of the agricultural ecosystem due to the roles they play in nutrient cycling, detoxification processes, and soil aggregate stability, among other functions (Lovaissa et al., 2017). We studied the impact of the bark extract formulations on soil biological quality. The substrate-induced respiration method was used for a determination of soil microbial biomass or SMC content. The determined microbial biomass in untreated soil samples fell in the range of between 0.40 mg kg^{-1} and 0.54 mg kg^{-1} . The results from soil samples which had been treated with a 2% spruce extract preparation, following the normal incubation period, did not differ significantly from the figures for untreated soil. Our data can be seen to be comparable with that which was obtained by Lovaissa et al (2017) in one year-old strawberry fields. The next step will be the determination of the impact of bark extract formulations on soil invertebrates and on *Spirodela polyrhiza* (L.) growth.

CONCLUSIONS

The newly developed formulations which are based on pine and spruce bark ethanol extracts did not negatively influence crop plants at concentrations of 1% and 2%. However, in some cases the amount of chlorophyll increases, although after two days this returns to its initial level.

The newly developed formulations which are based on pine and spruce bark ethanol extracts contain active substances in detectable quantities – coumaric acid, quercetin, epicatechin, and ferulic acid.

Some significant changes were observed in the amount of coumaric acid, epicatechin, and quercetin in strawberries after their treatment by different formulations, and in a significant increase of coumaric acid and quercetin in raspberries after their treatment with a spruce bark extract formulation of a 2% concentration.

The coniferous tree bark preparations which had been developed contained natural compounds that are present in the environment. Therefore their application did not leave any significant influence on soil quality levels that were stated using soil microbial biomass as an indicator. The next stage will be to explore the impact on soil invertebrates and on *Spirodela polyrhiza* (L.) growth.

ACKNOWLEDGEMENTS. We would like to acknowledge I Pugajeva, *Dr Chem*, from the Institute of Food Safety, Animal Health, and Environment (BIOR) for conducting the HPLC-MS/MS measurements, and Dr O Treikale and J Volkova Msc from the Latvian Plant Protection Centre for arranging and conducting the field studies. This study has been supported by ERDF 2010/0249/2DP/2.1.1.1.0/10/APIA/VIAA/168 and LU grant 2016/AZ81.

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Model for cost calculation and sensitivity analysis of forest operations

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Abstract. Forest operations include logging, off-road and road transport of round wood, harvesting residues and wood chips, soil scarification and pre-commercial thinning, as well as other less conventional operations like stump extraction and undergrowth removal before felling. The process of harvesting can involve different interfering phases with specific productivity parameters, which will have impact on the productivity of harvesting and delivery, as well on the prime cost of logs and forest biofuel. Detailed prime cost calculation allows to assess the impact of various factors on costs of the products, as well as to define threshold values for certain parameters affecting the productivity. The base model elaborated within the COST action FP0902 is complemented with standard economic methods and adopted to the harvesting process or any other forest or farming operation including systems consisting from several machines. The model is designed in a way, which is simple in use, easily extensible with additional parameters and machines and with possibility to change individual input data. The cost calculation section of the model consists from investments (base machines and equipment), labor costs (salaries, social charges, insurance and other payments) and operational costs (fuel, lubricants, maintenance, repair and other consumables). The average hourly cost is calculated according to forecast of number of working hours per year. Engine hours are used in calculation to synchronize input data with service statistics from dealers' centers. The parameters of the forest stands affecting productivity, like diameter or volume of an average extracted tree, number of relocations per year, average off-road transport distance, driving speed and other parameters are defined in the calculation. Productivity and load size can be set as fixed values or equations (in case if the sensitivity analysis should be done). The model calculates the hourly cost (productive, engine and proposed working hours) and the unit price for each phase of the work process. The sensitivity analysis demonstrates impact of various factors, like number of working hours per year, dimensions of the average extracted tree, forwarding and road transport distance, fuel price and fuel consumption as a default parameters or any other indicator, which can be added to the sensitivity analysis. The model is validated against the actual harvesting contracts and hourly cost of rental machines. Default parameters in the calculation are summaries of information provided by contractors or service companies.

Key words: cost calculation, forest operations, productivity.

INTRODUCTION

The harvesting process involves logging, off-road and road transport of round wood, harvesting residues and wood chips, as well as other less conventional operations

like stump extraction and undergrowth removal before felling (Uusitalo, 2010; Sarmulis & Saveljevs, 2015). This process can involve different interfering phases with specific productivity parameters, which will have impact on the productivity of the harvesting and materials' delivery, as well on the prime cost of logs and forest biofuel.

The prime cost of the harvesting is the total amount of utilized production resources expressed in monetary terms (Vītola & Soopa, 2002; Grīnfelds, 2004; Alsiņa et al., 2011). Detailed prime cost calculation allows to assess the impact of various factors on cost of the production, as well as to define threshold values for certain parameters affecting the productivity (Grīnfelds, 2004; Alsiņa et al., 2011).

Cost calculation models usually are complex tables consisting from the input and output sections. The input section of the model may consist from investments (base machines and equipment), labor costs (salaries, social charges, insurance, training and other payments), operational costs (fuel, lubricants, maintenance, repair and other consumables) and other input data, like productivity, characteristics of stands, forwarding and driving distance, road transport distance, average load size (FAO, 1992; Grīnfelds, 2004; Alsiņa et al., 2011; Ackerman et al., 2014). Production cost consists of direct and indirect costs. Direct production costs are directly related to creation of certain cost objects and depends from utilization rate of the machine or number of produced units. Generic or indirect production costs are not directly related to the production of the particular product but are conditionally linked to the production process and are included in the cost calculation of production using addition rate (Vītola & Soopa, 2002; Alsiņa et al., 2011). Determination and allocation of indirect costs by object of calculation is carried accordingly to the amount of production or period of production (Alsiņa et al., 2011). Output section of the cost calculation model usually represents hourly cost (productive, engine or proposed working hours) and the unit price for each phase of the harvesting process (Ackerman et al., 2014).

Sensitivity analysis is aimed on demonstration of impact of various factors, like number of working hours, dimensions of the average extracted tree, off-road and road transport distance, fuel price and fuel consumption or other factors.

Aim of this study is to create comprehensive cost calculation model, which can be used to evaluate multiple forest operations in different work conditions and to determine impact of changes in the system on the costs of production.

MATERIALS AND METHODS

The base model elaborated within the scope of COST action FP0902 (Ackerman et al., 2014) is complemented with standard economic methods and adapted for the harvesting process or any other forest operation including systems consisting from several machines.

The model is validated against actual harvesting contracts in state forests in Latvia and hourly cost of rental machines provided by the dealers' centres. The default utilization rate (engine hours per year) is also taken as average value of multiple machines utilized in state forests. Other default parameters in calculations are summaries of information provided by contractors, service companies or dealers.

Productivity data obtained in previous studies over the period 2013 to 2017 are used to create the default productivity equations for thinning and final felling in the prime cost calculation. To validate the model, it is assumed, that middle sized harvester (John

Deere 1270 with engine power 170 kW, boom max reach 10 m, operating weight 18 t and fuel consumption on average 12 L per E₁₅) with accumulating Moipu 300 felling head is used for harvesting. Middle class forwarder (John Deere 810 E with engine power 100 kW, operating weight 12.9 t, average load 7.9 m³, max crane reach 8.7 m, fuel consumption on average 12 L per E₁₅) or larger forwarder (John Deere 810 D with engine power 86 kW, operating weight 11 t, average load 5.4 m³, fuel consumption on average 12 L per E₁₅, with crane CF 1, max reach 8.7 m) is used for off-road transport of roundwood and harvesting residues. In validation of cost of roundwood delivery to the consumer logging truck (Volvo D13K with engine power 309 kW, average load 36.2 m³, fuel consumption on average 18 L per E₁₅) with trailer and Loglift 96 S crane is used. In biofuel delivery scenario costs are validated against mobile chipper of biomass Bruks 1001 (engine power 336 kW, fuel consumption 68 L per E₁₅) mounted on a forwarder Timberjack 1410 (engine power 136 kW, fuel consumption on average 12 L per E₁₅) and truck Volvo D13K (engine power 309 kW, average load 90 bulk m³, fuel consumption on average 18 L per E₁₅) with interchangeable containers is used to validate road transport cost of wood chips. The service costs, as well as default parameters for calculations are available from earlier studies (Kalēja et al., 2014; Lazdiņš & Zimelis, 2015).

It is also assumed in the model validation that the implemented forest operation is thinning in coniferous stand and conventional cut-to-length technology is applied.

Engine hours are used in calculation to synchronize input data with service statistics from dealers' centres. The engine hours are also used to synchronize all time elements in the calculation, respectively, it is mandatory parameter, which should be obtained during time studies.

Cost items of the calculation model include investment costs and labour costs (Brinker et al., 2002; Alsiņa et al., 2011; Ackerman et al., 2014). The purchase value of new machinery and equipment are used in the calculation by default, an example is shown in Table 1 (Uusitalo, 2010; Ackerman et al., 2014). Real figures available from studies or provided by contractors are used to validate the model.

Table 1. Example of calculation of the investment costs

	Harvester	Forwarder of round wood	Log truck	Forwarder of harvesting residues	Chipper	Chip truck
Base machine price, € per unit	350,000	250,000	171,429	246,000	185,714	171,500
Depreciation period, engine hours	25,000	20,000	20,000	19,000	14,000	20,000
Type of equipment	felling head	tracks	-	tracks	chipper	-
Price of equipment, € per unit	30,000	18,500	-	18,500	255,500	-
Depreciation period, engine hours	10,000	12,000	-	12,000	14,000	-

The time frame during which the machine productivity and operating costs are economically justified is defined as the economic life time and in the calculation model is expressed in working hours or years (FAO, 1992) which are synchronized with engine hours.

Equipment is considered as variable costs because depreciation period (in working hours) of the equipment can differ from base machine, respectively the equipment should be changed several times during life time of the machine (FAO, 1992; Ackerman et al., 2014).

Depreciation period (C) of machinery and equipment in years is calculated (Eq. 1) by dividing proposed working hours (economic life time) with the forecast of engine hours per year according to the productivity indicators (Brinker et al., 2002).

$$C = \frac{B}{SX} \quad (1)$$

where B – depreciation period in engine hours; SX – productive hours per year.

To calculate residual value (E , expressed as a percentage of the purchase value) of harvesters and forwarders after end of economic life regression Eq. 2 is used (Bright, 2004; Spinelli et al., 2011).

$$E = 0.836 - 0.281 \cdot \ln(C) \quad (2)$$

where C – depreciation period in engine hours.

For other machinery and equipment it is assumed by default, that the residual value (E) will be 15% of the purchase value.

Depreciation of machinery and equipment, calculated on a straight-line basis, is gradually attributed to the production costs (Alsiņa et al., 2011).

The residual value (F) estimated as a share of the purchase value of the machinery or equipment, is characterized by the expected resale values of the machine at the end of the economic life (FAO, 1992; Spinelli et al., 2011, Eq. 3).

$$F = A \cdot E \quad (3)$$

where A – base machine price, €; E – residual value, %.

Cost factor (G) is expressed in %. By default 5% depreciation rate (D) is used in the model to determine the annual investment cost of machinery and equipment (Eq. 4).

$$G = \frac{(D \cdot (1 + D)^C)}{((1 + D)^C - 1)} \quad (4)$$

where C – depreciation period, years; D – depreciation rate, %.

Annual costs of base machine and equipment (H) are calculated using Eq. 5.

$$H = G \cdot (A - F) \quad (5)$$

Labour costs consists of basic and supplementary wage of operator's, employer's compulsory social contributions and operator's benefits like training and insurance cost (Grīnfēlds, 2004; Ackerman et al., 2014).

In calculation of labour costs, the average gross salary rate of the industry operator is used. The calculation of the production cost includes the social tax paid by employer, which according to Latvian legislation is 24.09% (State Social..., 1997) and is calculated from the salary rate (Alsiņa et al., 2011). In salary calculation it is also possible to set operators' overtime with double payment rate (Labor Law, 2011).

Labour cost calculation also includes additional incomes, that means compensation for a travel to work (by default 0.2 € km⁻¹), daily allowance, by default 6.00 € per day (Procedures for..., 2010), trainings (186 € yr⁻¹) and other labour costs, like insurance and subsistence costs.

Relocation costs are considered for harvesters and forwarders using separate trailer and for chipper (and other machinery, if needed) on its own (by default trailer's speed is set to 40 km h⁻¹, relocation distance – 50 km in one direction and 50 moves per year.

The calculations also use indicators that characterize availability of the machine. The availability of the machine depends from time spent for repairs and maintenance (Uusitalo, 2010). By default availability is set to 80%. Working hours per year (*SZ*) of each machine are calculated using Eq. 6.

$$SZ = ((11 \cdot 20) \cdot 80\%) \cdot SH \cdot SJ \quad (6)$$

where *SH* – overtime per shift, hours; *SJ* – number of shifts per day.

Machine utilization rate shows the readiness of machine in productive work (Uusitalo, 2010) and by default this value is set to 85%. The last value differs a lot depending from working conditions and age of machines.

Productive working hours per year (*SX*) of each machine (except log truck and chip truck) are calculated using Eq. 7. Idle during machine movement is calculated by dividing the average machine movement distance (50 km) and average machine speed (40 km h⁻¹). Time for loading and unloading belongs to work time and is excluded from productive time. On average, machines are moved 50 times per year.

$$SX = (SV + SZ) \cdot 85\% - \left(\frac{50}{40}\right) \cdot 50 \quad (7)$$

where *SV* – working overtime, hours per year.

In the calculations it is assumed that one unit of machinery is serviced by 2 to 3 operators working on average 8 hours per shift for 11 months a year (on average, 20 working days per month).

Operators' driving distance per year (on average 30 km in shift (*SL*)) to access felling site and to return home (*SY*) of each machine operator (except log truck and chip truck) is calculated using Eq. 8.

$$SY = SL \cdot 2 \cdot SJ \cdot ((11 \cdot 20) \cdot 80\%) \quad (8)$$

where *SL* – trip to work (on average), km in shift; *SJ* – number of shifts, pieces per day.

In calculation it is assumed that the average compensation for each machine operator (except log truck and chip truck) for a trip to work is 0.2 € per km but daily allowance is 6 € per person per day. Annually 186 € per person are spent for training. Also other labour costs (approximately 1,500 € per person annually) are included in cost calculation (except operators of log truck, chip truck and biomass chipper). The cost calculation includes personal insurance, 357 € per person per year.

Operational costs are variable costs and they are closely related to the work load. These costs include fuel, lubricants, hydraulic oil, repairs, regular maintenance, relocations and other variable costs not listed above. Price of item included in calculation of operating costs is variable and depends on the situation on the market.

Working hours (*E₀*) in calculations corresponds to engine hours. Productive working time (*E₁₅*) is obtained by subtracting non-productive delay time from engine

hours. Yearly operational costs are calculated according to number of engine hours per year.

Table 2 shows examples of consumption of items included in operational cost calculation.

Table 2. Consumption of items included in operational cost calculation

	Harvester	Forwarder of round wood	Log truck	Forwarder of harvesting residues	Chipper	Chip truck
Fuel, L LV m ⁻³	-	-	-	-	0.7	-
Fuel, L E ₁₅ ⁻¹	12	12	18	12	68	18
Fuel, L 100 km ⁻¹	-	-	45	-	45	45
Fuel of trailer, L 100 km ⁻¹	45	45	-	45	-	-
Lubricant, g E ₁₅ ⁻¹	60	18	15	45	15	-
Lubricant for chain, g E ₁₅ ⁻¹	170	-	-	-	-	-
Fungicides, g E ₁₅ ⁻¹	3	-	-	-	-	-
Hydraulic oil, ml E ₁₅ ⁻¹	100	47	25	100	10	-

In order to make the prime cost calculation more accurate and adaptable to different conditions, specific productivity indicators and equations are used, like average size of extracted tree, harvester productivity, forwarder and truck load volume.

The indicators of the forest stands affecting productivity, like diameter or volume of an average extracted tree, average off-road transport distance, driving speed and other parameters can be set in the calculation.

Driving time (min) of roundwood forwarder and forwarder of harvesting residues (RI') is calculated using Eq. 9.

$$RI' = \frac{RH}{RE} + \frac{RH}{RF} \quad (9)$$

where RH – driving distance (one way), m; RE – average speed of forwarder (loaded), m min⁻¹; RF – average speed of forwarder (unloaded), m min⁻¹.

Calculation of log and chip transport (RI'' , min) is done using Eq. 10. In calculation it is assumed that average speed of log and chip truck is 40 km h⁻¹.

$$RI'' = \frac{(2 \cdot RG)}{40} \cdot 60 \quad (10)$$

where RG – driving distance (one way), km.

Time spent (RJ , min or min of E₁₅) for transportation of one load with roundwood or harvesting residues forwarder, or truck of log or chip is calculated using Eq. 11.

$$RJ = RA + RB + RI \quad (11)$$

where RA – loading time of forwarder, minE₁₅ per load; RB – unloading time of forwarder, min E₁₅ per load; RI – driving time, min.

Productivity (RM , expressed in m^3 per productive hour or RN , loose volume (LV) m^3 per productive hour) and load size (RL) can be set as fixed values or calculated using Eq. 13 or 14 (in case if the sensitivity analysis should be done).

$$RM = \frac{RL}{RK} \quad (12)$$

where RL – average load, m^3 ; RK – time per load, hours. E_{15} per load.

$$RN = \frac{RL}{RK \cdot 2.4} \quad (13)$$

To transfer solid cubic meter into loose volume (LV), the density coefficient 2.4 has been used by default. The default value for load size is based on results of productivity study.

The model calculates the hourly cost (productive, engine and proposed working hours) and the unit price for each phase of the harvesting process.

Sensitivity analysis includes a range of certain input data, from minimum to maximum value obtained during the studies, national statistics or the data provided by the contractors, for instance fuel consumption for the same type of machine, average forwarding or road transport distance, or applicable range of dimensions of extracted trees (usually obtained from time studies). These values are used to determine range of costs depending from value of the parameter. The model is validated against actual harvesting contracts in state forests and hourly costs of rental machines.

RESULTS AND DISCUSSION

Harvesting costs consist of forwarding, logging and road transport of roundwood, as well as the costs of biofuel extraction where applicable. Different models are used for prime cost calculation by researchers and enterprises (FAO, 1992; Väättäinen, et al., 2006; Ackerman et al., 2014), but there is still unfulfilled demand in a model giving detailed view of the prime cost of different forest operations, integrating productivity and costing parameters in dynamic calculation system.

In different cost calculation models various factors affecting costs are taken into account (FAO, 1992; Väättäinen et al., 2006; Spinelli et al., 2009; Harrill & Han, 2012; Ackerman et al., 2014). Logging, forwarding and roundwood delivery costs are heavily affected by dimensions of the average extracted tree, which needs to be represented in sensitivity analysis to see threshold values in expected range of the work conditions. The average productivity of logging, forwarding and road transport (the last 2 values are determined by load volume) are calculated for each diameter class and used in the calculation.

The cost calculation model allows to vary the factors affecting prime costs of several machines, choosing the type of preparation and delivery of roundwood and harvesting residues, planning work hours of forest machines, changing working conditions and forest machines (Fig. 1).

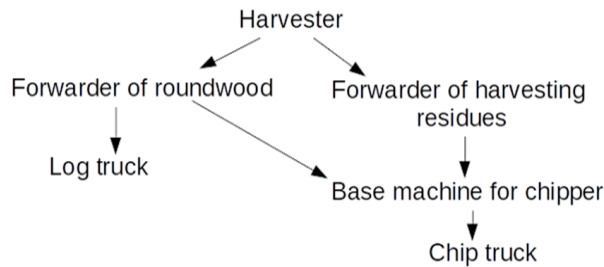


Figure 1. Modeling of harvesting system in forest operations.

Most of the cost calculation models predict calculate the cost of each separate forest machine, which do not represent how interaction of the machines and changing logging conditions can affect the cost of production and how to achieve higher economic efficiency (Ackerman et al., 2014). The following example (Table 3) shows how costs are analyzed in the proposed model.

Table 3. Example of output of cost calculation

Calculation items	Harvester	Forwarder of round wood	Log truck	Forwarder of harvesting residues	Chipper	Chip truck
Summary of costs, € per year						
Investment costs	51,725	39,058	15,206	41,246	71,194	15,212
Labour costs	62,637	62,637	72,692	62,637	60,765	72,692
Operational costs	103,896	53,056	31,207	51,102	172,673	39,150
Profit margin	10,913	7,738	5,955	7,749	15,232	6,353
Total	229,171	162,488	125,060	162,734	319,864	133,406
Productivity						
Roundwood with bark, m ³ E ₁₅ h ⁻¹	6.7	10.0	10.6	-	-	-
Biofuel, LV m ³ E ₁₅ h ⁻¹	-	-	-	37.5	96.5	23.9
Amount of roundwood and biofuel produced per each unit of machinery per year						
Total roundwood, m ³ per year	19,144	26,778	14,658	108,793	90,318	35,753
Logs, m ³ under bark	15,955	24,125	13,205	-	-	-
Biofuel (stem residues), m ³ per year	1,434	-	-	-	-	-
Biofuel (logging residues), m ³ per year	-	-	-	108,793	-	-
Bark and other residues, m ³ per year	1,755	2,654	1,453	-	-	-
Biofuel (wood chips), LV m ³ per year	3,443	-	-	261,103	216,762	85,807
Output						
Logs under bark, € per m ³	14.4	6.7	9.5			
Biofuel, € per LV m ³				0.6	1.5	1.6

Basic model version can be used to calculate if it is cheaper to deliver forest biofuel as logs or chips (Table 3); however, it can be easily adapted to different comparisons including system analysis.

According to the sensitivity analysis implemented in the model, the diameter of the average extracted tree significantly affects productivity. Similar or simplified approach can be used to determine, how the forwarding and road transport distance affects costs of production and to find threshold values for these parameters. Built in spreadsheet linear optimization functions can be used to determine the threshold values. Similar conclusions are also available in other studies (Väättäinen et al., 2006; Harrill & Han, 2012).

The model can be used to identify the factors affecting total harvesting and delivery cost under theoretical or real life conditions based assumptions (Figs 1 and 2).

Any other parameter considered in the cost calculation can be added to the sensitivity analysis. Where applicable, the sensitivity analysis should be combined with productivity models or equations. For example, change of dimensions of extracted trees should reflect in productivity of harvester, as well as on load size in off-road and road transport, reflecting in productivity of forwarder and log truck.

Sensitivity analysis of forwarder driving distance (Fig. 2) shows that increase of forwarding distance by 150 m in the conditions used for verification of the model increases the total production cost by 0.5 EUR per m³. Fuel consumption can also be differentiated in the model, for instance, different values of fuel consumption can be applied for driving loaded and empty, as well as for loading and unloading operations.

Sensitivity analysis of utilization rate (Fig. 3.) demonstrates that increase of the utilization of harvester significantly reduces total production cost. Similar effect is observed for all machines due to increase of indirect cost per working hour.



Figure 2. Sensitivity analysis of forwarder driving distance.

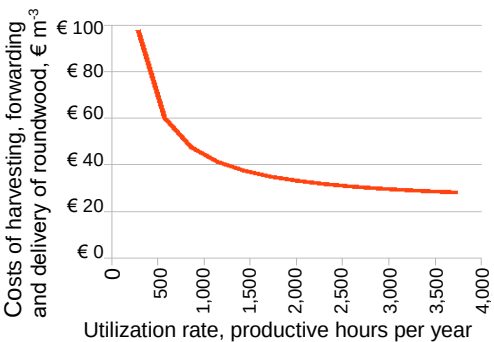


Figure 3. Sensitivity analysis of the utilization rate.

Comparison of the calculation results with actual harvesting costs in 2017 provided by the Joint stock Company ‘Latvia state forests’ and Central statistical bureau approves that the modeled values are within the uncertainty range of available statistical data; however there is still considerable potential for underestimation of harvesting costs by utilization of the study data due to overestimation of the utilization rate of forest

machines. This parameter was estimated using expert judgments in contrast to other parameters, where dealers' centers or contractors' information is available. Therefore, the calculation was tuned to conform to the real harvesting prices by changing the utilization rate. Other parameter significantly affecting cost of production is salary rate; some companies are paying fixed monthly salaries, some are paying per produced unit, some are combining these 2 methods. As a result, provided monthly or hourly salary rates differ a lot between companies, in spite the average annual income has no tendency of such a big variation. The model uses average hourly rate assuming full-time employment as a basic assumption, which can lead to overestimation of personnel costs in case of combined or per piecework payment scheme.

CONCLUSIONS

The elaborated model is simple in use, easily extensible with additional parameters, machines and equipment. It can be used in practice, at a company level to analyze and to predict machine costs, as well as in research for system and sensitivity analysis.

One of the largest benefits of the model is using of engine hour as a reference time unit providing opportunity to use machine service data in cost calculations without adaptation of the applied data.

The model contains internal system of quality assurance, like calculation of the net income of operators and a company, and the hourly cost of machine, which can be validated against the service data.

The model is supplied with the default input data, which are already validated in Latvia and can be easily adapted to other conditions providing at the same time opportunity to avoid logical mistakes in data entering, like use of non-realistic values for consumption lubricants or fuel.

The model allows to get an overview of the cost of the machine system in dynamic conditions, which, accordingly, allows to choose the most efficient combination of machines, threshold values for certain operations, like off-road transport distance, and stand parameters, like minimum dimensions of trees.

ACKNOWLEDGEMENTS. The study is implemented within the scope of the Forest Sector Competence Center project No. 1.2.1.1/16/A/009.

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The application of micro-wave treatment to reduce barley contamination

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Abstract. The goal of this work is to study the applicability of ultra high frequency electromagnetic field treatment for decontaminating barley grain used in brewing while preserving its technological properties. The germination rates and/or yield of the treated sample seed were compared with those of the untreated seed germinated under normal conditions. To determine optimal treatment conditions, a two-factor analysis was carried out, taking the mycological state of the grain into account. The heating rate and the duration of electromagnetic exposure were chosen as variables; these values varied from 0.4 to 0.8 °C s⁻¹ and from 30 to 90 s, respectively. It was found that germination of the treated barley seed was increased about 10.1–15.7% compared with that of the untreated seed. The microbial load decreased up to 80%. A heating rate of 0.4 °C s⁻¹ and treatment exposure time of 30 s showed the strongest effect of decontamination while preserving the viability of the barley grain.

Key words: microwaves, barley, *Alternaria*, malt, mycotoxin, the grain viability.

INTRODUCTION

As a key global food resource, the contamination of grains with insects or microorganisms is a persistent concern for the grain industry due to irreversible damage to quality and safety characteristics and economic losses. The reason for the poor quality of barley grain is a high susceptibility of this culture to phytopathogenic microorganisms.

Alternaria fungi are one of the most common components of the grain microbiome. The most dangerous result of infecting with *Alternaria* fungi is the accumulation of a large number of mycotoxins (Logrieco et al., 1990), (Milicevic, 2009). Mycotoxins entering the human body can cause diseases associated with violation of the gene and nervous system structure, acute chronic kidney disease, and the development of cancer (Hussein & Jeffrey, 2001). Studies carried out by scientists from different countries confirm that aflatoxin is a toxic, mutagenic, and carcinogenic compound (Webley et al., 1998; Scott, 2001; Logrieco et al., 2009; Janić Hajnal et al., 2015; Wu et al., 2016)

The problem of early grain blights is still relevant as it is widespread now both in Russia (Gavrilova et al., 2016; Gavrilova et al., 2017), and abroad (Müller et al., 2002; Scott et al., 2012; Müller et al., 2015). Therefore, it is rather difficult for enterprises engaged in grain processing and storing to ensure the stable quality of plant raw materials.

To create and organize the environmentally safe production of food products, it is necessary to use fundamentally new technologies, since traditional methods cannot guarantee, a decrease in grain contamination with microorganisms and activation of its growth in malting. Some traditional methods are also very energy intensive, require expensive equipment, and have a limited field of application (Yaldagard, et al., 2008; Wilson, et al., 2016; Los, et al., 2018).

One promising method for reducing grain contamination is the resource-saving technology based on electrophysical methods, in particular, microwave heating.

Currently, microwave heating is successfully used for treating raw materials with active enzymes, wherein some treatment modes contribute both to an increase in activity and inactivation of microflora. A positive effect from microwave heating of wheat grains was observed at a power of 700 W with a treatment time of 0–60 s (Qu et al., 2017). However, analysis has shown that it is necessary to strictly regulate the treatment parameters (Yaldagard et al., 2008; Wilson, et al., 2016; Los, et al., 2018) for preserving the technological properties of barley grain used in the brewing technology.

The stimulating effect of microwave energy is caused by the excitation of active enzyme centers involved in the germination of seeds, as well as the increase in the permeability of cell membranes due to the formation of free radicals, which contributes to a better oxygen and water supply to the cells. The features of the interaction of microwave energy with food raw materials and the pronounced bactericidal effect are of particular interest (Chen, et al., 2012; Dalmoro et al., 2015; Viliche Balint, et al., 2016; Motallebi, 2016.). Usually the most useful ways to improvement of barley quality are IR-radiation; γ -beams, ultrasound, electronic ionic technologies. However some of them only allow to reduce grain contamination (Wilson et al., 2016; Los, et al., 2018), others – positively influence only process of the grain viability (Yaldagard et al., 2008). However it is difficult to reach complex effect.

The purpose of this paper is to develop effective methods for decontaminating barley grain used in brewing while preserving its technological properties by using ultrahigh frequency electromagnetic field treatment.

MATERIALS AND METHODS

Sample collection and preparation

At the first stage, was studied the quality of raw grain materials and their applicability in the production of malt used in brewing, which grown in Russia in different areas of the Chelyabinsk, Kurgan, Sverdlovsk, and Tyumen regions (batches 1, 2, 3, 4 respectively). 100 samples were selected from each batch; the experiments were repeated at least 15 times. The control variables were grain contamination with *Alternaria* fungi and grain viability. Indicators were evaluated taking the conditions of growing barley grain into account. The coefficient of variation (v,%) was calculated using the mathematical statistics methods.

The test samples of grain were treated in the electromagnetic field – microwave treatment at a frequency of 2,450 MHz (Microwave oven SAMSUNG GE83KRS-3, Malaysia). The heating rate varied from 0.4 to 0.8 °C s⁻¹ and the duration of the electromagnetic exposure – from 30 to 90 s.

100 g weighed grain portions were selected for each treatment option. Further, the samples of barley grain were placed in specially folded standard paper bags and treated in set conditions.

Design of the experiment

After microwave treatment, was measured the heating temperature of the experimental batches of barley with a thermometer (Espada TA-288, Russia). Then, was analyzed grain contamination and grain viability.

Grain contamination with fungi was determined by the biological method based on stimulating the development and growth of microorganisms in contaminated grains. For this purpose, the grain was germinated in a wet chamber (thermostat TSO-1M, Russia) at a constant temperature (22–24 °C). Four working samples of 50 grains were used for analysis. The contamination results were analyzed four days after the grain was placed in the wet chamber.

The intervals of change for these variables were selected because at the chosen values of these variables, other things being equal, we obtain barley grain that is suitable for the production of malt. The main criterion for assessing the influence of each factor on the disinfection of barley grain was heating temperature. The minimum value depended on the environment, and the maximum value was limited to barley grain quality indicators.

At the next stage of the study, was evaluated the effect of the electromagnetic influence on the grain viability. In the process of barley grain decontamination and finding effective modes of microwave field influence thereon, our main task was to preserve grain viability. This quality index is the main indicator of the physiological usefulness of barley and characterizes the suitability of barley grain for the production of malt.

The grain viability was determined by germinating the pre-wet grains. For this purpose, an average sample with the weight of 50 ± 1 g of grains was first formed, from which two analytical samples of 500 whole grains were taken. The grain viability was determined 120 hours after the beginning of the experiment by visual evaluation.

Statistical data analysis

The experimental data was processed using traditional variation statistics methods and expressed as an arithmetic mean ($\bar{m}$) and standard error (m). To determine the statistically significant differences between the test and control groups was used the Mann-Whitney test (U). Results were expressed as an arithmetic mean and its standard deviation. The differences were considered significant at $p < 0.05$. Statistical interrelations were studied using the nonparametric correlation analysis by calculating the Spearman correlation coefficients (PC).

RESULTS AND DISCUSSION

Mycological analysis of the barley grain contamination revealed the presence of various species of fungi belonging to *Alternaria*, *Aspergillus*, *Fusarium*, *Bipolaris*, *Cladosporium*, *Penicillium*, *Mucor*, and other genera in the microbiota. The most

widespread among the detected micromycetes were the representatives of *Alternaria* – from 50 to 78% depending on the batch (Fig. 1).

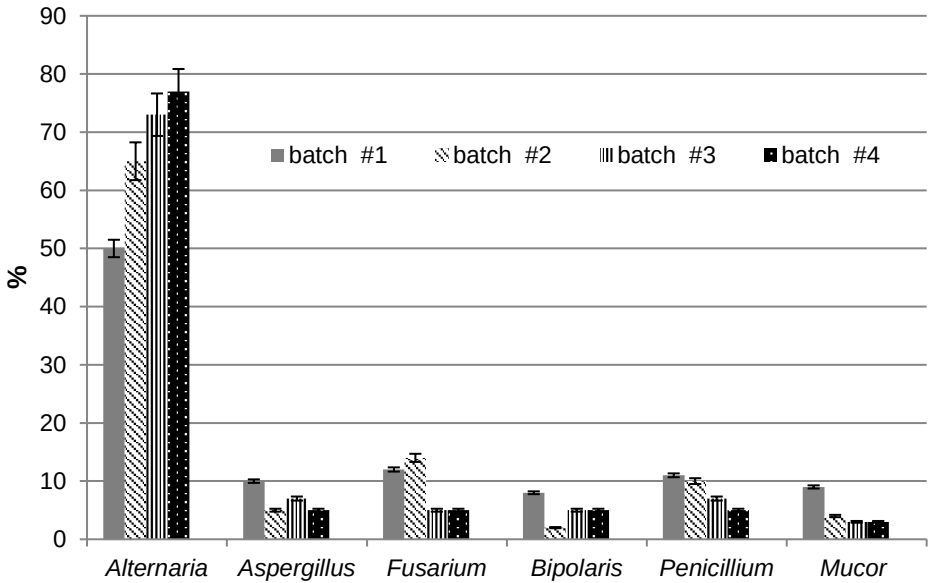


Figure 1. Percentage of fungi grain contamination in control batches from different areas of the Chelyabinsk, Kurgan, Sverdlovsk, and Tyumen regions of Russia.

The control batches of brewing barley had the grain viability equal to $80 \pm 2\%$ (Fig. 2), consequently, such barley grain is not suitable for malt production, since normally germinated barley has the grain viability should be not less than 95% (for barley of class I) and 90% (for barley of class II).

To determine optimal treatment conditions, a two-factor analysis was carried out. The heating rate (τ) and the duration V_t of the electromagnetic exposure were selected as variables. Monitored parameter: grain contamination and viability (Figs 3, 4). Was established that the type of electro physical exposure used at the heating rate of $0.6\text{--}0.8\text{ }^{\circ}\text{C s}^{-1}$ with a treatment exposure of 60–90 s leads to the disinfection of almost all types of fungal infection, including *Alternaria* fungi (Fig. 3).



Figure 2. The grain viability of control sample.

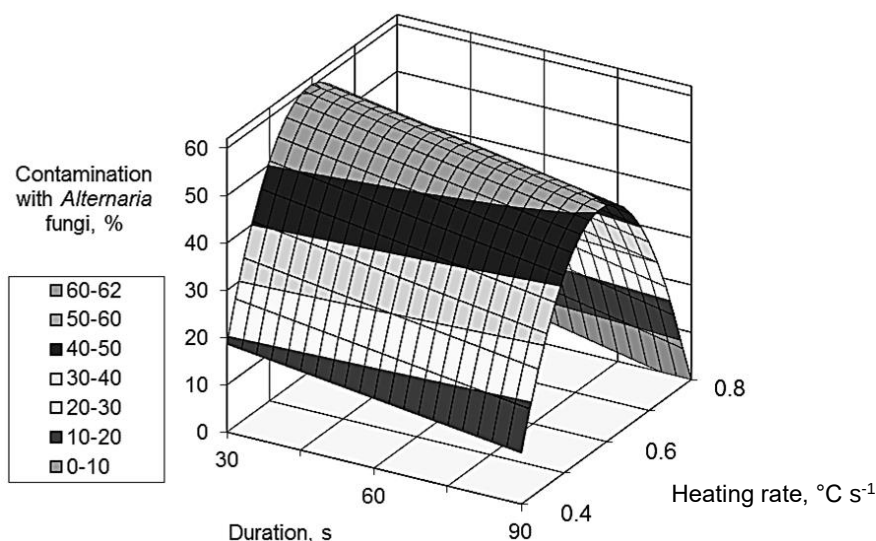


Figure 3. Influence of the microwave energy on the *Alternaria* fungi contamination of barley grain used for brewing.

At the minimum microwave field loads, there is a significant reduction in grain contamination with these fungi compared to other ways of processing grain (IR, cold plasma) (Wilson et al., 2016; Los et al., 2018), even at a low heating temperature (30 °C).

A further increase in the microwave field load (up to 0.6 °C s⁻¹), regardless of the treatment exposure time, leads to the active development of microorganisms; grain contamination by pathogens of these fungi reaches 50–60%. Under such conditions, the fungus mycelium develops abundantly, which can cause the darkening of the grain shell. Increase in the dissemination of fungi of *Alternaria* occurs not only due to the development of fungi already present on the grain and under its membrane (in the endosperm), but also because of the transition of spores to healthy grains, since sprouts of mycelial fungi freely penetrate to the embryo and grain tissues (Justé et al., 2011).

Provided that the heating rate is high (0.8 °C s⁻¹), and the exposure reaches 30–90 s, the barley grain contamination with *Alternaria* fungi disappears completely or falls to the permissible level.

The combination of these parameters of the electromagnetic field effect positively influences the cytology of the mycelial fungal cell, thereby reducing the number of mycotoxins produced.

The treatment mode of a heating rate of 0.4 °C s⁻¹ and an exposure time of 30–90 s, as well as the mode with a heating rate of 0.8 °C s⁻¹ and exposure time of 30–90 s can be considered effective for this genus of pathogens; the microbial load decreases up to 80%. Use of IR-radiation makes it possible to lower the microbial load of grain to 42% with a treatment duration of 180 s and a radiation intensity of 5.55 kW m⁻¹ (Wilson et al., 2016). Disinfection to the permissible levels can also be achieved by using high-voltage closed processing by atmospheric cold plasma – in this case, processing time is 20 minutes with a voltage of 80 kV (Los et al., 2018).

The results of the microwave effect influence on the grain viability of barley grain are shown in Fig. 4, Table. 1.

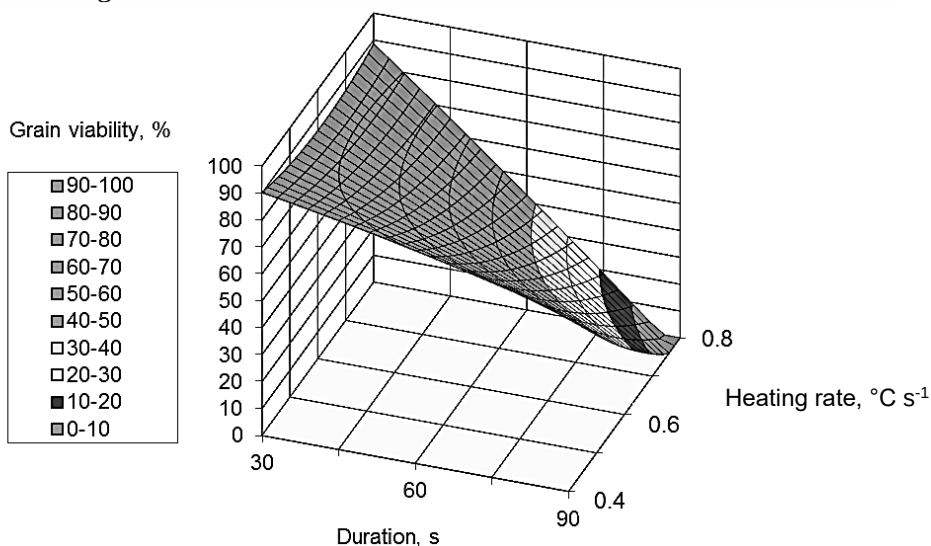


Figure 4. Influence of microwave heating on the grain viability.

The main goal of barley germination on malt is the synthesis and activation of enzymes, which is achieved by dissolving the mealy body of the grain. In this case, the grain structure changes, important biochemical changes occur: some enzymes pass from the inactive state to the active state, others are formed as a result of synthesis. The activity of enzymes changes the structure of endosperm cells, as a result of which the cell walls soften. Changes in the grain structure determine the degree of loosening of the grain endosperm – an important technological factor of malting. This factor is due to the action of a complex of cytolytic and proteolytic enzymes, the rate of accumulation of which determines the quality of preparation of malt. These data are consistent with those of other scientists (Woonton, et al., 2005).

The nature of metabolic processes in germinating grains can be influenced by the use of various physical factors of influence (Woonton, et al., 2005). In particular, using microwave treatment, it is possible to enhance the activity of hydrolytic enzymes in the process of barley germination to malt.

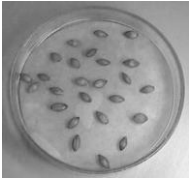
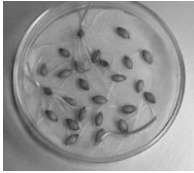
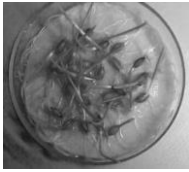
The moderate thermal effect of the microwave field (heating to 30 °C) creates a brewing condition for barley grain that coincides with the conditions for the beginning of embryo growth. This favorably influences the grain viability, which increases to 90.3%. These conditions lead to the decontamination of *Alternaria* fungi alone as well.

With a further increase in the microwave field load (heating rate up to 0.6 °C s⁻¹ and treatment exposure time of 30–60 s), the grain viability of brewing barley grain is reduced and amounts to 60–85% versus 90.3% (under the condition of ‘weak’ microwave field loads). These standard parameters of microwave heating make it possible to almost completely remove the fungal infection. When treatment exposure time is increased to 90 s, the grain viability decreases six times, and with an increase in the heating rate to 0.8 °C s⁻¹, the grain viability is equal to zero. Thus, modeoffered the strongest

decontamination effect while preserving barley grain viability: a heating rate of $0.4\text{ }^{\circ}\text{C s}^{-1}$ and treatment time of 30 s (the grain viability – 90.3%). This favorably influences the grain viability: compared to the control, this indicator increases by 10.1–15.7%. Compared to the use of ultrasound, germination of the treated barley seed was increased about 4–6% (Yaldagard et al., 2008) and this processing method doesn't provide the decontamination effect.

The Table 1 demonstrates that not all modes of processing of grain by the microwave field are effective.

Table 1. The typical examples of the microwave treatment influence on the grain viability

$(\tau = 90\text{ s}; V_t = 0.8^{\circ}\text{C s}^{-1})$ the grain viability – $0.0 \pm 0.5\%$	$(\tau = 30\text{ s}; V_t = 0.8^{\circ}\text{C s}^{-1})$ the grain viability – $87.7 \pm 0.7\%$	$(\tau = 90\text{ s}; V_t = 0.4^{\circ}\text{C s}^{-1})$ the grain viability – $71.0 \pm 0.5\%$
		
$(\tau = 30\text{ s}; V_t = 0.4^{\circ}\text{C s}^{-1})$ the grain viability – $90.3 \pm 0.6\%$	$(\tau = 90\text{ s}; V_t = 0.6^{\circ}\text{C s}^{-1})$ the grain viability – $12.7 \pm 0.7\%$	$(\tau = 30\text{ s}; V_t = 0.6^{\circ}\text{C s}^{-1})$ the grain viability – $86.7 \pm 0.5\%$
		
$(\tau = 60\text{ s}; V_t = 0.8^{\circ}\text{C s}^{-1})$ the grain viability – $37.7 \pm 0.5\%$	$(\tau = 60\text{ s}; V_t = 0.4^{\circ}\text{C s}^{-1})$ the grain viability – $73.7 \pm 0.5\%$	$(\tau = 60\text{ s}; V_t = 0.6^{\circ}\text{C s}^{-1})$ the grain viability – $67.3 \pm 0.5\%$
		

The different reaction of barley grains to the electrophysical effect allows us to assume the mechanism of action, which is largely determined by the parameters of the effect, i.e. speed and processing time. With some parameters, activation of seeds is observed (acceleration of their germination, increase in the rate of root growth), and in others – inhibition. An important consequence of such impacts may be a change in the dynamics of water absorption. At certain frequencies, due to the vibration of individual heterogeneous parts of the grain, the microstructure of the natural grain canals becomes favorable for the transport of water and nutrients. It is also possible to accelerate the processes of denaturation and untwisting of polypeptide chains in the embryo and endosperm during the water absorption stage, resulting in the formation of cavities into which additional osmotic water rushes. As a result, good conditions are created for the life of the embryo, the release and transport of enzymes into the cells of the aleuron layer, the synthesis of enzymes necessary for the cleavage of starch in endosperm cells

and nutrition of the embryo during germination (processing time – 30 s and heating speed $0.4\text{ }^{\circ}\text{C s}^{-1}$).

So, for example, the processing time 90 s and heating speed $0.8\text{ }^{\circ}\text{C s}^{-1}$ leads to an inactivation of enzymes, the grain viability decreases to 0% what is inadmissible at the malt production. The processing parameters: the processing time 60–90 s and heating speed $0.4\text{--}0.8\text{ }^{\circ}\text{C s}^{-1}$ also lead to decreasing of the grain viability. The processing parameters: processing time 30 s and heating rate $0.4\text{--}0.8\text{ }^{\circ}\text{C s}^{-1}$ and processing time 45 s and heating rate $0.4\text{--}0.44\text{ }^{\circ}\text{C s}^{-1}$ promote increase in activity of enzymes and grain viability.

Thus, the microwave field can have a stimulating, an inhibitory, and even a sparing influence on the biological activity of barley grain. Moreover, the exposure effect depends on the value of the variables.

The treatment mode with a heating rate from $0.4\text{ }^{\circ}\text{C s}^{-1}$ and exposure time of 30 s showed the strongest decontaminating effect while preserving barley grain viability.

CONCLUSIONS

Ultra high frequency heating can be used as a stimulating factor aimed at decreasing the fungal infection of barley grain contamination and increasing the activity of enzymes.

The use of the ultra high frequency electromagnetic field energy made it possible to reduce *Alternaria* fungi infection in grain from 80 to 10%, up to complete disinfection, and to increase viability by 10.1–15.7%.

ACKNOWLEDGEMENTS. This article was written with support from the Government of the Russian Federation (Resolution №211 of 16.03.2013), Agreement №02.A03.21.0011 and subsidies for the fulfilment of a fundamental portion of a state order, project №40.8095.2017/BCh. The work was supported by Act 211 Government of the Russian Federation, contract №02.A03.21.001, Project №19.8259.2017/BCh. Agreement № 02.A03.21.0011, and subsidies for the fulfilment of a fundamental part of a state order under Project № 40.8095.2017/BCh.

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Air-conditioning in the cabins of passenger cars

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Abstract. The objective of this paper is to analyse the current state of the constructional design and operational conditions of air-conditioning device in passenger cars. The research was focused on the function of air-conditioning equipment of passenger cars Skoda and KIA in various modes of operation during the winter, spring and summer season at different levels of air conditioning (without air-conditioning, minimum, medium and maximum level). Air temperature, air humidity, globe temperature, CO₂ concentration, dust concentration and noise inside the cabin were measured. Solar radiation plays a big role to rise up temperature inside the cabin. It resulted in the higher values of globe temperature than temperature of the air. The results of the measurements showed that CO₂ values were significantly lower than 2,500 ppm at minimum air-conditioning, lower than 600 ppm at medium and lower than 500 ppm at maximum level of air-conditioning. For all vehicles, dust concentration was greater when it measured with the air conditioning switched off than with the air conditioning system turned on. The measurements confirmed that the total dust concentration was not more than 47 µg m⁻³, PM₁₀ lower than 28 µg m⁻³ and PM₁ lower than 27 µg m⁻³. The noise levels ranged from 49.1 to 68.7 dB(A). The air-conditioning had very positive impact on the inside comfort in car cabins from all points of view during all periods of the year.

Key words: air humidity, carbon dioxide, drivers comfort, dust, noise, temperature.

INTRODUCTION

Drivers in all transport categories, in the course of their daily operations, are affected by microclimate, which is determined by air temperature, air velocity, relative humidity, carbon dioxide and thermal radiation. The objective of this research is to examine the microclimate value measured in the driver's cabins in the passenger cars. Microclimate in the driver's cabin significantly affects human thermal comfort; the cabin environment has an emphasis on thermal comfort not only for reasons of convenience, but also safety. It is necessary to ensure a suitable microclimate in the car cabin even in extreme operating conditions.

The recommended values of microclimate inside the cabin of the car, are according to publication Vlk (2003). The recommended values are as follows: air temperature 18–22 °C and relative humidity 40–60%; air velocity 0.1 m s⁻¹ at 18 °C and 0.4 m s⁻¹ at 24 °C; air exchange per person (clean air): 25–50 m³ h⁻¹ of fresh air; maximum concentration of pollutants: 0.17% CO₂, 0.01% CO and 1 mg m⁻³ of dust. Scientific studies, Anderson (1998) in the past have shown the effects of inappropriate working

conditions on fatigue, which significantly applies to prolonged driver's working hours. A suitable microclimate is very necessary for to improve the safety features of vehicles, and the systems must ensure it.

However, the increasing awareness of the strong influence of car microclimate on drivers and travellers leads to automobile users also being interested in the provision of actual and comprehensive characteristics of air quality inside the vehicle cabin, for their own knowledge. In this case, it is assumed that air quality evaluation should be performed quickly on-site, and should result in concise, easy to absorb information. Unfortunately, the outputs of a measuring system cannot be directly used for comprehensive description of air quality (Szcurek & Maciejewska, 2015).

The driver's cabin features a large flat windscreen. A small volume of air inside and relatively low heat insulation, resulting in a greater degree of influence on the operating conditions. If the temperature in a driver's cabin is below 17 °C, it provides comfortable zone inside the cabin; resulting in a reduction of efficiency and a risk of muscle fatigue. In addition, inaccuracy and constraints of movements are observed. If the temperature is above 25 °C, reactions slow down and the rate of physical tiredness accelerates. At a temperature above 30 °C, mental activity will worsen. Generally, drivers are sensitive to humid air because the human body uses evaporative cooling as the primary mechanism to regulate temperature. Under humid conditions, the rate at which perspiration evaporates on the skin is lower than it would be under arid conditions. Because human beings perceive the rate of heat transfer from the body rather than temperature itself, we feel warmer when the relative humidity is high than when it is low (Zewdie & Kic, 2016b).

Some drivers experience difficulty breathing in humid environments. Some cases may possibly be related to respiratory conditions, while others may be the product of performance anxiety disorder. In times of extreme stress, a driver may shake uncontrollably, hyperventilate (breathe faster and deeper than normal) or even vomit in response, causing sensations of numbness, faintness, and loss of concentration, among others (Gładyszewska, 2011). Air conditioning reduces discomfort in the summer not only by reducing temperature but also by reducing humidity. In winter, heating cold outdoor air can decrease relative humidity levels indoor to below 30% leading to discomforts such as dry skin, cracked lips and excessive thirst (Zewdie & Kic, 2015).

The interior of a vehicle is considered as a specific microenvironment. It is mainly experienced via quality of air and thermal conditions, noise levels, vibration inside the cabin. Air quality is a term that describes the physical, chemical and biological state of indoor air at some specific place at specific time. Mainly, it is characterized by physical and chemical parameters such as temperature (T), relative humidity (RH%) and airflows (Musat & Helerea, 2009).

Generally, transport technology improvement is correlated to safety. Microclimate composition rate is an important index factor affecting the contentment of drivers in the cabin. Numerous researchers used different measurements to assess the driver workload under diverse driving conditions. The conclusions reached by monitoring measures support an objective and continuous analysis in a dynamically changing microclimate situation (Zewdie & Kic, 2016a).

In the course of breathing, we exhale CO₂, which can displace O₂ in an indoor environment such as a vehicle cabin, leaving the environment O₂ deficient. Such high CO₂ and low O₂ concentrations can cause adverse human health effects. Various

independent studies, Galatsis et al. (2001) have also shown that through this process, the concentrations of O₂ and CO₂ may come to exceed safety limits. Interestingly, a study on fatal single-vehicle crashes highlights that the vehicle is more likely to have closed windows and a heater on than to have fresh air and air conditioning fitted, Maroni et al. (1995).

An O₂ deficient environment has been termed ‘hazardous’ when the O₂ concentration is less than 19.5%, Galatsis et al. (2000). Low O₂ levels can impair judgment, increase heart rate and impair muscular coordination. In the conclusion in their respective findings that thermal state of internal microclimate inside the drivers' cabins has a strong correlation with drivers' comfort which has an influence on the safety of drivers.

In vehicles that are parked, no ventilation and no air conditioning takes place. If a vehicle is exposed to direct solar radiation, an immediate temperature rise occurs. The high cabin air temperature can threaten children and animals that are left unattended in vehicles. In the USA, lethal heat strokes cause a mean death rate 37 children per year (Horak et al., 2017).

MATERIALS AND METHODS

The authors carried out the research on three different passenger cars equipped with air-conditioning (AC). For research implementation, the authors applied two Skoda cars (Skoda Octavia II and Skoda Octavia III Combi) and KIA Sportage. Main technical data and parameters of all three vehicles are summarized in Table 1.

Table 1. Researched cars

Type of Car	Production year	Kilometer reading	Engine
Skoda Octavia II	2007	217,745	1.9 TDI
Skoda Octavia III Combi	2016	10,000	1.6 TDI
KIA Sportage	2016	3,424	1.7 L I4(TD)

Both cars of the Skoda Octavia were equipped with an automatic Climatronic air-conditioning system. This system is a combination of automatically operating heating, ventilation and cooling equipment that ensures optimal passenger comfort. Climatronic automatically keeps the set temperature.

The first car Skoda Octavia II was regularly serviced, but the air conditioning system since 2010 has not been serviced. In 2013, they changed only cabin filter. The second car Skoda Octavia III Combi was a new car with perfect condition AC system.

The third tested car was KIA Sportage. It was also new car as written in above table kilometer reading was 3,424 km. The AC system of the car was perfect and it had well-functioning sensor system to keep comfortable zone inside the cabin.

Data on the microclimate conditions in all drivers cabin were collected from measurement devices which were installed on the passenger seat next to the driver in the cab of the respective vehicles.

Following instruments used for measurements: the thermal comfort in the cabin was continuously measured by globe temperature (measured by globe thermometer FPA 805 GTS with an operative range from –50 to +200 °C with the accuracy ± 0.01 K and diameter of 0.15 m) together with temperature and humidity of surrounding air measured

by sensor FH A646-21. The temperature sensor NTC type N with the operative range from -30 to $+100$ °C with accuracy ± 0.01 K and air humidity by capacitive sensors with the operative range from 5 to 98% with accuracy $\pm 2\%$ was installed. All installed equipment's had very good accuracy range.

The concentration of CO₂ was measured by the sensor FY A600 with operative range 0–0.5% and accuracy $\pm 0.01\%$. The informative noise level measurements were conducted by UNITEST Sound Level Meter 93411 capacitor microphone and measuring ranges: 30–100 dB(A) or 65–135 dB(A), resolution 0.1 dB(A), during the measurement used frequency evaluation filter A, time evaluation slow (1.5 s), frequency range 30 Hz – 12 kHz. The sound level meter is a produce of Ch. BEHA GmbH, Germany. All data were measured continuously and stored at intervals of one minute to the measurement instrument ALMEMO 2690–8 throughout the measurement process.

The research was focused on the verification of the function of air-conditioning equipment of passenger cars in various modes of operation outside the Prague city. Measurements were carried out on identical routes. The route was starting from Prague Suchdol via Uněťice and Tursko to Kralupy nad Vltavou and back. The total length of the route was 42.6 km. All cars were air-conditioned, but the tests were provided at different levels of air conditioning (without 0, minimum, medium 50% and maximum 100%). The measurements have been carried out during three principal periods of the year (winter, spring, and summer). During each measurement, outdoor climatic conditions were observed. The AC function was set at 22 °C during the winter and spring measurements, and on 24 °C during the summer.

Assuming steady conditions, with a uniform distribution of pollutants in space the required volume airflow for ventilation V_c is calculated according to (Szekyova et al., 2006) by the Eq. (1).

$$V_c = \frac{M_p}{c_i - c_e} \quad (1)$$

where: V_c – required volume airflow for ventilation, $\text{m}^3 \text{h}^{-1}$; M_p – mass flow of produced pollutant, uniformly leaking into the space, kg h^{-1} ; c_e – concentration of pollutant in inlet air, kg m^{-3} , (usually is $c_e = 0$); c_i – concentration of pollutant in outlet air, kg m^{-3} , (usually considered OEL – Occupational Exposure Limits or MEL – Maximum Exposure Limits).

The thermal conditions composed of internal globe temperature t_g , and internal temperature t_i as well as internal relative humidity RH_i are carefully collected for further analysis. The obtained results of airflow for ventilation V_c were processed by Excel software and verified by statistical software Statistica 12 (*ANOVA and TUKEY HSD Test*). Different superscript letters (a, b) mean values in common are significantly different from each other in the column (*ANOVA; Tukey HSD Test; $P \leq 0.05$*), e.g. if there are the same superscript letters in all the rows it means the differences between the values are not statistically significant at the significance level of 0.05.

RESULTS AND DISCUSSION

The mean values including standard deviation were calculated from the results of measurements of external temperature t_e (°C) and each internal microclimatic parameter internal temperature t_i (°C), internal relative humidity RH_i (%), internal globe

temperature t_g (°C) and noise level L_A (dB(A)) are summarized in the Tables 2, 4, 6 and 8.

The mean values including standard deviation were calculated from the results of measurements of total dust concentration, PM_{10} , PM_1 , concentration of CO_2 (%) and calculated volume of ventilation air flow V_c ($m^3 h^{-1}$) in the driving cabin are presented in the Tables 3, 5, 7 and 9.

Mainly external conditionings outside the cars influenced the first measurements in the cars with passengers (Table 2 and 3) during the parking (not working engine, without AC). The inside temperatures t_i and relative humidity RH_i are in relation to the external conditions according to psychometrics principle. The inside air temperature $t_i = 21.1$ °C in Octavia III was near to the optimal temperature 22 °C. The noise caused only by the external conditions were slightly higher in KIA during the summer measurement, probably because of the higher traffic in the surrounding, but the differences between the car's measurements are not statistically significant.

Table 2. Thermal state of the environment (external temperature t_e , internal globe temperature t_g , internal temperature t_i , relative humidity RH_i and noise level L_A) in tested vehicles without function of AC. Different letters (a, b) in the superscript are the sign of high significant difference (ANOVA; Tukey HSD Test; $P \leq 0.05$)

Car	t_e °C $\pm$ SD	t_i °C $\pm$ SD	t_g °C $\pm$ SD	RH_i % $\pm$ SD	L_A dB(A) $\pm$ SD
Octavia II	3.2 $\pm$ 0.1	14.8 $\pm$ 0.2	15.9 $\pm$ 0.6	51.6 $\pm$ 1.2	49.7 $\pm$ 4.9 ^a
Octavia III	12.7 $\pm$ 0.1	21.1 $\pm$ 0.9	21.7 $\pm$ 0.3	47.2 $\pm$ 1.5	49.1 $\pm$ 6.6 ^a
KIA	28.9 $\pm$ 0.2	33.8 $\pm$ 0.3	33.1 $\pm$ 1.4	38.8 $\pm$ 1.8	54.7 $\pm$ 6.1 ^a

SD – Standard deviation.

The dust and CO_2 concentrations (Table 3) were highest in comparison with the other measurements. As the AC was switched off, the CO_2 concentrations are extremely high and therefore the calculated ventilation rate V_c is very low (only natural air exchange by cars leakages). High concentrations of CO_2 and dust can cause adverse human health effects.

Table 3. Total dust concentration and concentration of dust fractions PM_{10} and PM_1 , concentration of carbon dioxide CO_2 and values of the intake fresh air V_c in tested vehicles without function of AC. Different letters (a, b) in the superscript are the sign of high significant difference (ANOVA; Tukey HSD Test; $P \leq 0.05$)

Car	Total dust $\mu g m^{-3} \pm$ SD	PM_{10} $\mu g m^{-3} \pm$ SD	PM_1 $\mu g m^{-3} \pm$ SD	CO_2 ppm $\pm$ SD	V_c * $m^3 h^{-1}$
Octavia II	53 $\pm$ 25	21 $\pm$ 6	5 $\pm$ 0	4,340 $\pm$ 1,190	8.5 $\pm$ 3.4 ^a
Octavia III	36 $\pm$ 13	13 $\pm$ 0	12 $\pm$ 1	3,420 $\pm$ 1,105	12.0 $\pm$ 5.9 ^a
KIA	48 $\pm$ 9	27 $\pm$ 2	23 $\pm$ 2	2,530 $\pm$ 844	24.4 $\pm$ 9.6 ^b

* – calculated value; SD – Standard deviation.

The AC switched on the minimum level (Tables 4 and 5) increased internal temperature ($t_i = 16.4$ °C) in winter (Octavia II), temperature $t_i = 23.2$ °C in Octavia III was slightly over the set AC temperature 22 °C. Very high internal globe temperature $t_g = 37.9$ °C in KIA was caused mainly by solar radiation through the windows. The inside air temperature $t_i = 32.6$ °C was also higher than the set AC temperature 24 °C,

which means that the AC in minimum level is not able to cool enough supply air in the hot summer season. The noise level ($L_A = 63.7 \text{ dB(A)}$) in old Octavia II was significantly higher than in other cars.

Table 4. Thermal state of the environment (external temperature t_e , internal globe temperature t_g , internal temperature t_i , relative humidity RH_i and noise level L_A) in tested vehicles with minimum ventilation rate (minimum level of AC). Different letters (a, b) in the superscript are the sign of high significant difference (ANOVA; Tukey HSD Test; $P \leq 0.05$)

Car	t_e °C ± SD	t_i °C ± SD	t_g °C ± SD	RH_i % ± SD	L_A dB(A) ± SD
Octavia II	3 ± 0.1	16.4 ± 1.1	15.7 ± 0.5	42.5 ± 6.7	63.7 ± 3.4^a
Octavia III	12.1 ± 0.2	23.2 ± 0.4	22.9 ± 0.3	36.4 ± 2.0	59.8 ± 2.6^b
KIA	29.5 ± 0.6	32.6 ± 1.3	37.9 ± 2.3	30.7 ± 0.9	59.3 ± 2.1^b

SD – Standard deviation.

Total dust concentration $47 \mu\text{g m}^{-3}$ in Octavia II was approximately twice higher than in other cars (Table 5). The concentration of PM_{10} was in all cars lower than $50 \mu\text{g m}^{-3}$. Increased airflow rate V_c caused the lower concentration of CO_2 , which stalled rather high in all cars. The worst situation was in old Octavia II. Minimum ventilation rate $V_c = 26.2 \text{ m}^3 \text{ h}^{-1}$ was not sufficient, therefore the inside concentration $\text{CO}_2 = 2,370 \text{ ppm}$ was so high. On the basis of the previous studies in this area, these measurements proved that this measured condition of microclimate inside the cabin can be the cause of performance anxiety and stress for drivers and travelers in the situation of traffic jam during their journey in the summertime.

Table 5. Total dust concentration and concentration of dust fractions PM_{10} and PM_1 , a concentration of carbon dioxide CO_2 and values of the intake fresh air V_c in tested vehicles with minimum ventilation rate (minimum level of AC). Different letters (a, b) in the superscript are the sign of high significant difference (ANOVA; Tukey HSD Test; $P \leq 0.05$)

Car	Total dust $\mu\text{g m}^{-3} \pm \text{SD}$	PM_{10} $\mu\text{g m}^{-3} \pm \text{SD}$	PM_1 $\mu\text{g m}^{-3} \pm \text{SD}$	CO_2 ppm ± SD	V_c * $\text{m}^3 \text{ h}^{-1}$
Octavia II	47 ± 21	15 ± 3	8 ± 1	$2,370 \pm 1,530$	26.2 ± 13.8^a
Octavia III	18 ± 6	14 ± 1	14 ± 2	$1,200 \pm 848$	99.0 ± 63.6^b
KIA	22 ± 6	20 ± 3	18 ± 1	$1,000 \pm 155$	67.4 ± 14.8^b

* – calculated value; SD – Standard deviation.

The AC switched on the medium level (Tables 6 and 7) increased internal temperature ($t_i = 22.8 \text{ °C}$) in winter (Octavia II), temperature $t_i = 23.4 \text{ °C}$ in Octavia III was slightly over the set AC temperature 22 °C . In case of KIA there was a little decrement in temperature $t_i = 27.9 \text{ °C}$ (summer) but it was still much higher than the set AC temperature 24 °C , but nevertheless, it was the positive result because at the medium operational condition of AC showed little cooling effect. The noise level ($L_A = 65.5 \text{ dB(A)}$) in the old Octavia was still higher than other cars.

Total dust concentration $37 \mu\text{g m}^{-3}$ in Octavia III was higher than other cars (Table 7). The concentration of PM_{10} was less than $40 \mu\text{g m}^{-3}$ in all cars. V_c caused a lower concentration of CO_2 . At medium operational condition of AC, it showed positive results as a decrement in CO_2 level inside the cabin. The minimum ventilation rate was

again in Octavia II ($V_c = 114.6 \text{ m}^3 \text{ h}^{-1}$) and maximum $V_c = 212.8 \text{ m}^3 \text{ h}^{-1}$ was in Octavia III. The difference between Octavia III and KIA was not statistically significant.

Table 6. Thermal state of the environment (external temperature t_e , internal globe temperature t_g , internal temperature t_i , relative humidity RH_i and noise level L_A) in tested vehicles with medium ventilation rate (medium 50% level of AC). Different letters (a, b) in the superscript are the sign of high significant difference (ANOVA; Tukey HSD Test; $P \leq 0.05$)

Car	t_e °C ± SD	t_i °C ± SD	t_g °C ± SD	RH_i % ± SD	L_A dB(A) ± SD
Octavia II	3 ± 0.1	22.8 ± 1.3	18.6 ± 0.9	25.8 ± 2.1	65.5 ± 2.6^a
Octavia III	12.1 ± 0.2	23.4 ± 1.8	23.9 ± 0.1	35.3 ± 0.4	61.3 ± 4.1^b
KIA	29.5 ± 0.6	27.9 ± 0.5	32.0 ± 0.7	36.1 ± 0.6	$62.5 \pm 2.9^{a,b}$

SD – Standard deviation.

Table 7. Total dust concentration and concentration of dust fractions PM_{10} and PM_1 , the concentration of carbon dioxide CO_2 and values of the intake fresh air V_c in tested vehicles with medium ventilation rate (medium 50% level of AC). Different letters (a, b) in the superscript are the sign of high significant difference (ANOVA; Tukey HSD Test; $P \leq 0.05$)

Car	Total dust $\mu\text{g m}^{-3} \pm \text{SD}$	PM_{10} $\mu\text{g m}^{-3} \pm \text{SD}$	PM_1 $\mu\text{g m}^{-3} \pm \text{SD}$	CO_2 ppm ± SD	V_c * $\text{m}^3 \text{ h}^{-1}$
Octavia II	23 ± 6	17 ± 2	14 ± 4	590 ± 50	114.6 ± 19.0^a
Octavia III	37 ± 13	16 ± 2	16 ± 3	470 ± 32	212.8 ± 50.3^b
KIA	28 ± 5	28 ± 4	27 ± 2	550 ± 52	211.0 ± 42.5^b

* – calculated value; SD – Standard deviation.

The AC switched on the maximum level (Tables 8 and 9) increased internal temperature ($t_i = 23.5 \text{ °C}$) in winter (Octavia II), temperature $t_i = 23.8 \text{ °C}$ in Octavia III was slightly over the set AC temperature 22 °C . According to the result of measurements of both Octavia cars (winter and spring), the maximum operating condition of AC system was very effective for increment in temperature for achieving desired comfortable temperature inside the cabin. In third car (KIA Sportage) result was also satisfactory. In KIA Sportage cabin temperature reached 26.6 °C (summer), there was little difference between cabin temperature and set temperature (24 °C). The noise level ($L_A = 68.7 \text{ dB(A)}$) in Octavia II was again higher than other cars and lowest was in Kia Sportage ($L_A = 66.5 \text{ dB(A)}$). Total dust concentration was higher in KIA Sportage ($24 \mu\text{g m}^{-3}$).

Table 8. Thermal state of the environment (external temperature t_e , internal globe temperature t_g , internal temperature t_i , relative humidity RH_i and noise level L_A) in tested vehicles with maximum ventilation rate (maximum 100% level of AC). Different letters (a, b) in the superscript are the sign of high significant difference (ANOVA; Tukey HSD Test; $P \leq 0.05$)

Car	t_e °C ± SD	t_i °C ± SD	t_g °C ± SD	RH_i % ± SD	L_A dB(A) ± SD
Octavia II	3 ± 0.1	23.5 ± 0.3	21.3 ± 0.6	24.6 ± 0.5	68.7 ± 1.1^a
Octavia III	12.1 ± 0.2	23.8 ± 0.2	24.3 ± 0.2	34.3 ± 0.6	$67.8 \pm 1.5^{a,b}$
KIA	29.5 ± 0.6	26.6 ± 0.3	32.6 ± 0.4	36.9 ± 0.5	66.5 ± 1.3^b

SD – Standard deviation.

At maximum operational condition of AC, the concentration of CO₂ was overall low but it was higher in KIA Sportage (440 ppm) as compared to other cars. The main reason for high concentration of CO₂ in KIA Sportage was low ventilation rate as compare to other cars. The value of PM₁₀ was less than 25 µg m⁻³ in all cars. On the behalf of measurements of maximum operating condition of AC system there is no doubt AC system is very effective to keep all recommended parameters of microclimate inside the cabin. The recommended values of parameters are mentioned above in introduction.

The result of the measurements in all cars shows that without AC operation the level of CO₂ was very high inside the cabins but after in operational condition with switched on AC system it decreased several times. The ventilation V_c rate increased according to operational conditions of AC system. It was highest in Octavia III V_c = 1,216.8 m³ h⁻¹ (Table 9). Relative humidity more effectively decreased in Octavia II by operation of AC system. It reached approximately on its half. The value of total dust decreased according to operational conditions of AC system in each car. It was measured lowest in Octavia II 15 µg m⁻³ (Table 9). The value of PM₁₀ was lower than the value of external limit 50 µg m⁻³. Due to AC, the value of PM₁ was also very low in all measured situations.

Table 9. Total dust concentration and concentration of dust fractions PM₁₀ and PM₁, the concentration of carbon dioxide CO₂ and values of the intake fresh air V_c in tested vehicles with maximum ventilation rate (maximum 100% level of AC). Different letters (a, b) in the superscript are the sign of high significant difference (ANOVA; Tukey HSD Test; $P \leq 0.05$)

Car	Total dust µg m ⁻³ ± SD	PM ₁₀ µg m ⁻³ ± SD	PM ₁ µg m ⁻³ ± SD	CO ₂ ppm ± SD	V _c * m ³ h ⁻¹
Octavia II	15 ± 4	10 ± 2	10 ± 4	380 ± 40	1,159.2 ± 397 ^a
Octavia III	22 ± 2	22 ± 10	18 ± 4	360 ± 19	1,216.8 ± 291 ^a
KIA	24 ± 2	18 ± 3	18 ± 1	440 ± 33	543.2 ± 284 ^b

* – calculated value; SD – Standard deviation.

According to the results of the measurement can be summarized, that the more modern model of Octavia III has better parameters of AC than the older model Octavia II. Nevertheless, those results can be influenced by many years of previous use of Octavia II.

CONCLUSION

Solar radiation plays a big role to rise up temperature t_i inside the cabin. It resulted in the higher values of globe temperature t_g than the temperature of the air t_i. The concentration of CO₂ and RH_i is depend on ventilation rate V_c, in other words, they are inversely proportional to ventilation rate V_c. Ventilation rate depends on the construction of AC system, its regulation, and control system and design of the cabin. Overall ventilation rate V_c was good in Octavia III Combi as well as in KIA.

AC system shows the best result on its maximum operational condition. AC system always increases little noise level L_A inside the cabin. Noise level L_A depends on the technical condition of the car. The lowest level of noise was in KIA Sportage car during the maximum operational condition of AC system.

CO₂ concentration and RH_i is the main challenge during winter and spring season inside the cabin. Total dust reduction depends on the operational condition of AC system. Level of total dust depends on material and condition of mats and carpet and seat covers of the car. Textile material more increases dust level inside the cabin than rubber material. The AC has a positive impact on the dust reduction inside the cars.

Time taken for achieving desired comfortable condition inside the cabin is also dependent on the positioning of blowers inside the cabin. Sensor system inside the cabin plays a big role to maintain desired comfortable condition inside the cabin.

It can be a generalized conclusion, that the AC has a very positive impact on inside comfort in cars cabins from all points of view during all periods of the year.

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Split water application for a water supply reduction in *Callistemon Citrinus* pot plant

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Abstract. Irrigation management in Greenhouse Nursery Production (GNP) is based on empiric methods based on farmer personal experiences with over-irrigation results. The effects of irrigation volume and daily application were studied in a pot experiment carried out on rooted cuttings in a greenhouse. The irrigation volume treatment was performed on Full and reduced Treatment. The treatment of water application was carried out with split supply and unsplit supply. The effects of the treatments were evaluated in terms of biomass accumulation and partitioning, leaf area, photosynthesis and stomatal response, chlorophyll content, and water productivity. *Callistemon* showed a good adaptation to the different treatments tested during the experiment. A positive relation was found between biomass accumulation and irrigation volume, moreover split water application increased plant Dry Weight.

Therefore, the highest biomass accumulation was registered in full irrigation volume in split application treatment, and this behavior was confirmed by the photosynthetic rate. No statistical differences were found, in terms of Relative Water Content (RWC), between the treatments. Stem water potential and stomatal conductance values suggest in *Callistemon* an anisohydric water stress response behavior.

Our results evidenced that, in *Callistemon* potted plants, an irrigation volume reduction is possible when a split application occurs during the daytime. A full irrigation volume amounts to 10.8 L per plant during the trial period of 90 days while the reduced volume amounts to 8.2 L per plant. Therefore, an increased water productivity can be obtained if the daily water requirement is split on two applications during the daytime. Our results highlighted a possible reduction in environmental impact of *Callistemon* greenhouse pot production, through the 25% reduction of the volume irrigation.

Key words: photosynthesis, drought, anisohydric, WUE, water productivity.

INTRODUCTION

The genus *Callistemon* is a woody aromatic tree or shrub (ca. 0.5 m to 7 m tall) belonging to the family Myrtaceae which comprises over 30 species. These plants were originally found in the temperate part of Australia and in south America and Asia and show remarkable adaptability to high temperature heat, sun, aridity and wind.

However, they are now found across the globe as flowering shrubs used in gardening and landscaping.

Callistemon leaves are lanceolate and very aromatic. The flower spikes of bottlebrushes form are made up of a number of individual flowers with prominent red stamens. Their petals are of greenish or pale color, tiny, inconspicuous and in some cases deciduous (Oyedeki et al., 2009). For its ornamental values, *Callistemon* is primarily produced as a potted plant, representing an important product in the Greenhouse Nursery Production (GNP) in the Mediterranean basin.

High environmental impact of ornamental GNP is also due to the high water consumption for plant irrigation. Nowadays, less attention is given to the water requirement of ornamental plants, therefore there is few available information about ornamental species. Consequently, irrigation management in most nurseries is based on farmers' personal experiences. This results over-irrigation and low Water Productivity (WP). WP may carry different meanings with respect to different water-using production sectors e.g. hydrology, irrigation engineering, field crops, etc. (Ali & Talukder 2008). In crop production, WP can be defined as the ratio between yield and water supply. It's also used to quantify Water Use Efficiency (WUE) on different scale, from individual leaf up to a hydrological basin (Feres et al., 2014). WP is strictly influenced by the relationship of three parameters (Ali & Talukder 2008; Feres et al., 2014) environment, genotype and water management. The environmental parameters are the result of a complex interaction among soil, temperature, air humidity and light availability. These interactions can represent a limitation for plant adaptations, especially when plants come from different climatic areas (Giovino et al., 2014). Whereas under GNP, environmental parameters can be controlled and considered constant in the short period. The genotype influence on WP performance can vary through an adequate choice (species, cultivars, etc.), depending on its drought tolerance. Finally, water management plays a key-role in the determination of WP. Excess of water in irrigation represents both economic and environmental loss (Ali & Talukder, 2008). Moreover, it can promote the occurrence of phyto-pathological adversities. Contrariwise, a deficit irrigation may be used, in potted ornamental plants, to improve plant quality, by reducing excessive vigor and promoting a more compact habit (Cirillo et al., 2013). Furthermore, it was demonstrated that a deficit irrigation promotes a better nitrogen use by plant reducing nitrogen loss (Mahdavi-Damghani et al., 2010). Drought in pot occur when water supply is scarce or inadequate.

To avoid excessive drying of the substrates, especially, in ornamental crops grown in pots with a small water capacity, deficit irrigation requires a precise scheduling (Álvarez & Sánchez-Blanco 2013). Splitting strategy could be used for irrigation scheduling to improve WP under pot condition. Indeed during the daytime, the evapotranspiration demand varies depending on plant's endogenous and exogenous (environmental) requirements. The split water application allows the water supply improving WP reducing the exogenous factors influence. Split application must allow physiological and biochemical process in plants reducing water loss trough the evaporation from the soil. The application of split water improves the water supply increasing WP and reducing the exogenous factors influence. Split application must allow physiological and biochemical process in plants by reducing water loss trough the evaporation from the soil. An important process for plant survival is related to restoration ability after photo-damage. Photo-damage frequently occurs during photosynthetic

processes (Werner et al., 1999), especially in climates, such as the Mediterranean one, where light intensity often exceeds the requirement of the plant. This damage affects the proteins of PSII that are usually restored by the 'PSII repair cycle' (Takahashi & Badger, 2011), under the availability of light and water. However, when there is excess light during photosynthesis, the restoration process is depressed and it does not depend to water availability. PSII repair process is also inhibited by the environmental stresses that induce stomatal closure (Aro et al., 2005). Previous investigations focused on the effect of irrigation water supply in relation to phenological stages under different environmental conditions (Patane et al., 2011; Yihun et al., 2013), whereas not much information is available on the effect of day time split water supply on potted plant.

In the light of the problem stated, this research work aim at evaluating the effect of 25% reduction in water supply on the growth rate of potted *Callistemon*. Furthermore, the authors seek to test the hypothesis that a daily split water amount application can improve *Callistemon* WUE and their performances growth.

MATERIALS AND METHODS

Plant materials and experimental conditions

A 90 days experiment was carried out on rooted cuttings of 6 months-old of *Callistemon citrinus* (Curtis) Skeels. The cuttings, grown in 7 x 7 x 7 cm pots, were transplanted into 3L plastic pots filled with a mixture of 30% peat 30% sand and 40% perlite (v:v:v) amended with 2 g L⁻¹ of Osmocote Plus Scotts® Australia (14:13:13 N, P, K plus micro element). Pots were placed inside an east-west-oriented greenhouse (540 m²) with a steel structure and methyl polymethacrylate cover, located in Bagheria (PA), Sicily, Italy (38° 5' 28" N, 13° 31' 18 E; 23 m above sea level). During a 10-day acclimatization period, pots were maintained at field capacity. All the plants were daily irrigated (water electrical conductivity was 0.8 dS m⁻¹) using drip irrigation system.

Treatments and statistical analyses

Irrigation volume treatment was performed on two levels, Full (F) and Reduced (R) corresponding to 100% and 75% of daily effective evapotranspiration (ET_e) respectively. Application treatment was performed on two levels, Unsplit (U) and Split (S) which correspond to irrigation volume of 1 and 2 applications per day respectively. ET_e was determined, on six plants, by weighing pots on a daily basis. The experiment was laid out in a split-plot design with irrigation volume as main factor and daily application as sub-factor, with three replicates. Each treatment was composed by 36 plants.

The experiment was performed on 12 plants randomly attributed to each treatment and each block. U daily irrigation was performed at 8:00 solar time and S daily irrigations were performed at 8:00 and 18:00 solar time.

Data were analyzed using two-way ANOVA computed using XLStat for Windows systems (XLSTAT-Base, version 10). Treatments means were separated with Tukey Test ($P \leq 0.05$).

Growth and physiological parameters

Growth and physiological parameters were measured at 0, 30, 60 and 90 Days After experiment Start (DAS) on 3 plants per replicate and per treatment.

Plants were separated into stems, leaves and roots recording fresh and dry weight, leaf number and leaf area. Leaf area was measured by a WInDIAS leaf area meter (Image Analysis System, DELTA-T DEVICES LTD, Burwell, Cambridge, England). Dry Weight (DW) was measured after oven-drying the samples at 60 °C until constant weight was achieved (around 48 h).

WUE was calculated as the rate of biomass accumulation and total water supplied. During the trial, plants were supplied with 119 mL day per pot (average of the trial period) for F treatments. The total water supply during the trial was 10.8 L per pot for full treatment. Relative Growth Rate (RGR) was calculated as:

$$\text{RGR} = (\ln \text{DW}_2 - \ln \text{DW}_1) / (T_2 - T_1) \quad (1)$$

where DW1 – initial dry weight; DW2 – final dry weight; T1 – starting time; T2 – final time.

Relative Water Content (RWC) was calculated as:

$$\text{RWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100 \quad (2)$$

where FW – fresh weight; TW – turgid weight determined after leaf submersion in distilled water at 6-8 °C in the dark for 24 h; DW – dry weight measured after oven drying at 60 °C for 48 h.

Relative Chlorophyll Content (RCC) was determined by a Minolta SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan) at the midpoint of two mature leaves per plant and three plants per treatment.

Stem Water Potential (WP_s) was measured at midday with pressure Scholander chamber (Soil Moisture Equipment Co., Santa Barbara, CA, USA). WP_s was measured using leaves that had been bagged with both a plastic sheet and aluminum foil for at least 1 h before measurement in order to prevent transpiration from leaves, in this way, leaf water potential equaled stem water potential (Begg & Turner 1970; Valladares & Pearcy, 1997; Navarro et al., 2009). Leaf stomatal conductance (g_s), net photosynthetic rate (P_n) and the Vapor Pressure Deficit (VPD) were determined using a gas exchange system (LI-6400, LI-COR Inc., Lincoln, NE, USA) at 9:00, 13:00 and 17:00 solar time during every sampling on one mature leave per plant and three plants per treatment. The VPD, was calculated by the difference between saturation and real air pressures according to the method reported by (Moura dos Santos et al., 2013).

RESULTS AND DISCUSSION

Growth parameters

At the end of the experiment (90 DAS), biomass accumulation was significantly influenced by the treatments (Table 1). Total, Leaf, Root and Stem DW decreased according to the irrigation volume reduction (107.0 ± 5.1 vs 81.0 ± 4.8 ; 26.1 ± 0.4 vs 21.3 ± 0.5 ; 68.2 ± 4.8 vs 48.2 ± 4.4 ; 12.8 ± 0.3 vs 11.5 ± 0.3 in F and R respectively). A similar pattern was registered in Leaf Area (1165.8 ± 17.0 vs 982.5 ± 22.8 cm² in F and R respectively) whereas no significant difference was registered in terms of leaves number. Also root shoot rate was not influenced by the irrigation volume treatment.

Total DW, Leaf DW and Root DW were influenced by the application treatments, higher values were measured on S treatment than on U treatment (107.5 ± 4.91 vs 80.5 ± 4.82 ; 24.8 ± 0.76 vs 22.5 ± 0.82 ; 70.0 ± 4.13 vs 46.3 ± 4.05 g respectively). The

same pattern was registered in terms of Leaf Area and R/S (1,118 vs 1,030 cm²; 1.86 vs 1.34 in S and U respectively). Whereas no significant differences were registered in terms of leaves number and above ground RGR. (Table 1). Roots RGR evidences a statistical interaction between the treatments with the highest value in FS treatment. A positive relation was found in terms of roots RGR in the irrigation volume treatment (0.0465 vs 0.0424 in F and R respectively). Roots RGR shown statistical differences also in the application treatment (0.0469 vs 0.0420 in S and U respectively). In terms of WUE (Table 1) statistical difference was found both in the irrigation volume treatment (146.62 g L⁻¹ vs 147.94 in F and R respectively) then in the application treatment (168.85 vs 125.69 in S and U respectively).

Table 1. Growth parameters in *Callistemon citrinus* pot plants under different irrigation management at 90 DAS

Irrigation Volume (IV)	Full Volume (F)				Reduced Volume (R)				IV	AP	IV*AP
Application (AP)	split (S)		Unsplit (U)		Split (S)		Unsplit (U)		Pr < F	Pr < F	Pr < F
Total dry weight, g	120.3	A	93.7	B	94.7	B	67.3	C	0.0004	0.0003	0.9224
Leaf dry weight, g	27.1	A	25.1	A	22.5	B	20.0	C	<0.0001	0.0019	0.6117
Root dry weight, g	80.0	A	56.3	BC	60.1	AB	36.3	C	0.0030	0.0012	0.9816
Stem dry weight, g	13.2	A	12.3	AB	12.0	AB	11.1	B	0.0283	0.0807	0.9406
Leaves, n.	311.0		286.0		319.7		271.7		0.8987	0.1381	0.6094
Leaf area, cm ²	1,199.5	A	1,132.2	AB	1,037.4	BC	927.6	C	0.0011	0.0304	0.5240
R/S	1.98	A	1.51	AB	1.74	AB	1.17	B	0.0786	0.0091	0.7367
RGR a.g., g day ⁻¹	0.0259		0.0210		0.0201		0.0230		0.1886	0.4644	0.1922
RGR roots, g day ⁻¹	0.0531	A	0.0400	B	0.0408	B	0.0441	B	0.0388	0.0185	0.0011
WUE, g L ⁻¹	164.8	A	128.4	B	172.9	A	122.98	B	0.0011	0.0017	0.2800

DW = dry weight; R/S = root shoot ratio; RGR a.g.: Relative Growth Rate referred to above ground biomass; RGR roots: Relative Growth Rate referred to roots biomass; WUE: Water use efficiency. R – Reduced irrigation volume (75% ETe); F – Full irrigation volume (100% ETe); S – Split irrigation corresponding to two applications per day of the irrigation volume at 8:00 and 18:00; U – Unsplit irrigation corresponding to one application per day of the irrigation volume at 8:00 A.M. Means (n = 3) within a column without a common letter are significantly different by Tukey test.

During the experiment, pronounced increases in terms of leaf DW and leaf area (Fig. 1 and Fig. 2) were observed from 60 DAS, when *Callistemon* flowering began (58 ± 3 DAS). No statistical differences among the treatments were observed in terms of flowering time, number of flowers, and flower DW (data not shown).

Statistical interaction was found at 30 DAS in terms of Leaf DW (Fig. 1). FS treatment shown the highest values until the end of the experiment, whereas no differences were found among the other treatments until 60 DAS (Fig. 1). In terms of leaf area, no statistical differences were registered until 60 DAS (Fig. 2).

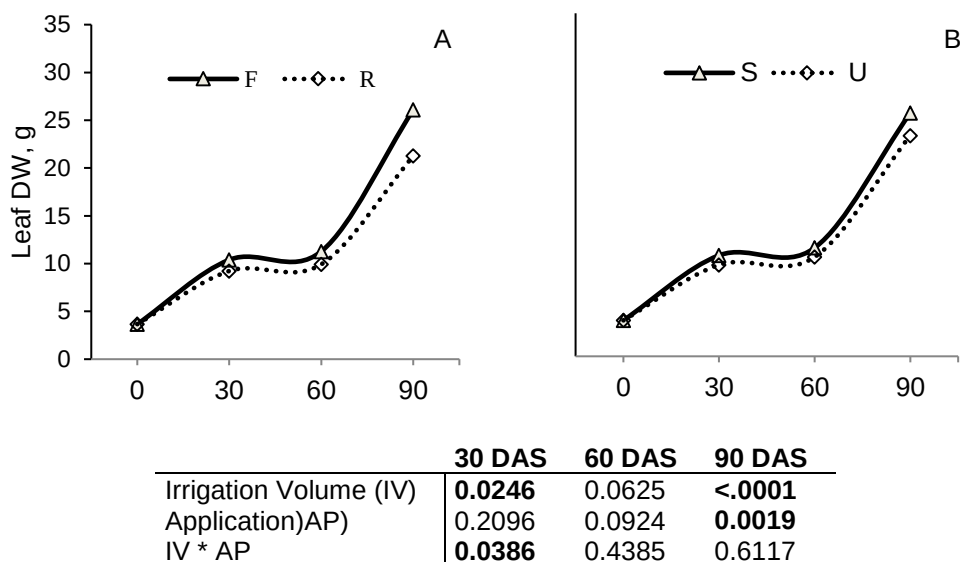


Figure 1. Leaf DW trend during the experiment in *Callistemon citrinus* pot plants under different irrigation management. R – Reduced irrigation volume (75% ETe); F – Full irrigation volume (100% ETe); S – Irrigation volume per day split on two applications at 8:00 and 18:00; U – Irrigation volume on single application per day at 8:00. Means (n = 3) are significantly different by Tukey test.

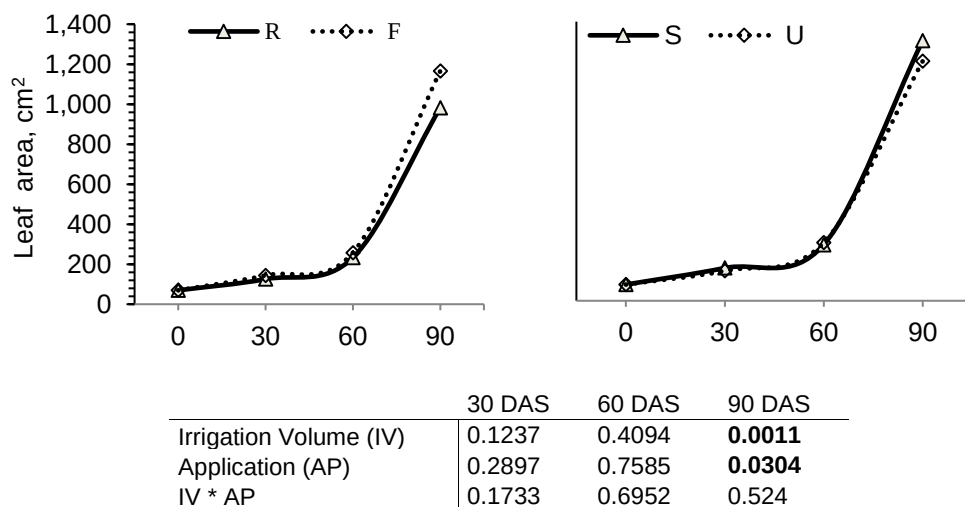


Figure 2. Leaf Area trend during the experiment in *Callistemon citrinus* pot plants under different irrigation management. Irrigation treatments were carried out in: R – Reduced irrigation volume (75% of ETe) marked with a circle; F – Full irrigation volume (100% ETe) marked with a triangle; S – Irrigation volume per day split on two applications at 8:00 and 18:00 marked with close signs; U – Irrigation volume on single application per day at 8:00 marked with open signs. Means (n = 3) are significantly different by Tukey test.

Root shoots rate evidences a similar trend, during the experiment, among the treatments (Fig. 3) with remarkable difference only at 90 DAS (Table 1).

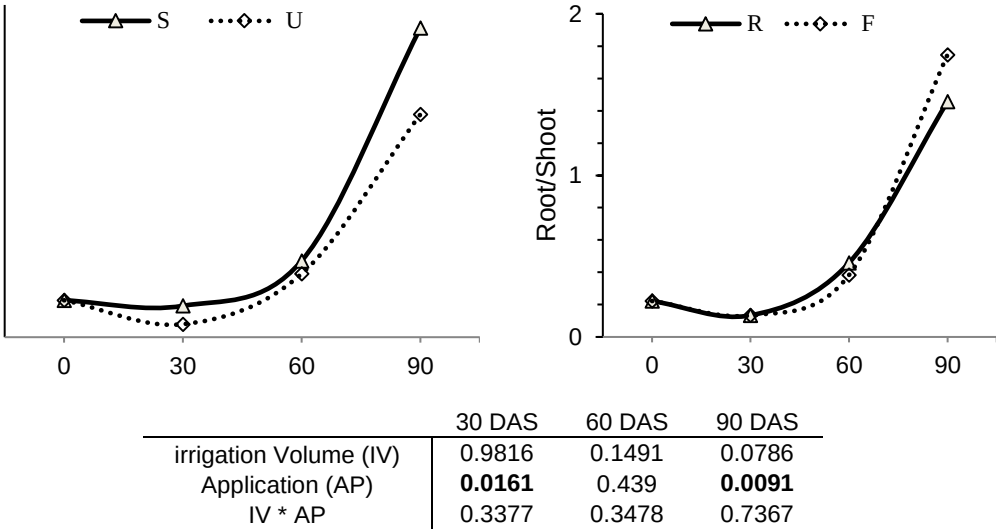


Figure 3. Root/Shoot ratio trend during the experiment in *Callistemon citrinus* pot plant under different irrigation management. R – Reduced irrigation volume (75% of ETe) marked with a circle; F – Full irrigation volume (100% ETe) marked with a triangle; S – Irrigation volume per day split on two applications at 8:00 and 18:00 marked with close signs; U – Irrigation volume on single application per day at 8:00 marked with open signs. Means (n = 3) are significantly different by Tukey test.

Physiological parameters

At the end of the experiment RCC highlights the lowest value in RU treatment (61.9 vs 63.2 respectively) while no statistical differences among the other treatments were registered (Fig. 4).

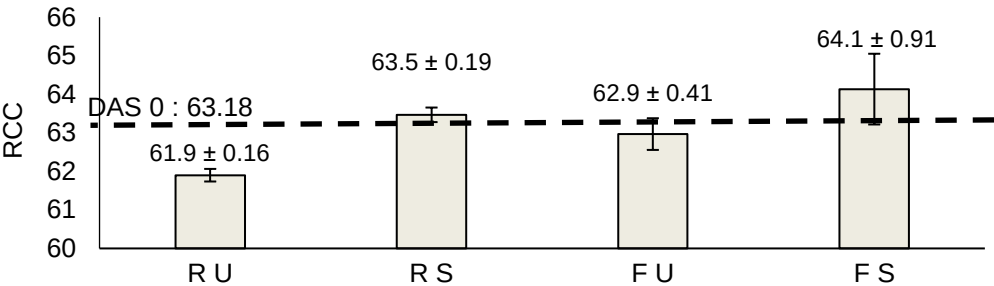


Figure 4. Relative Chlorophyll Content (RCC) in *Callistemon citrinus* pot plant under different irrigation management. R – Reduced irrigation volume (75% of ETe); F – Full irrigation volume (100% ETe); S – Irrigation volume per day split on two applications at 8:00 and 18:00; U – Irrigation volume on single application per day at 8:00. Means (n = 6) represented in columns without a common letter are significantly different by Tukey test, P ≤ 0.05 probability level. Dashed line represent the RCC means value at 0 DAS, columns represent the RCC means value at 90DAS.

During the experiment at 30 and 60 DAS, no statistical differences were registered among the treatments in terms of midday WP_s (Fig. 5); while, at 90 DAS, WP_s was significantly influenced by the application treatment (-2.76 vs -2.37 MPa in U and S respectively); volume irrigation did not affect the data. No statistical differences were registered in terms of RWC between the treatments (data not shown).

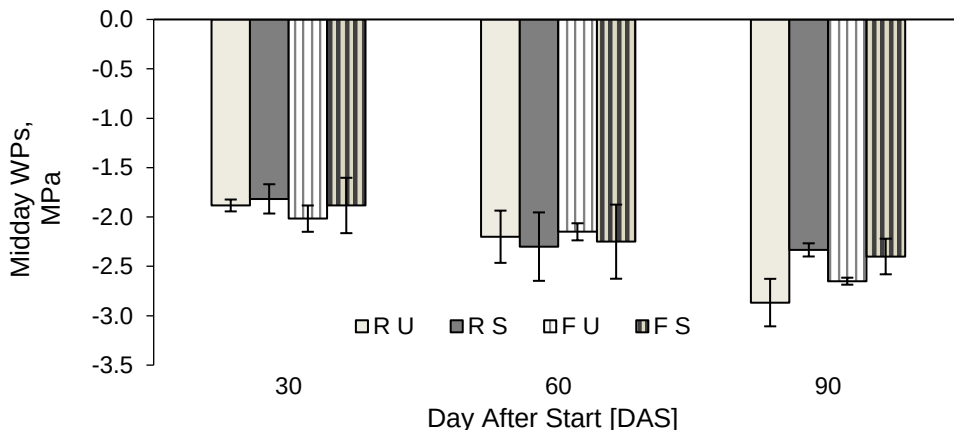


Figure 5. Stem Water Potential (WP_s) in *Callistemon citrinus* pot plant under different irrigation management. R – Reduced irrigation volume (75% of ETe); F – Full irrigation volume (100% ETe); S – Irrigation volume per day split on two applications at 8:00 and 18:00; U – Volume irrigation on single application per day at 8:00. Vertical lines represent standard error ($n = 3$).

Statistical differences were found in terms of photosynthesis and stomatal conductance (Fig. 6, A, B) between the treatments. Highest values of photosynthesis and P_n/G_s were measured on the split treatments (Fig. 6, A, C). Application treatment shown the higher values of photosynthesis and P_n/G_s , (Fig. 6, C) during the day when split application was adopted. In addition, the highest values of photosynthesis were registered in FS treatment.

A relation between stem water potential and stomatal conductance was determined (Fig. 7). The regression model highlights a typical anisohydric water potential regulation strategy.

Our results highlighted a different biomass accumulation due to the irrigation volumes applied during the experiment. Indeed, the volume irrigation reduction produced a significant reduction in terms of leaves, root and stem DW.

Generally, in field experiments under drought conditions, an increase in root growth has been reported by many authors (Kashiwagi et al., 2006; Blum, 2009; Hernández et al., 2009; Porcel et al., 2012). In our trial, pot plants responded with a vigorous root growth in full-irrigated treatments. Similar responses were observed in slight water stress conditions in *Populus*, *Erythrina*, *Eucalyptus* and *Avocado*, (Zollinger et al., 2006; Shao et al., 2008). A significant root reduction, under water scarcity, was reported on *Opuntia* species (Snyman, 2014). The author suggested as limited water availability reduces roots elongation process.

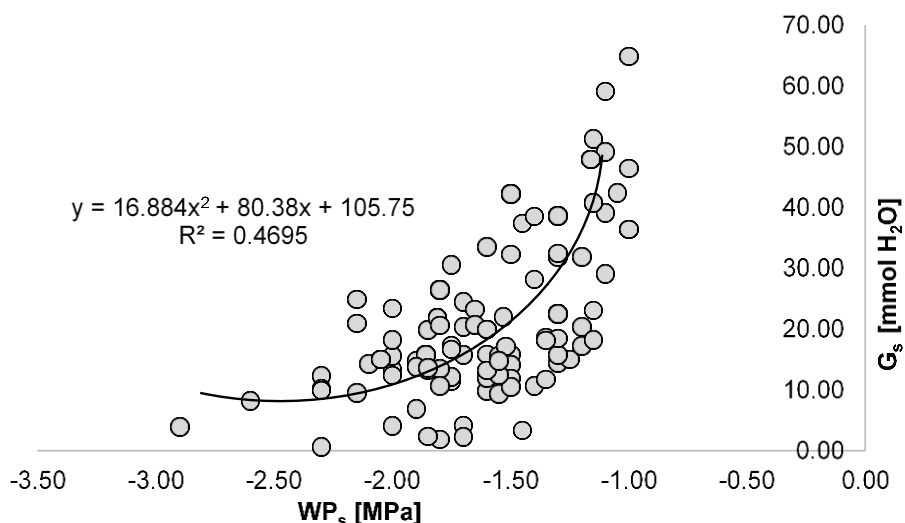


Figure 7. Stomatal conductance (G_s) and Stem water potential (WP_s) relation in *Callistemon citrinus* pot plant under different irrigation management. R – Reduced irrigation volume (75% of ETe); F – Full irrigation volume (100% ETe); S – Irrigation volume per day split on two applications at 8:00 and 18:00; U – Volume irrigation on single application per day at 8:00.

As for irrigation volume, the application treatment influenced biomass accumulation. Independently to the water amount, split application, increased the plant biomass accumulation. Important information arises from the interaction between volume irrigation and application treatment. Indeed, RS treatment gained a similar value of dry matter than FU treatment. This highlights the possibility to obtain good growth performance on *Callistemon* potted plant under deficit irrigation by water split application.

Although, no statistical differences were registered in terms of number of leaves among the treatments, some authors report that the effects of a periodic drought stress promote a physiological response in terms of loss of leaves to reduce the evapotranspiration phenomenon (Shao et al., 2008). Especially during the flowering time, when the meristematic activity needs a high water availability, the lower number of leaves and leaf DW would confirm that a water drought condition occurred in the unsplit treated plants.

Water use efficiency is largely influenced by biomass accumulation. Many authors reported that an irrigation reduction involves a growth reduction with a consequently WUE increase as it was seen in herbaceous (*Papaverum somniferum* (Mahdavi-Damghani et al., 2010), *Helianthus annuum* (Feres et al., 2014), *Solanum esculentum* (Savić et al., 2008) and woody plants [*Olea europea* (Feres et al., 2014), *Pistacia vera* (Iniesta et al., 2008)]. In our experiment, split showed higher WUE than unsplit application, independently by the volume irrigation. This behavior is due to the differences occurred during the experiment in terms of root and leaves dry weight accumulation.

Differently to the irrigation volume treatments, split application treatments affect this rate with higher values than those of the unsplit treatments. Two different Pn/Gs rate behavior were observed between split and unsplit application (Fig. 6). Under unsplit conditions, the lower and constant rate Pn/Gs showed that other factors likely affected the photosynthetic process. Probably, under these conditions, a water deficit condition did not allow for the photo restoration of PSII proteins. This was confirmed by the Pn/Gs higher values present in the split treatments during the last hours of the daytime.

The different relation between photosynthesis and stomatal conductance among the treatments, evidence a different strategy of water deficit avoidance actuated by plants depending on the stress level. This difference can be due to the occurrence of a water deficit adaptation in split irrigation treated plants. Indeed, no differences were measured in terms of RWC (data not shown) among the treatments, but different patterns were registered in terms of WPs and stomatal conductance. Different water potential regulation can be adopted by plants as a survival strategy to drought conditions (McDowell et al., 2008). Similarly to other anisohydric species (*Cistus*, *Myrtus* and *Olea*), also in this case a variation of WP occurs with no other adaptations (Tardieu & Simonneau 1998, McDowell et al., 2008; Quero et al., 2011).

Callistemon showed a typical anisohydric species relation of the ratio Gs/WPs. In these species, transpiration is not tightly regulated by stomatal closure. Under deficit irrigations, the stomatal conductance reduction had a big impact on photosynthetic rate. This is because plants need to drain the exceed of photon energy linked to the reduction of photosynthetic activity. During these stress conditions (Low conductance, low water availability, low photosynthesis) this 'impact' is represented by the photo damage of the PSII as consequence to the exceeding and not safely dissipated energy that cause changes in the functional state of the thylakoid membranes of the chloroplasts. The negative effects on photosynthesis due to this morphological adaptation, can be quantified in the leaves, estimating the inhibition or damage in the process of electron transfer in photosystem II (fluorescence) (Moura dos Santos et al., 2013). This characteristic of anisohydric plants explains the low differences in terms of biomass accumulation between the treatments because a very low reduction in terms of water availability occurred. During this low reduction of water amount, the photosynthetic reduction is primarily linked to the stomatal regulation; however this relation decreases when deficit level increases and when another biochemical regulation occurs (down-regulation) Flexas & Medrano (2002).

CONCLUSIONS

Our results evidence that, in *Callistemon* potted plants, a 25% irrigation volume reduction is possible. A full irrigation volume is 10.8 L per plant during the trial period (90 day) while the reduced volume is 8.2 L per plant.

The water reduction produced a plant biomass reduction, but split application improved performance growth and water use efficiency. Indeed, the plant response to reduced irrigation with split irrigation (RS) was comparable to the response of plant treated with full unsplit (FU) irrigation. Therefore, an increased water productivity can be obtained if the daily water requirement is split on two applications during the daytime. Deficit irrigation strategy for nursery *Callistemon* pot plants production can be used if water reduction is combined with split irrigation strategies reducing the environmental

impact of the production process. A further confirmation of this hypothesis could come from the study of the plant ABA accumulation during the daytime.

ACKNOWLEDGEMENTS. This research was supported by the National Operational Program ‘Research and Competitiveness’ 2007–2013 PON ‘R&C’ – Italian Ministry of Education, University and Research. We thank Ignazia Altobello and Domenico Platia for technical assistance.

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Tools for building production and woodworking made from the perforated steel wastes

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Abstract. The rising of efficiency of the building and construction production is an actual task. One of the possible ways to ensure higher efficiency is using innovative tools and facilities of small-scale mechanization, which increase productivity and enhance working condition. Most observable influence of such strategy is on concrete works, plastering and earthworks. Another important tendency in production engineering, building and construction production is recycling of the technological wastes, which sufficiently reduce cost of the products and improve ecology. The goal of the present paper is to offer new possibility for recycling of the technological wastes, i.e. perforated steel tapes achieved after stamping of fine parts, by producing from mentioned perforated tapes the building tools and facilities of small-scale mechanization. In particular, the technological wastes of the JSC 'Ditton' (Daugavpils, Latvia) – perforated steel tapes – received after stamping of the elements of driving chains for different apparatus were used in this research. The prototypes of the scrapers for the finishing building work, as well as cutting edges and circular coronas for the woodworking were elaborated and offered in this work. The results of approbation of elaborated prototypes of the tools are offered. It was proven, that proposed innovative tools could be used effectively in building production and woodworking.

Key words: perforated metallic waste, building tools, scrapers.

INTRODUCTION

Nowadays the perforated metallic materials (PMM) have wide and various applications in the building, mechanical engineering, chemical industry, agricultural machinery industry and others branches (O' Donnell & Associates, 1993). Most of all the steel PMM are used. A lot of companies commercially produce perforated steel bands and plates, grids, tubes, filters, sieves and others goods (Accurate Perforating Company, 2018; Euro Sitex Company, 2018). Shapes variety, small weight and high strength are the most important advantages of PMM. Fast achievement and distribution of the PMM is based on the modern technologies of sheet-metal stamping and high speed cutting. Thus, recently the CNC technology to perforate metal with 3D textures from one single sheet was developed (Ceilings Plus Company, 2018).

At the same time as the development of new materials production technologies, the recycling of wastes is becoming more and more urgent issue. Thereby the waste-to-resource vision (Durr, 2017; Stahel, 2017) also became more relevant.

In a number of published works the possibility of using the technological waste in the shape of steel PMM for producing new building goods and constructions is shown (Lisicins et al., 2011; Mironovs et al., 2013; Mironovs & Lisicins, 2015).

In author's opinion additionally to the known applications of the PMM such materials from alloyed steels, which are characterized by high mechanical properties, could be used for producing edged tools like comber-type tools and sawing tools. Thus, the comber-type tool in the shape of number of plates with teeth (Poletaev et al., 1997) is used for machining wood materials as well as for removing the paint and coatings. As a rule, such tool produce from the all-metal serrate leaf on which the teeth are notched.

Another possible application of PMM could be circular cutting coronas. Coronas with the larger diameter (more than 30 mm) are equipped with the centring drill (Fig. 1). The centring drill increase the work accuracy of the corona as well as increase the stiffness of the construction of tool, i.e. exclude possible skews and other deviations and damages. The technology of producing such tool is quit complicate and labor-consuming. As a result, the cost of tool is relatively high.

During exploitation of cutting coronas the careful control and maintenance are needed. Most significant is to ensure the sharpness and wholeness of teeth, as well as the cleanness of tool cavity. If the control and maintenance are not sufficient the wearing of cutting edges of corona will occur faster than expected, even damage is possible.

Using the technological wastes, i.e. perforated steel tapes achieved after stamping of fine parts, for producing edged tools like described above could decrease the cost of such tools as well as open up the new possibilities in development of edged tools. Some technical ideas are presented in the Author's patent (Mironovs & Lisicins, 2015).

Thus, the comber-type tool was produced from the perforated steel tape (PST) (base material: steel C50). This tool comprises the body (1) and perforated plate (2) with preliminary shaped teeth (Fig. 2, a). The teeth were produced by cutting of the perforated tape through the perforated holes, that's why the teeth step coincided with the perforation step of holes. The perforated plate (2) is mounted on the body (1) by the rivets (3). To change the cutting angle during cutting process the perforated tape plates with different angle α were produced (Fig. 2, b).



Figure 1. Cutting corona with the ribbon sawing disc and centring drill.

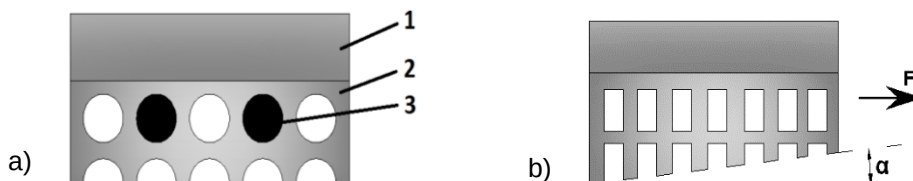


Figure 2. Design of the comber-type tool produced from the PST: with the equal length teeth (a) and with different length teeth (b); F – loading force.

For increasing the stiffness and reliability of tools, it was proposed to mount in the tool body (1) the number of parallel plates (2) as shown on Fig. 3.

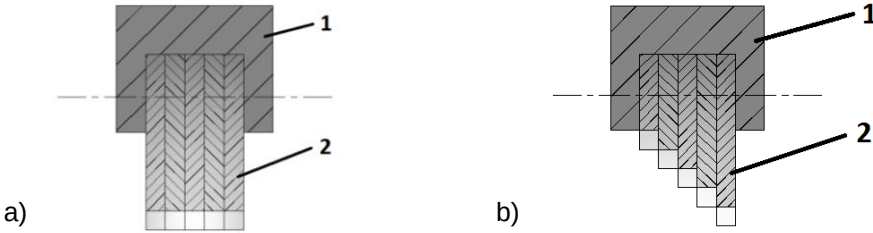


Figure 3. Anchorage scheme of PST as a block: with the equal length plates (a) and with different length plates (b).

On the other hand undoubtedly there is another important tendency in production engineering, building and construction production – the recycling of the technological wastes, which not only sufficiently reduce cost of the products, but improve ecology and raise the effectiveness of material usage as well.

The goal of the present paper is to offer new possibility for recycling of the technological wastes, i.e. perforated steel tapes achieved after stamping of fine parts, by producing from mentioned perforated tapes the building tools like scrapers for the finishing building work, as well as cutting edges and circular coronas for the woodworking.

MATERIALS AND METHODS

In general, the perforated steel tapes are received after stamping of the elements of driving chains for different apparatus. The base material as a rule is a steel with the carbon content from 0.1 to 0.5%. Chemical compositions of the appropriate carbon steel as well as mechanical properties are listed in the Table 1.

Table 1. Carbon content and mechanical properties of carbon steel used for producing driving chains

Steel grade	Carbon content, wt %	Tensile strength, N mm ⁻²	Strain, %
St08	0.05–0.12	275–390	8–33
St15	0.12–0.19	325–470	8–27
St20	0.17–0.24	340–490	7–28
St30	0.27–0.35	400–650	7–21
St50	0.47–0.55	540–720	6–14

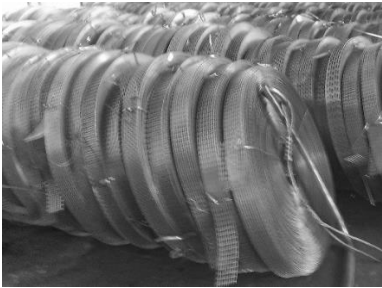
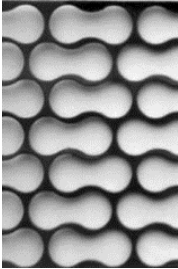
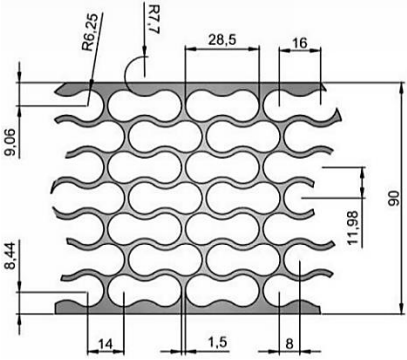
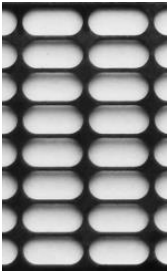
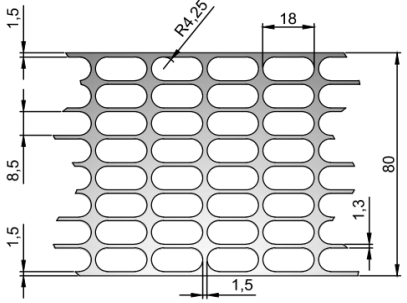


Figure 4. Technological wastes in the shape of perforated steel tapes (PST) (JSC 'DITTON Driving Chain Factory', 2018).

The technological wastes of the JSC 'Ditton' (Daugavpils, Latvia) – perforated steel tapes (PST) – received after stamping of the elements of driving chains for different apparatus were used in this research (Fig. 4).

Mechanical properties and geometrical parameters of PST samples (PST-1 and PST-2 types) used in current work are shown in Table 2.

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

Parameter	Value	Tape representation	Tape geometry
designation	PST-1		
steel grade	08пс-OM-T-2-K		
standard	GOST 503-81		
thickness, mm	1.50		
width, mm	90		
permeable area, %	75.32		
effective cross-sectional area, mm ²	26.43		
tensile load bearing capacity, kN	10.10		
tensile strength, MPa	406.81		
displacement, mm	2.25		
strain, %	1.21		
designation	PST-2		
steel grade	50-T-C-H		
standard	GOST 2284-79		
thickness, mm	1.20		
width, mm	80		
permeable area, %	70.50		
effective cross-sectional area, mm ²	14.44		
tensile load bearing capacity, kN	13.48		
tensile strength, MPa	933.43		
displacement, mm	2.43		
strain, %	1.40		

RESULTS AND DISCUSSION

As it was shown in the Table 2, the technological wastes in the shape of perforated steel tapes (PST) could be characterized as a material with high tensile strength (200–900 MPa) and surface hardness (about 1–2 GPa), as well as with sufficient plasticity for applying such material for producing flat or cylindrical tools like cutting edges and circular coronas etc. For instance, PST could be used for producing trowels for decorating works, tools for overlaying the glue or adhesives in the glaze mounting or tile paving works, etc.

The possibility of using pst for the manufacture of a rotating tool

When producing the circular cutting corona the PST was mechanically turned into tube (2) (Fig. 5, a), then welded and mounted on tool muff (1) (Fig. 5, b). Mounting inside the toll muff could be done by tube pressing from the outside (Fig. 5, c).

Obtained tools were tested on the vertical drill. The cutting properties and operability were in the focus of interest. Circular corona was produced with the diameter from 50 to 80 mm according to scheme presented on the Fig. 5, b from the PST with the thickness 1.2 mm. The aerated concrete and wood were chosen as testable materials. Testing parameters are shown in the Table 3.

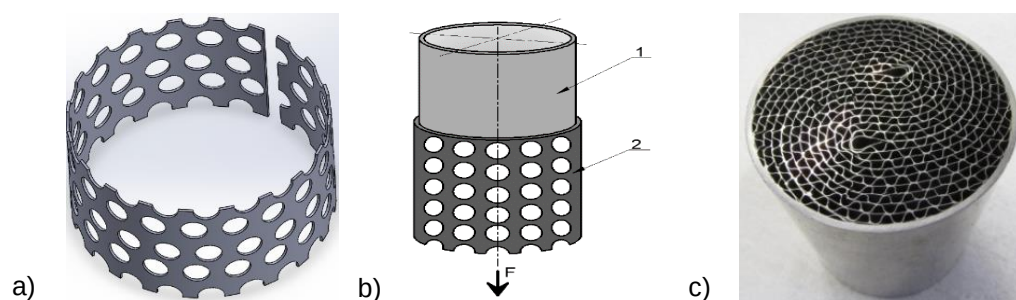


Figure 5. Cylindrical shape PST (a) and its fixation outside (b) and inside (c) of tool muff: 1 – tool muff; 2 – PST; F – loading force.

Table 3. Regimes of material processing by cylindrical cutting coronas, produced from PST

Type of material	Rpm	Cutting depth, mm	External diameter of a muff, mm	Drilling time, min
Aerated concrete D350.	300	350	50	2.2
Aerated concrete D450	300	200	50	4.5
Pine tree	600	100	80	1.2
Aspen	900	100	80	0.8

The testing results prove the operability of the cylindrical cutting coronas, produced from PST. At that, the best results in processing were achieved during cutting wood materials.

The strength of the plate on compression and its elastic properties, geometry of tools and other factors have a great influence on the durability of the cutting element, what requires a separate in-depth study.

The possibilities of using pst for the manufacture of a flat blade tool

As the second variant the PST was used for producing the scrapers for the finishing building work (Fig. 6, a, b) and the tools for overlaying the glue in the ceramic glaze mounting (Fig. 6, c, d).

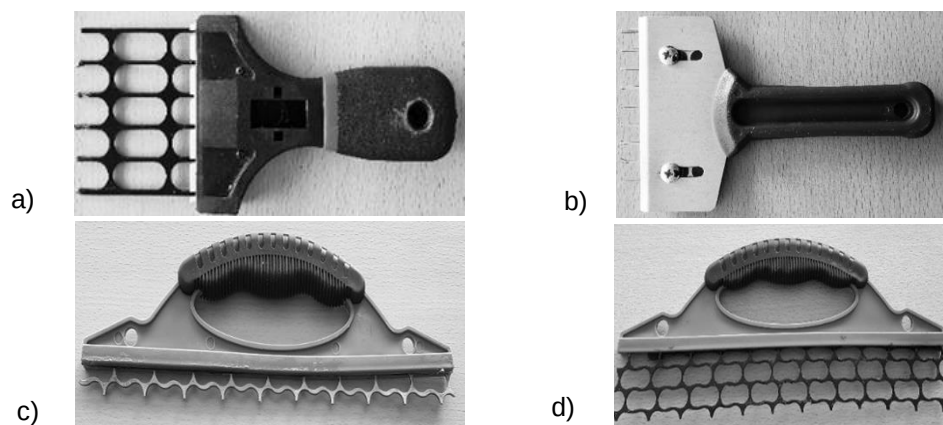


Figure 6. Tools for surface finishing works (a, b) and tiling (c, d) made from PST.

The approbation of both types of flat tools proves the appropriateness of using such tools for intended purposes. Thus, the tools for overlaying the glue in the ceramic glaze mounting provide the uniform and qualitative distribution of glue on the surface.

The experiment was conducted to evaluate the possibility of more effective application of glue or mortar to the cement base surface during tiling works. It is usually difficult to distribute adhesive masses of a given thickness using a solid plate spatula. In addition, experience shows that there is no need to apply glue or mass to the entire contact surface. Our experiments have shown that it is advisable to use spatulas with PST plates, which leave uniform voids in the mass. Using such spatulas, the largest coverage was achieved at a tooth height of $h = 3$ mm and step of 10 mm. This is 30–35% more than when using a spatula with a solid plate. The best quality of adhesive distribution was obtained at a tooth height of $h = 1.5$ mm and with a perforation step of 15 mm.

It is possible to apply glue coatings of various viscosities, using a different shape of the spatula teeth.

Strengthening of cutting tools by laser treatment method

It is clear that the hardness of surfaces of teeth is most important factor for providing operability and life time of cutting tools. For the increasing the hardness the Nd:YAG laser hardening was used according to the methodology described in (Mironov et al., 2017). Research done shown, that after laser hardening the micro hardness increases by 1.5–2.0 times and reaches 2.0–2.5 GPa in the surface layer with the depth 1 μ m.

CONCLUSIONS

The new possibility for recycling of the technological wastes, i.e. perforated steel tapes achieved after stamping of fine parts, by producing from mentioned perforated tapes the building tools like scrapers for the finishing building work, as well as cutting edges and circular coronas for the woodworking is offered in the current work. The results of approbation of elaborated prototypes of the circular are offered as well.

A significant effect may be achieved by the use of perforated materials, especially waste tapes, for the manufacture of blade tools – in particular working plates spatulas for applying glue or mastic on the surface.

It was proven, that proposed innovative tools could be used effectively in building production and woodworking. For the hardening the surface of cutting teeth the Nd:YAG laser hardening could be used successfully.

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Identification of yeast species involved in fermentation of the Kazakh camel dairy product–shubat

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Abstract. In certain countries of the world, camel's milk is used for food on a level with cow's milk. *Shubat* is a traditional food product based on camel milk in Kazakhstan. It is a fermented milk product obtained as a result of spontaneous fermentation of camel's milk under the influence of native microflora. Received dairy product from the southern region of Kazakhstan became the object of the investigation of the microflora of the fermented milk product *shubat*. The aim of the research was to study the microflora of camel milk, which causes its spontaneous fermentation. During the experiment, the dynamics of acid accumulation by the change in active acidity (pH) and titratable acidity (°T) was studied. In addition to lactic fermentation fermented product (*shubat*), alcoholic fermentation was noted, which has given the finished product an increased acidity and a high degree of gassing. To enumerate and identify microorganisms, *shubat* was sown to the following nutrient media: MRS, Malt wort-agar medium at 36 °C and 30 °C respectively both for 3 days. We suppose that the dominant component of the *shubat*'s microflora was yeasts: *Brettanomyces anomalus*, *Naumovozyma castellii*. Pathogenic microorganisms, such as *Salmonella*, *Shigella*, were not detected during the research, considering that the *shubat* is formed as a result of spontaneous fermentation and has poor hygienic characteristics in comparison with pasteurized milk. Identification of individual strains of bacteria allows us to simulate a starter microflora for the production of a safe fermented product based on camel milk on an industrial scale in Kazakhstan. The identified microflora, which causes spontaneous fermentation of camel milk and isolated strains of lactic acid bacteria, will make a significant contribution to the improvement of food safety in arid regions.

Key words: camel milk, *shubat*, spontaneously fermented dairy products, lactic acid bacteria, yeasts, *Brettanomyces anomalus*, *Naumovozyma castellii*.

INTRODUCTION

Nowadays camel's milk and products based on it are adequately consumed in food in various countries of the world (Rashid et al., 2007; Abdelgadir et al., 2008). The most popular are such products in arid and semi-arid regions, where climatic conditions are favorable for breeding camels and having a number of advantages for this type of farm animals. Historically, camel's milk is consumed as food to meet the nutrient and energy needs (Dirar, 1993; Ahmed et al., 2010).

According to several authors' researches, the composition of camel milk varies depending on its geographical origin, the physiological state of the animal, the conditions of keeping, feeding, lactation, heredity, health of camels, etc. (Shori, 2017).

The current demand for camel milk products is also due to the historically prevailing preferences of the population (Lore et al., 2005; El-Hadi Sulieman et al., 2006b; Shori, 2012; Yam et al., 2014). It is evident that camel milk and sour-milk products based on it were used to treat certain diseases (Mal et al., 2000; Yagil & Van Creveld, 2000; Mohamad et al., 2009). Many authors argue that camel milk has antimicrobial activity against pathogenic bacteria (Abbas & Mahasneh, 2014). According to the publications of the authors, it is known that camel's milk has the ability to ferment naturally to the fermented milk product, without preliminary heat treatment and without the addition of starter cultures (Wullschleger et al., 2013; Kaindi et al., 2018). The authors determined that the suppression or intensification of the microorganisms' development in dairy raw materials can be carried out, including means of ultrasonic action inside process pipelines (Suchkova et al., 2014).

It is known that the milk of farm animals is favorable environment for the growth of lactic acid bacteria and yeast (Jans et al., 2012). Among the lactic acid bacteria, probiotics are of particular importance, useful properties of them are the following: stimulating the reaction of the human immune system, preventing the development of pathogenic bacteria in the body, preventing the development of a number of diseases of the gastrointestinal tract, etc. (Borisova et al., 2008). Due to the development of fermented microflora, fermented milk drinks have a number of nutraceutical properties for the human body, in particular, they stimulate appetite, quench thirst, stimulate the release of gastric juice, enhance the peristalsis of the gastrointestinal tract, improve kidney function, have antibiotic properties, etc. (Lopatina et al., 1997; Glushanova, 2003). In addition, dairy products can be further functionalized using various biologically active substances (Zabodalova et al., 2014).

In different parts of the world, dairy products based on camel milk have unique names, for example in Sudan and Somalia, the *gariss* product is very popular, it is also known as 'hameedh' or 'humadah' (El-Hadi Sulieman et al., 2006b; Shori, 2012). In South Africa and Kenya, a similar product was named '*suusac*' (Lore et al., 2005). In Turkey, fermented milk drink is known as '*chal*' and otherwise referred to as a '*Turkic drink*' (Yam et al., 2014). The product '*shubat*' is widely consumed in Kazakhstan (Rahman et al., 2009b; Akhmetsadykova et al., 2014).

Traditionally, all these fermented milk products are produced by spontaneous fermentation as a result of the development of native microflora inherent to camel milk: lactic acid bacteria and yeast (Holzapfel, 2002; Lore et al., 2005). Lactic acid bacteria provide lactic fermentation of the milk base, resulting in the accumulation of lactic acid in the product. Yeast, in turn, causes alcohol fermentation in the fermented milk product,

which leads to the accumulation of a sufficiently high amount of carbon dioxide and ethanol (Madadlou et al., 2005; Oleshkevich et al., 2013). According to organoleptic indices, fermented milk products based on camel milk have a homogeneous, foaming, viscous consistency, characteristic white color peculiar to camel's milk, a specific smell and taste (Hassan et al., 2008).

The fermented product *shubat* was made from ancient times by the Turkic people. Modern Turkic peoples are numerous, including Kazakhs. Thus, at present, the dairy product *Shubat* is rightly recognized as a Kazakh national drink (Rahman et al., 2009b; Akhmetsadykova et al., 2014). According to the traditional technology, the product is prepared at home, most often from the milk of Bactrian camels, at room temperature by spontaneous fermentation for 3–4 days. The optimal temperature of milk ripening varies from 25 to 30 °C. It can be noted that camel's milk is not fermented at a temperature below 10 °C with a fermentation time of 72 hours or more (Rashid et al., 2007). To accelerate the fermentation of the *shubat* drink, some of it is mixed with a new portion of raw camel milk, resulting in an optimization of the time factor for obtaining the finished product (Saitmuratova & Sulaimanova, 2000). As noted earlier, mixed fermentation produces lactic acid and carbon dioxide, which leads to a significant decrease in the active acidity to 3.96 pH and an increase in titrated acidity to 181 °T (Rahman et al., 2009a).

In the study of the microflora of fermented milk products based on camel milk, scientists from different countries have found that *Lactobacillus* is a significant quantity, which is an acid-forming component, and to a lesser extent *Enterococcus*, which promotes aromatization in the final product (Abdelgadir et al., 2001; Gonfa et al., 2001; Narvhus & Gadaga, 2003; Sulieman et al., 2006a; Omar et al., 2007). The active acid formation of the lactic acid bacteria (LAB) is considered as one of the important factors of antagonism to a pathogenic microflora (Borisova et al., 2008). It has been proven that members of the genus *Lactobacillus* stimulate the suppressed immune system and do not affect the immune system that is in a normal state (Glushanova, 2003).

In this connection, a special interest, in our opinion, is the study of the qualitative, quantitative and specific composition of the microflora of a lactic acid drink—*shubat*, which is produced in a traditional way, in particular, through spontaneous fermentation of raw camel milk. The present work is aimed at studying the microflora of the national Kazakh *shubat* product and its safety performance indicators.

MATERIALS AND METHODS

Sampling

As objects of research used: raw camel milk (sample number 1) and sour milk product based on camel milk – *shubat* – (sample number 2), obtained from the southern region of Kazakhstan. To receive reliable results of the experiment, sample No. 1 was obtained from three different camels and subjected to physicochemical and microbiological methods of analysis no later than 2 hours after its preparation. Sample No. 2 was obtained as a result of spontaneous fermentation of assembled camel milk, after it was hermetically packed in thermal containers and transported at 4 ± 2 °C for further research in the laboratory of St. Petersburg and the Leningrad Region, in particular in the ITMO University laboratory; the laboratory of genetics of plant-microbial interactions of the All-Russian Scientific Research Institute of Agricultural

Microbiology and the laboratory of the St. Petersburg branch of the Research Institute of the baking industry. During the experiment the storage temperature of sample No. 2 was maintained within 5 ± 1 °C.

All studies of sample No. 1 and sample No. 2 were obtained in triplicate.

The physico-chemical analysis of sample No. 1 included a number of studies: on the determination of titrated acidity, active acidity, protein content, fat, lactose, ash, salts, density, freezing temperature. Investigation of the physicochemical properties of sample No. 1 was carried out on an ultrasonic milk quality analyzer *Klever-2M*, (Biomer, Russian Federation). The operation principle of this analyzer is based on passing ultrasound vibrations through the sample and recording the values of output signals depending on the values of measured milk's parameters of various types of farm animals. The device was calibrated on camel milk in order to minimize measurement errors under the manufacturer documentation and methodology.

The physico-chemical analysis of sample No. 2 was carried out by determining the titratable acidity, the active acidity and the ethanol content:

Determination of active and titratable acidity

The measurement of active acidity of each samples repeated three times, each time removing the electrodes from the sample and immersing them into the sample (GOST (Russian National State Standard) 32892–2014).

The results of the measurement of active acidity in milk and dairy products were obtained by taking the arithmetic mean of the results of three parallel determinations.

The titrimetric determination of acidity of the samples were accomplished according to the method described by Nadtochii (Nadtochii & Koryagina et al., 2014).

Determination of ethanol content

A 3 mL sample was steam distilled into acidified potassium dichromate solution. Unreacted dichromate was determined by titration with ferrous ammonium sulphate solution using phenanthroline as an indicator (Bradley et al., 1992).

The antibiotics analysis of sample No. 1 and 2. The content of antibiotics such as levomycetin, tetracycline group, streptomycin, penicillin was conducted according to Methodical Guidelines '4.2.026-95 – Express method for the determination of antibiotics in food products'.

The toxic elements analysis of sample No. 1 and 2. The study on the presence of toxic elements (Pb, Cd, As, Hg) was performed according to GOST 33824-2016 Food products and food raw materials.

The content of radionuclides of sample No. 1 and 2. The content of radionuclides (Cs, Sr) was investigated in accordance with GOST 32161-2013 'Food products. Method for determination of cesium Cs-137' and GOST 32163-2013 'Food products. Method for determination of strontium content Sr-90'.

The content of pesticides of sample No. 1 and 2. The samples with pesticides were determined according to the methodological guidelines for the determination of organochlorine pesticides in water, food products, feed and tobacco products by thin-layer chromatography. The method is based on chromatography of chlorine-containing pesticides in a thin layer of aluminum oxide, silica gel or Silufo plates in various systems of mobile solvents after their extraction from the samples and purification of the extracts. The mobile solvent is n-hexane or n-hexane mixed with acetone. The sites of localization of the drugs are detected after spraying the plates with a solution of silver ammine followed by ultraviolet irradiation or after irradiating ultraviolet light with Silufol-

containing o-tolidine plates. The determination of pesticides was carried out according to Methodical Guidelines 2142-80 'Guidelines for the determination of organochlorine pesticides in water, food, feed and tobacco products by thin-layer chromatography'.

The content of aflatoxins of sample No. 1 and 2. The method is based on the extraction of aflatoxins M1 from a sample of the product, purification of the extract from interfering substances, and measuring the mass concentration of aflatoxin M using thin-layer chromatography by visually determining the amount of substance in the spot. The range of measured contents in dairy products: 0.0005–0.005 mg kg⁻¹. The content of aflatoxins was investigated in accordance with GOST 30711-2001 'Food products. Methods for detection and determination of aflatoxins B (1) and M (1)'.

Microbiological analysis of sample No. 1. Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms (*QMAFAnM*) and pathogenic microorganisms were determined according to the standard method GOST 10444.15-94 and GOST 31659-2012 (ISO 6579: 2002), respectively.

Microbiological analysis of sample No. 2. Lactic acid microorganisms were determined by GOST 10444.15.94, bacteria of the group of *Escherichia coli*, in particular coliforms – GOST 32901-2014, pathogenic microorganisms – GOST 31659-2012, including *Staphylococcus Aureus* – GOST 30347-2016, mold and yeast – GOST 10444.12-13.

Enumeration of microorganisms of sample No. 2. Ten (10) mL of camel milk sample were homogenized with 90 mL of saline water (8.5 g L⁻¹) to make an initial dilution (10⁻¹). The suspension was used for making suitable serial dilutions up to 10⁻⁸ by incorporating 1 mL into 9 mL of sterile saline water in sterile tubes. Enumeration of LAB and yeast were determined using media MRS agar and Malt wort-agar, respectively. LAB strains were incubated in MRS media at 36 °C for 3 days and yeast incubated in malt wort-agar at 30 °C for 3 days (Tezira et al., 2005; Nurgul et al., 2009). After the incubation, the Petri dishes with a number of colonies from 30 to 300 were studied using colony counter (Goryaev's chamber) with a 1 cm grid and side lighting. The colonies were counted in at least 20 squares, determining their average number by one cm². The result was multiplied by the surface area of the medium in the cup (Eremina & Kriger, 2005).

Preparation and microscopy of preparations of sample No. 2. Preparation of microscopic preparations, including their staining, was carried out according to the standard technique described in the article by Babaeva and Rogacheva (Babaeva & Rogacheva, 2012).

Genomic DNA extraction

The Genomic DNA of two isolates was extracted from yeasts cultures according to the procedure of MicroSeq[®] 500 16S rDNA Sequencing Kit protocol.

One mL of each yeast culture was added into a sterilized micro centrifuge tube with adding sodium dodecyl sulfate and protein-degrading enzyme. The samples were centrifuged for 15 min at 14,000 rpm using centrifuge DiaCent-CW[®] (Bio-Rad). The supernatant was discarded by suction apparatus FTA-1[®] (Biosan) and the pellets were washed with buffer pH 8 and centrifuged for obtaining pure pellets. DNA purification from protein impurities was performed by extraction with phenol/chloroform, 500 µl into each tube. The supernatant which include the extracted DNA was transferred into new micro centrifuge tube for using in the following step. 550 µl of isopropanol was

added to precipitate the DNA and the samples were centrifuged for 15 min at 14,000 rpm. After supernatant removal the precipitate was washed with 1 mL of 70% ethanol, dried and dissolved in 30–50 µl of TE. The DNA solution was stored at 4 °C.

Polymerase chain reaction (PCR)

The amplification of extracted DNA was carried out in ICycler® (Bio-Rad). The reaction volume for thermal cycling is 30 µL and the program of polymerase chain reaction is presented in Table 1. The PCR products were stored at minus 20 °C until use in the following step.

Table 1. The program of polymerase chain reaction

Primers	Annealing	Denaturation	Elongation
ITS1F/ITS2	52 °C/90 sec	92 °C/60 sec	72 °C/50 sec

Sequencing the PCR product

Polymerase chain reaction products were purified for removing the unused dNTPs and primers from the PCR mixture before sequencing. For sequencing used a set of standard reagents for the sequencer ABI PRISM 3500® (Thermo Fisher Scientific). Finally, the data were compared with the known data in basic alignment search tool (BLAST) with the degree of homology not less than 99%.

Statistical analysis

All experiments were performed in triplicate and the results were shown as the mean value ± the standard deviation. All microbiological counts were converted to the base -10 logarithm of cfus per milliliter (mL) of samples (log cfu mL⁻¹), and from these, means and their standard deviations were calculated.

RESULTS AND DISCUSSION

At the first stage of the work to substantiate the safety studied samples' quality, a study was made of the microbiological indicators of camel's raw milk (sample No. 1), which is of significant importance for the traditional method of producing shubat (sample No. 2). According to the results of microbiological methods of analysis, no pathogenic microorganisms, including *Salmonella*, were found in sample No. 1, the QMAFAnM indices do not exceed the permissible norm. In sample No. 2: bacteria of the group of *Escherichia coli* (coliforms), pathogenic microorganisms, including *Salmonella*, *St. Aureus* and mold are not found.

The content of inhibitory substances, in particular antibiotics, was not found in the test samples. Toxic elements such as cadmium and arsenic are not detected. The content of lead and mercury does not exceed the permissible norm, in particular, not more than 0.01 and 0.005 mg kg⁻¹, respectively. Radionuclides of cesium 137 and strontium 90 are within the limits of permissible norms. The presence of pesticides and aflatoxins in the samples was also not detected.

The physico-chemical properties of camel's raw milk (sample No. 1) were studied as the basis for the production of a sour milk drink – *shubat* (sample No. 2). According to the conducted studies (data of Table 2), the chemical composition of camel milk (sample No. 1) is within the limits of identification indicators values. Moreover,

identification indicators values of the camel milk chemical composition are much higher than cow's milk.

Table 2. The chemical composition of camel's raw milk (sample No. 1)

Indicators	Identification indicators of raw milk *		Research results of sample No. 1
	Cow	Camel	
titratable acidity, °T	16.00–21.00	not more than 17.50	17.10 ± 0.15
total solids, %	not less than 11.00**	on average 15.00	$16.26 \pm 0.18^{**}$
protein, %	not less than 2.80	not less than 3.80	5.09 ± 0.17
fat, %	not less than 2.80	not less than 3.00	5.52 ± 0.06
density under temperature 20 °C, kg m ⁻³	not less than 1,027.00	not less than 1,032.00	$1,036.00 \pm 0.30$
freezing temperature, °C	not higher than -0.505	not standardized	-0.57 ± 0.50

* Technical Regulations of the Customs Union 'On the safety of milk and dairy products' (TR TS 033/2013) in accordance with the Unified Agreement principles and rules of technical regulation in the Republic of Belarus, the Republic of Kazakhstan and the Russian Federation on November 18, 2010.

** The calculation of dry matter of camel milk was performed according to the following formula: Total solids=MSNF+F.

The calculation of milk solids-non-fat in camel milk performed according to the following formula: Milk solids-not-fat (MSNF)= $0.25 \times D + 0.225 \times F + 0.5$, where: D – density (in degrees density hydrometer); F – fat content of raw milk, %.

In addition to the data presented in Table 2, other indicators of the chemical composition of sample No. 1 are also defined, in particular: ash – $0.75 \pm 0.12\%$; lactose – $5.15 \pm 0.10\%$; salts – $0.84 \pm 0.15\%$. The values of indicators of identification of milk obtained from individual milkings described in Table 2 may vary within wider limits than in the data of technical regulations. Obviously, raw camel's milk, obtained from three different camels, varies insignificantly in terms of chemical composition. The revealed variability can be connected with the physiological condition of the animal, as the feeding conditions of all camels were the same. According to the results of the study, it can be stated that the titratable acidity is at an acceptable level. The content of protein, fat and dry substances significantly exceeds the established normalized values, which indicates the high nutritional value of camel milk obtained in the southern region of Kazakhstan. In particular, the protein content in the samples No. 1 varied from 5.38 to 4.80%, the fat content from 5.12 to 5.93%, the lactose content from 4.70 to 5.61%. In all the samples studied, the value of milk density exceeded the identification indicators with fluctuations from 1,034 to 1,038 kg m⁻³, which indicates a high content of mineral salts in the studied camel milk: from 0.80 to 0.89%. As a result, camel milk, obtained from the southern region of Kazakhstan, showed compliance with the standards imposed on raw milk, which allowed further study of sample No. 2, obtained on the basis of sample No. 1.

At the second stage of the work, sample No. 2 was examined during storage. The parameters of the assessment at this stage of the experiment were changes in the active, titratable acidity and alcohol content for 21 days, with a storage interval of 7 days. The duration of the experiment is determined by the shelf life of the fermented product produced by the traditional method through spontaneous fermentation. The results of the study are presented in Table 3.

A high level of titrated acidity of sample No. 2 (Table 3) may be due to exposure to the microflora of camel's milk, which is actively developed in the process of spontaneous fermentation. Low values of active acidity of sample

No. 2 indicate a significant effect of lactic acid bacteria and yeast during spontaneous fermentation, which is confirmed by the organoleptic characteristics of the fermented product, in particular the formulation of the taste of the finished product as 'acidic'. It is obvious that camel's raw milk is an excellent medium for the development of native microflora inherent in this type of dairy raw materials.

For the initial detection of the microflora of the fermented milk product –*shubat*–a microscopic preparation of the freshly prepared sample No. 2 was prepared, the results of the experiment are shown in Fig. 1, a. The change in microbiological parameters of the sour milk drink (the number of lactic acid microorganisms, yeast) during the storage for 21 days is shown in Fig. 1, b. It is obvious that in the process of storage of sample No. 2, the amount of yeast increases, which leads to intense gas formation of the product, which is less pronounced in the freshly prepared fermented product.

Table 3. Change in active, titratable acidity and alcohol content during sample No. 2 storage

Storage days	Active acidity, pH	Titratable acidity, °T	Ethanol, %
0	4.81 ± 0.01	85 ± 0.15	0.68 ± 0.01
7	4.49 ± 0.01	168 ± 0.16	0.79 ± 0.01
14	4.24 ± 0.01	181 ± 0.16	0.91 ± 0.01
21	3.96 ± 0.01	191 ± 0.15	1.10 ± 0.01

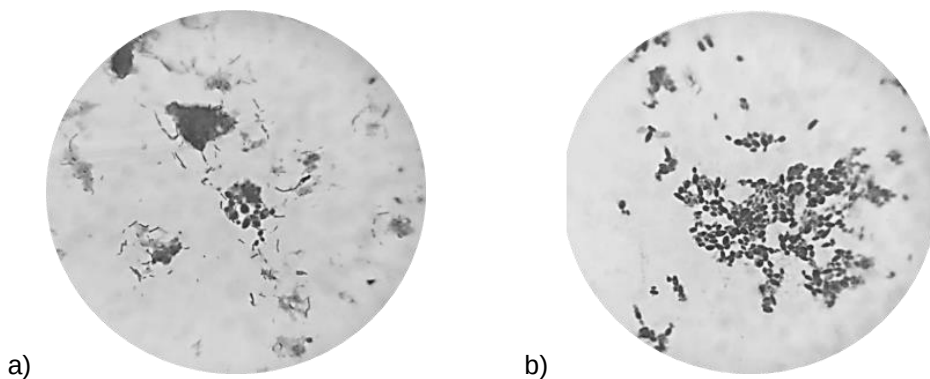


Figure 1. Microscopic preparations of sample No. 2 during storage, days: 0 day (a), for 21 days (b).

Based on the data in Fig. 1 it can be stated that the microscopic preparation of the freshly prepared product (Fig. 1, a) is represented mainly by lactic acid rods, which are located singly, in pairs and short chains, to a lesser degree there are yeast round and oval-ovoid in small groups. On day 21 of the experiment, a significant amount of yeast is developed in the microscopic preparation of sample No. 2, which develops during sample No. 2 storage (Fig. 1, b). However, this method of investigation is visual and requires confirmation by other methods.

In this connection, in the next stage of the study, the number of microorganisms in sample No. 2 was determined by the dilution method, culturing them on a nutrient medium followed by counting the grown colonies. A quantitative account of the browned up colonies was carried out in Goryaev's chamber with the purpose of revealing the

patterns of colony growth. The quantitative analysis of the grown colonies of the main groups of microorganisms of the finished fermented product on the 21st day of storage was conducted.

The quantity of lactic acid bacteria in sample No. 2 at the end of the shelf life is determined to be 1.93×10^6 CFU mL⁻¹ and the yeast is 1.53×10^6 CFU mL⁻¹, which indicates a sufficiently high number of microorganisms in *shubat* prepared in the traditional way. As is known, *shubat* is obtained as a result of mixed fermentation, as evidenced by the results of the experiment.

Within the framework of this work, yeast, which caused alcoholic fermentation of camel milk, was of particular interest. To identify the specific composition of yeast, evidently inherent in the native microflora of camel milk, studies were carried out to isolate pure yeast cultures and identify them. In the course of the experiment on the isolation of pure yeast cultures of a fermented beverage – *shubat* – a microscopic preparation was evaluated (Fig. 2), as a result of which two preferred yeast types of a white and gray colony were identified. Fig. 2 clearly shows the morphological differences of yeast colonies, in particular, the yeast of the white colony has a rotundity and spherical shape (Fig. 2, a), and the yeast of the gray colony has a rodlike shape (Fig. 2, b).

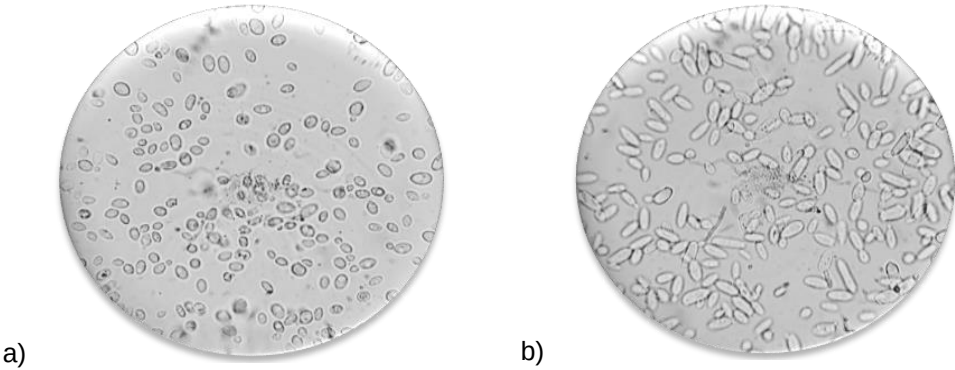


Figure 2. Microscopic preparations of pure yeast cultures isolated from sample No. 2.

The nucleotide sequences of two strains were analyzed by software application and data were compared with officially registered data. Molecular of the isolated pure yeast cultures of sample No. 2, in particular, the white and gray yeast colonies were genetically identified as *Brettanomyces anomalus* and *Naumovozyma castellii*, respectively. Specific features of the identified yeast are indicated in Table 4.

Table 4. Characteristics of the identified yeast colonies

White yeast colony	Gray yeast colony
<i>Brettanomyces anomalus</i>	<i>Naumovozyma castellii</i>
Optimal growth temperature: 31–32 °C	Optimal growth temperature: 24–26 °C
Aerobe	Aerobe
Strain: CBS 7654	Strain: CBS 4309

Yeasts of the genus *Brettanomyces anomalus* are characterized by increased degree of gassing, the production of high concentrations of ethanol, are thermotolerant. In the biotech industry, this kind of yeast is also used to produce bioethanol due their tolerance to low pH, nutrient-efficient metabolism and ability to produce high concentrations of ethanol (Passoth et al., 2007). *Brettanomyces* species can synthesize volatile phenolic compounds, including phenol, syringol (Heresztyn 1986) and several ethyl phenols (Chatonnet et al., 1997).

Yeasts of the genus *Naumovozya castellii* are wild yeasts not used in the food industry. It should be noted that this type of yeast is widely used for research in the field of genetics. Yeast of the genus *Naumovozya castellii* is widely used as a model organism in biological research. Due to the specific ability for genetic modification, this type of yeast becomes more and more interesting as a potential model of yeast for functional analyzes (Karademir et al., 2016).

Accordingly, of the two identified yeast species only the strain *Brettanomyces anomalus* can be used in the starter microflora for the production of fermented products produced under the action of mixed fermentation. The development of a complex starter based on strains of microorganisms isolated from camel milk will expand the range of fermented milk based products on an industrial scale.

CONCLUSIONS

Fermented dairy products based on camel milk are investigated to a lesser extent compared to products based on cow's milk. This is confirmed by the less informative regulatory framework for the milk of farm animals, with the exception of cow's milk and products based on it, the information of which are largely presented in the technical regulations. According to the results of a comprehensive research, it was proved that camel milk obtained in a number of farms in the southern regions of Kazakhstan has a high nutritional value and is assessed as a safe raw material resource. A fermented product based on camel milk was also studied, which showed compliance in terms of quality safety indicators. In consequence of the study, two species of yeast that cause the alcoholic fermentation of camel milk were isolated and identified. The experimental evidence on the identification of yeast colonies provide valuable insights of the microflora species diversity of camel milk. The present research will contribute to the development of new strains of microorganisms used in the production of starter microflora of fermented products in an industrial environment, thereby improving the quality and safety of fermented milk products.

Further research is planned on camel milk as a source of probiotic strains of lactic acid bacteria (LAB). The optimal approach in the future work will be a comprehensive assessment of camel milk microflora from various regions of Kazakhstan, which will provide extensive information on the strains of dairy microorganisms of camel milk and assess their role in the process of fermentation of dairy raw materials. In the future tense, it is scheduled to work on obtaining direct practice of starter microflora as a result of a combination of microorganisms' strains isolated from camel milk obtained in Kazakhstan.

ACKNOWLEDGEMENTS. This research work was financially supported by the Government of the Russian Federation, Grant RFMEFI58117X0020.

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Film agents as an effective means of reducing seed shattering in *Festulolium*

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Abstract. In the conditions of the forest steppe of the Central Chernozem region, methods were studied to reduce shatter losses in the pre-harvesting period for *Festulolium* during the period 2009–2011, applying the film forming agents, Elastik ($0.8\text{--}1.2\text{ L ha}^{-1}$), Bifaktor ($0.8\text{--}1.2\text{ L ha}^{-1}$), and Metylan Universal Premium ($1.4\text{--}3.8\text{ kg ha}^{-1}$). The agents were used at seed humidity levels of between 60–65%. Following anti-shattering treatment, seed moisture content gradually decreased. The application of film forming agents prevented seed shattering, and seeds were harvested by direct combining at a humidity of between 20–25% without heavy losses being suffered. Moreover, decreased seed losses due to film forming agents significantly reduced the cost of seed heap drying. In the control treatment, the seed yield amounted to 214.8 kg ha^{-1} , and 360.7 kg ha^{-1} was lost as a result of natural seed shattering in the process of ripening. The Elastik and Bifaktor preparations prevented seed shattering and contributed to the preservation of a seed yield of between $522.1\text{--}563.5\text{ kg ha}^{-1}$. The application of film forming agents contributed to a reduction in losses during harvesting within the range of 9.7–16.8%. Application of the Metylan Universal Premium glue in the studied doses provided a significant increase of seed yield. The highest seed yield ($490.1\text{--}495.2\text{ kg ha}^{-1}$) was obtained in the treatment which used a Metylan application at a dose of $3.0\text{--}3.4\text{ kg ha}^{-1}$, where seed shattering was reduced by 14.8–17.6%.

Key words: perennial grasses, shatter losses, film forming agents, seed humidity.

INTRODUCTION

The harvesting of seed herbage for many forms of agricultural crops is the most complicated procedure in the process of cultivation, because it always carries risks that primarily are due to its weather dependence, the blotchy ripening of seeds and their small size, and also due to the ability of many species to shatter seeds (Kulikov, 2010).

Rape, feed legumes, peas, soybean, and other crops are prone to seed shattering during ripening. Under the conditions required to reduce seed loss during ripening and harvesting on a working farm, adhesive preparations are used that are based on synthetic latex. The basic principle of their action is as follows: when they are sprayed onto the plant's surface, their drops spread and merge, creating a uniform elastic film. In the case of rape, this prevents pod shatter, promotes natural ripening, reduces seed humidity, decreases the negative impact of ultraviolet radiation and friction between the pods, and reduces the development of certain fungal diseases which, as a result, leads to higher seed yields and higher oil content in seeds (Fedotov et al., 2008).

Seed shattering (or scattering) is characteristic of most grasses and is one of the stages of the natural process of seed dispersal. The separation of ripe seed from the maternal plant occurs when it is abscised from the rachilla; usually this occurs during the final stage of ripening. In this period all of the inflorescences become straw-coloured, and the endosperm hardens completely (Perepravo et al., 2008).

No data is available on the use of adhesive preparations on perennial grasses. At present, it is recommended that easily-shattering perennial grasses be harvested [(perennial ryegrass (*Lolium perenne* L.) and Italian ryegrass (*Lolium multiflorum* L.), meadow fescue (*Festuca pratensis* L.), etc)] at the seed humidity level of 40–45%, ie. before wholesale seed shattering due to inflorescences begins. Dry matter accumulation in seeds during this period stabilises and reaches its maximum extent (Mikhaylichenko et al., 1994; Shchedrina et al., 2009).

Festulolium ($\times$ *Festulolium* F. Aschers. et Graebn.) is an artificially created fodder crop that was obtained by intergeneric hybridisation in the *Lolium* sp. and *Festuca* sp. genera system. The main objective behind the creation of this hybrid was to combine several economically valuable features of its parental forms in one plant. From various ryegrasses, *Festulolium* inherited excellent feeding qualities: high sugar, protein, and exchange energy content, as well as good palatability and the digestibility of feed, and the ability by the plant to intensively form a large number of folious vegetative shoots. During the vegetation period the hybrid quickly regrows after repeated mowing or grazing; at the same time, unlike ryegrass, it is less inclined to the formation of generative shoots and is responsive to the use of mineral nitrogen fertilisers and irrigation. From fescues the hybrid borrowed good winter hardiness, drought resistance, and resistance to long-term grazing and trampling. Depending on the selection of parental forms and their morphotypes, the hybrids that were obtained are being used for the preparation and conservation of various types of feeds, both in pure form and in grass mixtures on cultural hay-producing land and pasture, as well as in the creation of lawns, and the improvement of field aerodromes and sports grounds (Barnes et al., 2014; Kvasnovsky et al., 2014; Schiavon et al., 2014; Kubota et al., 2015).

The VIK-90 *Festulolium* variety was bred in V Williams All-Russian Fodder Research Institute. It is characterised by high productivity levels in terms of green mass (74.3–80 t ha⁻¹), dry matter (14.0–15.5 t ha⁻¹), longevity, good winter hardiness, the evenness of forage mass during the growing season, rapid spring aftergrowing, and after heavy grazing. However, the VIK-90 variety is not free of the shortcomings that are proper to its seed parent, the main being non-simultaneous ripening of seeds and their easy shattering in the pre-harvesting period (Vasko et al., 2010; Perepravo & Ryabova, 2003).

During the study of the dynamics of *Festulolium* herbage ripening with varying degrees of maturity, it has been reported that the delay in harvesting of the VIK-90 variety leads to significant seed loss due to natural seed shattering, starting with humidity levels for the seeds that is less than 40%, and increasing further during the seed herbage ripening process (Kulikov, 2010; Perepravo, 2011). It was revealed that at the stage of complete seed ripeness for the VIK-90 *Festulolium* variety (at seed humidity levels of 20–25%), losses due to seed shattering amounted to 244.6 kg ha⁻¹. The same results were obtained for other grass species (Griffiths et al., 1971; Cherniauskas et al., 1977; Mikhaylichenko, 1987; Lebedeva, 2010).

Therefore, it is obvious that any delay during harvesting leads to excessive seed loss (up to 40–55% of the entire yield). The low actual productivity levels of *Festulolium* seed herbage can be adequately explained by this fact, which is one of the main factors to serve to restrain extensive use of the culture on working farms in the Central Chernozem region (Obraztsov et al., 2013).

It can be seen that, while there are no *Festulolium* varieties that are completely resistant to seed shattering or which undergo simultaneous ripening of the seeds, one of the most important issues in its cultivation technology is the search for ways to reduce shatter loss in the seeds.

The objective of our studies was to develop methods for reducing *Festulolium* seed loss due to natural shattering during the ripening period and harvesting (including delayed harvesting).

MATERIALS AND METHODS

The experimental area of the study was carried out in field trials for the Department of Crop Science, Forage Production and Agricultural Technologies, Voronezh State Agrarian University, on the fields that are run by the ‘Agrotechnology’ Training, Research and Technological Centre (N51.7140416 E39.21545371) in 2009–2011.

The experimental design included the application of three adhesive preparations: Elastik and Bifaktor in dosages of 0.8, 1.0, and 1.2 L ha⁻¹, and Metylan Universal Premium in dosages of 1.4, 1.8, 2.2, 2.6, 3.0, 3.4, and 3.8 L ha⁻¹. Control crops were treated with clean water. Adhesive preparations were applied at the milk-ripe stage of *Festulolium* weevils when seed humidity was not less than 60–65%. Generative shoots were treated using the Hozelock Professional 4816 manual knapsack sprayer with a volume of sixteen litres. Spray material consumption was 200 L ha⁻¹. The area of the registration plot was 20 m². A four-replicate experiment was carried out, the placement of experimental plots was randomised.

Shatter loss in the seeds was defined by the seeds gathering in special containers over a total area of 1 m², with these points being located between the rows of the grass stand.

The harvesting of seeds was carried out by direct combining at humidity levels of 22–25%. The clipping height was increased in order to eliminate the cutting of green leaves in the *Festulolium* plants.

A germination test was carried out under the laboratory conditions between two-to-three months after harvesting. It was carried out using the rolling method (Fedotov et al., 2011). A hundred seeds were placed embryo-down on moistened filter paper 12 cm × 100 cm in size on a line drawn between 2–3 cm from the upper edge. They were then covered with a sheet of moistened filter paper of the same size. The strips were loosely rolled up and were placed in a vertical position into germination chambers with 50 mL of water. Seeds were germinated for seven days in the dark in a thermostat at a temperature of 20 °C. A germination test was carried out in four replications. On Day 3 the germinating power was determined. At the end of the germination period, laboratory germination was determined. In addition, the weight of 1,000 seeds was determined.

Mathematical processing of the data obtained was carried out by means of a variance analysis (Dospekhov, 1985). Economic efficiency was calculated on the basis of process flow charts using standard specifications for the prices of 2011.

RESULTS AND DISCUSSION

We offer to change the first paragraph of the Results and discussion.

Following the anti-shattering treatment of *Festulolium* plantings due to the application of adhesive preparations, a thin film was formed on the ears, polymer network with the effect of diffusion covered the entire surface of the plant without stomatal closure and disruption of gas exchange functions of plants. These factors helped to reduce shatter losses in the seeds.

The results indicated that following the anti-shattering treatment of *Festulolium* plantings due to the application of adhesive preparations, a thin polymer film was formed on the ears and on the surface of each plant without stomatal closure and disruption of gas exchange functions in plants. These findings compare favourably to those reported by Fedotov et al. (2008).

The proposed treatment helped to reduce shatter losses in the seeds.

Additionally, it is worth pointing the fact that the greater part of the leaves of the plantings remained green. A pre-harvesting treatment of *Festulolium* plantings by means of adhesive preparations allowed harvesting to be carried out at lower seed humidity levels and with minimum losses (Table 1).

Table 1. Productivity and *Festulolium* seed shattering rate impacted by the applied adhesive preparations at different concentrations (averaged for the 2009–2011 period). N = 4 observations

Treatment		Productivity, kg ha ⁻¹		Seed shattering rate	
Preparation	Dose	Biological	Real	kg ha ⁻¹	%
Control (water treatment)		575.6	214.8	360.7	62.0
Metylan	1.4	584.3	284.1	300.1	51.0
Universal	1.8	589.5	342.4	247.1	42.9
Premium,	2.2	581.3	382.3	199.0	35.4
kg ha ⁻¹	2.6	585.5	448.9	136.6	24.1
	3.0	578.7	495.2	83.5	14.8
	3.4	590.0	490.1	99.8	17.6
	3.8	593.4	453.9	139.5	24.1
Elastik,	0.8	620.3	522.1	98.3	16.8
L ha ⁻¹	1.0	627.2	543.9	83.4	14.0
	1.2	630.2	563.9	66.4	11.2
Bifaktor,	0.8	615.6	524.8	90.8	15.4
L ha ⁻¹	1.0	617.4	547.4	70.0	11.9
	1.2	619.7	563.5	56.2	9.7
LSD ₀₅		9.8	5.1	3.1	1.8

We registered high levels of efficiency in the preparations that were being studied in regard to *Festulolium* plantings. In the control treatment the real seed yield was 214.8 kg ha⁻¹, and seed shatter losses during the ripening process were at 360.7 kg ha⁻¹.

Elastik and Bifaktor preparations decreased seed shattering and therefore increased the real yield of *Festulolium* seeds to 522.1–563.5 kg ha⁻¹. Film-forming substances decreased harvesting losses from 62% (in the control treatment) to between 9.7–16.8%.

The application of Metylan Universal Premium glue at the recommended rates also provided a significant increase of seed yield. The highest seed yield (490.1–495.2 kg ha⁻¹) was produced under application of the glue at a dose of 3.0–3.4 kg ha⁻¹, which decreased seed shattering by 14.8–17.6%. The spray material of Metylan Universal Premium glue at the maximum dosage (3.8 kg ha⁻¹) was characterised by high viscosity. Its application caused a strong clogging of the sprayer nozzles, which resulted in the poor quality of treatment of generative shoots and increased seed loss (up to 24.1%).

The application of adhesive preparations did not degrade the sowing qualities of *Festulolium* seeds (Table 2). In the control treatment, laboratory germination amounted to 92.2%, whereas in the experimental variants it was at 92.2–95.7%. The average mass of 1,000 seeds reached 2.88–2.92 g.

Table 2. The sowing qualities of *Festulolium* seeds impacted by the applied adhesive preparations (averaged for the 2009–2011 period). N = 4 observations

Treatment Preparation	Dose	Weight of 1,000 seeds, g	Germinating power, %	Laboratory germination, %
Control (water treatment)		2.89	74.6	92.2
Metylan	1.4	2.92	73.7	94.7
Universal	1.8	2.90	76.1	93.4
Premium,	2.2	2.88	74.5	92.4
kg ha ⁻¹	2.6	2.94	72.8	92.2
	3.0	2.91	72.1	93.1
	3.4	2.92	76.3	93.6
	3.8	2.90	75.3	93.1
Elastik,	0.8	2.92	70.9	95.7
L ha ⁻¹	1.0	2.91	73.9	94.8
	1.2	2.89	69.6	93.5
Bifaktor,	0.8	2.88	73.0	94.1
L ha ⁻¹	1.0	2.90	68.5	93.2
	1.2	2.89	72.4	94.2
LSD ₀₅		1.6	2.8	4.3

Material and total costs for *Festulolium* cultivation in the control treatment amounted to RUB 29,300 per hectare. When applying adhesive preparations this indicator amounted to RUB 29,900–31,800 per hectare. In economic terms the anti-shattering treatment for *Festulolium* plantings with film forming agents was effective. When applying Elastik and Bifaktor the lowest production costs for 100 kg of seeds (RUB 5,400–5,900 per hectare) and the highest profitability levels (104–123%) were observed (Table 3).

Table 3. The economic efficiency of applying film-forming preparations on seed plantings of *Festulolium* (averaged for the 2009–2011 period)

Treatment		Value of production	Costs per	Cost of	Net income,	Profitability
Preparation	Dose	per 1 ha, RUB	1 ha, RUB	1 c, RUB	RUB	level, %
Control (water treatment)		25,780	29,291	13,634	-3,511	-12.0
Elastik,	0.8	62,648	30,737	5,888	31,911	103.8
L ha ⁻¹	1.0	65,264	30,930	5,687	34,334	111.0
	1.2	67,664	30,612	5,429	37,052	121.0
Bifaktor,	0.8	62,980	30,390	5,893	32,050	103.6
L ha ⁻¹	1.0	65,692	30,172	5,512	35,520	117.7
	1.2	67,620	30,392	5,393	37,228	122.5
Metylan	1.4	34,096	29,966	10,547	4,130	13.8
Universal	1.8	41,088	30,158	8,808	10,929	36.
Premium,						2
kg ha ⁻¹	2.2	45,880	30,351	7,938	15,528	51.1
	2.6	53,872	30,544	6,804	23,328	76.4
	3.0	59,424	30,737	6,207	28,687	93.3
	3.4	58,816	30,930	6,311	27,886	90.2
	3.8	54,468	31,798	7,005	22,670	71.3

The study resulted in the execution of Russian Federation Patent 2420050: ‘A method for the pre-harvesting treatment of *Festulolium* seed crops’ (Obraztsov et al., 2011).

CONCLUSIONS

On the basis of studies conducted in 2009–2011 during seed harvesting in the conditions of the forest-steppe of the Central Chernozem region, and devoted to the seed productivity of *Festulolium* depending on the application of adhesive preparations, the highest real yield of *Festulolium* seeds was registered when applying Bifaktor, amounting to 524.8–563.5 kg ha⁻¹ (averaged across three years of studies).

The application of Metylan Universal Premium glue revealed a significant increase in seed yield due to shatter loss reduction. When applying a dose of 3.0–3.4 kg ha⁻¹, the seed yield amounted to 490.1–495.2 kg ha⁻¹ and the seed shattering rate did not exceed 14.8–17.6%.

The pre-harvesting treatment of *Festulolium* plantings by Bifaktor preparation was economically reasonable (the production costs for 100 kg of seeds was RUB 5,500 per hectare with a profitability level of 118%). In the application of Metylan Universal Premium glue, production costs for 100 kg of seeds was RUB 6,200–8,800 per hectare and the profitability level was between 14% and 93%.

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The effect of herbicides on seed productivity of *Festulolium*

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Abstract. An artificially developed intergeneric hybrid *Festulolium* ($\times$ *Festulolium* F. Aschers, et Graebn.) is the best forage crop with high energy and protein nutrition value which can be used for green fodder when creating cultural haylands and pastures. The advantages of this crop are high regrow capacity, high content of sugars and good winter hardiness. Wide use of this new crop in fodder production is constrained, firstly, because the crop is yet new and little known and, secondly, because there is a lack of seeds due to imperfections in the production technology. There are still very few scientific studies on the biology and technology of *Festulolium* cultivation in the forest-steppe of the Central Chernozem Region of Russia. In our works we were the first to study the biological features of *Festulolium* and develop the main technological methods of growing and harvesting its seeds. The work was carried out in long-term studies of the Department of Crop Science, Forage Production and Agricultural Technologies of Voronezh State Agrarian University in 2009–2011. The soil in the experimental plot was leached medium loamy chernozem. The experiments involved the VIK-90 *Festulolium* variety with the preceding crop being the vetch-oat mixture harvested for green fodder. The soil preparation was conventional for seed herbage of perennial grasses in the Central Chernozem Region. The associated records and observations were made according to conventional methods adopted in the seed production of perennial grasses. A high efficiency of the Aurorex (0.55 L ha^{-1}) and the Dicamba (0.15 L ha^{-1}) herbicides in the suppression of annual and perennial dicotyledonous weed plants has been identified. The application of these herbicides has significantly reduced (by 40–73%) the weed infestation of seed herbage in the first year of vegetation and, as a result, has improved its structure and crop quality. The use of the developed agricultural techniques allows reducing the energy costs and receiving an average of $433\text{--}496 \text{ kg ha}^{-1}$ of certified seeds.

Key words: seed herbage, types of weed plants, shortfall in seed production, efficiency of herbicides, weed infestation.

INTRODUCTION

It is known that perennial grasses are the most efficient and least energy-demanding fodder crops. They allow obtaining fodders with well-balanced protein content, preserve the soil fertility, and increase the ecological safety and sustainability of fodder production (Lazarev et al., 2007; Perepravo, 2007; Shamsutdinov, 2010).

Grass species conventional for the Central Chernozem Region of Russia (*meadow fescue*, *cock's-foot*, *smooth brome*, *timothy grass*, etc.) are characterized by

insufficient content of water-soluble carbohydrates, extensive regrowth after regular disposal cycles, and growth depression in summer.

An important reserve for improving the level of fodder production industry in Russia is the creation of new species and varieties of non-conventional forage grasses on the basis of various selection methods, including distant hybridization. Such newly created species should have improved economic traits and should be introduced into production in order to increase the crop yield and the quality of produced fodders (Perepravo et al., 2011).

An intergeneric hybrid *Festulolium* ($\times$ *Festulolium* F. Aschers. et Graebn.) is a forage crop with high energy and protein nutrition value. It can be used for green fodder, hay, silage, and haylage, as well as in the creation of cultural haylands and pastures. The advantages of this crop over other Poaceae species are high content of sugars, good regrow capacity, winter hardiness, and drought resistance (Kocourkova et al., 2008; Barnes et al., 2014; Kvasnovsky et al., 2014; Schiavon et al., 2014; Kubota et al., 2015).

Festulolium shoots appear slowly and are heavily inhibited by strong stature weeds (Marchenko, 1996; Obraztsov & Fedotov, 2013). A high content of weed seeds in the crop harvest (more than 120 thousand pcs per 1 kg, even with repeated cleaning on seed-cleaning machines) does not always allow obtaining a seed material of high grade by contamination, which leads to large crop losses (Zolotaryov, 1991). Therefore, it is impossible to obtain a stably high yield of seeds without applying efficient means of protection against weed plants.

Weeds not only compete with perennial grasses for nutrition, light, and moisture, in many cases consuming more nutrients and water than cultivated plants, but also complicate the harvesting and post-harvest treatment of seeds. For instance, according to Vadopalas (1982), a shortfall in seed production of perennial grassland grasses with an average weed infestation is 10–20%, and with the basic seed cleaning the loss of seeds reaches another 12–20%. There are a number of weeds, the seeds of which are practically impossible to be removed from the seeds of the cultivated crop. Therefore, in addition to the agrotechnical and chemical means of weed control it is important to use herbicides in the preparation of soil for sowing perennial grasses, especially in their first year of vegetation, and also to improve the mechanical cleaning of seed material. According to Zolotaryov (2015), the agrotechnical and chemical treatments in the presowing period and in the year of sowing of herbs allow obtaining seeds of high grade by contamination and increasing the yield by 30% or more.

Herbicides allow eliminating the sprouted weeds, which ensures a better development of slowly growing plants of fodder crops. As a rule, the aftereffect of applying herbicides in the year of sowing persists on perennial grass seed plantings through all the years of their use. It is especially efficient for wide-row crops in combination with inter-row cultivation. This allows decreasing the contamination of seed herbages with vegetating weeds by 85–95% and reducing the amount of weed seeds in the crop harvest by 2–3 times (Mikhaylichenko, 1987).

At different times the Russian and foreign scientists were involved in the studies of harmfulness of weed plants in the herbages of perennial grasses and the development of measures of their suppression.

Bochkarev et al. (2012) recommended to treat perennial grasses against dandelion with the Agritox herbicide at the rate of 0.6–0.8 L ha⁻¹ in the second year of use after the first mowing.

Chuvilina et al. (2014) established that the application of the Magnum herbicide in its pure form and in tank mixtures significantly reduced the contamination of old-aged perennial grass herbage. The greatest efficiency (91%) in the control of weed plants was obtained by applying a tank mixture of Magnum and Dialen Super. At the same time, the fresh yield of perennial grasses increased by 810 kg ha⁻¹. In the plantings treated with herbicides the feeding mass of perennial grasses exhibited a decrease in ash content (by 0.6–1.2%), sugars (by 1.1–1.8%), fiber (by 2.1–4.1%), and nitrates (by 31–54 mg kg⁻¹), and an increase in carotene content (by 2.1–6.3 mg kg⁻¹) compared to the control.

In the studies of Goliński et al. (2009) the treatment of plantings of the *Felopa* variety of *Festulolium* with herbicides in the first year of use resulted in the death of 41–71% of weeds. The largest increase (36.8%) in the seed yield was obtained when applying the tank mixture of the Lontrel 300 SL + the Chwastox Extra 300 SL herbicides. All the applied herbicides exerted a positive effect on the structure of seed herbage and the yield of seeds.

Due to the availability of a wide range of efficient and environmentally less harmful herbicides, a research on the selection and efficient use of new preparations now deserves special attention.

In our studies our objective was to consider the species composition of weed plants in the plantings of *Festulolium* and to develop a chemical method for combatting them having selected optimal doses of modern herbicides.

MATERIALS AND METHODS

Experimental part of the study was performed in 2009–2011 in field trials of the Department of Crop Science, Forage Production and Agricultural Technologies of Voronezh State Agrarian University named after Emperor Peter the Great on the fields of the Training, Research and Technological Center ‘Agrotechnology’ (N51.7140416 E39.21545371).

The soil in the experimental plot was leached medium loamy chernozem containing 4.56–5.50% of humus, 78–129 g kg⁻¹ of labile phosphorus (P₂O₅), 109–118 mg kg⁻¹ of exchangeable potassium (according to Chirikov), pH_{salt} was from 4.9 to 5.1, the total absorbed bases was from 21.3 to 22.2 mg-eq. per 100 g of soil, and the degree of base saturation was of 74–86%.

The preceding crop for *Festulolium* was the vetch-oat mixture harvested for green fodder. The preparation of soil for sowing was conventional for creating the seed herbage of perennial grasses in the Central Chernozem Region. The experimental design included the control variant (no herbicides applied) and the application of three herbicides at different concentrations in the first year of vegetation of seed herbage: Lontrel Grand (0.120; 0.125; 0.130 kg ha⁻¹), Dicamba (0.10; 0.15; 0.20 L ha⁻¹), and Aurorex (0.50; 0.55; 0.60 L ha⁻¹). The application of herbicides was performed once in the tillering phase using the 16-Litre Hozelock Professional 4816 knapsack sprayer. The efficiency of herbicides was determined by measuring weed infestation. Weed infestation was recorded using the quantitative weight method according to *The Guidelines for Evaluation of Herbicides Applied in Crop Farming* (Larina, 2009) prior to treatment, 30 and 45 days after treatment, and before wintering. The mass of weed plants per 1 m² was weighed in two replicates 30 and 45 days after the application of

herbicides. Seed plantings of *Festulolium* were harvested by the Sampo-130 harvester at the seed moisture of 40–45% with split-plot yield accounting and its subsequent recalculation on the basis of 12% moisture and 100% seed purity. The experiment was laid in 4 replicates with the randomized location of the plots. The area of the registration plot was 20 m². Experiments, relevant records and observations were carried out according to standard *Methodological Instructive Regulations...* (1986) for seed production of perennial grasses.

RESULTS

The growth of *Festulolium* shoots is considerably suppressed by strong-stature weeds (Zolotaryov, 1991). However, there are still no herbicides allowed for application in *Festulolium* plantings.

The weed contamination of seed plantings of *Festulolium* was determined in the first and second years of vegetation. In the year of creation of *Festulolium* seed herbage there were more than 16 weed species in the plantings belonging to 7 different families: *Chenopodiaceae*, *Poaceae*, *Asteraceae*, *Brassicaceae*, *Convolvulaceae*, *Geraniaceae*, and *Euphorbiaceae*. In different years the following weeds were dominant: perennial weeds – *Convolvulus arvensis* L., *Cirsium arvense* L., *Sonchus arvensis* L., *Agropyron repens* L., *Taraxacum officinale* Wigg., *Barbarea vulgaris* R. Br., *Euphorbia virgata* Waldst. & Kit., *Artemisia absinthium* L.; spring weeds – *Chenopodium album* L., *Capsella bursa-pastoris* L., *Panicum crus galli* L., *Thlaspi arvense* L., *Galium aparine* L., *Setaria glauca* L.; annual and biennial wintering weeds – *Matricaria inodora* L., *Erodium cicutarium* L.

It was found that in 2009 the annual and biennial weeds represented 54.1% of weeds with the domination of wintering species (25.9%) (Table 1).

Table 1. Groups of weeds in the seed herbage of *Festulolium* in the first year of vegetation, %

Groups of weed species	2009	2010	2011
Annual and biennial, in total, including:	54.1	60.3	54.4
spring early	7.7	8.6	5.1
spring late	20.5	29.4	25.1
wintering	25.9	22.3	24.2
Perennial, in total, including:	45.9	39.7	45.6
soboliferous	36.2	30.6	34.5
rhizomatous	3.3	1.2	2.2
taproot	6.4	7.9	8.9

In 2010 the annual and biennial weed species were dominant (60.3%), mainly *Matricaria inodora* L., *Panicum crus galli* L., *Thlaspi arvense* L., and *Capsella bursa-pastoris* L. Among perennial species the *Sonchus arvensis* L. and *Convolvulus arvensis* L. were dominant. Their population varied throughout the vegetation season. The amount of annual and biennial weeds increased by 14.1%, whereas the amount of perennial weeds increased only by 4.1%. Such insignificant increase in the population of weeds in 2010 was due to the drought, especially in the 2nd and 3rd decades of May.

In the conditions of 2011 the weed contamination was represented by 45.6% of perennial and 54.4% of annual and biennial weed species. *Thlaspi arvense* L., *Setaria glauca* L., and *Matricaria inodora* L. were dominant among the annual and biennial weeds, while *Convolvulus arvensis* L., *Cirsium arvense* L. and *Taraxacum officinale* Wigg. were dominant among perennial weeds.

Our research showed that the application of the Aurorex herbicide in a dose of 0.50 L ha⁻¹ ensured a decrease in the total amount of weeds by 56.2% (including a decrease in the amount of perennial species 57.6%) already in 30 days. The efficiency of this herbicide against annual and biennial weeds during the first post-treatment period was not very high (61.2%) due to the presence of resistant grass weeds in *Festulolium* plantings (namely *Panicum crus galli* L., *Setaria glauca* L. and *Agropyron repens* L.). After 45 days the density of annual and biennial weeds decreased from 75.4 to 30.8 plants per 1 m², i.e. by 59.2%, and by the end of vegetation the total weed count in this variant was 49 plants per 1 m², which was 63.4% lower compared to the control variant (Table 2).

The Aurorex herbicide in a dose of 0.55 L ha⁻¹ decreased the total weed count to 60.5 plants per 1 m² after 30 days and to 36.1 plants per 1 m² by wintering. The total weed count decreased by 64.3% after 30 days, and by 73% by the end of vegetation season compared to control. The highest mortality (64.7%) was observed in perennial weeds after 30 days and reached 70% by wintering. The mortality of annual and biennial weeds was slightly lower compared to perennial, but it was also rather high: 64.1% after 30 days and 71.1% by wintering. The total weed mortality was 64.3–70.6%. The application of the Aurorex herbicide in a dose of 0.60 L ha⁻¹ yielded the highest herbicidal effect. The total decrease in the annual, biennial and perennial weed count in the plantings reached 69.0–77.5%. The maximum herbicidal efficiency against perennial weed species was 75.3%.

The Dicamba herbicide in a dose of 0.10 L ha⁻¹ decreased the total count of all weed species by 50% 30 days after its application. Throughout the vegetation period the weed mortality increased and reached 57.5% by wintering. A higher decrease was observed in perennial weed count: by 46.9% in the first recorded period and by 52.4% by the end of vegetation season. Thirty days after the treatment of plantings with the Dicamba herbicide in a dose of 0.15 L ha⁻¹ the annual and perennial weed count decreased by 66.8% and 61.1%, which was significantly more efficient than the dose of 0.1 L ha⁻¹.

Increasing the dose of the Dicamba herbicide up to 0.20 L ha⁻¹ increased the total weed mortality that reached 68.9% 30 days after the application and 74% by the end of vegetation season. This was much more efficient compared to the dose of 0.15 L ha⁻¹. The highest decrease in weed count was observed for annual and biennial weeds, i.e. by 72.2% 30 days after treatment and by 77.1% by wintering.

An increase in the dose of the Dicamba herbicide up to 0.20 L ha⁻¹ led to a further decrease in the weed count of *Sonchus arvensis* L., *Taraxacum officinale* Wigg., and *Cirsium arvense* L. At the end of vegetation season a complete death of *Convolvulus arvensis* L. was observed.

Table 2. The effect of herbicides on weed count and mass of weeds in *Festulolium* plantings in the first year of vegetation (averaged for the 2009–2011 period)

Types of weeds	Term after treatment	Control, pcs per 1 m ² or g per 1 m ²	Aurorex, L ha ⁻¹						Dicamba, L ha ⁻¹						Lontrel Grand, kg ha ⁻¹					
			0.5		0.55		0.6		0.1		0.15		0.2		0.12		0.125		0.13	
			a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Weed count																				
Annual and biennial	after 30 days	89	40	55	32	66	25	73	41	53	29	69	24	74	62	30	59	37	57	39
	after 45 days	75	31	59	24	70	20	75	28	62	22	73	17	78	54	29	50	38	48	40
	before wintering	72	28	61	19	74	16	79	25	65	20	74	16	79	47	35	40	47	37	51
Perennial	after 30 days	81	34	58	29	63	22	71	43	47	32	59	28	63	49	40	43	44	38	51
	after 45 days	70	27	62	20	69	20	70	33	52	26	60	20	68	38	46	35	47	30	55
	before wintering	62	21	66	15	73	14	75	29	52	22	62	18	69	30	52	27	52	24	59
Total	after 30 days	170	74	56	60	65	47	72	84	50	61	65	53	69	111	35	103	40	95	45
	after 45 days	145	57	60	44	70	39	73	62	58	48	67	38	74	91	37	84	42	77	47
	before wintering	134	49	63	35	74	30	77	54	59	41	69	34	75	77	43	68	49	61	55
Mass of weeds																				
Annual and biennial	after 30 days	39	19	52	15	61	14	66	16	75	13	81	10	85	30	64	27	69	26	70
	after 45 days	32	14	56	11	67	9	71	13	82	11	85	8	88	24	73	22	75	20	78
Perennial	after 30 days	43	20	52	15	64	13	69	24	65	16	77	13	81	23	71	22	72	20	75
	after 45 days	40	17	58	13	68	12	69	18	74	12	82	11	84	21	76	20	76	17	79
Total	after 30 days	82	39	52	31	63	27	67	40	40	29	58	24	65	54	17	49	25	46	29
	after 45 days	72	31	57	24	67	22	70	31	56	23	67	19	73	44	39	42	42	37	49

a – weed count, pcs per 1 m²; mass of weeds, g per 1 m²; b – decrease in weed count in % compared to control.

The Lontrel Grand herbicide in a dose of 0.120 kg ha⁻¹ decreased the contamination of *Festulolium* plantings by 26% for annual and biennial weeds and by 39.9% for perennial weeds 30 days after treatment. This herbicide was highly efficient against *Sonchus arvensis* L., *Taraxacum officinale* Wigg., *Convolvulus arvensis* L., and *Cirsium arvense* L. In the dose of 0.125 kg ha⁻¹ its efficiency increased significantly. The total mortality of weeds 30 days after treatment and at the end of vegetation season reached 38.1% and 47.1%, respectively. The highest efficiency of the Lontrel Grand herbicide was registered in a dose of 0.13 kg ha⁻¹.

The Aurorex herbicide appeared to be more efficient in *Festulolium* plantings. In the variants where it was applied in the year of seed harvesting the structure of yield components of seed herbage was considerably improved (Table 3).

Table 2. The effect of herbicides on the structure of deed herbage and seed yield of *Festulolium* the second year of vegetation (averaged for the 2010–2011 period)

Herbicide	Dose, L·ha ⁻¹ , kg·ha ⁻¹	Count generative shoots, pcs per 1 m ²	Ear length, cm	Number of seeds per 1 ear, pcs	Seed yield, kg ha ⁻¹
Control	0.00	739	15.7	60.0	432.7
Aurorex	0.50	798	16.0	59.5	480.5
(a.i. 21 g L ⁻¹ carfentrazone-ethyl + 500 g L ⁻¹ complex 2-ethylhexanol ester 2,4-D)	0.55 0.60	810 785	16.1 15.8	57.0 59.0	496.4 472.0
Dicamba	0.10	766	16.1	57.5	459.5
(a.i. 480 g L ⁻¹ dicamba acid)	0.15 0.20	774 756	15.8 15.7	60.0 56.5	464.8 449.6
Lontrel Grand	0.120	741	15.9	59.5	433.3
(a.i. clopyralid 750 g kg ⁻¹)	0.125 0.130	729 716	15.8 15.4	55.5 56.5	428.8 419.5
LSD ₀₅		27.1	0.14	3.3	19.5

For instance, the average generative shoot count over three years in the variant with Aurorex application was 785–810 pcs per 1 m² compared to 739 pcs per 1 m² in the control. This herbicide was characterized by a broad spectrum of activity and eliminated the weeds in the herbage when applied in a dose of 0.55 L ha⁻¹.

The number of vegetating weeds decreased to 45%, which resulted in the highest yield of *Festulolium* seeds in the experiment (496.4 kg ha⁻¹). The Dicamba herbicide was efficient against weeds in a dose of 0.15 L ha⁻¹. Having provided the mortality of 67.2% of weeds, it increased the yield of *Festulolium*, which reached 464.8 kg ha⁻¹.

The treatment of plantings with the Lontrel Grand herbicide resulted in a noticeable depression of *Festulolium* plants, especially in the first days, and the level of their depression increased with the dose of the herbicide. However, in the subsequent period the plants developed normally. Nevertheless, when this herbicide was applied in the doses of 0.125 and 0.130 kg ha⁻¹ there was a certain trend towards the decrease in *Festulolium* yield compared to control, although it was insignificant.

The cost of the obtained seeds per 1 hectare was determined using data on sales prices of *Festulolium* seeds (as of the end of 2011) and the crop yield. The cost of the obtained seeds was RUB'000 51.90 on the control variant, and ranged from RUB'000 50.34 to 59.52 on other variants (Table 4).

Table 4. Economic efficiency of producing *Festulolium* seeds depending on the application of herbicides (averaged for the 2010–2011 period)

Herbicide	Dose, L ha ⁻¹ , kg ha ⁻¹	Value of production, RUB'000 per 1 hectare	Costs, RUB'000 per 1 hectare	Cost of 100 kg of seeds, RUB'000 per 1 hectare	Net income, RUB'000 per 1 hectare	Level of profitability, %
Control	0.00	51.90	29.18	6.74	22.71	78
Aurorex	0.50	57.66	30.19	6.28	27.46	91
(a.i. 21 g L ⁻¹	0.55	59.52	30.29	6.10	29.22	97
carfentrazone-ethyl + 500 g L ⁻¹ complex 2-ethylhexanol ester 2,4-D)	0.60	56.64	30.39	6.44	26.24	86
Dicamba	0.10	55.14	30.29	6.59	24.84	82
(a.i. 480 g L ⁻¹	0.15	55.74	30.19	6.54	25.34	83
dicamba acid)	0.20	53.88	29.55	6.58	24.32	82
Lontrel Grand	0.120	52.02	30.09	6.85	22.05	75
(a.i. clopyralid	0.125	51.42	29.36	7.01	21.62	71
750 g kg ⁻¹)	0.130	50.34	29.92	7.13	20.14	68

Total costs per 1 hectare of planted area depended on the doses of applied herbicides and their cost. For instance, the lowest costs were observed on the control variant (RUB'000 29.18). The maximal costs (RUB'000 30.39) were observed on the plantings treated with the Aurorex herbicide in a dose of 0.60 L ha⁻¹.

The cost of the obtained seeds on the control variant was RUB'000 6.74 per 100 kg. The maximal cost (RUB'000 7.13) was noted on the plantings treated with the Lontrel Grand herbicide in the dose of 0.13 kg ha⁻¹, while the minimal cost (RUB'000 6.10) was observed on the plantings treated with the Aurorex herbicide in a dose of 0.55 L ha⁻¹.

The highest net income (RUB'000 29.22) and the maximal level of profitability (97%) were obtained with the application of the Aurorex herbicide in a dose of 0.55 L ha⁻¹.

Thus, in order to create a highly productive seed herbage of *Festulolium* it is necessary to apply herbicides in the year of sowing in order to eliminate the dicotyledonous weeds. The application of Aurorex in a dose of 0.55 L ha⁻¹ or Dicamba in a dose of 0.15 L ha⁻¹ proved to be the most efficient. It ensured the mortality of 69.5–73.0% of weeds and the yield of 464.8–496.4 kg ha⁻¹ of high-quality *Festulolium* seeds.

CONCLUSIONS

The following conclusions can be derived on the basis of our studies conducted in 2009–2011 during seed harvesting in the conditions of the forest-steppe of the Central Chernozem Region and devoted to winter hardiness, growth, development and seed productivity of *Festulolium* depending on application of herbicides.

The application of the Aurorex herbicide in a dose of 0.55 L ha⁻¹ in *Festulolium* plantings ensures the elimination of 73% of weeds. The mass of weed plants decreases by 67.3%. The yield of *Festulolium* seeds exceeded the control variant by 63.7 kg ha⁻¹ and provided the highest economic efficiency (the net operating profit amounted to

RUB'000 29.22 per hectare, the prime cost of 100 kg of seeds did not exceed RUB'000 6.10, and the profitability reached 97%).

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Effect of nitrogen fertilization on sorghum for biomass production

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Abstract. Two field experiments were carried out in 2005 and 2006 in central Italy in order to evaluate the effects of different nitrogen (N) application rates (0, 50 100 and 150 kg ha⁻¹) on flowering date, plant height, biomass production and partitioning (leaves, panicles and stems) and biomass quality of a sorghum hybrid (H133). Sorghum showed a high potential in terms of biomass production without N fertilization (18.5 t ha⁻¹ of d.m. in 2005 and 26.6 t ha⁻¹ of d.m. in 2006). The rate that maximized the biomass production was 100 kg ha⁻¹ of N, increasing the biomass dry weight by 23.8% in 2005 and 18.8% in 2006, with respect to unfertilized sorghum; higher N rates are not advisable in order to avoid increasing fertilization costs and environmental impact without benefit of greater biomass production. The two highest N rates when combined with low water availability appeared to increase the rate of plant development, causing earlier flowering and increasing the percentage of panicles in total biomass. Higher heating value (HHV), lower heating value (LHV) and ash concentration of biomass varied among N rates, with values of HHV and LHV lower for unfertilized sorghum (17.6 and 16.7 MJ kg⁻¹ d.m., respectively) than when N was applied (from 19.0 to 19.7 and from 18.1 to 18.8 MJ kg⁻¹ d.m., respectively); on the contrary, ash concentration was greater for unfertilized sorghum (7.5% d.m.) than for fertilized sorghum (from 5.8 to 6.7% d.m.). This research showed the high potential of sorghum in terms of biomass production also when cultivated with limited irrigation and fertilization inputs. The biomass dry yield obtained by one hectare of sorghum crop without N nitrogen fertilization (i.e. 22.6 t ha⁻¹ of d.m., average of 2005 and 2006 values) produces the same energy, by thermal utilisation, of 9.3 toe, that is equivalent to energy produced by 10,385 L of diesel fuel or 11,097 m³ of methane fuel. This aspect increases the certainty of the energetic and environmental sustainability of sorghum crop.

Key words: ash content, biomass quality, biomass yield, heating values, energy crop, N rate.

INTRODUCTION

Sorghum [*Sorghum bicolor* L. (Moench)] can be classified as grain, sweet, forage and biomass types (Dogget, 1998; Almodares et al., 2009). It is a widely adapted crop with potential for bioenergy production thanks to its relatively low input requirements, drought and salinity tolerance, ability to use water efficiently and to maintain high yields under a wide range of soil and environmental conditions (Regassa & Wortmann, 2014; Shakeri et al., 2017). Sorghum is used to obtain the most disparate products: food, forage, paper pulping, plastics, sugar for bioethanol and biomass for energy use

(Zegada-Lizarazu & Monti, 2012; Pannacci & Bartolini, 2016). In general, sweet sorghum types are richer than forage or biomass ones in the content of non-structural carbohydrates (sucrose, glucose, fructose and starch) (Almodares et al., 2011); while the biomass and forage types are predominantly composed by structural carbohydrates (hemicellulose, cellulose, and lignin) and their biomass can be used for combustion and 2nd generation biofuels (Zegada-Lizarazu & Monti, 2012). Sorghum is a C4 crop with a high biomass yield and good N use efficiency (Gardner et al., 1994). N is essential for plant growth and it is one of major factors limiting crop yield (Zhao et al., 2005). However, in this context, the evaluation of N requirement is crucial in order to quantify the rate of application needed to ensure high biomass production without waste. In fact, N fertilizer production is associated with significant CO₂ emissions and energy consumption, decreasing the net energy obtained by the crop (Lewandowski et al., 1995). Furthermore, the high mobility of N in the soil can increase the potential risk of aquifer pollution (Jaynes et al., 2001; Celik et al., 2017). Biomass sorghum potentially is a good feedstock candidate for the biofuel energy industry, but management information for the crop is still limited (Shahandeh et al., 2015). Currently, most management practices for energy sorghum production are based on interpolations from forage, grain and sweet sorghum production guidelines (Buxton et al., 1999; Han et al., 2012; Kering et al., 2017). The biomass yields of sweet sorghum have been reported to vary across a range of N fertilizer rates, cultivars, and plant populations (Uchino et al., 2013; Olugbemi & Ababyomi, 2016). Instead, information on the response of biomass sorghum to N fertilizer in conjunction with other management factors is still scarce, although is slowly accumulating. Maughan et al. (2012) observed responses up to 150 kg N ha⁻¹ in 2009 and 224 kg N ha⁻¹ in 2010 for biomass sorghum in southern Illinois, while recently, Hao et al. (2014) determined that optimal N fertilizer rates for yield and efficiency for photoperiod-sensitive sorghums in the Texas High Plains were 183 and 78 kg N ha⁻¹, respectively, in 2010, and 148 and 90 kg N ha⁻¹ in 2011. The objectives of this study, carried out under environmental conditions of central Italy, were to evaluate the effects of different N application rates on flowering date, plant height, biomass production and partitioning (leaves, panicles and stems) and biomass quality of a biomass sorghum hybrid.

MATERIALS AND METHODS

Two field experiments were carried out in 2005 and 2006 in central Italy (42°57'N, 12°22'E, 165 m a.s.l.) on two adjacent fields (one for each year), with similar characteristics in terms of agronomic practices and soil composition (clay-loam soil, 22% sand, 35% clay and 43% silt, 1.5% organic matter). The sorghum hybrid H133 was used (Table 1).

Experimental design was a randomized complete block with four replicates and plot size of 32 m² (4 m width). The experimental treatments were represented by different N rates: 0, 50, 100 and 150 kg ha⁻¹ of N. Each plot was established from eight rows, six central rows for measurements and two border rows on the perimeter of each plot to reduce potential border effects. The main agronomic practices are shown in Table 1. The trials were carried out in accordance with good ordinary practices, as concerns soil tillage, seedbed preparation and weed control (Bonciarelli & Bonciarelli, 2001), adopting low input in terms of irrigation. In both years, wheat, as preceding crop,

was not fertilized with N in order to reduce greatly the soil nitrogen content and then available for the subsequent sorghum crop, with the aim to obtain the zero nitrogen level in the field experiment. A pre-plant fertilization based on phosphorus and potassium was applied on sorghum (Table 1).

Table 1. Agronomic practices in the field experiments

Year	2005	2006
Preceding crop	Wheat	Wheat
Pre-plant fertilization (kg ha ⁻¹)	75 P ₂ O ₅ ; 75 K ₂ O	75 P ₂ O ₅ ; 75 K ₂ O
Sowing date	17 May	15 May
Sorghum hybrid	H133	H133
Density (plants m ⁻²)	31	31
Spacing between rows (m)	0.5	0.5
N fertilizer at sowing	23 May	17 May
Emergence date	25 May	19 May
Irrigation: m ³ ha ⁻¹ (n.)	600 (3)	1,150 (4)
Pre-emergence weed control	Terbuthylazine (750 g ha ⁻¹)	Terbuthylazine (750 g ha ⁻¹)
Harvest	13 October	25 September

Measurements and statistical analysis

The plant height of sorghum was measured at the height of the last leaf on 30 plants per plot at 92 and 95 days after emergence (DAE), in 2005 and 2006, respectively. Flowering time was the date (reported as DAE) at which 50% of plants in each plot were flowered and was used to evaluate the effect of N on the length of the growth cycle. The fresh and dry weight of biomass of sorghum and the moisture concentration were determined at harvest (141 DAE in 2005 and 129 DAE in 2006). At harvest, identified around 3 weeks after the soft dough stage of grain filling, the plants from the six rows in the central part of each plot (21 m⁻²) were cut, subdivided in stems, leaves and panicles and their weight was evaluated. A sample from each plant part (20% of total fresh biomass) was taken, weighed fresh and oven dried at 105 °C to a constant weight in order to assess moisture concentration, dry weight of biomass and then an equivalent yield (t ha⁻¹) for each plot. Furthermore, in 2006, a sample of total dry biomass was obtained collecting a sub-sample of biomass (taking stems, leaves and panicles at the quantity of 10% of their respective weight) from each pot and then compositing the samples across replicates within a nitrogen treatment so that there were a total of four composite samples, i.e., one for each nitrogen treatment. These samples were analysed to determine lower and higher heating values (LHV and HHV) and ash concentration of biomass, using, respectively, a calorimeter (AC-350 Leco) and a thermo-gravimetric analyzer (TGA-701 Leco).

All data (except HHV, LHV and ash concentration, because without replications) were subjected to ANOVA using the EXCEL® Add-in macro DSASTAT (Onofri & Pannacci, 2014). The year and treatment (N rate) were treated as fixed factors, with replication being a random factor. The differences between treatment means were separated using Fisher's protected LSD at P = 0.05 level when ANOVA was significant. A combined analysis of data showed that the interactions 'years x N rate' were significant (P < 0.05); therefore, the results were shown and discussed separately for each year.

Meteorological data (daily maximum and minimum temperature and rainfall) were collected from a nearby station. The average decade of daily values was calculated and compared with multi-annual average values (from 1921) (Fig. 1).

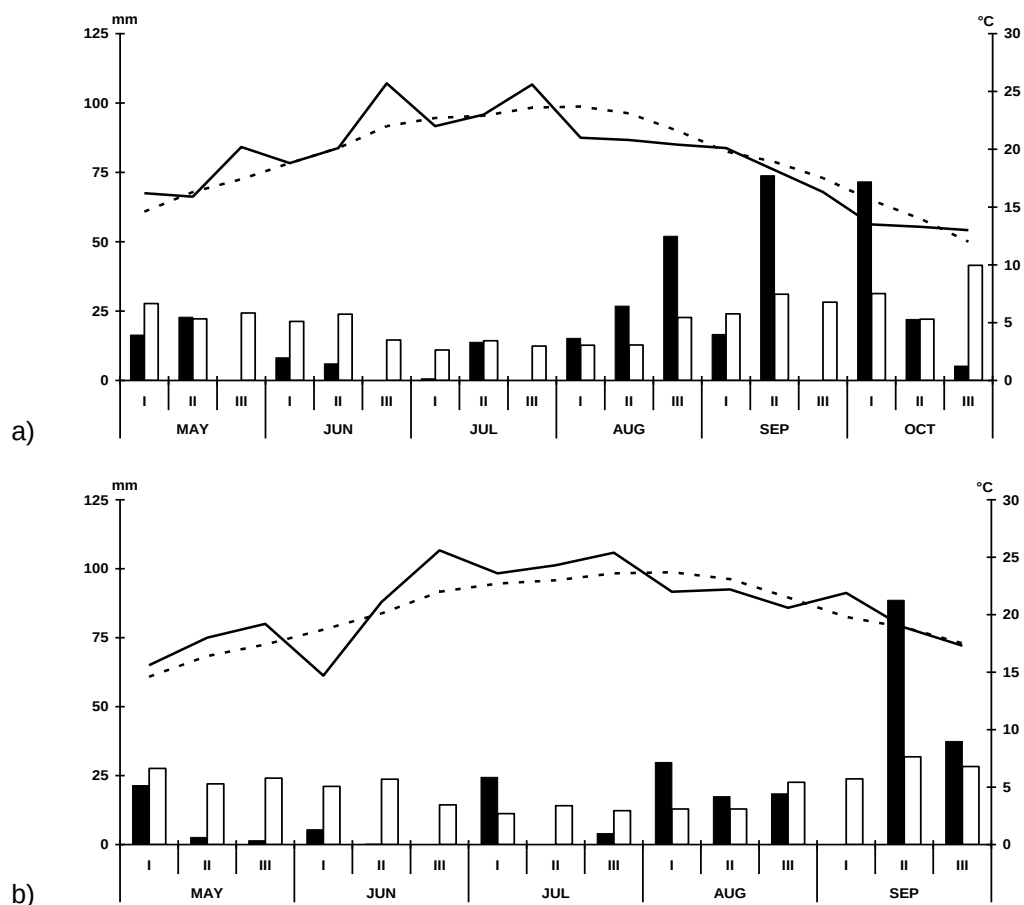


Figure 1. Average ten days values of rainfall (mm; bold bar) and temperature (°C; solid line) recorded during the experimental trial in 2005 (a), 2006 (b), compared to multi-annual (from 1921) averages (rainfall: mm, empty bar; temperature: °C, sketched line).

RESULTS AND DISCUSSION

In 2005, all response variables were significantly affected by N rates (Table 2).

In particular, N rates of 100 kg ha⁻¹ and 150 kg ha⁻¹ increased rate of plant development, shortening time to flower by 6 and 7 days, respectively, relative to the unfertilized crop (Table 2). N rates affected plant height, with unfertilized plants being taller than those in 100N and 150N plots (Table 2). Biomass production of sorghum fertilized with 100 and 150 kg N ha⁻¹ was greater than unfertilized sorghum (0N), but there was no difference between the two highest N rates (Table 2). The rate with maximum numeric biomass production was 100 kg ha⁻¹ of N, and biomass dry weight was 23.8% greater for this treatment than unfertilized sorghum (Table 2). The moisture

concentration of biomass ranged from 67% for 100N and 150N, to 71% for 0N (Table 2). Total dry biomass yield partitioning showed that only data of stems and panicles were significantly different among N rates (Table 3).

Table 2. Differences among the N nitrogen rates in terms of flowering time, plant height, dry weight and moisture content of biomass in 2005

N rate (kg ha ⁻¹)	Flowering time (DAE)	Plant height (m) (92 DAE)	Dry weight of biomass, t ha ⁻¹ (141 DAE)	Moisture concentration of biomass, % (141 DAE)
0	93.4 a	3.01 a	18.5 b	71.2 a
50	90.1 ab	2.85 ab	19.7 ab	68.5 ab
100	87.0 b	2.79 b	22.9 a	66.9 b
150	86.6 b	2.69 b	22.7 a	67.1 b
Average	89.3	2.84	21.0	68.4
LSD (p=0.05)	3.9	0.19	3.4	2.8

DAE: days after emergence. In each column, values followed by the same letter are not significantly different according to the Fisher's protected LSD test (P = 0.05).

In particular, N increased significantly the percentage of panicles on behalf of stems in the partitioning of total biomass with respect to the unfertilized sorghum (Table 3).

In 2006, only biomass production was affected by N rates (Table 4). This can be explained by the greater irrigation volume in 2006 than in 2005 that reduced the effects of N rates in terms of flowering time and plant height, maintaining the effects on dry weight of biomass, as already observed by Moghaddam et al. (2007). In particular, flowering time was 102 DAE (average value) with the plant height of 3.34 m (average value at 95 DAE).

Table 3. Total dry biomass yield partitioning as determined by the weight of stems, leaves and panicles at the different N rates in 2005

N rate, kg ha ⁻¹	Total dry biomass partitioning, %		
	Stems	Leaves	Panicles
0	70.7 a	18.8	10.5 b
50	62.8 b	16.6	20.6 a
100	61.5 b	18.7	19.8 a
150	61.9 b	20.3	17.7 a
Average	64.2	18.6	17.2
LSD (p=0.05)	5.3	n.s.	6.6

n.s. = no significant differences. In each column, values followed by the same letter are not significantly different according to the Fisher's protected LSD test (P = 0.05).

Table 4. Differences among the N rate in terms of flowering time, plant height, dry weight and moisture content of biomass in 2006

N rate, kg ha ⁻¹	Flowering time (DAE)	Plant height, m (95 DAE)	Dry weight of biomass, t ha ⁻¹ (129 DAE)	Moisture content of biomass, % (129 DAE)
0	102	3.33	26.6 a	68.2
50	102	3.31	29.1 ab	68.7
100	102	3.40	31.6 b	68.7
150	101	3.30	29.4 ab	69.3
Average	102	3.34	29.2	68.7
LSD (p=0.05)	n.s.	n.s.	3.16	n.s.

DAE: days after emergence; n.s. = no significant differences. In each column, values followed by the same letter are not significantly different according to the Fisher's protected LSD test (P = 0.05).

The dry weight of biomass showed significant differences among N rates, confirming as 100 kg ha⁻¹ of N seems to be near the optimal rate in order to maximize biomass production. In fact, in both years (see Tables 2 and 4), dry weight of biomass (dependent variable y) showed a quadratic response to N rate (independent variable x), according to the follow equations:

$$y = -0.0001x^2 + 0.0525x + 18.226 \text{ (R}^2 = 0.899\text{) in 2005,} \tag{1}$$

$$y = -0.0005x^2 + 0.0928x + 26.353 \text{ (R}^2 = 0.914\text{) in 2006,} \tag{2}$$

whose relationships are showed in Fig. 2.

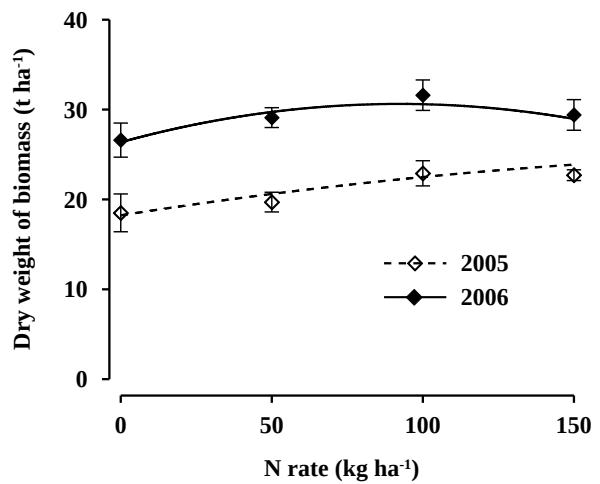


Figure 2. Relationships between N rate and dry weight of sorghum biomass observed in 2005 and 2006. Error bars represent ± standard errors of the means (n = 4).

In 2006, the total dry biomass yield partitioning was not affected by N rates (Table 5). In particular, there was a reduction in proportion of panicles and an increase in stem proportion in 2006 relative to 2005.

HHV, LHV and ash concentration of biomass showed different values among N rates, with values of HHV and LHV lower for unfertilized sorghum (17.6 and 16.7 MJ kg⁻¹ d.m., respectively, see

Table 6) than in the cases of N applications (from 19.0 to 19.7 and from 18.1 to 18.8 MJ kg⁻¹ d.m., respectively); while, on the contrary, ash concentration was greater for unfertilized sorghum (7.5% d.m.) than for fertilized sorghum (from 5.8 to 6.7% d.m.) (Table 6).

Table 5. Total dry biomass yield partitioning as determined by the weight of stems, leaves and panicles at the different N rates in 2006

N rate, kg ha ⁻¹	Total dry biomass partitioning (%)		
	Stems	Leaves	Panicles
0	76.6	16.6	6.8
50	75.5	17.7	6.8
100	76.7	17.1	6.2
150	75.6	18.7	5.7
Average	76.1	17.6	6.4
<i>LSD (p = 0.05)</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>

n.s. = no significant differences.

The faster rate of sorghum development when subjected to higher N rates supports the report of Uchino et al. (2013) who evaluated sweet sorghum in the semi-arid tropical zone of India. The extended duration of the growth cycle for the unfertilized plants (0N), means that these plants grow faster than fertilized plants (100N and 150N), as shown by plant height at 92 DAE (Table 2). The delay of flowering

time in the 0N implies an extension of the growth cycle and an increase in time available for plant growth, resulting in taller plants. On the other hand, this study confirms the positive effects of N on biomass production, identifying 100 kg N ha⁻¹ as an optimum rate in terms of biomass production while minimizing fertilizer costs and environmental impact. Furthermore, considering the average values 2005–2006 of sorghum biomass production, 100 kg N ha⁻¹ is resulted to be the most economic N rate, thanks to the highest difference between profit to biomass sale and cost of N rate (Table 7). Similar results, in term of biomass production, were obtained by Cozzolino et al. (2007) with the same sorghum hybrid (H133) at the same N rate (100 kg ha⁻¹), in the Mediterranean area with similar weather conditions. However, the previous authors tested sorghum under different N rate, in the southern Italy and only for one year. For these reasons, this research, thanks to two years of experimentation, allowed to confirm definitely the potentiality of sorghum as biomass crop, in response to different N rates, in central Italy. Furthermore, the quadratic response between dry weight of biomass and N rate found in this study was reported also by Kering et al. (2017) investigating the effect of N fertilizer on five sweet sorghum varieties in mid-central Virginia.

Table 6. Heating values (HHV and LHV) and ash concentration of total dry biomass for the different N rates in 2006

N rate, kg ha ⁻¹	HHV, MJ kg ⁻¹ d.m.	LHV, MJ kg ⁻¹ d.m.	Ash concentration, % d.m.
0	17.6	16.7	7.5
50	19.0	18.1	6.1
100	19.7	18.8	5.8
150	19.0	18.1	6.7
Average	18.8	18.0	6.5
<i>S.E.</i>	<i>0.43</i>	<i>0.44</i>	<i>0.37</i>

Table 7. Evaluation of the most economic N rate (average 2005–2006)

N rate, kg ha ⁻¹	Dry weight of biomass t ha ⁻¹ (Average 2005–2006)	Profit of biomass sale*, € ha ⁻¹ (A)	Cost of N rate**, € ha ⁻¹ (B)	Difference, € ha ⁻¹ (A–B)
0	22.6	523.2	0	523.2
50	24.4	566.1	37.5	528.6
100	27.3	632.2	75	557.2
150	26.1	604.4	112.5	491.9

* calculated considering the actual price of sorghum biomass equal to 23.2 € t⁻¹; ** calculated considering the actual cost of urea nitrogen equal to 0.75 € kg⁻¹.

The biomass yield results for sorghum showed its high potential for biomass production in central Italy, confirming the data obtained by the same authors in the same area (Pannacci & Bartolini, 2016) and by other authors in the same country (Habyarimana et al., 2004; Quaranta et al., 2010; Marsalis & Bean, 2010). On average, the values of biomass production, plant height and flowering time of sorghum were greater in 2006 than in 2005. This was likely due to greater irrigation volume in 2006

than 2005 (Table 1), since the weather conditions during the sorghum growth cycle (from May to September) were similar in 2005 and 2006 with rainfall of 252 mm and 251 mm, respectively (Fig. 1). This is in accordance with Montemurro et al. (2002) who found increasing N Use Efficiency (NUE) increase when irrigation increased up to 100% of crop evapotranspiration (ETc). Similarly, Marsalis & Bean (2010) indicated that in irrigated environments with high yield potentials, N application as high as 269 kg N ha⁻¹ may be needed, while little to no N fertilizer may be required under dry land conditions.

The moisture concentration of biomass was greater at 0N than at 100N and 150N, and this is in accordance with the extending of the growth cycle at low N rates, resulting in a greater moisture concentration of sorghum biomass at harvest (141 DAE). Similarly, concerning total dry biomass yield partitioning, the percentage of panicles was greater in fertilized than unfertilized sorghum due to the earlier flowering induced by N that allowed the proportion of panicle to increase until harvest, as already reported for sweet sorghum (FAO, 2017). Furthermore, the reduction of panicle proportion in favor of increased proportion of stems in 2006 relative to 2005, is likely due to greater irrigation volume in 2006 that prolonged the growth cycle, delaying the flowering time and as a consequence reducing panicle growth before harvest (occurred at 129 DAE in 2006 and 141 DAE in 2005).

The results of quality of biomass (HHV, LHV and ash concentration) were comparable to those of Pannacci & Bartolini (2016) and Monti et al. (2008). However, the quality of biomass can be influenced by management practices and environmental conditions as reported by Pannacci et al. (2009) and Singh et al. (2012). Furthermore, Monti et al. (2008) observed that leaves and panicles have greater ash concentration than stems, while Obernberger et al. (2006) reported that low ash concentration is preferred for solid biofuels in order to avoid high deposit formation, corrosion and fly ash emissions during thermal utilization. In order to reduce the leaf component in the total biomass yield, Pannacci & Bartolini (2016) suggested to separate the leaves at harvest time, using only the stems as biofuel. Leaves could then be incorporate into the soil with the aim of reducing loss of nutrients and potentially increasing organic matter in the soil.

Overall, this research showed the high potential of sorghum in terms of biomass production when cultivated with limited irrigation and fertilization inputs, as demonstrated by dry weight of biomass without N fertilization of 18.5 t ha⁻¹ of d.m. in 2005 and 26.6 t ha⁻¹ of d.m. in 2006. Furthermore, considering the LHV value at 0N (16.7 MJ kg⁻¹ of d.m.) (Table 6), one tonne of dry biomass of sorghum corresponds to 16,700 MJ. Since 1 tonne of oil equivalent (toe) = 41,868 MJ, one tonne of dry biomass of sorghum may be expressed as 0.41 toe. As a consequence, the biomass dry yield obtained by one hectare of sorghum crop without N fertilization (i.e. 22.6 t ha⁻¹ of d.m., average of 2005 and 2006 values) produces the same energy, by thermal utilisation, of 9.3 toe, that is equivalent to energy produced by 10,385 L of diesel fuel or 11,097 m³ of methane fuel. The above mentioned aspect increases the certainty of the energetic and environmental sustainability of sorghum crop, as already reported by Venturi & Venturi (2003). In fact, in the sorghum crop for biomass production, the mineral fertilization is the highest input (60.3% of the total energy consuming) to consider in the energy balance (Bartolini, 2008). This research was able to point out as sorghum for biomass production needs to N in order to maximize yield. However, this crop seems to be sustainable both from an energetic and environmental point of view, thanks to its highly documented

drought tolerance and low input requirements that allows it to maintain high yields also with low N input and low water supply.

ACKNOWLEDGEMENTS. This study was supported by EU Project (reg. CE 2182/2002) ‘Analysis and evaluation of alternative crop systems in the areas subject to tobacco reconversion-Co.Al.Ta.2’ with technical supervision of MiPAAF (Italian Ministry for Agriculture, Food and Forestry). The authors would like to thank Prof. Gino Covarelli as coordinator of the project and for the suggestions in the research.

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Inoculation technology for legumes based on alginate encapsulation

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Abstract. The main purpose of seeds inoculation is to provide the sufficient number of viable efficient bacteria that are able to actively colonize the plant roots immediately after germination. One of the promising forms of bacterial preparations is cells encapsulation in the polymer gel. Advantages of using alginate microspheres are slow, controlled release of bacteria, biodegradation in the soil and an increased shelf life. As a result of this study the effectiveness of using capsulated biopreparation was established to increase the nitrogen-fixing potential of legumes. The advantage in colonization activity is shown in comparison with other forms of the biopreparations due to the slow release of rhizobium from the capsules. The optimal composition for formulation is established which ensures the storage of biopreparation for more than 1 year. The prospect of using encapsulated biopreparations under adverse environmental conditions and for joint application with chemical pesticides and agrochemicals is analyzed.

Key words: biopreparations, encapsulation, sodium alginate, legume crops, inoculation.

INTRODUCTION

Immobilized preparative forms of microorganisms in biofertilizers are perspective for use in sustainable agriculture and attracting the attention of researchers and farmers in present time. In this form bacterial cells more resistance to aggressive environmental factors (Digat, 1991; Amiet-Charpentier et al., 1999; Lebsky et al., 2001; Bashan, 2014) as well, a number of researchers (Bashan & Gonzalez, 1999) point to the possibility of increasing the shelf life of bacteria in alginate granules up to 14 years.

Especially this technology can be relevant in creation and use of biopreparations based on nitrogen-fixing microorganisms, as the most effective method for productivity increasing of crops, yield quality and effectiveness of inoculants for legume plants (Burton, 1976; Jung, 1982; Chen & Huang, 1988; Carrillo-Gracia et al., 2000).

The main criteria for choosing of carrier for nodule bacteria is the cheapness, ease for use, moisture capacity and non-toxicity (Lewis & Papaviz, 1985; DeLucca et al.,

1990; Smith, 1992; Cassidy, 1996; Fenice et al., 2000). Peat has been used for a long time for these purposes but the main drawback of peat is the high variability of the samples depending on their origin. The organic polymers (carrageenan, agar, gelatin, chitosan and alginate) can be used as an alternative to peat, organic waste, inert materials.

The technology of inclusion in alginate gels refers to the soft methods of immobilization – cells remain alive and can carry out polyenzyme processes. The positive property of gel is the cells ability to multiply, as well as its ability to dissolve when the pH and temperature changes. The polymer can be sterilized by autoclaving and, in addition, the immobilization process is reversible, and processed by addition of a Ca^{2+} binding agent (eg EDTA, citric acid, monovalent cations or complex anions – citrates, phosphates, lactates). This makes it possible to isolate viable cells and facilitate to study of their properties (Thompson, 1980; Stormo & Crawford, 1992; Trevors et al., 1992; Carrillo & Bashan, 1997; Amiet-Charpentier, 1998). The advantages of alginate using are slow, controlled release of bacteria, biodegradation in the soil, and an increased shelf life.

MATERIALS AND METHODS

Materials

Sodium alginate was purchased from Sigma Aldrich (Alginic acid sodium salt, low viscosity) as 2 wt % solution. Other chemicals were reagent-grade products (Fluka) used without further purification. *Rhizobium* cells were cultured in YMB medium (Yeast Mannitol Broth). The standard medium includes mannitol, sucrose or glycerol as the carbon source, yeast extract as a source of nitrogen, growth factors, and mineral salts.

Bacterial strains

Mesorhizobium ciceri ST-282 and *Bradyrhizobium japonicum* M8 strains received from work collection of laboratory were used in this work.

Immobilization procedures

For the formation of alginate granules various compositions are used, as indicated in Table 1. In all experiments sodium alginate was used in an amount of 2.0% from total volume. Clays of kaolin, bentonite, gelatin and pectin were used as modifiers on the basis of literature data. (Ajayi et al., 2012; Devi & Kakat., 2013; Belscak-Cvitanovic et al., 2015; Batista et al., 2017) The composition also included glycerin (as osmoprotector) and sucrose for better dissolution of alginate and as additional source of nutrition. All ingredients were dissolved in distilled water and sterilized at 121 °C for 20 minutes. After sterilization centrifuged suspension rhizobial bacteria in amount of 20% of total volume of preparation was added to the mixture and mixed on shaker for 30 minutes. To determine the viscosity of the alginate matrix viscometric method was used, to determine the acidity the pH was measured using pH-meter. Then granules were formed with a mechanical dispenser in a 1.0–3.0% sterile calcium chloride solution and held for 30–40 minutes with slow stirring. Water-soluble sodium alginate was converted to water-insoluble calcium alginate. The resulting granules were washed with sterile 0.85% NaCl solution and stored in sealed bags. Diameter of granules was determined using microscope with occlusal ruler.

Table 1. Labeling of samples and main analytical data

Sample	Encapsulation mix	Mean bead Diameter, mm	Bacteria cell Density, 10 ⁸ CFU g ⁻¹	Cell leakage after 24 h
1	Na alginate + gelatine	3.8 ± 0.2	82	39%
2	Na alginate + pectin	4.9 ± 0.4	78	42%
3	Na alginate + kaolin	3.5 ± 0.6	86	10%
4	Na alginate + bentonite	3.7 ± 0.4	83	28%

Microorganism viability evaluation during immobilization treatments

1 g of microspheres loaded with microbial cells was destruction by adding EDTA 5% and homogenized with 10 cm³ of distilled water to obtain complete and homogeneous dispersion of cells. The cell density of *Mesorhizobium ciceri* ST-282 and *Bradyrhizobium japonicum* M8 was evaluated by plate count on yeast mannitol agar. Plates incubated for 7 days at 28 °C. The viability was determined for both free and entrapped cells, in alginate beads. The results were expressed as cfu living cell per 1 g of microspheres.

Cell leakage and colonization activity

Seeds of chickpea Volgogradskiy-10 cultivar selected in N.I. Vavilov Research Institute of Plant Industry, Russia were used. Chickpea seeds were surface-sterilized in a mercuric chloride solution for 7 min, washed in sterile water to remove mercuric chloride and germinated for 2 days in Petri dishes with moist vermiculite at 28 °C under sterile conditions. After that, chickpea seeds were placed in specially prepared gnotobiotic conditions (Simons et al., 1996). Sprouted seeds were sown on stainless steel grids placed into sterile glass vessels containing 100 mL of deionized water, mineral salts and 6.5 g of microspheres whiz bacteria *M. ciceri* ST-282. Incubated in a growth chamber at 16–18 h day light/darkness cycle and a temperature of 23–18 °C for 7 days.

Microbial cells leak from the microspheres was determined by plating on YMA media. The amount of bacterial cells was determined every 24 hours, for 19 days in the medium where chickpea plants were grown.

Fluorescent *in situ* Hybridization (FISH) and Confocal Laser Scanning Microscopy (CLSM)

Samples were fixed in 4% paraformaldehyde solution mixed with phosphate buffer in the volumetric proportion of 3:1 (Sambrook, 2001). For further treatment separate microsections of chickpea roots were placed in sterile 1.5 mL tubes. Fixed samples were hybridized with rRNA specific oligonucleotide probes following (Shcherbakov et al., 2013). We used for hybridization an equimolar mixture of universal bacterial samples EUB338, EUBII338, EUBIII338 (Amann et al., 1990; Daims et al., 1999). In the end the samples were mounted on an object slide and covered with a ProLong Gold Antifade reagent (Invitrogen, Germany). The preparations were studied on the Leica TCS SPE confocal microscope (Leica Microsystems, Germany). To detect oligonucleotide probes labeled with 6FAM, Cy3 and Cy5 fluorochromes, lasers with awave length of 488, 532 and 635 nm were used. Fluorescence was registered in the range of 508–566 nm, 665–607 nm and 657–709 nm, respectively.

Biodegradation of alginate microspheres

Microspheres with immobilized bacteria were placed in glass vessels containing moist soil for growing plants, 30 microspheres per vessel and were buried 5 cm below the soil surface in the natural soil described below. The soil was aged at 30 °C for 15 days and kept slightly below saturation with water, adding distilled water if necessary. Every 3 days, they were pulled out of the soil, and each of the beads was examined under stereoscopic microscope (Stemi 508, Zeiss, Germany). The diameter of all microspheres was measured immediately, the capsules were placed back into the soil after the measurements.

Inoculation of chickpea and soybean plants with microbead inoculant containing *Mesorhizobium ciceri* ST-282 and *Bradyrhizobium japonicum* M8

Field tests of 2016 were carried out on the experimental field of ARRIAM Research Institute, St. Petersburg, Pushkin. The area of experimental plots of 25 m², four-time repetition of experiments. For field experiment we used chickpea and soybean seeds var. Krasnokutsky and var. Lidiya. The seeding rate was 500,000 seeds ha⁻¹.

Biopreparation granules mixed with the seed at the rate of 1% by weight of the seeds. Options which used liquid preparation was treated as previously written. The seeds were sown when the soil temperature 7–8 °C, to the depth of 6–7 cm.

Statistical analysis

Results for nodulation, growth parameters, cell number in biopreparations were subjected to variance analysis. All measurements in each experiment were performed three times independently, producing similar results. Three independent experiments were conducted for each variant. They were considered as biological replications.

The means were compared by the least significant difference (LSD) test at $P = 0.05$ with the Diana Software (ARRIAM, St Petersburg, Russia).

RESULTS AND DISCUSSION

Development of biopreparation composition for immobilized bacteria

Immobilization by adsorption and incorporation into the spatial structure of alginates is the most mild and preferred method for fixation living bacterial cells. The polysaccharide chains are first joined together by hydrogen bridges when the alginate gel is formed, and then these chains form the cellular structure by binding to calcium ions. In the middle of each cell is the calcium ion (Idris & Suzana, 2006; Cruz et al., 2013).

On Fig. 1 shown the forms of preparations containing bacteria *Mesorhizobium ciceri* ST 282. The diameter of alginate granules was 3–4 mm.

As a result of preliminary studies it was found that the survival of bacterial cells in a liquid culture is extremely low, the titer drops significantly during 1–1.5 months by 3–6 orders. The composition No.1 with gelatin and No.2 with pectin, had a lower viscosity compared with compositions containing clays. The high acidity of pectin made it difficult to form the polymer matrix, so in the No.2 variant the granules did not have a spherical shape. Granules from composition No.3 with kaolin and No. 4 with bentonite

had a spherical shape. According to literature data bentonite and kaolin use as structure-forming agent by increasing the viscosity of alginate to maintain the spherical shape of the granules, while the spherical shape is maintained even at lower concentrations of alginate. The addition of clays increased the viscosity and improved the stability of granules and their mechanical strength, in addition, the clays possess sorption properties. Variation of bentonite content in biopreparations does not effect on effectiveness of immobilization and the number of ‘trapped’ cells does not change (Bashan, 1986b; Paul et al., 1993; Gonzalez & Bashan, 2000). Clays have a lower cost compared to pectin and gelatin.

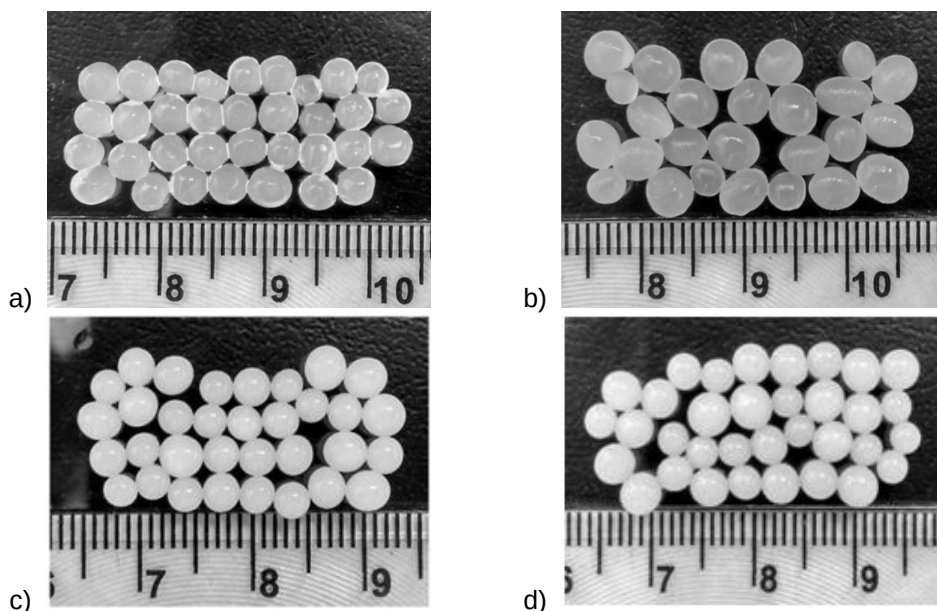


Figure 1. The granular form of biopreparation with immobilized cells *Mesorhizobium ciceri* ST-282: a – alginate + gelatin; b – alginate + pectin; c – alginate + kaolin; d – alginate + bentonite.

For further studies the composition containing 2% kaolin as a modifier of biopreparation was chosen (Table 1). The samples of biopreparation with immobilized microorganisms *Mesorhizobium ciceri* ST-282 and *Bradyrhizobium japonicum* M8 was stored in sterile polyethylene bags with airtight clasps at a temperature of 4 °C. Each month during the year the number of cells in Ca-alginate granules was determined.

Survival of bacterial cells in alginate granules

The alginate granules were used with initial concentration of bacterial cells *Mesorhizobium ciceri* ST-282 (8.6 ± 0.3)·10⁹ CFU g⁻¹ and *Bradyrhizobium japonicum* M8 (10 ± 0.4)·10⁹ CFU g⁻¹. The Fig. 2 shows the changes in the cell number of bacteria in biopreparation during storage period (1 year).

As the result of studies it was determinate the survival of bacteria in alginate granules, it was found that the titer of immobilized cells decreased smoothly during the first 3 months of storage (Fig. 2). There was a slight decrease in the titer of living cells at the subsequent period of storage from 4 to 12 months, the number of bacteria was

maintained stably within the error of measurement. The cell number of *Mesorhizobium ciceri* ST-282 became $19.5 \cdot 10^8$ CFU g⁻¹, and *Bradyrhizobium japonicum* M8 – $29.5 \cdot 10^8$ CFU g⁻¹ by the end of the year of storage. Thus, the developed formulation containing nodule bacteria immobilized into the granules of the alginate gel is able to stably preserve the bacteria during long-term storage which create more opportunities for its use in agriculture.

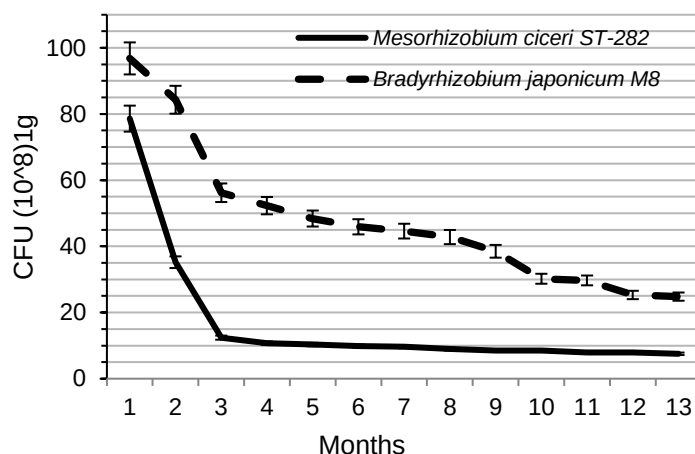


Figure 2. Cell number of bacteria *Mesorhizobium ciceri* ST-282 and *Bradyrhizobium japonicum* M8 in alginate granules depending on the storage time.

Degradation of Ca-alginate granules in soil

The microbiological and fermentative activity of the soil is active factor of hydrolytic and degradation processes. It is known that many soil microorganisms degrade natural polymers using them as a substrate for growth (Vassilev et al., 1997; Wan et al., 1992). There is the gradual degradation of the alginate gel under the action of the soil microorganisms and also due to substances that bind Ca²⁺ ions (Fravel et al., 1985; Sadasivan & Neyra, 1985; Kenney, 1997; Roger et al., 2006) in the soil. Study the biodegradation process noted that the presence of capsules in the soil (as additional substrate) activated the microorganisms while the processes of mineralization of organic matter were more active in experimental soil samples than in the control (soil without polymer). The investigated microbiological soil background is generally characteristic of rich chernozem soils. The total number of microorganisms from experimental soil samples was on the average 1.5 and 1.3 times higher than in control. The number of microorganisms of hydrolytes and oligotrophs significantly increased in the presence of alginate capsules what indicates more intensive processes of destruction of organic substances in experimental soil variants compared to the control. Thus, microorganisms actively reacted to the introduction of the polymer, as an additional nutrient substrate, into their habitat by its decomposition and utilization.

Changes in the shape and structure of alginate beads were noticeable after 7 days. The size of beads was significantly reduced in comparison with control (Fig. 3) after 14 days from beginning what indicate the biodegradation process of alginate capsules.

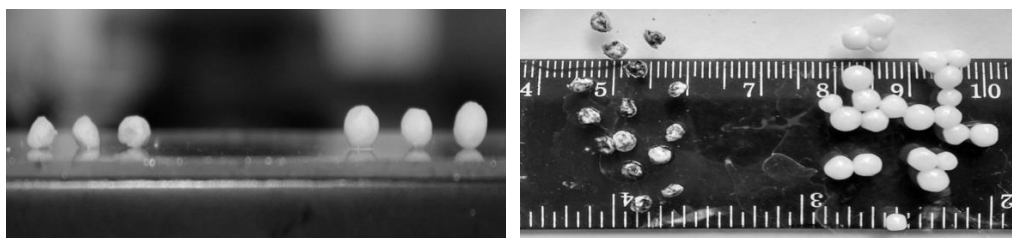


Figure 3. Visual assessment of biodegradation: A – after 7 days of biodegradation process, B – after 14 days of biodegradation process (on the left – alginate capsules, from vessels with soil, on the right – capsules that were kept in sterile Petri dishes).

The release dynamics of bacterial cells from granules into environment

Dynamics of bacterial release into environment has been slow at initial period (Fig. 4). During the first 10 days the release of bacteria into external environment was low at the level of 5–8% from initial content. After 20 days of experiment the content of nodule bacteria in media increased up to $19.5 \cdot 10^8$ CFU g⁻¹ for *Mesorhizobium ciceri* ST-282 and $29.5 \cdot 10^8$ CFU g⁻¹ for *Bradyrhizobium japonicum* M8.

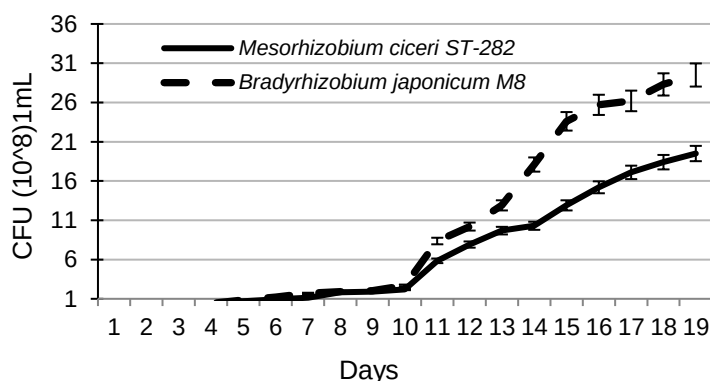


Figure 4. The release dynamics of bacteria *Mesorhizobium ciceri* ST-282 and *Bradyrhizobium japonicum* M8 from alginate granules.

The increased exudation of plant roots with organic acids, which stimulates the multiplication of rhizobium cells and accelerates the destruction of the polymer matrix, thereby facilitating the release of bacteria into the environment from alginate granules. The sharp increase in the number of bacteria coincides with period of active growth and development of root system which promotes active bacterial colonization of plant roots. As is known, a significant part of nodule bacteria is killed before the germination of plant as adverse conditions and lack of nutrition adversely affect them. The introduction of nodule bacteria in the alginate gel provides a sustained release of bacteria and retains them for successful colonization (Shcherbakova et al., 2017).

Colonization activity and spatial localization of bacteria on the roots

The colonization potential of nodule bacteria from alginate granules was rather high, bacteria are well established on the surface of chickpea roots. The maximum values

of root survival were recorded for the variant using alginate granules and amounted to $(8.3 \pm 0.7) \cdot 10^5$ CFU g⁻¹, in the control variant the number of bacterial cells was an order of magnitude lower $(6.4 \pm 0.4) \cdot 10^4$ CFU g⁻¹. It should be noted that the growth-stimulating effect on the variant where the alginate granules were used was the best.

The cells of *Mesorhizobium ciceri* ST-282 was localized on the surface and inside the root using specific oligonucleotide probes allowed the visualization. The roots for hybridization were selected on the 14th day of experiment. The Fig. 5 illustrates the location of bacterial microcolonies which were found both on the rod and on the lateral roots.

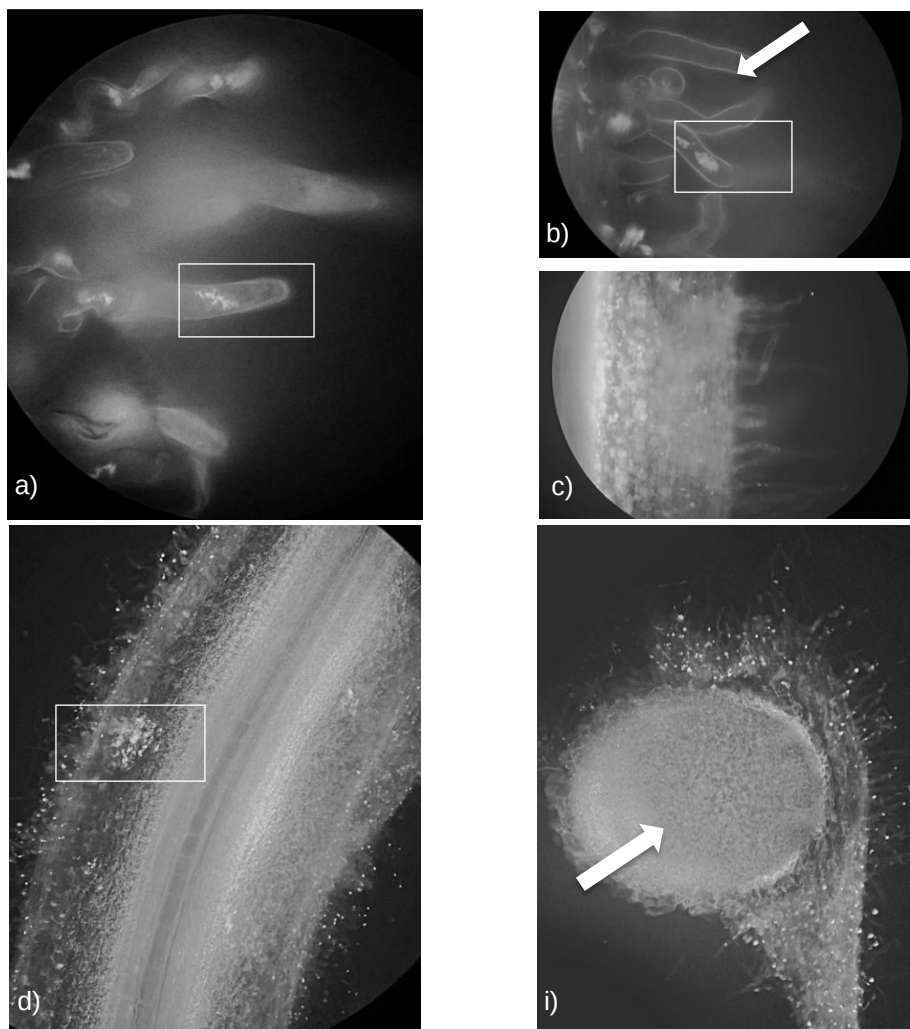


Figure 5. Localization of *Mesorhizobium ciceri* ST-282 on the surface and inside the root of chickpea plants using fluorescent *in situ* hybridization and epiluminescence microscopy: a and b – the root hairs with introduced bacteria, 400X; c – the general view of the root part with bacteria colonized its surface, 200X; d – formation of nodule, bacterial cells and initiation bacteroides development, 40X; e – longitudinal section of nodule, 40X.

The highest density of microbial populations was found on the lateral roots of the plant in the places of so-called ‘feeding points’ where the roots exudate nutrients. As is known, infection of legume plants with nodule bacteria begins with the reaction of bacteria to the appearance of signaling and nutrients released by host roots. The cells are attach to the root surface. The twisting and deformation of root hairs induced by bacteria (Fages, 1990; Elsas et al., 1992; Hernández-Carmona et al., 1999) can be seen on Figs 5, a and b (root hairs with introduced bacteria, the places of penetration of bacteria into the root hair are visible). Fig. 5, c shows the general view of the root part of the root with bacteria colonized on its surface.

Nodule bacteria penetrate the twisted root hairs at the point of greatest bending and introduced into them in the form of tubular structure called infectious filament. These tubes carry the rhizobium cells usually in the single chain to the base of the root hair (to the basal cell) (Bashan & Holguin, 1994; Grube et al., 2009). Immediately after release bacteria encapsulated by the cytoplasmic membranes of the host cells and never come into direct contact with the cytoplasm of host cells (Bashan & Levanony, 1989; Puente et al., 1999).

On Fig. 5, d present the beginning of formation the future nodule and localization of bacterial cells, initiation of bacteroides formation. Fig. 5, i shows the longitudinal section of nodule, the compaction nodule part adjacent to the root hair is seen, from the side of outer wall of nodule the localization of bacteroids and more loose structure is observed what indicates the further growth of nodule.

Field experiments with capsulated biopreparations for chickpea and soybean

The inoculation experiments were carried out on the experimental sites to assess the effectiveness of the microspheres modifier (with chickpea and soybean). Microspheres by themselves are not demonstrate any improvement in plant growth compared to non-inoculated control agents (Table 2). However, the inoculation of both chickpea and soybean plants with *Mesorhizobium ciceri* ST-282 and *Bradyrhizobium japonicum* M8 encapsulated in the alginate microspheres described in this study significantly increased the number and weight of nodules (Fig. 6, a and b).

Table 2. Influence of encapsulated biopreparations on nodules number and weight of chickpea and soybean plants in field experiments

Crop	Sample	The number of nodules in one plant	Node weight in one plant, g in one plant
Chickpea	Control (non inoculation)	6.4 ± 0.43	1.23 ± 0.08
	Inoculation <i>Mesorhizobium ciceri</i> ST 282	18.8 ± 0.28	3.61 ± 0.11
	Inoculation <i>Mesorhizobium ciceri</i> ST 282 encapsulated in the alginate microspheres	27.5 ± 0.32	6.64 ± 0.16
Soybean	Control (non inoculation)	11.8 ± 0.43	1.13 ± 0.08
	Inoculation <i>Bradyrhizobium japonicum</i> M8	24.6 ± 3.13	4.52 ± 2.7
	Inoculation <i>Bradyrhizobium japonicum</i> M8encapsulated in the alginate microspheres	43.5 ± 0.32	8.64 ± 4.19

The main advantages of alginate preparations are their non-toxic nature, soil degradation, slow release of microorganisms into the soil (van Elsas & Heijnen, 1990; Bashan 1998) and almost unlimited shelf life (Bashan & Gonzalez, 1999; Bashan & Davis, 2000). The preparation of microalginate granules of 1–3 mm in diameter containing bacteria is quite easily and is a multi step (Bashan, 1986a; Kobayashi et al., 1997). Several preparations based on alginate were evaluated for agricultural purposes, including encapsulation of rhizobial bacteria, able to enter into symbiosis with leguminous plants. This technology was also used to encapsulate *Mesorhizobium ciceri* and *Bradyrhizobium japonicum*, which were successfully used to inoculate the plants of chickpea and soybean in field conditions. Encapsulated rhizobia, showed significantly improved survival rates on non-encapsulated cells. What influenced the formation of nodules on the roots of plants. So when using bacteria encapsulation the number of nodules increased by 46 and 76% and the weight of nodules by 80 and 90% in comparison with non-encapsulated rhizobia.

CONCLUSIONS

This work defines some aspects of safe and general immobilization technology of symbiotic microorganisms for agricultural use. In particular, the study confirms the efficiency of the capture of bacteria in the polymer matrix. The method of production of sodium alginate beads with the use of a coal concentrate makes it possible to obtain granules with a narrow distribution of diameters and gradual release of trapped cells. The viability of immobilized cells is shown, the leakage is gradual and biological activity is retained. These positive effects are the ability to extend the stability of rhizobial bacteria and the range of their use. Encapsulation of rhizobia also allows effective protection of bacteria against ultraviolet and other adverse effects. As a result of the studies for encapsulation, a combination of alginate + kaolin was chosen as the most physically stable combination for obtaining clusters, supporting the maximum number of bacterial cells. Microspheres with a diameter of 3–5 mm can contain $> 10^8$ bead CFU g⁻¹, therefore even one ball of this diameter will be sufficient to inoculate the family. Wet microspheres are poorly suited for agricultural use, since very few farmers have the technical capacity to cover in the field, but future developments may allow the use of these wet biopreparations, such as fertilizers in the drip irrigation system.

ACKNOWLEDGEMENTS. This work is partially financially support by Project №14.607.21.0178 at 26.09.2017 (RFMEFI60717X0178) ‘Creation of microbiological preparations for expanding the adaptive potential of crops for nutrition, resistance to stress and phytopathogens’. Work of E.N. Shcherbakova, A.V. Shcherbakov, S.A. Mulina, L.M. Yahina, Yu. V. Laktionov, V.K. Chebotar was supported by grant of Federal Agency of Scientific Organizations N0664-2018-0028. The research leading to these results has received funding from the Ministry of Education and Science of the Russian Federation (project ID: RFMEFI60117X0016).

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Analysis of gamma rays induced variability in lentil (*Lens culinaris* Medik.)

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Abstract. In this study, a lentil variety, Idlib-3, was subjected to 100 Gy (LD₅₀) gamma-ray irradiation. At M₂, mutant families were characterized for the most beneficial agronomic traits. High genotypic coefficient of variation, broad sense heritability and genetic advance of the traits such as seed yield per plant and hundred-seed weight indicated expression of additive gene action and confirmed the response at early generation selection. Total number of pods per plant had positive correlation and the highest positive direct effect on seed yield per plant and hence the preference should be given for this trait during selection. The novel mutant families identified with early flowering, early maturity (families 5 and 90) in cluster I, and more first pod height (families 10,70 and 82) in cluster II could be utilized to breed short duration lentil varieties suitable for machine harvest.

Key words: cluster analysis, correlation, genetic advance, mutation breeding, path analysis.

INTRODUCTION

Lentil is an important cool season food legume crop which is cultivated predominantly in the Indian subcontinent, the Middle East, Northern America, Southern Europe, and Eastern and Northern Africa for food (Gupta et al., 2011). Algeria is the second largest lentil producer in North-Africa (FAOstat, 2016). Lentil cultivation has been promoted by the Algerian Ministry of Agriculture since 2007–2008. However, Algerian lentil production fluctuated more in the past four decades; it was around 8,876 tons in 1976, dropped very much to 194 tons in 2000, increased to 8,215 tons in 2011 and more than 10,071 tons in 2016 (FAOstat, 2016). In fact, Algeria is currently importing lentil to meet its domestic demand. In 2015, it imported about 91,136.5 tons (88 million US\$) of lentil from Argentina, Australia, Canada, USA, Turkey and Egypt (Ministry of agriculture 2016, FAOstat, 2016).

The main problem associated with lentil production in Algeria is lack of varieties adapted to drought and heat stress, resistant to diseases and suitable for machine harvesting. The cultivated lentil in Algeria possess narrow genetic base which results low level of genetic variability, poor adaptability and vulnerability to several pests and

diseases. Several authors have also declared that the cultivated lentil has narrow genetic base (Durán et al., 2004; Lombardi et al., 2014; Khazaei et al., 2016). This is an important issue in lentil breeding that needs immediate attention from the breeders.

Broadening the narrow genetic base through intra-varietal, inter-varietal and inter-specific hybridization techniques would bring a possible solution to this issue. But, the tiny size of the lentil flower makes crossing more difficult (Rana & Solanki, 2015). Alternatively, mutation breeding appears to be an efficient method to create new source of genetic variability in lentil. Among various mutagens, gamma rays are highly preferred to induce variability in lentil (Singh et al., 2011). It was successfully employed for the improvement of qualitative and quantitative traits such as high yield, earliness in flowering and maturity and resistance to various diseases (Rajput et al., 2001; Sadiq et al., 2008).

In any mutation breeding programmes, powerful statistical techniques are necessary to analyse the data, ensure its validity and reproducibility. Assessment of the genetic variability is the first important step in mutation breeding. Next, heritability estimates gives information on the magnitude of heritable attributes (Roychowdhury et al., 2012) and the heritability estimates along with genetic advance helps in predicting the gain under selection (Bisne et al., 2009). In addition, understanding the correlations between seed yield and its component traits would facilitate the breeder develop a best selection criterion (Ali et al., 2008). More to this point, it would be useful to understand both the direct and indirect relationships between yield and the other plant characters in order to select the lentil genotypes with high yield potential (Karadavut, 2009). Finally, getting the knowledge of genetic diversity through cluster analysis would facilitate in parent selection (Abna et al., 2012).

With this background, the present investigation was done to (1) characterize M₂ population of lentil for ten morphological traits and (2) evaluate the genetic variability, heritability, character association and diversity through various biometrical analyses for future crop improvement.

MATERIALS AND METHODS

All experiments were carried out at the National High School of Agriculture (ENSA), Algiers, Algeria. A microsperma lentil type, Idlib-3 (ILL6994) which is a derivative of a cross between ILL 99, a Moroccan landrace and ILL5588, a Jordanian landrace (El-Ashkar et al., 2004) was used as a parent in this study. ILL6994 is a small red seeded lentil variety (microsperma type). It was originally developed at the International Center for Agricultural Research in the Dry Areas (ICARDA), and was released at Algeria; it was chosen to promote the cultivation of microsperma lentils in Algeria where the cultivation of macrosperma types is predominated. For the development of pure lines of the ILL6994, seeds from the advanced breeding material were raised in long rows, increased at ICARDA. It was collected from the ICARDA and multiplied at the Technical Institute of Field Crops, Algeris, Algeria, where the source seed of ILL6994 was obtained and utilized in this study.

After determining the lethal dose of irradiation (Tabti et al., 2018), a sample of 10,000 homogeneous, air-dried seeds of Idlib-3 were treated with 100 Gy gamma radiation in order to raise M_1 generation, All treated as well as untreated seeds (10,000) were sown during 2013–2014 cropping season. From M_1 , a sum of 140 single plants (4,200 seeds), which had at least 30 seeds per plant were selected and sown (40 cm $\times$ 30 cm spacing between plants and rows respectively) in a randomized complete block design with three replications during 2014–2015 cropping season to raise M_2 generation. Each row represents one family (10 plants/replication); one row of untreated seeds is sown after each 10 rows of mutants (14 rows in each replication; 10 plants/row) (Tabti et al., 2018).

In M_2 generation, observations were made on ten beneficial agronomical characters including days to first flowering (DF), days to 95% maturity (DM), plant height (PH) (cm), number of primary branches (NPB), number of pods per peduncle (NPP), number of seeds per pod (NSP), total number of pods per plant (TPP), seed yield per plant (SY) (g) and hundred-seed weight (HSW) (g). In order to carry out a selection for machine harvestability, an additional trait, the height of the first pod (HFP) (cm) was added in the study. According to Saxena (2009), the height of first pod was calculated from the base of the plant up to the first node, bearing the first pod.

The ANOVA was done to study the genotypic differences among the families by Genstat software version 18 (VSN International, 2015). Phenotypic and genotypic variances (σ^2_p and σ^2_g) were calculated according to Singh (2000). Phenotypic and genotypic coefficients of variation (PCV and GCV) were calculated to estimate the variability of each trait according to Singh & Chaudhary (1985). The PCV and GCV were classified as low (< 10%), moderate (10–20%) and high (> 20%) (Sivasubramanian & Madhavamenon, 1973). The broad sense heritability (h^2) was estimated by Hanson et al. (1956). The heritability (h^2) percentage was categorized as low (< 30%), moderate (31–60%) and high (> 60%) (Robinson et al., 1949). According to Johnson et al. (1955a) the expected genetic advance as percentage of mean (GA %) was estimated and it was categorized as low (< 10%), moderate (10–20%) and high (> 20%) (Johnson et al., 1955b).

The correlation coefficients (r) among the measured traits were computed using SPSS software, version 20 (IBM Corp. 2011). Path coefficient analysis was carried out using correlation of days to first flowering, days to 95% maturity and yield components on seed yield per plant as illustrated by Dewey & Lu (1959). Standard path coefficients which are the standardized partial regression coefficients were obtained. The indirect effect of the i^{th} variable *via* j^{th} variable on dependent variable 0 (SY) was attained as $P_{0j} \times R_{ij}$; where, P_{0j} is the direct effects of variables j on the dependent variable 0 and R_{ij} is the possible correlation coefficient between independent variables. For cluster analysis, data were analysed to determine the Euclidean distance based on paired group method to determine dissimilar groups of the mutant families using PAST-multivariate software (version 3.13).

RESULTS AND DISCUSSION

Analysis of variance (ANOVA)

Analysis of variance (ANOVA) revealed significant genetic differences across the mutant families for all traits ($p < 0.001$ level) indicating the presence of genetic

variability across the traits. The results shown in Table 1 indicated a wide range of variability which was found for days to first flowering (86–95 days), plant height (22–30 cm), height of the first pod (7–11 cm), total number of pods per plant (15–112 pods), seed yield per plant (0.03–4.83 g) and hundred-seed weight (1.66–3.79 g). Many authors found broad range of variability for total number of pods per plant, hundred-seed weight and seed yield per plant in lentil through induced variability using gamma rays (Tyagi & Khan, 2010; Roy et al., 2013).

Table 1. Mean squares (MS), mean and range of different morphological, phenological, and yield attributes in 140 M₂ families of Idlib-3 lentil variety

Traits	MS	Mean $\pm$ SE	Range
DF	39.65***	89.08 $\pm$ 0.76	86.47–95.76
DM	37.24***	125.40 $\pm$ 0.54	122.80–127.40
PH	60.93***	26.57 $\pm$ 1.05	22.66–30.69
HFP	17.65***	9.75 $\pm$ 0.73	7.59–11.67
NPB	1.73***	2.67 $\pm$ 0.21	2–3.19
NPP	0.78***	1.29 $\pm$ 0.13	0.88–1.81
NSP	0.05***	1.03 $\pm$ 0.02	0.67–1.12
TPP	8350***	39.65 $\pm$ 10.86	15.26–112.52
SY	22.1***	1.28 $\pm$ 0.46	0.03–4.83
HSW	3.26***	2.81 $\pm$ 0.16	1.66–3.79

*** indicates significance at $p < 0.001$ level; SE standard error; DF: Days to first flowering; DM: Days to 95% maturity; PH: Plant height; HPF: Height of first pod; NPB: Number of primary branches; NPP: Number of pods per peduncle; NSP: Number of seeds per pod; TPP: Total number of pods per plant; SY: Seed yield per plant; HSW: Hundred seed weight.

Phenotypic coefficient of variation, genotypic coefficient of variation, broad sense eritability and genetic advance as percentage of mean

The genetic parameters such as phenotypic and genotypic coefficients of variation (PCV and GCV), broad sense heritability (h^2), and genetic advance as percentage of mean (GA %) of the traits are shown in Table 2.

Table 2. Estimates of genetic parameters of 10 different morphological, phenological, yield components, seed yield recorded among 140 M₂ families of Idlib-3 lentil variety

Traits	σ^2_p	σ^2_g	PCV	GCV	h^2	GA %
DF	18.93	10.36	4.89	3.61	54.70	5.51
DM	15.27	10.98	3.12	2.64	71.92	4.62
PH	31.38	14.78	21.08	14.46	47.09	20.45
HFP	11.27	3.19	34.4	18.3	28.31	20.06
NPB	1.02	0.36	37.78	22.38	35.11	27.32
NPP	0.42	0.18	49.69	32.77	43.50	44.52
NSP	0.02	0.02	14.77	12.94	76.78	23.36
TPP	3962.00	2194.00	157.68	117.34	55.38	179.87
SY	9.51	6.29	239.47	194.79	66.17	326.41
HSW	1.35	0.96	41.17	34.71	71.07	60.27

DF: Days to first flowering; DM: Days to 95% maturity; PH: Plant height; HPF: Height of first pod; NPB: Number of primary branches; NPP: Number of pods per peduncle; NSP: Number of seeds per pod; TPP: Total number of pods per plant; SY: Seed yield per plant; HSW: Hundred seed weight.

High and moderate PCV and GCV were observed in plant height and height of the first pod respectively. High PCV and GCV (> 20%) were found for seed yield per plant, total number of pods per plant, number of primary branches, number of pods per peduncle and hundred-seed weight. On the other hand, low PCV and GCV (< 10%) were identified for traits such as days to first flowering and days to 95% maturity. High PCV and GCV were already reported for seed yield per plant (Singh & Srivastava, 2013; Rana & Solanki, 2014) and number of primary branches (Tyagi & Khan, 2010) in lentil.

High difference between PCV and GCV for plant height, height of the first pod, number of primary branches, number of pods per peduncle, total number of pods per plant, seed yield per plant and hundred-seed weight is indicating high influence of environmental factors over the expression of traits. But, the low difference between PCV and GCV for days to first flowering, days to 95% maturity and number of seeds per pod indicates the least influence of environment and a greater contribution of genetic factors on expression of these traits (Gautam et al., 2014).

Nevertheless, GCV is not sufficient enough to determine the heritable variation of the trait. Heritability (h^2) and genetic advance as percentage of mean (GA %) would be useful to study the scope of improvement of the trait through phenotypic selection (Bisne et al., 2009). Further, heritability (h^2) estimates along with GA % and high GCV was helpful in predicting the gain through selection (Chatterjee et al., 2012). Such information is very limited in pulse crops, particularly in lentil. Our results found high h^2 with high GA % with high GCV for seed yield per plant and hundred-seed weight which indicates the expression of additive gene action and confirm the responsiveness of these traits under selection (Chatterjee et al., 2012). But, the high h^2 , high and low GCV of traits such as number of seeds per pod notified that expression of non-additive gene action and phenotypic selection will be less effective for such traits.

Correlations and Path analysis

Seed yield is associated with usually many quantitative traits. Information on correlations between seed yield and other quantitative traits would help to establish a suitable selection criterion (Raturi et al., 2015). In this current study, seed yield per plant was positively correlated with plant height, height of the first pod, number of primary branches, number of pods per peduncle, total number of pods per plant, number of seeds per pod and hundred-seed weight, but it showed significant negative correlation with days to first flowering and days to 95% maturity (Table 3). Early studies by Latif et al. (2010) and Tyagi & Khan (2010) showed similar results in lentil.

Table 3 showed correlations between yield components and phenological traits; total number of pods per plant showed positive and significant correlation with number of seeds per peduncle, number of primary branches and number of pods per peduncle; in other hand plant height showed negative and significant correlation with days to first flowering and days to 95% maturity.

More traits in the correlation often cause difficulty to ascertain the characters which are really contributing to the seed yield. While finding the direct contributions of traits as well as the indirect contribution of the traits through other traits, path coefficient analysis is helpful in making right decisions to perform selection. Direct and indirect effects of the observed characters on seed yield per plant are presented in Table 4.

Table 3. Correlation coefficients of 10 different morphological, phenological, yield attributes, seed yield recorded among 140 M₂ families of Idlib-3 lentil variety

Traits	DF	DM	PH	HFP	NPB	NPP	TPP	NSP	SY
DM	0.251**	1.00							
PH	-0.313***	-0.206*	1.00						
HFP	-0.170*	-0.161	0.595***	1.00					
NPB	-0.083	-0.446***	0.463***	0.312***	1.00				
NPP	-0.151	-0.545***	0.419***	0.449***	0.45***	1.00			
TPP	-0.235**	-0.777***	0.434***	0.253**	0.457***	0.699***	1.00		
NSP	-0.141	-0.366***	0.195*	0.086	0.173*	0.332***	0.489***	1.00	
SY	-0.194*	-0.568***	0.365***	0.175*	0.291***	0.574***	0.852***	0.543***	1.00
HSW	-0.166*	-0.389***	0.020	0.074	0.236**	0.327***	0.394***	0.155	0.452***

*, **, ***: correlation significant at 0.05, 0.01 and 0.001 level of probability respectively; DF: Days to first flowering; DM: Days to 95% maturity; PH: Plant height; HFP: Height of first pod; NPB: Number of primary branches; NPP: Number of pods per peduncle; NSP: Number of seeds per pod; TPP: Total number of pods per plant; SY: Seed yield per plant; HSW: Hundred seed weight.

Table 4. Direct and indirect effects of nine different morphological, phenological, yield attributes, on seed yield recorded among 140 M₂ families of Idlib-3 lentil variety

Traits	DF	DM	PH	HFP	NPB	NPP	NSP	TPP	HSW	Indirect effect
DF	0.01	0.0638	0.0022	0.0017	0.0073	0.0041	-0.0230	-0.2275	-0.0292	-0.2000
DM	0.0025	0.254	0.0014	0.0016	0.0392	0.0147	-0.0597	-0.7521	-0.0685	-0.8200
PH	-0.0031	-0.0523	-0.007	-0.0060	-0.0407	-0.0113	0.0318	0.4201	0.0352	0.3730
HFP	-0.0017	-0.0409	-0.0042	-0.01	-0.0275	-0.0121	0.0140	0.2449	0.0130	0.1850
NPB	-0.0008	-0.1133	-0.0032	-0.0031	-0.088	-0.0122	0.0282	0.4424	0.0415	0.3790
NPP	-0.0015	-0.1384	-0.0029	-0.0045	-0.0396	-0.027	0.0541	0.6766	0.0576	0.6010
NSP	-0.0014	-0.0930	-0.0014	-0.0009	-0.0152	-0.0090	0.163	0.4734	0.0273	0.3790
TPP	-0.0024	-0.1974	-0.0030	-0.0025	-0.0402	-0.0189	0.0797	0.968	0.0693	-0.1150
HSW	-0.0017	-0.0988	-0.0014	-0.0007	-0.0208	-0.0088	0.0253	0.3814	0.176	0.2740

In diagonal: direct effect on seed yield; DF: Days to first flowering; DM: Days to 95% maturity; PH: Plant height; HFP: Height of first pod; NPB: Number of primary branches; NPP: Number of pods per peduncle; NSP: Number of seeds per pod; TPP: Total number of pods per plant; SY: Seed yield per plant; HSW: Hundred seed weight.

The direct effect of total number of pods per plant on seed yield per plant was the highest among others, confirming that yield can be increased both directly and indirectly by increasing the number of pods per plant. Jain et al. (1991) indicated that number of pods per plant is the best selection criterion for yield improvement in lentil. Chakraborty & Haque (2000) and Tyagi & Khan (2010) also reported positive direct effect of total number of pods per plant on seed yield. The lowest significant direct effect on seed yield per plant was observed by number of seeds per pod. The highest indirect effect on seed yield per plant was observed in number of pods per peduncle. The direct effect of number of primary branches on seed yield per plant was negative and significant; whereas its indirect effect via total number of pods per plant was positive. Hundred-seed weight had positive and significant direct effect on seed yield per plant but with low indirect effects via other traits. Though the direct effect of number of pods per peduncle was insignificant, its indirect effect via total number of pods per plant was positive and high. Days to first flowering and days to 95% maturity had positive direct effect on seed yield per plant and negative indirect effect via number of seeds per pod, total number of pods per plant and hundred-seed weight. Plant height and height of the first pod had negative direct effect on seeds per pod whereas they had positive indirect effect via number of seeds per pod, total number of pods per plant and hundred-seed weight. Chakraborty & Haque (2000) reported positive direct effect of number of seeds per pod and negative direct effect of days to flowering and days to maturity. Whereas, Latif et al. (2010) found positive direct effect of days to maturity, plant height, and negative direct effect of days to flowering on seed yield. Reddy (2013) also reported positive direct effect of plant height on seed yield per plant.

Cluster analysis

Cluster analysis using all the ten morphological traits grouped 140 mutant families and control into four major groups at a genetic distance of 48 (Table 5). Cluster IV was the largest one with 72 families (51.06%) followed by cluster II with 35 families (24.82%) including control and cluster III with 31 families (21.99%). The smallest group identified was cluster I with only 3 families (2.13%). The mutant families included in the cluster I were characterized with high mean values for all traits except height of the first pod, days to flowering and days to maturity. The mean value of cluster II was high for the height of the first pod. The mean values of Cluster III were not found noteworthy for all traits. Likewise, cluster IV had lowest mean values for all traits except for days to first flowering and days to maturity. In particular, mutant families 5 and 90 in cluster I would be useful for the development early flowering (86.61 days) and early maturing (123.3 days), short duration lentil varieties. Such varieties would be beneficial for the areas more prone to terminal drought and heat stress. Mutant families such as 5, 42 and 90 in cluster I had high seed yield per plant (4.83 g). Likewise, mutant families such as 10, 70 and 82 in cluster II had more than 11 cm first pod height would be useful to breed lentil varieties suitable for machine harvesting. The clusters might be consisting of diverse mutant families for various qualitative traits. The selected families from various clusters would be useful for the genetic improvement of lentil.

Table 5. Mean values of ten different phenological and morphological characters, yield attributes, and seed yield for four groups revealed by cluster analysis among 140 mutant families and the control

Cluster	(%)	DF	DM	PH	HFP	NPB	NPP	TPP	NSP	SY	HSW
1	2.13	88.13	124.13	28.94	9.88	2.89	1.54	98.22	1.09	3.92	3.03
2*	24.82	88.84	124.26	26.90	9.96	2.78	1.44	58.42	1.05	1.95	2.91
3	21.99	89.16	125.08	26.54	9.66	2.66	1.35	41.18	1.03	1.17	2.79
4	51.06	89.22	126.26	26.11	9.53	2.56	1.21	26.65	1.01	0.55	2.63
mean	-	89.08	125.4	26.57	9.75	2.67	1.29	39.65	1.03	1.28	2.81

* Idlib-3 position; DF: Days to first flowering; DM: Days to 95% maturity; PH: Plant height; HPF: Height of first pod; NPB: Number of primary branches; NPP: Number of pods per peduncle; NSP: Number of seeds per pod; TPP: Total number of pods per plant; SY: Seed yield per plant; HSW: Hundred seed weight.

CONCLUSION

On the whole, mutation induction through gamma rays was found useful to create new source of variability and circumvent the bottleneck in lentil breeding. The induction of mutation by 100 Gy gamma rays on Idlib-3 was effective to generate significant variability for most of the quantitative traits studied. The high genotypic co-efficient of variation, broad sense heritability and genetic advance found for seed yield per plant and hundred-seed weight guaranteed early phenotypic selection and high expected genetic gain. Both correlation and path analysis suggested providing high preference to total number of pods per plant during selection due to its strong relationship and direct effects on seed yield per plant. Cluster analysis results confirm the presence of genetic dissimilarities among the mutant families. The identified mutant families such as 5, 42, 90, 10, 70 and 80 could be used as parents in the future breeding program of Algeria. Moreover, the mutants generated in this study could offer opportunity to perform molecular screening through TILLING (Targeting Induced Local Lesions in Genomes) in the immediate future.

ACKNOWLEDGEMENTS. We thank CRNA (M. Yefsah) of ALGIERS, for their help in the realisation of this work and Ms. Saoudi and Yahiaoui.

We express our sincere thanks to reviewers from the journal for their valuable suggestions on the manuscript.

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A comparative analysis of functional traits in semi-natural grasslands under different grazing intensities

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Abstract. The reduction of traditional management practices is a major threat for the conservation of permanent grasslands in many European marginal areas. The ecological importance of grasslands is acknowledged by the European Habitats Directive 92/43/EEC (1992) which includes many natural or semi-natural grassland types, and by the growing attention of society towards functions and services provided by these ecosystems. Nonetheless, the efficiency of conservation policies is questioned also for the lack of local-scale information on trends and state of grasslands hampers the definition of local-tailored schemes. The main objective of this work is to assess the potential of a set of functional traits in discriminating between different management intensities and their capacity to describe the dynamics occurring in semi-natural grasslands. The research was carried out in a hilly area of Tuscany (Italy) on four grassland sites characterized by similar environmental features (soil, climate, topography), and by different management practices for 10 or more years. The survey concerned collection and analysis of different functional traits related to foliar features, litter and botanical composition. The functional traits were able to differentiate the four sites under different management practices, but their effectiveness was different. Results support the possibility to perform a rapid appraisal of grassland successional stages based on leaf functional traits of dominant species and by the assessment of presence of a reduced number of species among those occurring in the community.

Key words: life forms, leaf dry matter content, leaf nitrogen concentration, litter, specific leaf area.

INTRODUCTION

Grasslands are one of the most widespread ecosystems at global level which are represented by many vegetal communities adapted to a range of different environmental conditions (Dixon et al., 2014). The importance of grasslands is not only related to forage production (Conant et al., 2016), but also to the provision of a number of other ecosystem services (Primi et al., 2016) such as protection of vegetal biodiversity (McAllister et al., 2014; Rossetti et al., 2015), conservation of open space for wildlife (Crosby et al., 2015; Cervasio et al., 2016), maintenance of areas for touristic activity (Dossche et al., 2016), and landscape preservation (Argenti et al., 2011; Schmid et al., 2017). Their ecological

importance is also acknowledged by the European Habitats Directive 92/43/EEC (1992) which includes many natural or semi-natural grassland types.

In the last decades, complex socio-economic changes have caused the abandonment of traditional management practices such as mowing or grazing in many European marginal areas (Török et al., 2016). That has triggered significant successional dynamics of the herbaceous ecosystems (Dengler et al., 2014). Currently, shrub encroachment and natural reforestation are the main vegetation dynamics which entail negative consequences on biodiversity and landscape aesthetics in marginal grasslands (Argenti et al., 2012; Koch et al., 2015; Braunisch et al., 2016). For these reasons, the Common Agricultural Policy includes measures to encourage the utilization of marginal environments. However, these measures are not always effective for the conservation of grassland biodiversity because they are not able to target efficiently the territorial heterogeneity of mountain areas and they do not strictly focus on the management of grasslands (Burrascano et al., 2016). Consequently, the availability of local-scale information on the grassland conditions is necessary to define conservation strategies able to optimize the efficiency of interventions (Lengyel et al., 2012). Indicators assessing the structure of plant communities in relation to land use and sensitive enough to suggest appropriate grassland management may be used to improve the effectiveness of the agro-environmental programs (Kampmann et al., 2012). However, indicators usually require specific expertise including an advanced knowledge of species taxonomy and local context. Indeed, availability of experts and labour costs are acknowledged among the main hurdles to improve the information base for the policy design (Targetti et al., 2014). The possibility to improve the availability of local-scale information, on the other hand, is necessary to enhance the efficiency and legitimization of agri-environmental schemes (Armsworth et al., 2012; Pe'er et al., 2014).

Functional traits include different plant features, such as morphological, ecophysiological and reproductive ones (Harrington et al., 2010). Some traits concern leaf characteristics of dominant species, whereas others are derived from vegetation composition as growth forms, flowering features or biomass partition (Schweiger et al., 2017). Relationships among these indicators and environmental and management factors are well established and -hence- their employment in ecological modeling is suggested to be potentially useful (Yang et al., 2015). For instance, plant functional traits (PFT) have been used as a key to link plant communities to ecosystem services and are considered able to reveal variations in local environmental conditions (Schweiger et al., 2017). PFT have been also tested in comparative analyses, to underline dynamics related to disturbance regimes, to assess relations among ecosystems services and management and to describe successional stages (Liu & Su, 2017). Nonetheless, constraints concerning data collection have been raised because the collection of functional traits is very often time-consuming and difficult in particular in marginal areas (Musavi et al., 2016).

Main questions arising from previous analysis of the state of the art are those concerning the efficiency of functional traits in explaining vegetation characteristics and dynamics and in the choice of such traits among a large amount of different possible alternatives. Trying to answer to these issues, the objective of the present paper is to assess the potential of a selected set of functional traits to describe the vegetation dynamics in semi-natural grasslands and to test their capacity of discriminating sites with different management history. The research was carried out in a hilly area of Tuscany

(Italy) on semi-natural grasslands characterized by similar environmental features (soil, climate, topography) and presenting different management intensities (animal stocking rate) for 10 or more years.

MATERIALS AND METHODS

The case study area is located at an altitude of 700 m asl in Mugello (North Apennines, Tuscany), a rural region characterized by high presence of semi-natural grasslands. The survey was carried out in semi-natural grasslands assigned to the *Festuco-Brometea* (Br.-bl. et Tuxen) phytosociological class (Ellenberg, 1988). The area is characterized by mean annual temperature of 11.3 °C, with precipitations concentrated during autumn and winter. The mean annual rainfall is about 1,000 mm with an annual potential evapotranspiration of 950 mm. The soil of the study area is classified as silty-loam with a neutral reaction.

Information collected by means of interviews with local farmers allowed to identify the most common management practices and the grassland sites where it was possible to retrieve information on past management of the last ten years. Subsequently, environmental conditions, ecological characteristics and landscape homogeneity were considered for the selection of the grassland sites.



Figure 1. Images from the study site grassland plots.

The selection allowed to identify four sites covering a gradient of grazing intensity (Fig. 1):

- high intensity (site A), mown in spring (usually May) and then grazed with an average stocking rate higher than 0.65 livestock unit (LU) ha⁻¹ yr⁻¹ in the last ten years;

- medium-intensity (site B), stocking rate between 0.45 and 0.65 LU ha⁻¹ yr⁻¹ in the last ten years, no mowing performed;
- low-intensity (site C), stocking rate between 0.25 and 0.45 LU ha⁻¹ yr⁻¹ in the last ten years, no mowing performed;
- abandoned area (site D), where no utilization (grazing or mowing) was performed in the last 25 years and presenting a vegetation mosaic of grasslands and shrub species.

A reference period of 10 years featuring the same or very similar pastoral management practice and stocking rate for each site was considered sufficient to balance floristic composition and functional characteristics in response to livestock grazing regime. All the pasture sites were fenced and grazed with horses as horse breeding is an economic alternative to traditional cattle grazing in the area due to progressive abandonment of dairy and cattle farming over the last decades (Targetti et al., 2013).

At each site, two permanent plots (25 m x 4 m) were randomly selected in order to reflect average environmental conditions and to avoid edge effects. To take into account climatic variability, field sampling covered two years (2006 and 2007) and two growing seasons (May and September) in each year. Vegetation composition was assessed in each plot: a permanent transect line (25 m-long) was established and vascular plant species were recorded every 50 cm according to the point quadrat method (Daget & Poissonet, 1969). Floristic nomenclature of all identified species refers to Pignatti (1982). The percentage contribution of each species (specific contribution, SC) in the sward was quantified according to the formula:

$$SC_i = \frac{SF_i}{\sum_{k=i}^n SF_i} \times 100 \quad (1)$$

where SF_i is the specific frequency of a given species, *i.e.* the number of times that a single species is recorded along the transect (Argenti & Lombardi, 2012).

In the present work, we considered three different categories of functional traits:

- leaf functional traits: specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen content (LNC) (based on field survey);
- vegetation traits: life forms, start and duration of flowering (based on existing database);
- characteristics of litter layer: dry weight, average weight per area, and density (based on field survey).

Leaf functional traits (SLA, LDMC, LNC) were investigated on three different grass species, namely *Brachypodium rupestre*, *Bromus erectus* and *Festuca gr. rubra* (following Pignatti, 1982) which were the most frequent in the plots (average presence between 7 and 24% in the four sites) and in agreement with other researches that emphasized the possibility of functional traits evaluation only on a reduced number of species among those occurring in the grassland community, particularly if the traits are weighted (Ansquer et al., 2005). We considered these species as a useful guide for interpreting the dynamic of the plant communities as response to environmental and management changes (Grime, 2001). SLA measures the light interception per leaf biomass unit, and it is often correlated with the potential growth rate of the species. LDMC is related to the average density of tissue and it is a measure of stress tolerance,

as it has important consequences for leaf energy and water balance (Cornelissen et al., 2003). LNC is the total amount of nitrogen per dry leaf mass unit and it is strongly correlated to the concentrations of leaf nitrogen compounds involved in photosynthesis (Garnier et al., 2004).

Vegetation samples for SLA, LDMC, and LNC measurements were collected close to the line transects at 5, 10, 15, 20 and 25 m interval. Specific leaf area and leaf dry matter content were performed at maximum biomass following a standard protocol proposed by Garnier et al. (2001). SLA was calculated as the ratio of leaf area to dry mass and LDMC was calculated as the ratio of leaf dry mass to fresh mass on young and fully expanded leaves. LNC was determined on 240 samples of dry fully expanded leaves of each species. For further details, refer to Targetti et al. (2013).

The average weighted values for Leaf Functional Traits (LFT) on the three target species were calculated as follows:

$$wLFT_{i,s} = \left(\frac{p_{i,s}}{\sum_i p_{i,s}} \right) \times LFT_i \quad (2)$$

where $wLFT_{i,s}$ is the weighted leaf functional trait of species i in transect s ; $p_{i,s}$ is the relative abundance of species i in transect s ; LFT_i is the average leaf functional trait of species i .

Based upon existing database (Pignatti, 1982), functional traits including life forms (*sensu* Raunkiaer, 1934), and start and duration of flowering were also assessed. These traits were selected with the aim to evaluate the performance of easy to measure traits which were available for a wide range of species and, therefore, transferable to sites with different species composition (Kahmen & Poschlod, 2008). Raunkiaer's life forms is an integrative trait comprising plant species strategies to survive to adverse seasons. We considered 6 categories of life form: therophytes, geophytes, hemicryptophytes, chamaephytes, nano-phanerophytes and phanerophytes. Concerning the start and duration of flowering period, three categories for the trait 'start of flowering period' (March or early, April or May, and June or later) and three categories for the trait 'duration of flowering period' (1 month or less, between 2 and 3 months and more than 4 months) were considered. The contribution of these traits was assessed for the complete set of species for each transect. Since these traits are categorical, the contribution of each trait category was calculated as the sum of the relative contribution of each species within that category.

The parameters referring to the litter layer were adopted since grazing pressure may affect plant characteristics primarily via biomass and litter removal (Mapfumo et al., 2002). Five litter layer heights (cm) were randomly measured in a sampling quadrat (0.25 m x 0.25 m) and repeated three times for each plot and sampling period. A sample of the standing phytomass was collected in the same quadrat. Each sample was transported and dried in laboratory at 60 °C for 48 hours until a steady weight was reached. Then the samples were weighted to determine dry weight (g), average litter weight per area (g m⁻²), and density (g m⁻³).

Linear discriminant analysis (LDA) was performed after standardization of data to assess the effectiveness of the measured functional traits in discriminating the four studied pasture sites. LDA is a multivariate statistical technique which classifies a set of

variables in terms of their capacity to ‘discriminate’ different groups of observations. The technique is usually used for dimensionality reduction in data mining and for the interpretation of the importance of a given set of variables (R Development Core Team, 2012). The general condition of normality of data for the discriminant analysis was checked using the Shapiro-Wilk test (Shapiro & Wilk, 1965).

RESULTS AND DISCUSSION

The three grass species selected for the measurement of the leaf functional traits (*Brachypodium rupestre*, *Bromus erectus*, and *Festuca gr. rubra*) were by far the most frequent in the four plots, except in site B where *Arrhenatherum elatius* was more frequent than *Festuca gr. rubra*. In Fig. 2 the relative frequency, expressed as specific contribution, and variability of the three selected species in the four different sites is presented.

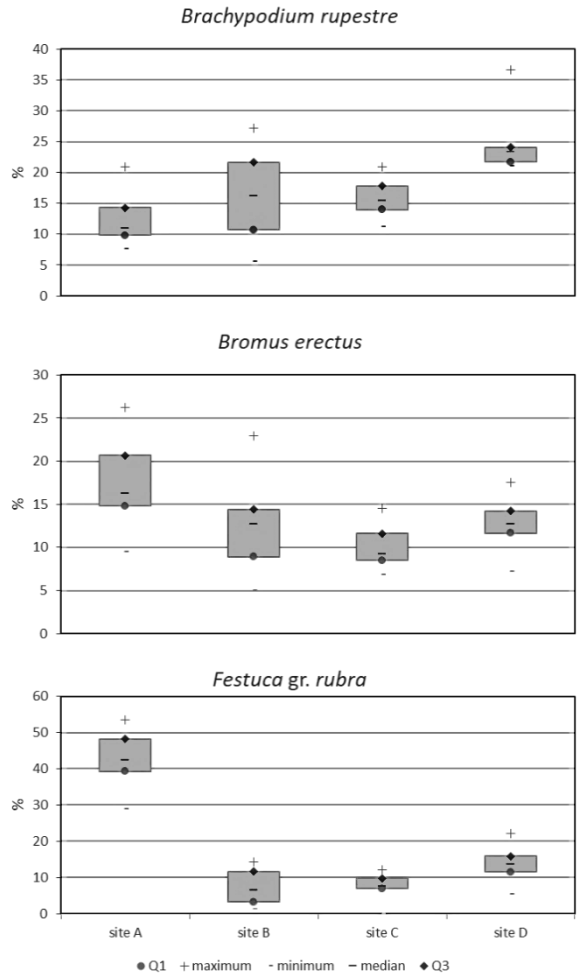


Figure 2. Boxplot charts concerning variability of specific contribution of the three most common species in the four different sites. Q1: 1st interquartile; Q3: 3rd interquartile.

Brachypodium rupestre tended to increase its abundance as grazing management decreased; on the contrary, *Bromus erectus* displayed an opposite tendency from site A to C. *Festuca gr. rubra* showed a more controversial trend between sites with an increase of its presence under high grazing pressure (site A) and in abandoned plots (site D).

The weighted leaf functional traits did not show a linear relation to management intensity (Fig. 3). The variables wLDMC and wLNC reflected the gradient of management intensity from site A to site C. All the three PFT highlighted noticeable differences between sites C and D.

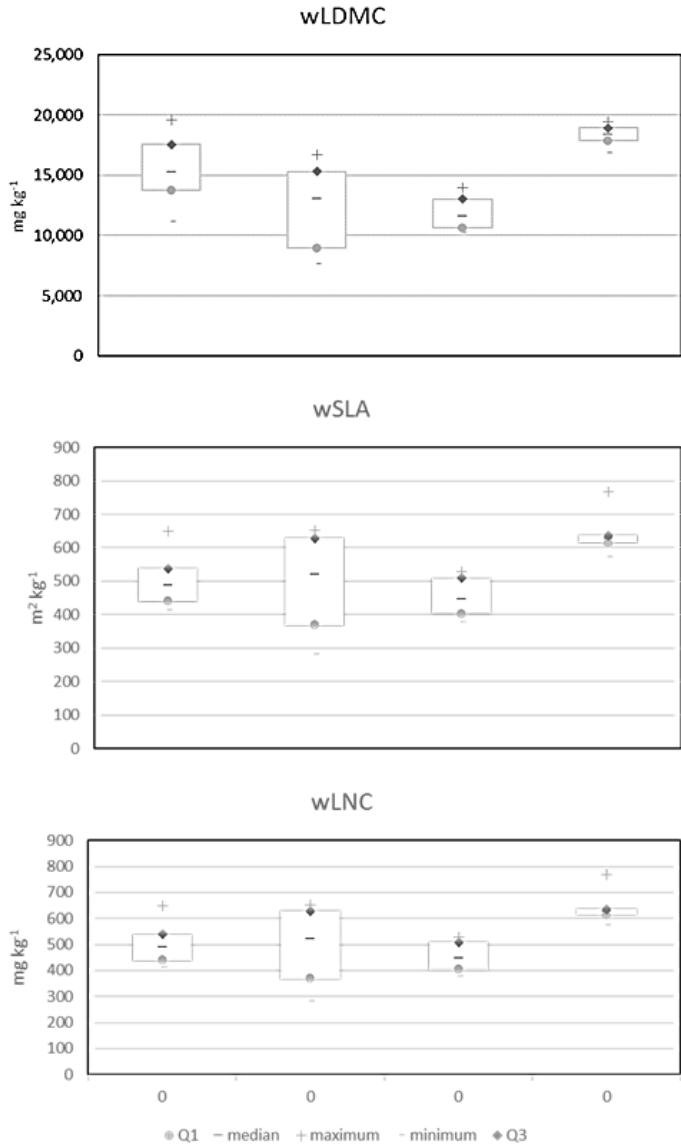


Figure 3. Boxplot charts concerning variability of the weighted leaf functional traits (wLDMC, wSLA, wLNC) for the three species in the four different sites. Q1: 1st interquartile; Q3: 3rd interquartile.

Results show different performances of the considered variables as proxies for management intensity, and in some cases are consistent with previous studies (e.g. Martin et al., 2009). In particular, some functional traits seem to have a linear relation with intensity of management, whereas others highlight a gradient that does not link clearly to management. Interestingly, variables that could be assessed with a rapid botanic survey such as the wLDMC and the frequency of *Brachypodium rupestre* seem to be effective indicators of management intensity. The use of wLDMC would however be feasible only if LDMC values for the most frequent species are available as the contextual collection and measurement of that parameter is not affordable in a day-by-day assessment. The increase of *Brachypodium rupestre* and the reduction of *Bromus erectus* from site A to site D was the main cause of the highlighted trend of wLDMC. The behaviour of *Festuca gr. rubra* was less linear because it reached higher frequencies at the two management extremes (sites A and D) and a low frequency in sites B and C. That species has a wide ecological spectrum (Gaucherand et al., 2006) and probably takes advantage of the heterogeneous ecological conditions in site D. Despite this fact, wLDMC calculated on these three species was the parameter recording the highest coefficient in the linear discriminant analysis (see hereafter) and outlines a relevant potential as indicator related to management intensity.

The effectiveness of wSLA in differentiating the sites would likely be more evident when comparing high intensive grasslands with low-input and abandoned grasslands as reported by Kahmen & Poschlod (2008). Indeed, the relation between disturbance and higher SLA is more evident in other studies including a wider range of species and management intensities (Garnier et al., 2007). The oligotrophic character of the four sites induced to discard species typical of more fertile environments like *Lolium multiflorum* or *Dactylis glomerata*, which are characterized by high values of SLA (Ansquer et al., 2009). The high SLA value of *Brachypodium rupestre* has probably had a masking effect on the wSLA in our plots. Similar results have been presented by Saar et al. (2012) and Timmermann et al. (2015) where high SLA values in abandoned sites were related to litter accumulation and the creation of mesic conditions which facilitates tall and high SLA species, as pointed out also by Giarrizzo et al. (2017).

Raunkiaer life forms highlighted a trend following the management intensity with a gradual reduction of hemycryptophytes and terophytes from site A to site D, and with a parallel increase of phanerophytes from site A to site D (Fig. 4). The other life forms classes evidenced a non-linear relation with the different management intensities. Geophytes frequency was between 2% in site B and 0.1% in site C, whereas no geophytes was recorded in site A. Chamaephytes were present in all sites, except site B. The highest concentration of chamaephytes was recorded in site C and D (8.9 and 5.6% respectively). The differences of frequency of nano-phanerophytes across the four sites was not relevant.

As expected, management rate reduction was related with frequency of chamaephytes (shrub-species) in site C and phanerophytes (woody-species) in site D. Higher stocking rates and soil disturbance in sites A and B had a clear inhibitive effect on these life forms due to two main effects: the mechanical effect of large herbivores trampling and the annual mowing in site A, and the exceptional low grazing selectivity

which is a typical feature of horses in comparison to cattle (Menard et al., 2002). Effects of grazing intensity on different vegetal functional group found in our study are consistent with previous research conducted in Mediterranean environment (Papanikolaou et al., 2011).

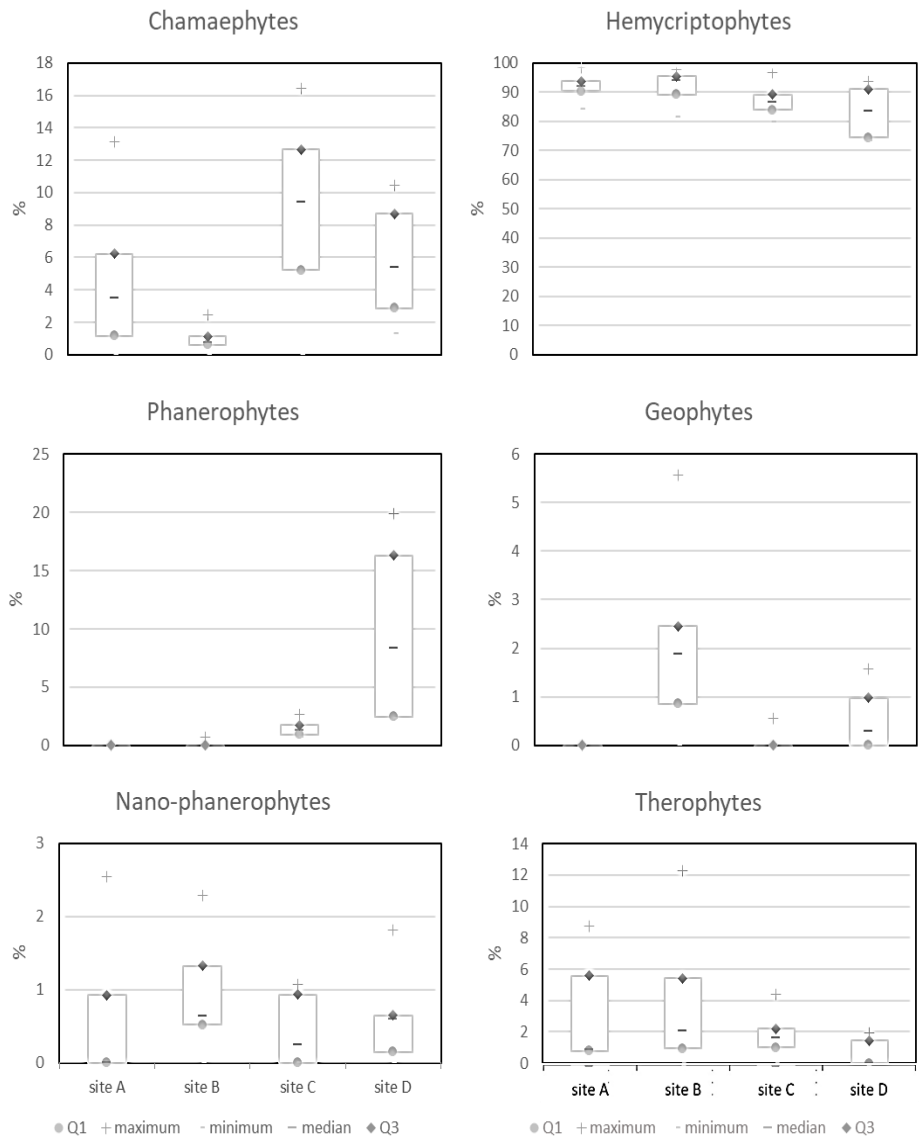


Figure 4. Relative frequency of the Raunkiaer biological forms (according to Pignatti, 1982) in the four different sites. Q1: 1st interquartile; Q3: 3rd interquartile.

Species flowering in April and May were by far the most present in the four sites (Fig. 5).

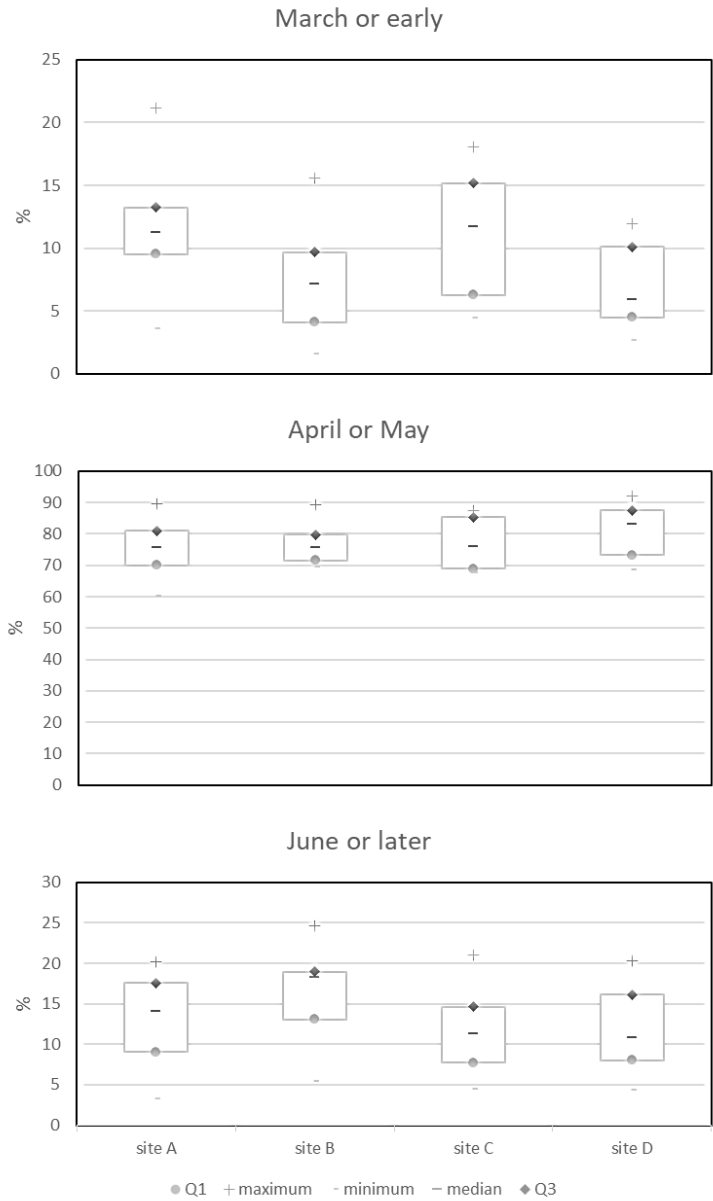


Figure 5. Relative frequency of the start flowering class (according to Pignatti, 1982) in the four different sites. Q1: 1st interquartile; Q3: 3rd interquartile.

Even though a slightly higher frequency of early flowering species was found in sites A and C, the functional trait related to the starting of flowering period did not show clear differences between the four surveyed sites and did not seem to be linked with the

management intensity. The duration of flowering seemed to be more useful as an indicator to differentiate the management intensity (Fig. 6). The frequency of species with short duration of flowering period decreased from more intensive to extensive and abandoned sites. On the contrary, the frequency of species with medium duration of flowering showed the opposite behaviour increasing from the intensive to the abandoned site even though the relation was less clear.

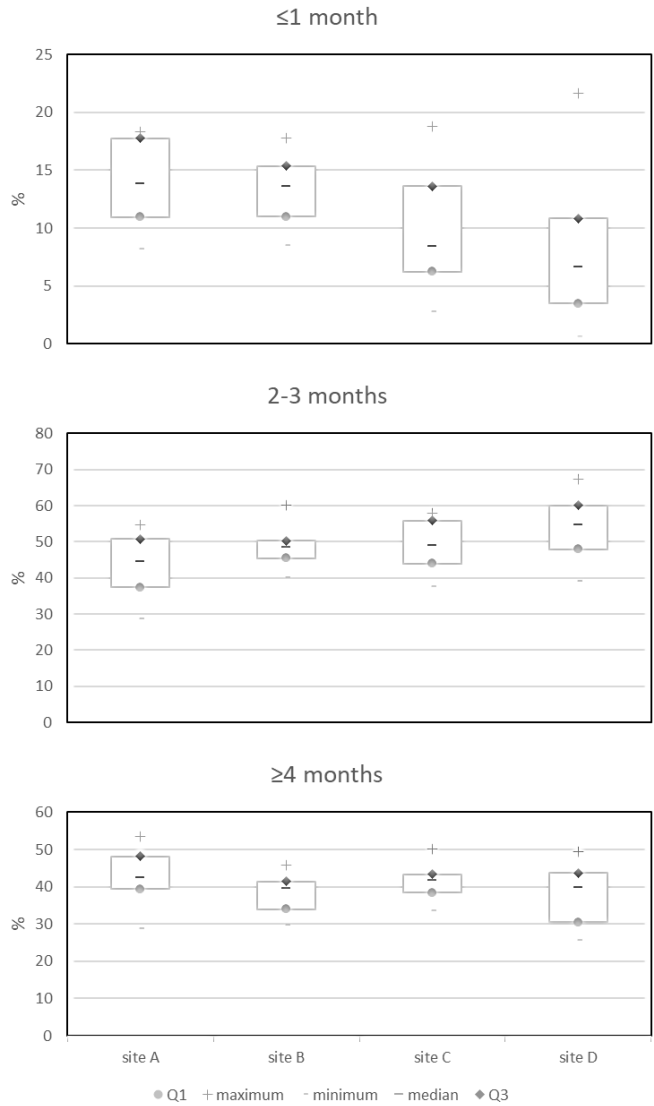


Figure 6. Relative frequency of the duration of flowering class (according to Pignatti, 1982) in the four different sites. Q1: 1st interquartile; Q3: 3rd interquartile.

In our study, functional traits linked with species phenology (start and duration of flowering) showed a marginal importance in relation with management and this is consistent with other studies (Farnsworth 2007; Kahmen & Poschold, 2008; Storkey et

al., 2013). Nevertheless, duration of flowering revealed an increasing trend of short flowering duration species from abandoned to high-disturbed sites. Similarly to findings of Köhler (2001), this suggested an advantage for species able to concentrate the reproductive period in areas under more severe utilization. On the contrary, delay and anticipation of flowering did not give any specific trend across the study sites.

The sites A and B recorded the highest values of litter density whereas the dry weight and thickness was higher in the site D (Fig. 7). Both the dry weight and the thickness highlighted a clear gradient from the more intensive plots to the abandoned ones.

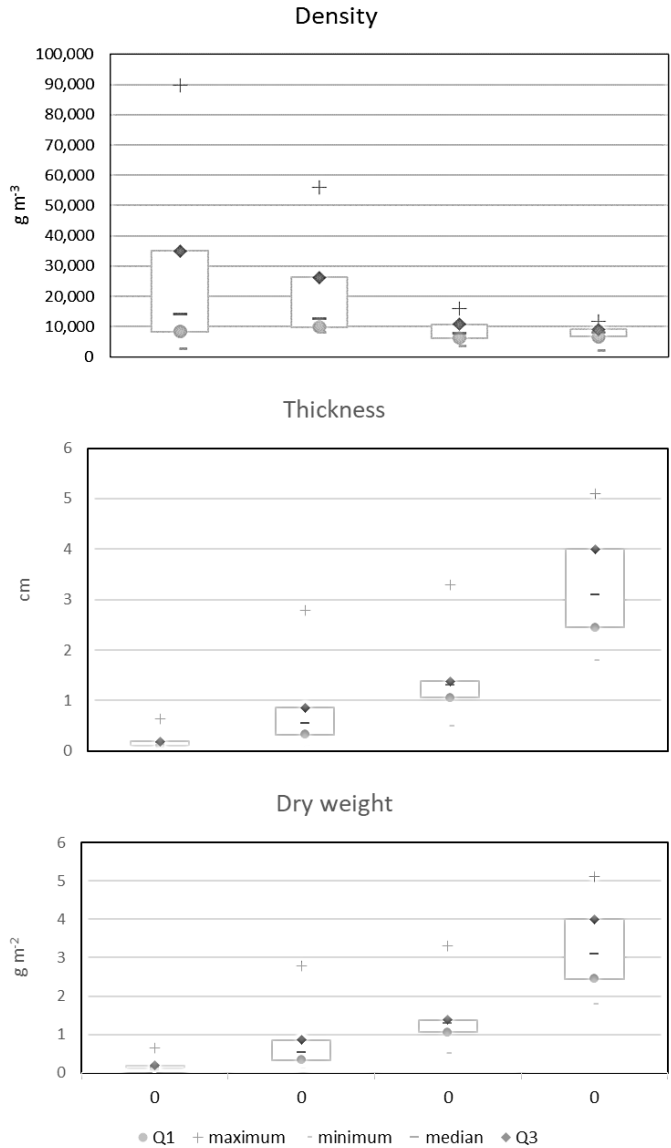


Figure 7. Thickness, dry weight, and density of litter layer in the four different sites. Q1: 1st interquartile; Q3: 3rd interquartile.

Similarly to other findings (Catorci et al., 2014), land abandonment favoured oligotrophic species such as *Brachypodium rupestre*. The litter accumulation in the abandoned site seems therefore related to the combined effect of the reduced biomass removal by grazing animals and the higher presence of grass species producing large amount of litter (Bonanomi et al., 2013).

The linear discriminant analysis (LDA) outlined clearly the difference between the four plots (Fig. 8).

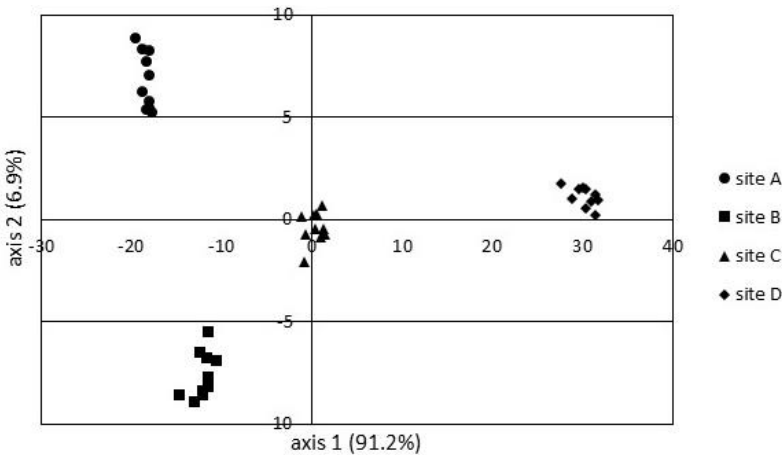


Figure 8. Linear discriminant analysis (LDA): position of observations on the first two axes (in brackets the proportion of variability explained by each axis).

The first axis accounted for more than 90% of variability and outlined the difference between site A and D. The second axis accounted for a reduced amount of explained variability (about 7%) but it seemed useful to differentiate within the managed sites (A, B, and C). By far, the first axis coefficient of *wLDMC*, *wSLA*, and the frequency of *Brachypodium rupestre* were the highest (Table 1). This result highlights the relevance of these variables in discriminating the four plots in comparison to the other considered variables. The coefficients of *Brachypodium rupestre* and *wLDMC* were high also in the second axis, where also the frequency of *Festuca gr. rubra* and *Bromus erectus* recorded the highest coefficients. The coefficients of the other functional trait groups were clearly lower along all the three axes.

Table 1. Linear discriminant analysis: variables scores on the first three axes

Leaf functional trait/vegetation trait/characteristics of litter layer'	Axis 1	Axis 2	Axis 3
	Coefficients of linear discriminants		
SC of <i>Brachypodium rupestre</i>	30.43	-81.76	72.09
SC of <i>Bromus erectus</i>	12.89	-47.59	33.02
SC of <i>Festuca gr. rubra</i>	1.48	-47.60	26.08
<i>wLDMC</i>	40.79	74.66	-33.96
<i>wSLA</i>	-38.24	24.31	-49.24
<i>wLNC</i>	-23.96	4.97	9.52
Chamaephytes	-1.52	-5.34	-6.58

Table 1 (continued)

Hemicryptophytes	-2.11	-9.04	-8.56
Phanerophytes	-1.29	-7.07	-6.82
Geophytes	0.06	-2.20	-0.43
Nano-phanerophytes	0.80	-0.45	-1.32
Therophytes	-0.88	-3.98	-3.25
Flowering Season A	0.48	-0.04	-0.62
Flowering Season B	-0.19	-0.17	0.04
Flowering Season C	-0.07	0.42	0.29
Flowering Duration A	-0.20	-0.39	0.49
Flowering Duration B	0.06	0.30	-0.13
Flowering Duration C	0.07	-0.07	-0.18
Litter thickness	-0.93	2.08	0.53
Litter dry weight	0.32	0.78	1.05
Litter density	1.08	-1.49	0.25

The discriminant analysis supported the interpretation of the results and showed the effectiveness of the considered parameters in discriminating the four study sites. In particular, sites B and C were clearly separated from the ‘extreme’ sites (A and D) on the first axis. This evidence suggested a good attitude of functional traits in the discrimination of the effect of abandonment in site D from the effect of the grazing/mowing management in site A. On the contrary, the studied traits were less effective in the characterization of the different grazing rates between sites B and C. Our results confirmed the potential of functional traits for the characterization of semi-natural grasslands as reported in other studies (Cruz et al., 2010).

CONCLUSIONS

In our study, functional traits were able to differentiate the four sites related with different management practices but they showed different effectiveness. In particular, results support the possibility to base the assessment of management intensity on a rapid appraisal consisting on the estimation of the frequency of a reduced set of abundant species together with the calculation of wLDMC. That involves the need of further studies to assess the feasibility and validity of such approach to different areas and at dissimilar scales. In particular, results concerning wLDMC encourage further developments for the implementation of the existing database (*e.g.* TRY database) to be used in combination with a rapid botanic assessment. This is an important knowledge-based support for land managers and local decision makers to define areas where concentrate the available resources for conservation. Nevertheless, the inclusion of other sites with different management options like inorganic inputs is particularly required for understanding the potentialities of the method as an operational tool.

ACKNOWLEDGEMENTS. The authors acknowledge a grant from the Italian Ministry for University and Research PRIN-2005 ‘Indicators of evolutionary states for oligotrophic grasslands in Apennine Mountains’.

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The effect of soil conditioner on the spatial variability of soil environment

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Abstract. The aim of the study was to assess and evaluate the effect of soil conditioner on the spatial variability of soil environment. Activator PRP-SOL conditioning soil properties was selected as a field of study. Assessment of soil environment was done through the evaluation of selected soil properties, namely, tensile resistance of the soil and soil infiltration ability. Two dose of PRP-SOL application was done twice in year 2015 (Autumn and Spring) and once in 2016 (Spring) with application rates 150 kg ha⁻¹ and 140 kg ha⁻¹, respectively. The area was divided into blocks where stimulators were applied and none treated as a control. The evaluation of recorded values showed that treatability and tillage itself was significantly better on the area which was treated by application of PRP-SOL activators. In addition, tensile resistance was decreased by 5.71% in comparison with non-treated area of experimental field. Since the infiltration ability is among the very important soil properties which have an effect on soil moisture regime as well as surface runoff and therefore soil erosion. The evaluation of recorded values has revealed the effect of treatment by PRP-SOL activators on soil infiltration ability and therefore it results in increases infiltration of precipitation as well. Overall increase of infiltration was recorded at value 2 mm h⁻¹. It can be concluded that application of soil activators may increase the soil conditions and therefore not only conserve soil fertility but even increase it from the long term perspective.

Key words: soil conditioning, activators, PRP-SOL, infiltration, tension resistance, tillage.

INTRODUCTION

Worsening situation in crop production in Slovakia, where quality of agricultural land started to decline rapidly due to impacts of using more and more heavy and complex machinery, gradually forced both professionals and laymen to look for and propose new possibilities of solving of adverse effects and impacts of machinery technique on soil culture, what finally results in low crop yield. Soil compaction is one of the soil properties in question. Strudley et al. (2008) stated that soil tillage practices can affect soil hydraulic properties and processes dynamically in space and time with consequent and coupled effects on chemical movement and plant growth. It leads to loss in crop yield (Ahmadi & Ghaur, 2015), since the compaction prevents plants' root system to penetrate through to deeper soil layers to reach water/nutrients (Šařec & Novák, 2017a and 2017b). Soil compaction has also negative impact on the environment (Ball et al., 1999; Chyba et al., 2014) due to the reduced ability of the soil to absorb water (Angulo-

Jaramillo et al., 2000). Chyba et al. (2014) verified significantly higher water infiltration rate in the non-compacted soil than in the compacted soil. Soil compaction, erosion and creation of spell of drought are factors which affect and cause soil degradation to the greatest extent, while they significantly influence its properties (Šařec & Novák, 2017a and 2017b). It for example increases soil bulk density, and thus leads to a reduction of soil water infiltration rate (Chyba et al., 2014). According to Strudley et al. (2008) understanding of soil pore geometry and structure is fundamental to identification of tillage effects on soil physical and hydraulic properties. Kay & Angers (2002) provided standard definitions (e.g., weak to strong soil structure), classifications (e.g., macropores > 75 µm), and a useful discussion of factors affecting soil structure, including texture and mineralogy, organic matter, inorganic materials, pore fluid, microorganisms, soil fauna, plants, climate, and management. The organic matter may be applied in various forms (Šařec & Novák, 2016; 2017a; 2017b). Manure or compost is commonly used, but it is possible to use also other forms (Wang et al., 2015). Especially on heavy and decarburized soils, there is a problem with the decomposition of applied organic matter (Fontaine et al., 2003; Sarec et al., 2017). It remains in the soil without decomposition and does not affect positively other properties of the soil, e.g. the physical and chemical ones (Steinbeiss & Gleixner 2005). On numerous locations, negative effects are underestimated and their interaction causes irreversible damage to soil fertility (Wilhelm et al., 2007). As a result, there is also a decrease in biomass yield of crops and grasslands (Shahzad et al., 2012). Of course, the problem of soil organic carbon loss may be stopped by applying sufficient quantities of organic matter (Johnson et al., 2006). Manure (or other forms of organic matter) can be supplemented by activators of biological transformation (Šařec & Novák, 2016). The use of activators for the decomposition of organic matter was also recommended by Parr et al. (1986). In their case, activators were applied within the composting. Barzegar et al. (2002) confirmed a positive impact of the compost treatment of as well, i.e. increased wheat yields and improved soil physical properties. Soil compaction primarily affects the physical properties of soil, either in the short or long term (Šařec & Novák, 2017a; 2017b). For example at higher soil moisture levels, passes of farm machinery can lead to excessive soil compaction (Kroulík et al., 2009). The results of Vero et al. (2012) indicate that higher soil moisture deficits (SMD) at the time of machinery trafficking resulted in smaller changes to soil characteristics and more rapid recovery from surface deformation than when trafficking occurred at lower SMD. According to the results of Ahmadi & Ghaur (2015), gradual increase in soil water content generally resulted in an increase in soil bulk density after tractor wheeling. The negative effect of soil compaction is manifested through increased bulk density, soil cone index, and other variables (Blanco-Canqui et al., 2017). This all leads to reduction in porosity, hydraulic soil properties, stability and other variables (Alakukku, 1996). All these parameters are connected together and influence crop yields (Indoria et al., 2016). Celik et al. (2010) confirmed organic applications to significantly lower the soil bulk density and penetration resistance.

Currently, the main objective becomes increasing of soil fertility in line with selection of minimization technologies of soil treatment taking into account soil conditions (Peltre et al., 2015). Suitable selection of these technologies combined with their rational application also contributes to disruption of these adverse effects significantly (Šařec & Novák, 2017a and 2017b). Gradual use of obtained knowledge resulted in designing and development of protective measures which effectively not only

restrain these adverse effects, but may also have positive impact on soil structure under certain conditions (Kay & Angers, 2002), so they directly affect fertility and expected yields of agricultural crops. Each soil structure has its own typical values of bulk density, porosity, hydraulic characteristics and other variables (Six et al., 1999; Šařec & Novák, 2017a). For example, sandy-loam soils have higher cumulative infiltration rate than clay-loam soils, the lowest values are observed in turn with clay soils (Ekwue & Harrilal, 2010). Liu et al. (2012) confirmed this positive effect in their further study where they showed a beneficial effect on maize growth, soil organic matter content, nutrients levels, and water-storage capacity in sandy soils. The most important thing for the future is to keep up the rising trend in this area and to search for new methods that could successfully eliminate these adverse effects in order to prevent further deterioration of soil environment (Ahmadi et al., 2015). Effect of the use of substances for soil amendment (activators) on soil properties is a relatively unexplored phenomenon (Šařec & Novák, 2017a; 2017b). Impact can be mainly expected on the physical and chemical properties of soil. Kroulík et al. (2011) suggested a beneficial effect of incorporation of organic matter on the physical properties of soil, on water infiltration into the soil and on partial elimination of the consequences of soil compaction beneath the tracks. It can be also assumed that changes in soil properties will be reflected in the long term rather than immediately after application (Šařec & Novák, 2017a; 2017b). According to Podhrázká et al. (2012), repeated conventional tillage and application of PRP Sol did not demonstrate any improvement in soil physical properties (density, porosity, soil compaction, reduced water content in soil). Another factor that influences the variables mentioned is soil structure and soil aeration. If the soil is loosened, water capacity is higher compared to the untilled soil (Ekwue & Harrilal, 2010). Each soil structure has its own typical values of bulk density, porosity, hydraulic characteristics and other variables. For example, sandy-loam soils have higher cumulative infiltration rate than clay-loam soils, the lowest values are observed in turn with clay soils (Ekwue & Harrilal, 2010). In terms of economy and operation, energy demand of soil tillage is one of the crucial elements (Liang et al., 2013). Tillage is the base operation in agricultural systems and its energy consumption represents a considerable portion of the energy consumed in crop production (Larson et al., 1995). McLaughlin et al. (2002), Liang et al. (2013) and Peltre et al. (2015) reported manure amendments to have significant effect on reduction in tillage implement draft. Prolonged application and higher rates brought advanced reduction.

The current pressure and need of increasing soil structure, its conservation and increase of soil fertility leads to increased research efforts and various soil biological activators were developed. According to this efforts it was addressed a field experimental research. The aim of the study was to assess and evaluate the effect of soil conditioner on the spatial variability of soil environment.

MATERIALS AND METHODS

Our research was carried out in 2015 – 2016 on one plot divided to two parts in the selected agricultural farm Agrodružstvo TP, ltd. Palárikovo, which consists of 57 soil units and a structure of soil fund represents 2,420 ha of agricultural land. One part was treated by the material (48.041713, 18.042425) for soil structure treatment and the other part was a control part (48.038541, 18.043567). PRP-SOL material for soil structure

treatment was applied to the plot widely. In terms of the objective defined, field experiments were carried out in this selected location and measurements were carried out under operational conditions. A term material for soil structure treatment means activator of soil vital functions PRP SOL. Auxiliary soil materials do not remove consequences, but create favourable conditions for biological life in soil what reflects in lower consumption of used agricultural chemistry. Therefore, PRP SOL is suitable for minimization which supports biological life in the upper part of soil horizon. Infiltrometer (Fig. 1) consists of polycarbonate tube with a diameter of 31 mm and height of 327 mm which is divided into two parts. Both parts are filled with water. Upper part serves for setting of air suction. Water filled in the bottom part infiltrates to soil through semi-permeable stainless steel diaphragm. Suction of air can be set according to soil type. There is a scale in the bottom part of the tube of infiltrometer on which a value of water volume is read in ml after 30 seconds. Measured results are processed by PC. It is important to choose a suitable place for measurement. An important pre-requisite is to make measurement on surface of soil without cracks. After selection of a suitable place, it is important to prepare surface of soil for measurement carefully, because it must be flat and smooth, so the whole diaphragm will be in contact with soil surface (Decagon Devices, 2005). Speed of infiltration v_i is expressed by a ratio of water quantity absorbed through a unit of area of soil surface per a time unit (Velebný et al., 2000).

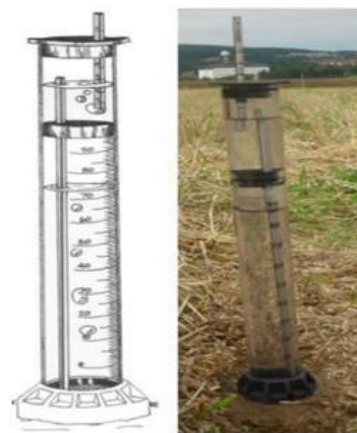


Figure 1. Infiltrometer Mini disc.

$$v_i = \frac{dV}{A \cdot dt}, (\text{mm s}^{-1}) \quad (1)$$

where dV – elementary volume of infiltrated soil per time unit dt (m^3); A – area through which water volume dV is infiltrated (m^2).

The result is not often expressed by height of water layer infiltrated to soil per time unit (mm s^{-1}). Total amount of water V infiltrated to soil per time Δt through unit of area of soil surface from the beginning infiltration is called cumulative (total) infiltration i (Velebný et al., 2000). Cumulative infiltration per time t can be expressed as follows:

$$i = \frac{V(t)}{A}, (\text{mm s}^{-1}) \quad (2)$$

where $V(t)$ – water volume (m^3); A – area through which water volume dV is infiltrated (m^2).

Tension force was measured during ploughing by 5-mouldboard plough to the depth of 22 cm by a tensometric apparatus in two parts of the plot, while one part was treated by a material and the other part was a control. In technical practice, tensometric measurements present effective method for detection of actual operating tensions. In addition, tensometric apparatus is also widely used in design of sensors of force, pressure, torque, etc. A set for measurement of tension resistance contained measuring

instruments and devices (Tractor John Deere 8300, fifth-wheel plough 5PHX 35 and measuring system Hottinger Baldwin Messtechnik Spider-8). Measurements were performed on the plot - both on treated part and control part. The apparatus records values of tension force in N in intervals of 0.2 seconds. Results were recorded during the same time of run in two repetitions with identical plough setting. HBM Spider 8 (Fig. 2) actuating device meets requirements of operating systems with the aim to ensure transfer of data and its operational accuracy. In case of trailing ploughs, dynamometer (tensometer) is located between a tractor and plough. Force measured on dynamometer directly corresponds to tension force $FT = FX$ (N).



Figure 2. Mechanical part of a measurement apparatus used for measuring of a tension force.

RESULTS AND DISCUSSION

The measurements were conducted on field trial held in Agrodružstvo TP, Ltd, Palárikovo. The field conditions were characterised as mostly flat relief with maximum elevation difference 1.1 m. The field has significant soil heterogeneity with a very good production potential and fertility. Majority of the field is formed by Chernozem and it is considered as heavy or very heavy soils. The measurements were conducted in years 2015–2016. Fig. 3 shows the climatic condition during the seasons of observation. As it is indicated at Fig. 3 the climatic conditions during the vegetation period was relatively stable and warm, however in case of precipitation year 2015 was significantly drier than year 2016 where the volume of precipitations were recorded significantly lower. Indoria et al. (2016) reviewed how the different management technologies like integrated nutrient management, tillage practices, mulching, addition of clay, surface compaction, conservation tillage, use of polymers, etc. can favourably modify the soil physical properties like bulk density, porosity, aeration, soil moisture, soil aggregation, water retention and transmission properties, and soil processes like evaporation, infiltration, run-off and soil loss for better crop growth and yield. Moreover, it was suggested that if appropriate soil management technologies are adopted in rainfed areas for the improvement of soil physical health, the productivity of rainfed crops can be significantly improved (Indoria et al., 2016).

During the first year of measurements the soil analysis was performed in order to define starting conditions of experiments and subsequent comparison of soil profile structure (Fig. 4). In the analysis of soil structure the soil clods diameters and distributions was considered according to Shaojie et al. (2016). The samples were

collected from two horizons in depths from 0.00–0.15 and 0.15–0.30 m in three replications. Every structural fraction was weighted individually and the percentages were calculated subsequently. For evaluation was introduced and calculated the coefficient of soil structure which characterise the relation between valuable from point of agronomical view (0.25–10 mm) and less valuable structural elements (> 10 and < 0.25 mm) shown in Table 1.

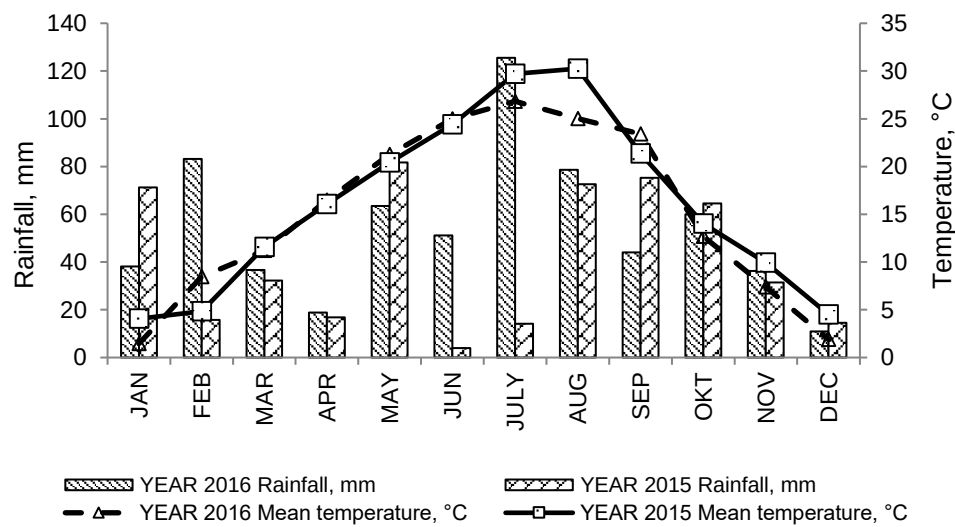


Figure 3. Rainfall represented monthly as sum of precipitations and temperature records for Palárikovo, Slovakia.



Figure 4. Example of comparison of soil profiles (Control and PRP Sol) from year 2015.

Table 1. Comparison of structural elements – in Palárikovo, autumn 2015

Variant	Depth, m	Structural elements, %						Coefficient of structure
		> 10	5–10	2–5	0.5–2	0.25–0.5	< 0.25	
Control	0.00–0.15	38.22	20.22	16.98	15.95	1.69	5.32	1.32
	0.15–0.30	42.54	25.63	17.81	11.31	1.32	2.41	1.23
	Average	40.38	22.92	17.40	13.63	1.50	3.86	1.27
PRP SOL	0.00–0.15	24.75	30.70	22.53	17.50	1.62	3.90	2.60
	0.15–0.30	30.51	34.21	13.95	17.90	0.85	2.40	2.80
	Average	27.63	32.45	18.24	17.70	1.23	2.56	2.34

From the observed values it can be concluded that the soil conditioner PRP Sol has a positive effect on soil properties and arability and can positively affect the compaction of arable horizon down to 0.30 m. The greatest differences in case of soil structural analyses were observed at soil structural elements above 10 mm where in case of PRP Sol conditioner was calculated value 27.63% in comparison with control part at 40.38% which means the difference 12.75% in average. In case of smaller soil structural elements from 0.25 to 0.5 mm the differences in both parts of experimental areas were only small and not significant. Similar observation was obtained also in case of soil structural elements below 0.25 mm. In the case of utilization of biostimulators also biochar is considered as an alternative. For example, Blanco-Canqui (2017) has reported that biochar generally reduces soil bulk density by 3 to 31%, increases porosity by 14 to 64%, and has limited or no effects on penetration resistance. Biochar increases wet aggregate stability by 3 to 226%, improves soil consistency, and has mixed effects on dry soil aggregate stability. It increases available water by 4 to 130%. Further study shows that saturated hydraulic conductivity decreases in coarse-textured soils, and increases in fine-textured soils following biochar application (Blanco-Canqui, 2017). In addition, Sajjadi et al. (2016) investigated the relations between infiltration rate and soil texture, moisture and compaction and it was shown the effect of soil properties and their relations on infiltration rate by using non-linear regression.

Later on in our study the selected physical properties of soil were observed and are shown in Table 2.

Table 2. Selected physical properties of the soil

Variant	Depth of soil, m	Density red., g cm ³	Porosity, %	Current content		Max. capillary capacity % volume	Min. air capacity
				Water	Air		
Control	0.0–0.1	1.27	51.77	10.66	40.87	37.56	14.77
	0.1–0.2	1.45	44.28	21.50	23.21	35.21	8.95
	0.2–0.3	1.51	40.75	24.92	14.99	35.12	5.90
	Average	1.41	45.60	19.02	26.35	35.96	9.87
PRP SOL	0.0–0.1	1.29	51.30	18.59	31.67	38.85	10.99
	0.1–0.2	1.55	43.95	25.21	18.98	34.61	8.10
	0.2–0.3	1.41	45.90	22.42	24.25	37.10	9.69
	Average	1.42	47.05	22.07	24.96	36.85	9.59

Measurements focused on comparison of speed of infiltration of water into soil. The plot area was 21 ha with 10 monitoring points where each monitoring point was calculated from ten repetitions. Volume of infiltrated water was measured in both parts of the plot, it means the part treated by PRP SOL material (48.041713, 18.042425) and control part (48.038541, 18.043567), and in ten repetitions during the same time period in the depth of 10 cm. Values of soil humidity were also measured in the control soil probes in 10 cm intervals. A measurement of volume soil humidity in surface zone to 10 cm was carried out in five repetitions for control.

Table 3 shows the statistical comparison of observed values for cumulative soil infiltration rate between untreated and treated part of the field experimental area by soil conditioner PRP Sol.

Table 3. Descriptive statistics of cumulative infiltration

Variant	Average	Max.	Median	Min.	Percentile 25	Percentile 75	Standard deviation
Control	0.14	0.23	0.15	0.03	0.10	0.19	0.06
PRP SOL	0.18	0.26	0.18	0.06	0.13	0.23	0.07

Result of measurements was a difference in average percentage of soil humidity content in the part of the plot treated by PRP SOL material with a value of 37.7% compared to the value of 39.4% in control part. Easy to say, water in treated area moved downwards vertically to lower zones of soil profile during the same time from the last rain more quickly. Speed of infiltration set according to retention curves and their trend lines from average measured values is graphically displayed in the Fig. 5.

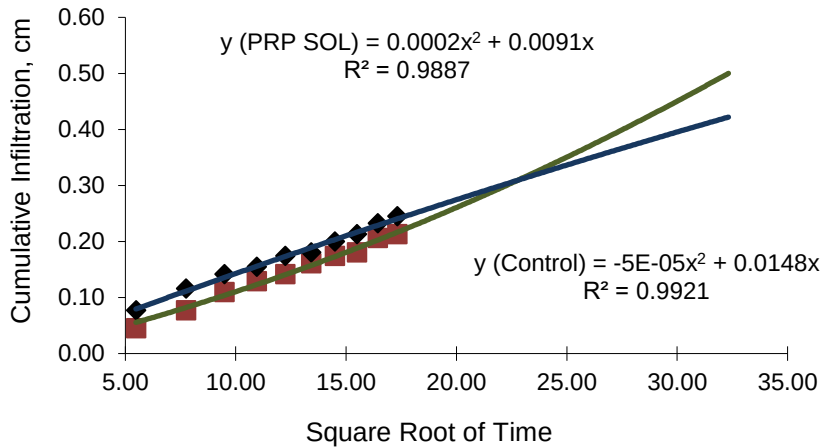


Figure 5. Speed of infiltration.

Blanco-Canqui et al. (2017) has reported that tillage treatments affected pounded infiltration only. Mouldboard plough significantly increased pounded infiltration rate by 21.6 cm h^{-1} at 5 min and by 8.8 cm h^{-1} at 60 min compared with no-till. However, when compared with disk and chisel, mouldboard plough increased pounded infiltration rates at all measurements times, which lasted 3 h. Regarding cumulative infiltration,

mouldboard plough increased cumulative infiltration by 26.9 cm to 39.0 cm after 3 h compared with other tillage systems. Similarities in tension infiltration suggest that the higher pounded infiltration for mouldboard plough was most likely due to the presence of voids or fractures (> 125 µm) created by full inversion tillage. Total porosity, saturated hydraulic conductivity, and water retention among the treatments did not differ (Blanco-Canqui et al., 2017). In these relations, tillage affects the infiltration speed in soil levels and application of soil activators may also positively affects the speed of infiltrations as well.

It was also concluded by Šařec & Žemličková (2016) that concerning soil bulk density, a drop in values can be discerned with the application of cattle manure, and with majority of variants using pig manure where there are high dosage rates, but the drop was found also with PRP Sol alone. Moreover, Strudley et al. (2008) concluded that differences in temporal variability depend on spatial locations between rows, within fields at different landscape positions, and between sites with different climates and dominant soil types. Most tillage practices have pronounced effects on soil hydraulic properties immediately following tillage application, but these effects can diminish rapidly. Long-term effects on the order of a decade or more can appear less pronounced and are sometimes impossible to distinguish from natural and unaccounted management-induced variability (Golchin et al., 1994).

Set values of retention curves and their trend lines point to the fact confirming a difference of soil humidity measurement in surface zone of soil profile that rain water in the surface treated by PRP SOL material was infiltrated more quickly. Difference in speed of infiltration to the depth after treatment by PRP SOL material was 2 mm per 1 hour. Sarec et al. (2017) observed the favourable effect of soil activators on the bulk density and other physical soil properties during the measurement of the physical properties of soil. Vegetation indices were another consideration for rating. They suggest a beneficial effect of application of bio-activators. The following values were measured by tensometer force sensors which recorded value (Table 4) of tension force and a measurement unit Hottinger Baldwin Messtechnik Spider-8. During driving of machinery, total measured tension force stopped on the value of 83,735 N in the plot which was used as a control part (Fig. 6). We measured lower values on the treated plot. Maximum tension force measured while driving on the plot treated by the material was 78,911 N (Fig. 7). Also Šařec & Žemličková (2016) has demonstrated the beneficial effect of substances for soil (PRP Sol) and manure amendment (PRP Fix) and of organic fertilisers of various origins on soil bulk density, cone index and on implement draft force reduction.

Table 4. Descriptive statistics of tension force

Variant	Average	Max.	Median	Min.	Percentil 25	Percentil 75	Standard deviation
Control	64.03	83.73	66.25	-5.94	63.11	68.84	12.55
PRP SOL	54.89	78.91	56.19	-0.40	50.88	62.10	13.10

In addition, Šařec & Novák (2017b) concluded that the impact of the manure and the activators on the value of saturated hydraulic conductivity is difficult to precisely define. One of the factors may be the duration of the experiment. Another, probably more relevant, is the soil texture of the trial field. All the variants treated with manure

demonstrated increase of saturated hydraulic conductivity, namely with PRP Sol applied as well. Moreover, Bagarello et al. (2006) reported that difficulties of measuring saturated hydraulic conductivity on light soils were found. At high levels of conductivity, the effects of soil tillage, fertilization or the influence of cultivated crops cannot be clearly demonstrated. In accordance with authors' assumptions, Celik et al. (2010) confirmed organic applications to significantly lower the soil bulk density and penetration resistance. However, the assumption was not verified by the results so far. Beneficial effect of activated organic matter on soil properties and on production potential was confirmed by Barzegar et al. (2002). Bernal et al. (1998) pointed to the gradualness of changes in the soil and to the need for long-term exposure to carbon fixation and microbial activity.

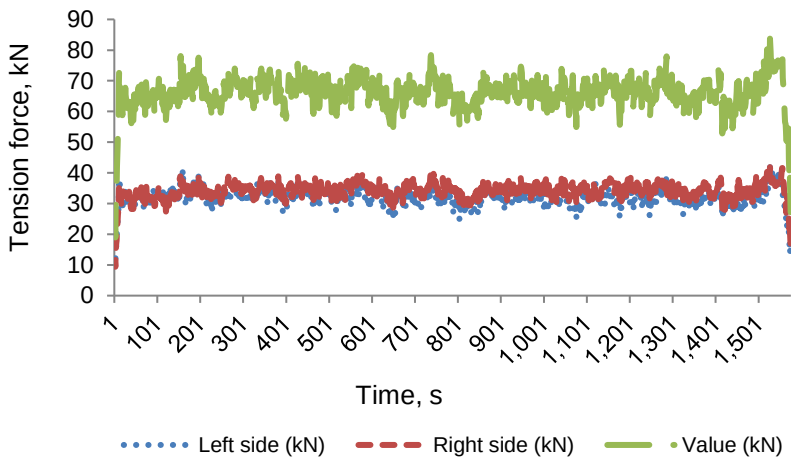


Figure 6.Record about measurement of tension force during ploughing in the control part (maximum generated force is 83.7 kN).

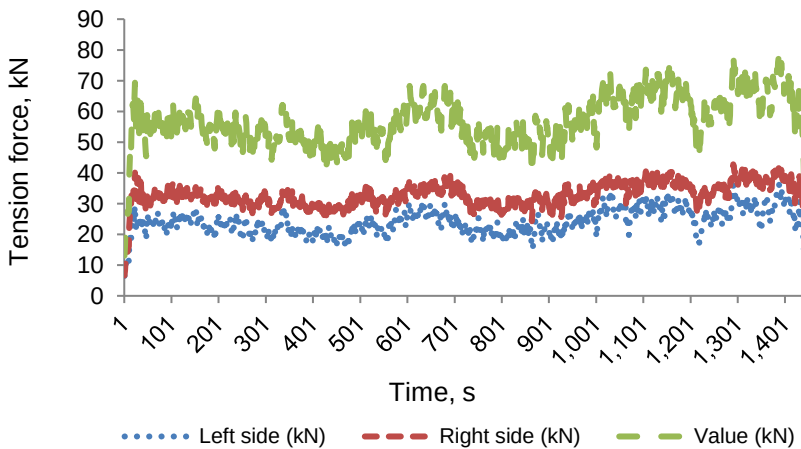


Figure 7.Record about measurement of tension force during ploughing on the plot after application of the material (maximum generated force: 78.9 kN).

By comparison of measured and calculated values of tension force caused by tools used in the soil and total need of work it was proven that degree of workability of soil is significantly better on the plot treated by the material than in the control part. Simple analysis of these results confirm a fact that improved function of biological activity and so structure of soil caused decrease of tension force for 5.71% compared to untreated plot (Table 4). According to Strudley et al. (2008) development of soil structure and aggregation are dynamic properties that depend upon soil parent material in addition to climate and management factors. Shrink/swell clays may play an important role in both the natural variability of soil structure and potential responses of soil hydraulic properties to management practices (Horn et al., 1994; McGarry et al., 2000). Changes in soil pore structure in swelling clays have been evidenced in studies of gas and water flow (Angulo-Jaramillo et al., 2000; Horn & Smucker, 2005) and solute transport (Bouma & Woesten, 1979). Swelling clays may also account for some reversal of soil disturbances, such as self-healing of cracks (Eigenbrod, 2003; McDonald et al., 2006) and re-formation of surface cracks upon drying (Radford et al., 2000). Smiles (1995; 2000) provided reviews of the physics of swelling soils, noted here for general reference. Moreover, as Strudley et al. (2008) pointed out, Smiles (2000) has never been cited before, which points to the lack of active advances in this area, with the exception of the few studies cited here on interactions of tillage with the shrink/swell behaviour of soils.

CONCLUSIONS

Tension force measurements were performed during ploughing by 5-mouldboard plough 5PHX 35 to the depth of 22 cm. The experiment itself was carried out in both parts of the plot, namely in the part treated by material and control part. The value of tension force was recorded by tensometer sensors and data were recorded by a measurement unit Hottinger Baldwin Messtechnik Spider-8. During driving of machinery, total measured tension force was 83,735 N in the plot which was used as a control part. We measured lower values on the treated plot. Maximum tension force measured while driving on the plot treated by the material was 78,911 N.

By comparison of measured values of tension force caused by tools used in the soil and total need of work it was proven that degree of workability of soil is significantly better on the plot treated by the material than in the control part. Simple analysis of these results confirms a fact that improved function of biological activity and so structure of soil caused decrease of tension force for 5.71% compared to untreated plot.

Infiltration of soil can be measured by several methods. One of the fastest and simplest methods is measurement by Minidisk infiltrometer. We can state that by using of infiltrometer we found out that speed of infiltration depends on compactness of soil, where some layers of soil infiltrate water faster, and parts of soil with compacted layer infiltrate water more slowly.

ACKNOWLEDGEMENTS. This work was supported by AgroBioTech Research Centre built in accordance with the project Building 'AgroBioTech' Research Centre ITMS 26220220180; and V4 funds project No. 21730049 'Creating a platform to address the techniques used in agriculture and economic groundwater mngmt'.

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Fatty acid composition in pork fat: De-novo synthesis, fatty acid sources and influencing factors – a review

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Abstract. Fats are among the basic nutrients the human organism needs as a source of energy, as well as to grow and regenerate cells, tissues, and organs. Particularly animal fats, with their higher proportion of saturated fatty acids and low content of n-3 fatty acids, are often seen by the public as relatively undesirable food components. Fatty acid (FA) composition of pork is affected by many factors: genotype, breeding, gender and feeding methods. Numerous research teams, therefore, have searched for means of effectively manipulating the chemical composition of animal fats. This paper reviews existing knowledge and means of effectively influencing the fatty acid composition in pig fat, which is a significant component of human food in European countries due to their high consumption of pork. The findings of various authors demonstrate that not only altering of fatty acids sources in animal diets but a range of other factors as well can significantly influence the composition of fatty acids in pig fat and consequently pork quality.

Key words: pig, fatty acid, nutrition, carcass, PUFA.

INTRODUCTION

Although pork remains today the meat most consumed in developed European countries, it increasingly is regarded among consumers as a relatively undesirable food due to the public's awareness of potential health problems related to consuming animal fats. The nutritional quality of pork is a significant factor affecting consumers' health, and this is particularly relevant to consumers worldwide for whom pork is the primary source of meat (Romano et al., 1995; Kouba et al., 2003; Wood et al., 2004; Jiang et al., 2017).

The content and composition of saturated and unsaturated fatty acids (FA) (i.e. the main components of fat) in human food are significant in terms of human health (Nuernberg et al., 2005). Compared to chicken and beef, pork has a lower content of unsaturated fatty acids in intramuscular fat. This implies a less favourable ratio of unsaturated and saturated fatty acids. The n-6/n-3 ratio of polyunsaturated fatty acids is unfavourable for pork and its fat (Woods & Fearon, 2009; Liu & Kim, 2018). Nutritionists often point out that conventional pork products provide too much n-6 while lacking n-3 polyunsaturated fatty acids (Wood et al., 2008; Kouba & Mouron, 2011).

Animals' muscle and fat tissues are the main sources of fat in human food, and therefore it is in the interest of food producers to modify the nutritional value of these tissues (Vehovský et al., 2015).

A high content of saturated fatty acids in food and an unfavourable ratio of n-6 and n-3 groups of polyunsaturated fatty acids may be the cause of a number of diseases, in particular cardiovascular diseases (Kouba & Mourot, 2011; Liu & Kim, 2018). Recommendations for healthy eating state that fats consumed in food should contain saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids in the ratio of about $< 1 : 1.4 : > 0.6$. In terms of health, n-3 polyunsaturated fatty acids are the most important fatty acids. These are lacking in animal fats even as groups of n-6 polyunsaturated fatty acids are abundant. Fats contained in feedstuffs fed to pigs can have a significant effect on the resulting fatty acid profiles in pork fat. This means there exists an opportunity to manipulate the composition of fatty acids in pork fat by means of pigs nutrition, and thus to influence the quality of the resulting meat and fat (Čítek et al., 2015; Liu & Kim, 2018).

The aim of this review is to provide a comprehensive overview of the composition of fatty acids and their synthesis in pork meat. Furthermore, to provide overview of exogenous sources influencing the composition of fatty acids and consequently the quality of pork meat.

FAT AND FATTY ACIDS IN SWINE ADIPOSE TISSUE

Lipid composition of pork differs depending on the type of fat, muscle and muscle fibres, (see Table 1.) and it is affected by many factors. These factors include gender, genotype, breeding, and feeding methods. A current tendency in agricultural production is to feed animals so as to maximize production of lean meat and eliminate excessive fat development.

Table 1. Fatty acid composition (% of total fatty acids) in different pork tissue (Adapted from Sobol et al., 2016; Velišek & Hajšlová, 2009)

Fatty acid	IMT	Backfat	Visceral tissue	Neck	Shoulder	Loin	Ham	Belly
Lauric acid	0.21	0.09						
Myristic acid	2.54	1.62	1.6	0.137	0.085	0.087	0.059	0.188
Palmitic acid	28.68	26.82	26.7	2.76	1.74	1.78	1.17	3.87
Palmitoleic acid	5.48	2.7	2.2	0.244	0.176	0.165	0.122	0.335
Stearic acid	8.67	15.94	10.2	1.85	1.08	1.15	0.721	2.53
Oleic acid	31.98	33.5	36.4	4.38	3.00	2.81	1.97	6.26
Linoleic acid	12.11	10.03	16.1	1.55	1.02	0.899	0.676	1.95
α -linolenic acid	4.09	5.79	1.2	0.518	0.305	0.312	0.193	0.697
Arachidic acid	2.92	0.13	0.3	0.052	0.044	0.038	0.034	0.068
Eicosenoic acid				0.109	0.070	0.073	0.046	0.163
Eicosadienoic acid	0.1	0.45						
Docosahexaenoic acid			1.8	0.019	0.018	0.011	0.009	0.025

Carcass composition of pigs of modern genotypes has been changed in recent decades to favour a higher proportion of lean meat and lower fat content while generally breeding pigs with increased attention to the quality of pork and fat tissue (Hallenstvedt et al., 2012).

Physical and chemical properties are greatly affected by the length of carbon chains, degree of saturation, and isomeric form of fatty acids (Rossi et al., 2010). The ratio of saturated and unsaturated fatty acids is reflected in the fat consistency, and fat quality is thus determined by the composition of fatty acids (Wood et al., 2004; Suzuki et al., 2006). Unsaturated FA provide the primary substrate for oxidative processes, and increasing of n-3 PUFA content, in particular, may reduce the oxidative stability of pork and consequently diminish its sensory properties (Kristinsson et al., 2001; Karolyi et al., 2012).

The pig's own diet is one of the significant factors influencing the structure of pork and lard. Even as nutrition may affect the fat composition amount of fat deposited in the body and also its fatty acid profile (D'Sousa et al., 2010), feeding strategies also can modify such sensory properties of both meat and fat as softness and tenderness. (Wood et al., 2008; D'Sousa et al., 2010; Skiba et al., 2012). Iodine value a measure of unsaturation fatty acid, is one method used by pork processors for assessing pork fat quality. Increases in fatty acid unsaturation or IV are associated with negative impacts on pork fat quality. This can lead to problems with belly slicing efficiency, fat smearing, and reduced shelf life because of oxidative rancidity (Wood et al., 2004 and 2008; Paulk et al., 2015).

Fatty acid composition in animal products is influenced both by biosynthesis of fatty acids in animal tissues and by lipids present in feedstuffs consumed by livestock. The effect of nutrition is more important in monogastrics than in ruminants because ruminants are capable of hydrogenating FA in the rumen (Wood et al., 2004; Kouba & Mourot, 2011). Thus, by modifying pigs' feed, daily intake of unsaturated FA's can be increased which will increase unsaturated FA content of pork products (Woods & Fearon, 2009). The differences in digestion between monogastric animals and ruminants is the reason that the recommended dietary PUFA/SFA ratio is 0.4 in swine and other monogastrics and is significantly higher compared to that for ruminants. On the contrary, the n-6/n-3 PUFA ratio is more favourable in ruminants. The nutritional recommendation of this indicator is $R \leq 4$ (n-6/n-3 PUFA ratio) (Warnants et al., 1999). These monitored ratios are considerably affected by external factors, and the choice of diet, in particular, can affect them significantly. A number of research teams have experimented with manipulating the FA composition in pig fat through feeding components (Enser et al., 2000; Wood et al., 2004; Corino et al., 2008; Haak et al., 2008; Bečková & Václavková, 2010; Raj et al., 2010). Corino et al. (2008) report a linear relationship between dietary PUFA intake and PUFA in intramuscular fat (IMF) and subcutaneous fat. Alonso et al. (2012) observed the lowest n-6/n-3 ratio in subcutaneous fat as an effect of soybean oil in the diet, while that ratio was highest in IMF within the same group and compared to groups supplemented with animal fats and the control group. This also suggests different incorporation of FA in swine tissue, and in particular of α -linolenic acid. D'Arrigo et al. (2002) state that changes in the profile of n-3 fatty acids manifest themselves more in neutral lipids of the reserve tissue than in the structural lipids of muscles (IMF).

According to Wood et al. (2008) as well as Bečková & Václavková (2010), pigs have a far higher level of the pivotal FA linoleic acid in IMF and subcutaneous fat than do cattle and sheep. The relationship between backfat thickness and FA composition is the focus of papers by Suzuki et al. (2006) as well as Wood & Enser (1997). Those authors report a positive correlation between backfat thickness and SFA content and they observed negative correlations between backfat thickness and palmitoleic acid (C16:1) and linolenic acid (C18:2) contents.

Duran-Montgé et al. (2010) studied the degree of essential FA utilization, monitoring the influence of fats fed to pigs on the levels of FA retained and the level of de novo FA synthesis in the pig carcasses. Fatty acids of the intramuscular fat and backfat mirrored those FA in the diet while visceral fat had a higher share of long-chain PUFA. The authors note that this suggests higher utilization of long-chain FA for the visceral fat.

Fatty acid in intramuscular fat

The specific taste, juiciness, and other sensory characteristics of pork are in large measure determined by intramuscular fat (IMF). In contrast to beef, IMF in pork (sometimes with the exception of meat from the Duroc breed (Suzuki et al., 2003; Dashmaa et al., 2011)) is not visually perceptible (Mourot & Hermier, 2001). As a result of intensive selection for a high proportion of lean meat in finished swine, this component in pork has been reduced significantly. Genetic selection has diminished the proportion of IMF in raw meat – which is the carrier of tenderness and juiciness – to less than 1% versus the 2–4% occurring in the past (Alonso et al., 2010). In general, the term intramuscular fat (IMF) refers to lipids and lipids related substances in lean muscle which can be extracted by organic solvents. Nevertheless, it is necessary to distinguish lipids of cell membranes, in particular phospholipids and neutral lipids composed of triacylglycerols localized in adipocytes along muscle fibres. The share of phospholipids as membrane components is relatively fixed so that the properties of cell membranes are maintained, and these exist in the range of 0.2–1% of the muscle weight (De Smet et al., 2004). Content of muscle triacylglycerols is closely associated with the total fat content and ranges between 0.2% and 5% and more of the muscle weight. Although the PUFA content of triacylglycerols can be influenced by dietary factors, particularly in monogastrics, it is diluted by de novo fatty acid synthesis consisting of SFA and MUFA, thus causing a decline in the PUFA/SFA ratio with increasing fat deposition (Alonso et al., 2012).

IMF composition in pork carcasses can vary considerably, and that composition is a trait with a medium to high level of heritability ($h^2 = 0.4\text{--}0.6$) (Bečková & Václavková, 2010). IMF content is greatly influenced by the sufficiency of energy content in pigs' diets (Alonso et al., 2012).

FACTORS AFFECTING FATTY ACIDS COMPOSITION IN PIG ADIPOSE TISSUE

Differences in FA composition depending on gender

In relation to carcass value, the effect of gender in slaughtered animals is reflected primarily in the amount of fat tissue. It also is known that FA composition of adipose tissues in pigs is associated with the expression of lipogenic enzymes that are

considerably influenced by gender (Doran et al., 2006; Missotten et al., 2009). Thus, castration significantly affects FA composition of swine fat (Missotten et al., 2009). This was confirmed in a study by Nuernberg et al. (2005), which showed that gilts had significantly higher proportions of PUFA in IMF and backfat compared to surgically castrated barrows, regardless of the source of fat in the diet. This also was confirmed by results from a study conducted by Alonso et al. (2009), in which gilts of three hybrid combinations had higher proportions of PUFA in IMF compared to barrows. The influence of gender on FA composition was dealt with also by Halenstvedt et al. (2012), who recorded in their study a demonstrably higher content of PUFA in fattened young boars compared to fattened gilts while a significant higher level of MUFA was recorded in gilts. The authors observed no difference in SFA content in relation to gender.

Current trends in pig farming also include immunological castration (immunocastration) of fattened young boars. Pauly et al. (2009) conducted a study on immunocastration of fattened young boars in which they compared fattened young boars, barrows, and immunological castrates. Regarding FA composition, the authors recorded demonstrably the highest proportion of PUFA and lowest proportion of SFA in fattened boars compared to both surgically and immunologically castrated boars. A study conducted by Mörlein & Tholen (2015) dealt with the influence of the most important components of boar taint (androstenone, skatole, and indole) on FA composition in boar fat. In the boars with higher levels of boar taint components in backfat compared to boars characterized by lower levels of androstenone and skatole, the authors recorded in boar backfat a considerably higher content of SFA and a lower content of PUFA. According to those authors, the trend above all resulted from higher concentrations of linoleic acid and α -linolenic acid in boars with lower concentrations of androstenone and skatole in fat. Androstenone and skatole are correlation with fatty acid composition. The correlation between fatty acid composition and androstenone is higher than with skatole (Liu et al., 2017). MUFA were not significantly affected by concentrations of the aforementioned components. The literature generally thus indicates an apparent effect of gender and its expression of lipogenic enzymes on the composition of FA in pig fat. Some authors (e.g. Flachowsky et al., 2008) nevertheless state that FA proportions in pig fat are much more influenced by the dietary FA source than by gender.

Interbreed differences in fatty acid composition

Interbreed differences are reflected in the carcass value of slaughtered animals, including the proportions of fat and its composition. A number of authors have reported on the effect of breed on the concentration and composition of FA (Table 2.), proteins and fat in pig carcasses (Kouba & Mourot, 1999; Wood et al., 2004; Raj et al., 2010; Choi et al., 2016). Compared to the more crossbred pigs the authors recorded higher concentrations of PUFA in fat (above all C18:2) in the Pietrain breed, even though this breed typically is characterized by a higher content of protein and lower content of fat in the carcass. Monin et al. (2003) recorded more SFA and MUFA in the fat of the Large White breed and, on the contrary, less PUFA compared to the Pietrain breed. Alonso et al. (2009) studied the influence of hybrid combination on FA composition and meat quality, evaluating the effect of parental breeds Pietrain, Duroc, and Large White. Although the authors state that there were no significant differences in meat quality, the hybrid combination with Duroc as parental breed showed the highest proportion of IMF. Pietrain as parental breed manifested a significantly higher proportion of PUFA, as well

as higher ratios of PUFA/SFA and of n-6/n-3 in MLLT and semimembranosus muscles. This reflects the fact that linoleic acid and PUFA generally occur at higher concentrations in the fat of leaner swine breeds than in the fat of breeds with higher carcass fat contents.

Raj et al. (2010) explain this phenomenon as the *de novo* synthesis of FA being lower in leaner breeds, which breeds have lower proportions of endogenous FA. In such breeds, there is lower dilution of exogenous linoleic acid by synthesized saturated FA. Also relevant to this subject is the conclusion from Alonso et al. (2009) that exogenous FA are utilized more in IMF.

Table 2. Difference of fatty acid contents (% of total fatty acids) of longissimus lumborum muscle from pig breeds (Adapted from Raj et al., 2010; Subramanian et al., 2016; Dashmaa et al., 2011)

Fatty acid	Duroc	Pietrain	Large white	Hampshire	Belgian landrace	Berkshire	LxLWxD
Myristic acid	2.23	1.21	1.81	1.27	1.28	2.27	1.76
Palmitic acid	29.49	21.34	26.96	22.41	20.32	34.8	29.61
Palmitoleic acid	4.5	1.91	4.33	1.9	1.26	5.13	3.14
Stearic acid	15.3	11.42	14.07	11.88	11.67	17.14	15.21
Oleic acid	40.19	35.26	38.18	37.31	38.01	29.85	38.06
Linoleic acid	6.65	21.87	12.87	18.53	19.22	8.2	12.69
α -linolenic acid	0.57	1.05	0.59	0.92	1.21	0.76	0.1

LxLWxD (Landrace x Large White x Duroc).

DE NOVO SYNTHESIS AND EXOGENOUS SOURCES OF FATTY ACIDS IN PIG NUTRITION

Traditionally fats are included in pig diets in such forms as cereals rich in oil, oilseeds, and fish oil as high-energy feed ingredients. Nutritionally, they are concentrated sources of energy, providing essential fatty acids that are the building blocks for hormone-like compounds and are carriers for the liposoluble vitamins A, D, E, and K. Compared to other feed nutrients, the use-efficiency as metabolized energy of lipids is very high and with a minimal heat increment (Woods and Fearon, 2009; Rossi et al., 2010; Krogh et al., 2017).

Fatty acid synthase is a key enzyme catalyzing the *de novo* synthesis of long-chain FA from acetyl-CoA and malonyl-CoA. Fatty acids (FAs) are essential constituents of lipids involved in membrane biogenesis and are critical substrates in energy metabolism (Mendez & Lupu, 2007; Guo et al., 2017). An absence or excess of some FA – in particular long-chain FA – influences the pig’s organism in relation to *de novo* synthesis of FA mediated by elongase and desaturase enzymes (Kouba et al., 2003). Several authors have reported that activity of desaturases, and in particular Δ -9 desaturase, increases with the proportion of dietary SFA, while a diet rich in oleic acid reduces the activity of this desaturase (Klingenberg et al., 1995; Kouba et al., 2003; Pascual et al., 2007). Pascual et al. (2007) mention a lower inhibition of Δ -9 desaturase in the Duroc breed compared to the Large White and Landrace breeds.

Hallenstvedt et al. (2012) are among those who have studied the effect of FA in swine nutrition. They describe retention of FA in subcutaneous fat in proportions similar to those in the diets fed but increased *de novo* FA synthesis when low fat diets were provided. The latter case resulted in mainly saturated and monounsaturated FA (C16:0, C18:0 and C18:1) being synthesized and the total proportion of PUFA in final product being thus reduced. According to Duran-Montgé et al. (2010) as well as Alonso et al. (2012), dietary fat limits *de novo* synthesis and feeding low-fat diets thus causes higher concentrations of SFA and MUFA to occur in pig fat. Kouba & Mourot (1999) state that n-6 PUFA inhibit activity of stearoyl-coenzymeA desaturase, the key enzyme in the process for desaturating stearic acid to MUFA. Therefore, higher concentrations of exogenous linoleic acid in feed contribute to an increase in PUFA with concurrent decrease in the MUFA portion (Kouba & Mourot, 1999; Alonso et al., 2012). This theory is confirmed by results presented by Riley et al. (2000) and by Enser et al. (2000), who observed higher proportions of MUFA – in particular oleic acid – in pig fat when a higher proportion of saturated fats was included in the diet.

Warnants et al. (1999) compared retention in fat of PUFA, MUFA, and SFA from a diet enriched with soybeans. In that study, PUFA were observed to be incorporated most effectively in pig fat while MUFA and SFA in the diet had a smaller effect on the pig fat composition.

In a study as to the effect of various FA sources in the diet and their influence on the *de novo* synthesis of FA in pigs, Duran-Montgé et al. (2010) observed that when a diet not enriched with fats and oils was fed palmitic acid, stearic acid, and oleic acid were *de novo* synthesized in the ratio of 1.6 : 1.0 : 3.0. When feeds supplemented with oil were provided, the *de novo* synthesis was influenced by the type of oil supplemented and its FA composition.

SOURCES OF FATTY ACIDS

Several studies have been conducted to modify the FA composition of pork fat tissues by nutritional means, including by supplementing specific oils, oilseeds, or forages in the animals' diets (see Table 3).

Table 3. Updated summary of data known for different feed supplement which affected fatty acids composition in pig tissue

Reference	Feed supplement	Effect of FA composition
Bee et al., 2002	Soybean oil/ Tallow	Soybean oil or tallow increased proportion of PUFA that has been compensated by reduced (SFA) and (MUFA) proportions.
Apple et al., 2009	Soybean oil	Soybean oil increased proportion of PUFA.
Alonso et al., 2012	Soybean oil/ Tallow / Palm oil	Soybean oil decreased proportion of MUFA and increased PUFA without negative effect of meat quality.
Warnats et al., 1999	Soybeans	Soybeans in the final 6 weeks before slaughter increased PUFA/SFA ratio in backfat.
Park et al., 2009	Soybean oil	Soybean oil increased PUFA without effect on growth performance and carcass traits.

Table 3 (continued)

Missotten et al., 2009	Linseed oil/ Fish oil	Maternal diet with fish oil or linseed oil increased the level of n-3 PUFA in muscle of piglets.
Enser et al., 2000	Linseed oil	Linseed oil increased levels of EPA and DHA in adipose tissue.
Sheard et al., 2000	Linseed oil	Linseed oil increased α -linolenic acid levels, with increases in total n-3 PUFA content and reduced n-6 PUFA.
Kouba et al., 2003	Linseed	Linseed increased the content of n-3 PUFA in plasma, muscle, and adipose tissue.
Bečková & Václavková, 2010	Linseed oil	Linseed oil reduced the n-6/n-3PUFA ratio and SFA/PUFA ratio.
D'Sousa et al., 2010	Linseed oil / Canola oil /Soybean oil/ PUFA commercial oil	Linseed oil improved lean tissue accretion (lean meat and loin area in the carcass) and meat quality.
D'Arrigo et al., 2002	Linseed oil	Linseed oil improved n-6/n-3 ratio due to increase in α -linolenic.
Romans et al., 1995	Linseed	Linseed improved FA composition after 7 days feeding.
Jing et al., 2017	Linseed oil/ Soybean oil	Linseed oil improved meat tenderness compared to soybean oil.
Huang et al., 2008	Linseed	Linseed increased the content of n-PUFA in muscle and adipose tissue, stimulated IMF accumulation, and promoted the hypertrophy of the <i>longissimus dorsi muscle</i> , <i>quadriceps femoris muscle mass</i> , and <i>semitendinosus muscle</i> .
Luo et al., 2009	Linseed oil	Linseed oil could be effected IMF content, FA profile and gene expression in tissue.
Okrouhlá et al., 2013	Linseed	Linseed increased PUFA content and PUFA/SFA ratio, especially through increasing the n-3 PUFA content, and decreased the MUFA content, the MUFA/PUFA, MUFA/SFA, and n-6/n-3 PUFA ratios and the thrombogenic index.
Čítek et al., 2015	Linseed/ Maize	Maize and linseed reduced the PUFA/SFA ratio and improved atherogenic and thrombogenic indexes without negative effect on technological characteristics of meat and backfat.
Vehovský et al., 2015	Linseed / Maize	Linseed significant increased PUFA content compared to additional maize.
Sobol et al., 2016	Mixture (Linseed, Rapeseed, Fish oil)	Mixture of linseed, rapeseed and fish oils improved the FA content in pork.
Della Casa et al., 2010	Maize	Maize showed wide differences in linoleic acid due both to total lipid content and to fatty acid profile.
Opapeju et al., 2006	Maize (2 variety of corn)	Maize increased linoleic acid and decreased stearic acid in pig fat greater concentration of PUFA.

Table 3 (continued)

Rossi et al., 2002	Maize/ Tallow/ Rapeseed oil	Tallow increased SFA in backfat, rapeseed oil increased the proportion of linolenic acid.
Han et al., 2005	Maize/ Wheat	No differences were show between wheat and maize groups in FA composition in backfat and backfat colour.
Hoz et al., 2003	Sunflower oil	Sunflower oil increased proportion of PUFA, proportion of n-6 PUFA, and the decreased proportion of n-3 PUFA.
Boselli et al., 2008	Sunflower oil	Sunflower oil increased triacylglycerols containing oleic acid in the raw meat, and a decreased of both linoleic and SFA.
Mitchaothai et al., 2007	Sunflower oil/ Tallow	Fat type had no significant effect on carcass traits, sunflower oil increased content of linoleic acid and PUFA.
Realini et al., 2010	Sunflower oil/ Linseed oil/Tallow/ fat bland / oil bland	Sunflower oil and linseed oil showed high percentages of n-6 and n-3 FA, PUFA/SFA ratios were increased by feeding all supplements.
Corino et al., 2002	Rapeseed oil / Sunflower oil/ Maize	Rapeseed oil increased linoleic acid content of total lipid.

Park et al. (2012) evaluated the effect of various oils in the diet and concluded that the type of fat in the feed mix, including soybean oil, did not have a demonstrable effect on the chemical composition and quality of meat or on the fattening capacity parameters. Fat in the diet did, however, influence FA composition in the pig fat. Including soybean oil in the diet for pigs significantly increases the proportion of PUFA (Bee et al., 2002; Apple et al., 2009; Alonso et al., 2012). Incorporation of FA in fat tissue and their subsequent elimination upon withdrawal of the dietary fat source was the subject of a study by Warnants et al. (1999). They found that healthier fat with favourable PUFA/SFA and n-6/n-3 PUFA is produced by pigs fed with soybeans during fattening. The experiment evaluated the time needed for feeding with a source of PUFA in order to achieve the desired incorporation in pig fat. The authors concluded that the content of important PUFA (linoleic, α -linolenic, eicosadienoic, and arachidonic acids) and total PUFA in the loin and backfat increased when feeding a diet enriched with PUFA during pig fattening. Experimental groups fed a PUFA source during the final 6–8 weeks prior to slaughter showed the same FA composition in IMF and backfat as did an experimental group fed the PUFA source all through the fattening period (16 weeks). The authors observed the greatest increase of PUFA in backfat during the first two weeks following incorporation of the source into the diet. A similar trend was observed in the experiment upon excluding soybeans from the feeding diet. An experiment by Warnants et al. (1999) demonstrated that the PUFA/SFA ratio in backfat can be increased from 0.34 to 0.55 by addition of soybeans in the final 6 weeks before slaughter. Park et al. (2009) evaluated the effects of soybean oil supplementation in pigs' diets. Pigs on the soybean oil diet had poorer average daily gain compared to a control group receiving supplemental tallow, but only up to live weight of 80 kg. Thereafter, the group with addition of soybean oil compensated for the earlier slower growth in the final phase of fattening. There were no overall differences in growth between the two groups. Extending the period of feeding soybean oil caused the proportions of α -linolenic and docosahexaenoic acids to increase.

Supplementing the diet with soybean oil boosted n-3 PUFA without negatively influencing either growth or carcass value of the fattened pigs. Alonso et al. (2012) conducted a study examining the effect of oil in the diet of young boars. Groups of animals were fed a control diet with no added fat or diets supplemented with tallow (1%, 3%), palm oil (1%), or soybean oil (1%). The 1% supplementation with soybean oil throughout fattening was sufficient to cause a significant increase in the contents of linoleic and α -linolenic acids, as well as of total PUFA (including the n-3 family). A higher concentration of n-6 PUFA was the cause of a slightly higher n-6/n-3 ratio in the group of young boars fed with soybean oil.

According to some studies, higher doses of unsaturated FA can lead to excessive fattening of animals while having a negative impact on quality of pig fat (by influencing its consistency). In a study by Duran-Montgé et al. (2010), feeding tallow, which characteristically has a low content of PUFA, resulted in decreasing the total proportion of fat in the swine carcass. Nuernberg et al. (2005) examined the effect of feeding components with higher proportion of PUFA. When they added 5% linseed oil and 5% olive oil to the pig ration, they observed no effect on carcass value and meat quality.

The most important sources of PUFA include linseed, due to its favourable FA composition, and the linseed oil produced from it. If this oil plant is fed, particularly the content of linoleic acid and α -linolenic acid increases. Conversely, the content of SFA decreases and favourable decreases the ratio of n-6/n-3 PUFA. This has been confirmed in studies by Enser et al. (2000), Sheard et al. (2000), Kouba et al. (2003), Huang et al. (2008), Bečková & Václavková (2010), and D'Sousa et al. (2010), Sobol et al. (2016). The maximum increase content of n-3 PUFA was observed by D'Arrigo et al. (2002), as well as Romans et al. (1995) during first weeks of a linseed diet. D'Arrigo et al. (2002) stated that changes in the profile of n-3 FA due to pig nutrition manifest themselves more in neutral lipids of the reserve tissue than in the structural lipids of muscle (IMF). Jiang et al. (2017) concluded that the inclusion of linseed oil as a substitute for soybean oil in pig diets, in combination with organic selenium, altered the FA profile such that there was a lower omega 6:omega 3 ratio in the organic selenium + linseed oil treatment. Although the dietary treatments showed no notable influence on the oxidative stability of pork, the organic selenium supplementation combined with linseed oil did substantially reduce drip loss (by 58–74%) and increased tenderness.

Huang et al. (2008) observed linear growth in the IMF content with increasing period of feeding linseed before slaughter. The same paper reported increase in α -linolenic acid and total n-3 PUFA, both in IMF and backfat. With increasing period of feeding, the authors noted decreasing content of SFA and the n-6/n-3 ratio.

In an experiment testing various dietary FA sources in the forms of 2% soybean oil, rapeseed oil, linseed oil, or a commercial PUFA oil, D'Sousa et al. (2010) observed no changes in pigs' growth rates. They did, however, note demonstrable increase in the proportion of meat in the carcass and loin area with the diet containing 2% linseed oil. FA composition in intramuscular fat (MLLT) reflected the composition of oil added to the feed mix. Linseed oil in the feed mix for the final stage of fattening increased gain in muscle (lean meat and loin area in carcass body).

The effect of a higher ratio of unsaturated fatty acids was examined by Woods et al. (2003), who studied the effect of a diet containing 6% ground linseed. The study's results demonstrate the effect of linseed meal causing a higher proportion of n-3 PUFA in blood plasma, in IMF, and in backfat unless the level of docosahexaenoic acid (an important n-3 PUFA) is increased. The authors reported an effect of duration of feeding linseed meal on n-3 PUFA in pig tissue. Higher PUFA levels were observed in pig tissues within the group of pigs slaughtered 60 days after the start of supplementing the diet with linseed meal compared to the groups slaughtered 20 and 100 days after the start of linseed meal supplementation. Luo et al. (2009) observed under the influence of a diet including linseed a positive correlation between the IMF content and the content of linoleic acid, α -linolenic acid, EPA, docosahexaenoic acid, total PUFA, and n-3 PUFA. In a study by Okrouhlá et al. (2013), supplementation with 15% of extracted linseed meal had a positive effect on increasing PUFA. It improved the n-6/n-3 PUFA ratio without any negative effects on the physical and chemical properties of the pork. Thus, linseed has been shown to have favourable effects on FA composition in pig fat. Due to its high content of PUFA, however, which may negatively influence the stability and consistency of fat in pork products, it is appropriate to investigate further what would be the optimal amount of this ingredient to include in feed mixes for fattening pigs.

Maize is today widely grown and utilized as a livestock feed. Although there is a great variability in the content of linoleic acid in modern maize hybrids, the average content of this acid can reach as high as 59.7% of the total FAs in maize grain. That means maize grain is among the most pronounced sources of PUFA (Della Casa et al., 2010). Products from pigs fed a diet based on maize grain can be regarded by some markets as being of low quality, however, compared to the products from pigs fed with commercial mixtures based on barley or wheat. This may be due to a difference of meat and fat colour inasmuch as maize contains a higher content of carotenoids and these are readily incorporated into swine tissues. The higher unsaturated fatty acids content in maize grain may cause pigs to synthesize fats of soft consistency that are susceptible to rapid oxidation and that can be regarded negatively. Opapeju et al. (2006) concluded that a diet containing maize grain as the main energy component was the cause for a 20% increase in linoleic acid and demonstrable decrease in stearic acid in pig fat. Those authors mention, too, that a maize diet does not have a negative effect on the firmness of either backfat or lard.

The studies by Della Casa et al. (2010), Opapeju et al. (2006), and Rossi et al. (2002) demonstrated decrease in total SFA and MUFA and concurrent increase in total PUFA in pigs fed with a diet including maize compared to the control group. The total content of n-3 PUFA was reduced, thus resulting in a demonstrable increase in the ratio of n-6/n-3 PUFA. A worsening of this ratio is attributed in particular to a significant increase in linoleic acid (Della Casa et al., 2010). Han et al. (2005) and Morales et al. (2003) report overall less favourable carcass value parameters in pigs fed with an addition of maize to the diet. Doing so increased the proportion of fat in pig carcasses, and particularly in backfat.

Various genotypes of modern maize hybrids show differences both in the content of fat and its FA composition. By selecting the appropriate maize hybrids, however, the content of these feed ingredients can be controlled at a particular level. Differences as small 0.3% in linoleic acid content between maize hybrids can result in considerable differences in the FA composition in swine fat (Della Casa et al., 2010).

Sunflower also stands among those crop plants containing a higher PUFA content. Compared to other oil plants with high PUFA content, however, sunflower oil is characterized by a high content of n-6 PUFA, particularly due to a high proportion of linoleic acid and low content of α -linolenic acid within the total FA content. In an experiment conducted by Hoz et al. (2003), sunflower oil supplement in the pig diet was associated with the significant highest proportion of PUFA, the highest proportion of n-6 PUFA, and the lowest proportion of n-3 PUFA in *psaos major* muscle. Boselli et al. (2008) observed an effect of feeding Italian heavy pigs with high-oleic sunflower. This was associated with significant increase in oleic acid at the expense of linoleic acid and saturated fatty acids, particularly triacylglycerols. Fatty acids in phospholipids were less affected by animal nutrition. Conversely, a study conducted by Mitchaothai et al. (2007) showed that diets with added sunflower oil increased content of saturated and polyunsaturated fatty acids at the expense of monounsaturated fatty acids. Addition of sunflower oil was reflected most significantly in an increased content of linoleic acid in various tissues while having no other effect on the meat properties and quality. In a feeding study involving gilts, Realini et al. (2010) observed the influence of sunflower oil in the diet to be a higher proportion of fat and a lower proportion of lean muscles in carcasses from gilts.

Rapeseed oil is among the oils with the highest proportion of MUFA. In particular, it contains the highest amount of oleic acid relative to other commonly used oils. Its proportion of PUFA is not inconsiderable (Table 4). A study by Corino et al. (2002) also dealt with the issue of different fats in feed rations. The authors observed the effects of feeding animal fat, sunflower oil, and rapeseed oil in the amount of 3% or 2.5%, in later stages of fattening. In the group supplemented with rapeseed oil, the authors observed the highest content of α -linolenic acid in fat. No significant differences between the groups were observed in terms of body weight, pH, fat, and juiciness 45 min *post mortem*, and subsequently no differences in the contents of fat and protein of *longissimus lumborum* muscle. Oxidative stability of IMF was not affected negatively by rapeseed oil after 60 mins of forced oxidation. In animals fed with a supplement of unsaturated oils (rapeseed oil, corn oil), poorer oxidative stability was seen only after 300 min of forced oxidation. The results presented in the paper by Corino et al. (2002) indicate that long-term pig nutrition with the stated amount of rapeseed oil in a feeding diet have no or only minimal negative effect on quality and sensory properties of pig meat. Via inclusion of 5% or 3% of rapeseed oil in a feed mix, Raj et al. (2010) and Rossi et al. (2002) achieved increase in α -linolenic content and decrease in linoleic acid content. This also resulted in decreasing the ratio of n-6/n-3 PUFA in the feed.

Table 4. Fatty acid composition of oils containing α -linolenic acid (% of total fatty acids; ranges of values reported for major oils and fat), from Velíšek & Hajšlová (2009)

Fatty acid	Rapeseed oil	Soybean oil	Linseed oil	Sunflower oil	Palm oil	Corn oil	Fish oil	Tallow
Lauric acid	0	0.0–0.1	–	0.0–0.1	1	0.0–0.3	–	1
Myristic acid	0.0–0.2	0.0–0.2	–	0.0–0.2	5	0.1–0.3	3–10	1.4–7.8
Palmitic acid	3.6–6.0	8.0–13.3	4.0–7.0	5.0–8.0	32–59	10.7–16.5	13–25	17–37
Palmitoleic acid	0.1–0.6	0.0–0.2	–	< 0.5	1	0.0–0.3	5–8	0.7–8.8
Stearic acid	1.1–2.5	2.4–5.4	2.0–5.0	2.5–7.0	1–8	1.6–3.3	1–4	6–40
Oleic acid	52.0–66.9	17.7–25.1	12.0–34.0	13.0–40.0	26–52	24.6–42.2	9–22	26–50
Linoleic acid	16.1–24.8	49.8–57.1	7.0–27.0	40.0–74.0	5–14	39.4–60.4	1–2	0.5–5.0
α -linolenic acid	6.4–14.1	5.9–9.5	35.0–65.0	< 0.3	2	0.7–1.3	0.6–2	< 2.5
Arachidic acid	0.2–0.8	0.1–0.6	–	< 0.5	1	0.3–0.6	0.3–0.5	< 0.5
Eicosenoic acid	0.1–3.4	0.0–0.3	–	< 0.5	0.1	0.2–0.4	9–15	< 0.5
Eicosadienoic acid	0.0–0.1	–	–	–	–	–	0.5–0.7	–
Docosanoic acid	0.1–0.5	0.3–0.7	–	0.5–1.0	–	–	–	–
Docosenoic acid	0.0–2.0	0.0–0.3	–	0.0–1.8	–	0.0–0.1	12–27	–
Docosadienoic acid	0.0–0.1	–	–	–	–	–	0.4–1	–
Docosapentaenoic acid	–	–	–	–	–	–	0.5–1.3	–
Docosahexaenoic acids	–	–	–	–	–	–	4–10	–
Lignoceric acid	0.0–0.2	0.1–0.4	–	0.2–0.3	–	0.1–0.4	–	–
Tetracosenoic acid	0.1–0.4	0	–	–	–	–	–	–
Behenic acid	–	–	–	–	–	0.1–0.5	–	–

CONCLUSIONS

Many studies have confirmed that adding vegetable oils and/or fatty acids to pig diets can have a positive effect on the n-6/n-3 PUFA ratio and overall nutritional value of pork. A subject of current research is to evaluate the influence of feed mixes enriched with PUFA, and particularly n-3 PUFA. For manipulating PUFA and particularly its n-3 group in pig fat, oilseeds or their oils directly (linseed, rapeseed, soybean, etc.) are most often mentioned as the appropriate sources of fatty acids. Fish oil, too, has a favourable effect on FA composition in fat of animal products, but it also bears the risk of tainting the products with fish odour. Further studies are needed, however, to determine the most suitable oil additive and the correct dosing for each pig age category.

ACKNOWLEDGEMENTS. This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. MSM 6046070901) and Internal Grant Agency of the Czech University of Life Sciences Prague (CIGA) (Project No. 20172005).

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Volatile combustible release in biofuels

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Abstract. Plant biomass consists of varied materials. Biomass is used for different purposes, but it is most frequently burnt in modern combustion devices for heat production. The quality of solid biofuels depends on the total content of combustibles while the volatile combustible content affects the combustion process. The aim of the paper is to determine the exact content of the biofuel components by the means of the gravimetric method – namely volatile combustible, ash and moisture content – and to evaluate the process of volatile combustible release as a function of temperature during the experiment. The device Nabertherm L9/11/SW/P330 type with accessory was used to carry out the experiments. Various biofuel samples were examined, namely wood (9 kinds), wood cuttings and wood chips (2 kinds), pellets (4 kinds), sawdust (1 kind), compared to less traditional fuels (DDGS and RME – 2 kinds) and wood coal (1 kind). The tables and graphs present the experimental results, which allow evaluation of the components content in different biofuels and provide characteristics of the process of volatile combustible release in analysed fuels. Spruce wood without bark showed the highest content of combustible (99.89%). Sawdust of fruit trees contains the highest proportion of volatile combustible (93.978%) and releases the combustible at the highest rate (15.25 mg h⁻¹).

Key words: ash content, wood, pellets, combustible content, Scheffe test.

INTRODUCTION

Biomass as a source of heat energy is nowadays gaining in importance. Biomass is organic matter, which arose from photosynthesis, or material of animal origin. The plant biomass used for energy purposes represents a renewable source of energy. From the available alternative resources (wind, water, solar power etc.), meant to reduce greenhouse emissions, biomass is the only carbon-based sustainable option (Khan et al., 2009). Demirbas (2004) claims that the biomass energy is one of the earliest sources of energy for humankind, particularly in rural areas, where it is often the only available and affordable source of energy. Globally, biomass ranks fourth among energy resources, providing approximately 14% of the world's energy demand. All human and industrial processes produce waste – normally unused and undesirable products of a specific process. Liquid biofuels are also a common type of alternative fuels. These types of fuels are made from renewable sources and their combustion process produces low emissions, mainly particulate matter (Uhrinová et al., 2012). The third group of alternative fuels includes gaseous biofuels. Gaduš & Giertl (2016) carried out research focused on the gaseous biofuels.

Akhmedov et al. (2017) states that the use of solid biofuels made of different types of biomass became a viable alternative to conventional fuels in many countries. Numerous benefits, such as low cost of the final product that meets the standards of quality, financially undemanding production, possibility of producing briquettes or pellets from almost any agricultural waste or combination of raw materials speak in favour of biomass-based fuels.

Solid, liquid and gaseous biofuels can be classified according to chemical composition. The quality of solid biofuels depends on moisture and volatile combustible content as a portion of total combustible content (Mikulová et al., 2014). Obernberger et al. (2006) presents that the chemical properties of the different types of solid biofuels affect their thermal utilization and thus combustion and flue gas cleaning technologies. Coniferous and deciduous woods contain relatively low amounts of N, S and Cl. Straw, cereals, grasses, grains and fruit residues may contain relatively high levels of N, Cl and S, which is of special relevance in respect to NO_x, HC, PCDD/F and SO_x emissions as well as corrosion.

Combustion process is an oxidizing process resulting in conversion of energy content to heat due to oxidation of combustible in fuel with atmospheric oxygen. The quality of biofuel depends on the quantity and quality of combustible and ballast content (moisture and ash content). Biomass contains higher portion of volatile combustible compared to fossil fuel (Vitázek et al., 2014; Nosek & Holubčík, 2016). Biomass combustion does not pollute the environment by the excessive production of CO₂. Biomass offers a wide variety of raw materials and is universally used in energetics (Jandačka et al., 2015). The modern combustible devices use biomass for heat and electricity production. The different properties of raw materials result in varied properties of the biofuels. Holubčík et al. (2015) discussed the composition of solid raw materials (straw, wood, corn) in terms of combustion.

This paper deals with the determination of the exact proportion of biofuel components by means of the gravimetric method. Volatile combustible content and volatile combustible release rate (milligram per minute) were calculated within the given time interval. The paper presents the comparison of different biofuels. The five tested groups contained wood, pellets, sawdust, alternative biofuel based on distillery waste and wood coal. In addition, comparison of these biofuel types in terms of statistical significance is also provided. The research papers previously published in this field deal solely with the composition of biofuels, namely with the proportion of combustible, moisture and ash (Jandačka et al., 2012 and Kantová et al., 2017) and non-volatile combustible release rate. We focused on this issue to present new findings in the field.

MATERIAL AND METHODS

The total content of combustible, volatile combustible content and the volatile combustible release rate during the combustion are the most important factors affecting the combustion of biofuels. The ash is the residual after the fuel combustion. The examined samples were classified into groups as follows: wood (9 kinds), wood cuttings and wood chips (2 kinds), pellets (4 kinds), sawdust (1 kind). They were compared with less traditional fuels (DDGS – distiller's dried grain with solubles and RME – pressing refuse of rapeseed methyl ester – 2 kinds) and wood coal (1 kind).

The samples of solid biofuels were combusted in a Nabertherm L9/11/SW/P330 furnace (Fig. 1). Data of weight loss rates during the time intervals were measured by Kern digital scales with the accuracy 0.1 mg and recorded in a personal computer. This measurement equipment enables determination of moisture, combustibles and ash content in the tested biofuel samples. The individual components of biofuel samples were identified according to the weight changes during the process.



Figure 1. Measurement equipment consisting of furnace Nabertherm L9/11/SW/P330 type and laboratory scales Kern.

Proportions of particular components of biofuel samples were calculated as follows: ash content (A') according to standard STN ISO 1171, ash content without the moisture (pps) according to standard STN EN 14775, moisture content (w) and combustible content (h') according to standard STN EN ISO 18134-2.

The standard STN EN 14774-2 prescribes the heating of analysed sample to $105\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ and drying for 120 minutes. Weight loss in an interval of 0–180 minutes was accounted for by the removed moisture.

The mass residue at the end of the experiment was made up of ash. The residual amount of dry matter was the combustible (p_{hs}). The volatile combustible released until the time interval of 240 min, i.e., until the temperature reached $500\text{ }^{\circ}\text{C}$. The solid biofuel composition is schematically shown in Fig. 2.

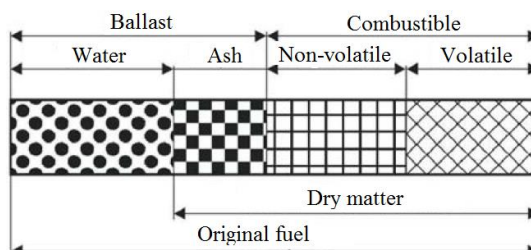


Figure 2. The scheme of solid biofuel composition.

RESULTS AND DISCUSSION

The fuel was dried at $105\text{ }^{\circ}\text{C}$ during 180 minutes (the time interval consisted of 60 minutes of the heating starting from ambient temperature to $105\text{ }^{\circ}\text{C}$ and 120 minutes of drying process). At temperatures above 150° , volatile matter began to release. After exceeding temperatures from $260\text{ }^{\circ}\text{C}$ to $410\text{ }^{\circ}\text{C}$, the release of volatile matter was significantly accelerated. The weight loss in the interval of $180\text{--}500\text{ }^{\circ}\text{C}$ was accounted for by the volatile combustible (the third interval). The solid portion of combustible began to oxidize at a temperature of about $500\text{ }^{\circ}\text{C}$. Temperature of $815\text{ }^{\circ}\text{C}$ led to a complete oxidation of the solid residue (Mikulová & Vitázek, 2016).

The results of gravimetric measurements of analysed samples are shown in Table 1. The highest moisture content was recorded in case of wood chips, which were combusted immediately after processing in a forest and therefore water did not have time to evaporate from this biofuel type. On the other hand, the lowest moisture content was observed in black locust wood, because this biofuel type was stored in dry conditions and therefore water was released before the combustion process. Brunerová et al. (2017)

carried out research on moisture content of tropical waste biomass. This work confirms the observation that the moisture content also depends on the biofuel type.

Table 1. The values of moisture content, ash content and combustible content in analysed samples

Biofuels	Parameter				
	w, %	A, %	h, %	p_{ps} , %	p_{hs} , %
Black locust wood (C1)	6.20	0.24	93.56	0.24	99.76
Apricot wood (C2)	7.32	1.63	91.05	1.76	98.24
Plum wood (with bark) (C3)	8.44	0.68	90.88	0.74	99.26
Cherry wood (C4)	11.91	0.45	87.64	0.51	99.49
Walnut wood (C5)	24.87	2.35	72.78	3.16	96.84
Spruce wood (without bark) (C6)	8.32	0.10	91.59	0.10	99.90
Apple tree wood (C7)	11.40	1.06	87.54	1.13	98.87
Hardwood (C8)	7.89	0.11	92.00	0.12	99.88
Maple wood (C9)	6.90	0.24	92.86	0.27	99.73
Cuttings from coniferous trees (C10)	41.67	6.17	52.16	11.30	88.70
Wood chips (C11)	58.19	0.68	41.13	1.61	98.39
Sunflower pellets (C12)	9.84	3.59	86.58	3.96	96.04
Spruce pellets (90% spruce wood, 10% fir) (C13)	10.33	0.55	89.12	0.62	99.38
Spruce wood pellets (C14)	7.53	0.38	92.09	0.38	99.62
Pellets from waste – Agrobio (C15)	7.35	5.12	87.53	5.65	94.35
Sawdust (fruit trees) (C16)	9.21	2.40	88.39	2.65	97.35
DDGS (C17)	10.43	4.32	85.25	4.64	95.36
RME (C18)	11.29	6.33	82.37	7.13	92.87
Wood coal (C19)	4.49	18.21	77.30	19.08	80.92

The content of combustible in dry matter (p_{hs}) was calculated from the measured values obtained from three repetitions of the experiment (Table 2). One measurement lasted 7 hours. Average value and standard deviation for each biofuel were calculated from three measured values.

Table 2. Measurement repetition, average value and standard deviation of combustible in different biofuels

Biofuels	Value, %	Average value of p_{hs} , %	Standard deviation, %	Biofuels	Value, %	Average value of p_{hs} , %	Standard deviation, %
C1	99.745 99.796 99.737	99.76	0.032	C11	98.368 98.407 98.389	98.39	0.019
C2	98.243 98.197 98.274	98.24	0.039	C12	96.021 96.013 96.085	96.04	0.039
C3	99.259 99.284 99.246	99.26	0.019	C13	99.388 99.367 99.391	99.38	0.013
C4	99.508 99.480 99.488	99.49	0.014	C14	99.631 99.592 99.649	99.62	0.029
C5	96.869	96.84	0.166	C15	94.473	94.35	0.494

Table 2 (continued)

	96.656				93.801		
	96.984				94.764		
C6	99.893	99.90	0.007	C16	97.357	97.35	0.094
	99.889				97.246		
	99.902				97.433		
C7	98.808	98.87	0.168	C17	95.173	95.36	0.188
	99.061				95.349		
	98.744				95.548		
C8	99.877	99.88	0.010		92.862	92.87	0.071
	99.869			C18	92.939		
	99.889				92.798		
C9	99.743	99.73	0.074		80.931	80.92	0.078
	99.651			C19	80.842		
	99.797				80.998		
C10	89.421	88.70	0.627				
	88.402						
	88.278						

Tables 3–5 show the comparison of all biofuels to state the differences in combustible content in terms of statistical significance according to the Scheffe test. Number 0 means the highest statistical significance of difference, whereas 1 indicates the lowest. Grey cells mark the statistical significance of differences in average values of combustible content at significance level lower than 0.05.

Table 3. The comparison of biofuels C1 – C6 to state the statistical significance of differences in combustible content (p_{hs})

Biofuels	C1	C2	C3	C4	C5	C6
C1		0.000023	0.934768	0.999963	0.000000	1.000000
C2	0.000023		0.020988	0.001028	0.000127	0.000003
C3	0.934768	0.020988		0.999996	0.000000	0.655365
C4	0.999963	0.001028	0.999996		0.000000	0.991950
C5	0.000000	0.000127	0.000000	0.000000		0.000000
C6	1.000000	0.000003	0.655365	0.991950	0.000000	
C7	0.097610	0.651644	0.994074	0.684774	0.000000	0.021331
C8	1.000000	0.000004	0.700090	0.994998	0.000000	1.000000
C9	1.000000	0.000034	0.962187	0.999994	0.000000	1.000000
C10	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
C11	0.000196	1.000000	0.111633	0.007769	0.000015	0.000028
C12	0.000000	0.000000	0.000000	0.000000	0.229158	0.000000
C13	0.996216	0.004597	1.000000	1.000000	0.000000	0.914314
C14	1.000000	0.000159	0.997792	1.000000	0.000000	0.999956
C15	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
C16	0.000000	0.093379	0.000000	0.000000	0.919239	0.000000
C17	0.000000	0.000000	0.000000	0.000000	0.000041	0.000000
C18	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
C19	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000

Table 4. The comparison of biofuels C7 – C12 with all biofuels to state the statistical significance of differences in combustible content (p_{hs})

Biofuels	C7	C8	C9	C10	C11	C12
C1	0.097610	1.000000	1.000000	0.00	0.000196	0.000000
C2	0.651644	0.000004	0.000034	0.00	1.000000	0.000000
C3	0.994074	0.700090	0.962187	0.00	0.111633	0.000000
C4	0.684774	0.994998	0.999994	0.00	0.007769	0.000000
C5	0.000000	0.000000	0.000000	0.00	0.000015	0.229158
C6	0.021331	1.000000	1.000000	0.00	0.000028	0.000000
C7		0.025973	0.130163	0.00	0.948717	0.000000
C8	0.025973		1.000000	0.00	0.000035	0.000000
C9	0.130163	1.000000		0.00	0.000296	0.000000
C10	0.000000	0.000000	0.000000		0.000000	0.000000
C11	0.948717	0.000035	0.000296	0.00		0.000000
C12	0.000000	0.000000	0.000000	0.00	0.000000	
C13	0.916577	0.934768	0.998591	0.00	0.030428	0.000000
C14	0.323113	0.999983	1.000000	0.00	0.001319	0.000000
C15	0.000000	0.000000	0.000000	0.00	0.000000	0.000002
C16	0.000021	0.000000	0.000000	0.00	0.016899	0.000498
C17	0.000000	0.000000	0.000000	0.00	0.000000	0.508932
C18	0.000000	0.000000	0.000000	0.00	0.000000	0.000000
C19	0.000000	0.000000	0.000000	0.00	0.000000	0.000000

Table 5. The comparison of biofuels C13 – C19 to state the statistical significance of differences in combustible content (p_{hs})

Biofuels	C13	C14	C15	C16	C17	C18	C19
C1	0.996216	1.000000	0.000000	0.000000	0.000000	0.000000	0.00
C2	0.004597	0.000159	0.000000	0.093379	0.000000	0.000000	0.00
C3	1.000000	0.997792	0.000000	0.000000	0.000000	0.000000	0.00
C4	1.000000	1.000000	0.000000	0.000000	0.000000	0.000000	0.00
C5	0.000000	0.000000	0.000000	0.919239	0.000041	0.000000	0.00
C6	0.914314	0.999956	0.000000	0.000000	0.000000	0.000000	0.00
C7	0.916577	0.323113	0.000000	0.000021	0.000000	0.000000	0.00
C8	0.934768	0.999983	0.000000	0.000000	0.000000	0.000000	0.00
C9	0.998591	1.000000	0.000000	0.000000	0.000000	0.000000	0.00
C10	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.00
C11	0.030428	0.001319	0.000000	0.016899	0.000000	0.000000	0.00
C12	0.000000	0.000000	0.000002	0.000498	0.508932	0.000000	0.00
C13		0.999992	0.000000	0.000000	0.000000	0.000000	0.00
C14	0.999992		0.000000	0.000000	0.000000	0.000000	0.00
C15	0.000000	0.000000		0.000000	0.025057	0.000041	0.00
C16	0.000000	0.000000	0.000000		0.000000	0.000000	0.00
C17	0.000000	0.000000	0.025057	0.000000		0.000000	0.00
C18	0.000000	0.000000	0.000041	0.000000	0.000000		0.00
C19	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	

According to the experimental results, the following values were calculated: the content of the volatile combustible from the total amount of combustible in dry matter during the time interval from 180 to 240 minutes, total amount of oxidized combustible to 300 minutes of experiment duration and the volatile combustible release rate

expressed in milligrams per minute of 1 gram of dry matter of the sample. The results are listed in Table 6. Graphical representations of the released combustible content are shown in Figs 3 and 4. Considering the wood biomass (Table 3, column 2), the black locust wood (C1) is related to spruce wood without bark (C6), hardwood (C8), maple wood (C9) and spruce wood pellets (C14). On the other hand, less traditional fuels (DDGS and RME) and pellets from waste (C15) show the significant differences with all researched biofuels. These facts are important for replacement of biofuels by each other. Besides the combustible and ash content, the moisture content is also very important factor affecting the combustion process in boiler with specific burner type.

Table 6. The content of volatile combustible and the volatile combustible release rate

Biofuels	Volatile combustible (180–240 min), %	Oxidized combustible (180–300 min), %	Volatile combustible release rate, mg min ⁻¹
Black locust wood (1)	64.08	85.08	10.65
Apricot wood (2)	68.85	83.74	11.27
Plum wood (with bark) (3)	71.55	83.87	11.84
Cherry wood (4)	72.17	88.54	11.97
Walnut wood (5)	73.70	91.18	11.90
Spruce wood (without bark) (6)	73.97	94.61	12.32
Apple tree wood (7)	74.05	93.40	12.19
Hardwood (8)	76.25	96.52	12.69
Maple wood (9)	78.51	97.06	13.05
Cuttings from coniferous trees (10)	83.35	97.59	12.28
Wood chips (Vráble) (11)	89.58	97.84	14.69
Sunflower pellets (12)	67.17	79.89	10.75
Pellets (mix) (13)	68.02	85.74	11.27
Spruce wood pellets (14)	70.18	85.87	11.65
Pellets from waste – Agrobio (15)	73.48	87.67	11.57
Sawdust (fruit trees) (16)	93.98	95.76	15.25
DDGS (17)	73.55	85.52	11.67
RME (18)	61.85	76.77	9.57
Wood coal (19)	54.25	89.79	7.32

The overall proportion of combustibles in the tested samples is presented in Table 1. Proportion of volatile matter in dry matter at the interval from 180 minute to 240. minute is shown in Table 6, column 2 (the heating from 105 °C to 500 °C). Values are ascending from the lowest to the highest (54.25% – 93.98%) in case of all groups, therefore indicating a high content of the volatile combustible in solid biofuels. The heating endurance at 500 °C was during the next time interval (from 240 minute to 300 minute). In Table 6 (column 3), the time interval from 180 minutes to 300 minutes presents the portion of released combustible. It was no longer regarded as volatile matter, but as a proportion of total oxidized combustibles. The course of the experiment for wood coal also confirmed this fact, because the content of the total and the volatile combustible was significantly lower than in case of another (raw) fuels. The biofuels contained a high content of total and also volatile combustible. Table 6 shows that 97.6%

of total combustible was released at 500 °C. The biofuels with the highest content of total combustible may not contain the highest portion of the volatile combustible.

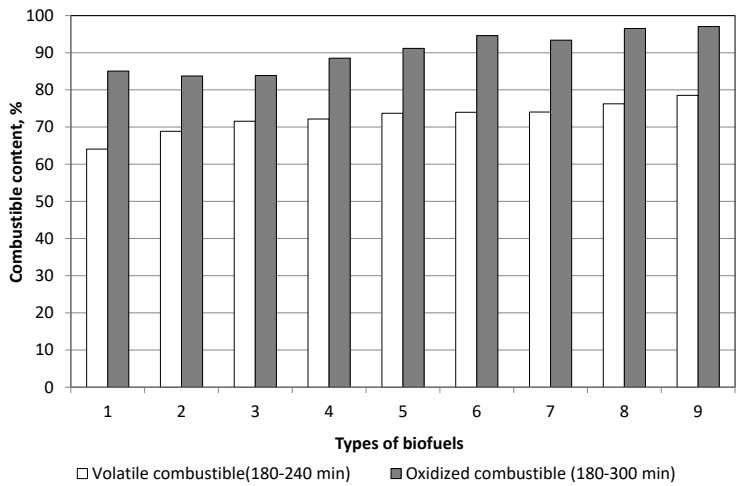


Figure 3. The content of released combustible in biofuels no. 1–9.

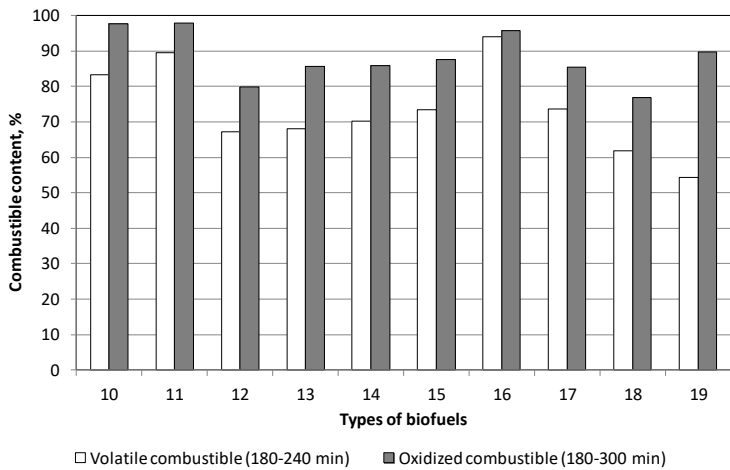


Figure 4. The content of released combustible in biofuels no. 10–19.

The highest content of combustible in dry matter was present in the sample of spruce wood without bark (99.90%, Table 1). On the other hand, the lowest was found in the cuttings from coniferous trees (88.70%) with the ash content of 11.30%, apart from the wood coal with the content of combustible in dry matter of 80.92%. The data presented in Table 6 shows that the highest content of volatile matter was present in the sawdust (fruit trees) sample (93.98%). The case of cuttings from coniferous trees is particularly interesting, because it indicated the highest ash content and the total content of oxidized combustible reached 97.59% (Table 6, column 3). The rate of volatile combustible release is calculated per 1 gram of the sample dry matter (Table 5,

column 4) and expressed by mg per minute. The sawdust (fruit trees) reached the highest value $15.25 \text{ mg min}^{-1}$.

The values of total combustible content are listed in Table 1. However, these results did not allow determination of the volatile part. Therefore we analysed the weight loss of biofuel samples during given time intervals. The results are presented in Table 6. The data shows that biofuels with the highest content of combustible in dry matter (p_{hs}) do not also contain the highest proportion of volatile combustible, which is being released at the highest rate. Comparison of the data of Table 6 indicated that the smallest difference between the intervals is found in biofuel sawdust (fruit trees). In the case of wood, this difference was more apparent and stable.

Graphical views of volatile combustible release rate are shown in Figs 5 and 6.

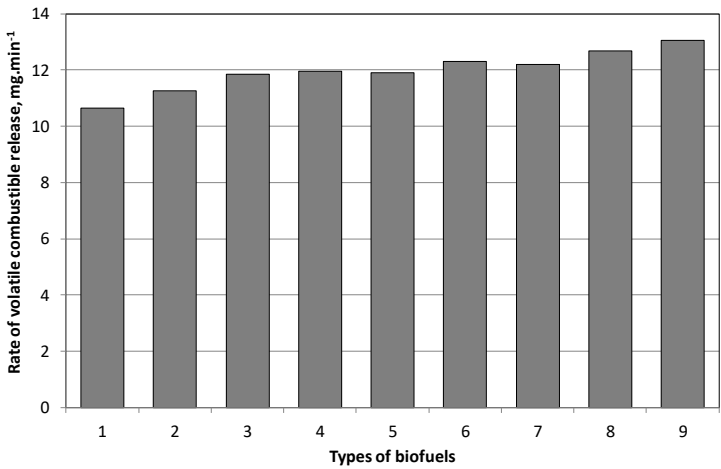


Figure 5. The volatile combustible release rate in biofuels no. 1–9.

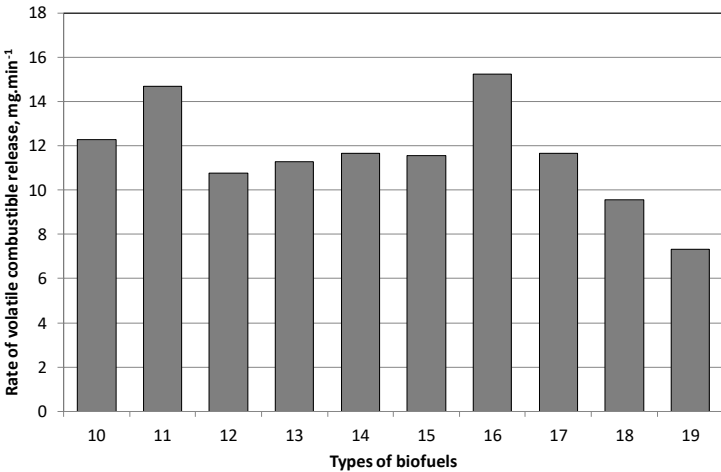


Figure 6. The volatile combustible release rate in biofuels no. 10–19.

The content of combustible in biomass depends on the fuel type. The moisture content of 14÷15% is common value required for safe long-term storage. The combustion process does not yield the maximum amount of biomass energy. Conversion of biomass to another fuel type which allows yielding the maximum energy is much more effective. Technical conversion is the most widespread type of various conversion processes (Gaduš & Giertl, 2016). It enables to produce liquid, gas or solid fuels of higher quality from the biomass. The converted fuel properties allow for reaching a high amount of biofuel energy and increasing the combustible content in biofuel. Biomass processed this way is characterised by low moisture with an impact on caloric value and the combustion process (Beláková et al., 2017). The thermal decomposition of spruce wood was studied using the thermogravimetric analysis in air atmosphere and the dependence of the apparent activation energy on the degree of conversion was determined (Ondro et al., 2018).

Ash content is affected by various additives – our results indicated that the bark content is an important factor. The influence of bark on the properties of biofuels was examined by Nosek et al. (2016). The ash content of spruce wood is presented by Radačovská et al. (2017) – this fuel has ash content of 0.26%, while our results indicated 0.1%. In general, spruce wood contains very low proportion of ash. Radačovská et al. (2017) examined also spruce wood with bark where the ash content reached the value of 0.55%. This result confirmed the statement that bark affects the ash content. Difference in our results was presumably caused by the bark removal in case of the biofuel sample tested by Radačovská et al. (2017). The differences in values found in all tested biofuels may be influenced by the composition of the fuels. These differences were the most apparent in pellets, because they did not in many cases contain raw wood.

The lowest content of released combustible in observed intervals was found in RME (61.85% and 76.77%). DDGS and RME samples confirmed that waste from processing is suitable for use as an alternative fuel. Our results supported the findings obtained by Dand et al. (2014).

The paper of Mikulová & Vitázek (2016) showed the graphic representations of combustible release rates in equal time intervals. The highest content of released combustible was found in the cherry wood sample (88.32%). In our experiment, cherry wood sample yielded 88.54%.

The influence of moisture content on the heat value is dealt with by Nosek & Holubčík (2016) and Vitázek et al. (2013). The examined samples indicated that biomass in a boiler room in Vráble was combusted even at an initial moisture content of 58.2%. The particular boiler enabled this process. The obtained results confirmed the high combustible content in pure wood (only slight differences were observed). In the case of cuttings from coniferous trees the combustible content in dry matter was lower than 90% (content of various additives). The course of combustion is, naturally, influenced by the initial moisture content, which in one case reached 58.19%. The highest content of released combustible in the interval up to 240 min. was found in sawdust (93.98%), the lowest in black locust wood (64.08%). The highest content of released combustible in the interval up to 300 min was found in wood chips (97.84%), the lowest in RME (76.77%). Hard wood yielded lower values; therefore the content of non-volatile combustible is higher. Rate of combustible release is average during the

entire time interval. This rate is initially higher in fuels with a higher volatile combustible content. Wood coal is presented just for comparison, it is thermally processed biomass.

The quality of solid biofuels depends on physical properties, too (Križan et al., 2017). Basic physical properties include form of fuel, particle size distribution of fuel, etc. These properties influence the proper design of combustion devices. The usage of additives has a significant impact on the properties of wood pellets, which include combustion and production of emissions (Jandačka et al., 2012; Kantová et al., 2017).

Unsuitable temperature in combustion chamber and high content of combustible in solid biofuel can cause ash sintering, even when using the new boilers equipped with innovative technologies. It can cause permanent damage of combustion devices (Radačovská et al., 2017). Therefore, the knowledge of the combustible content in biofuel, combustible release rate and other thermophysical properties is of significant importance. The presented findings are a follow-up to the results published in (Vitázek et al., 2018).

CONCLUSION

The different materials under different conditions (moisture content) were processed in different shapes and forms (pellets, wood chips, brown coal). These materials have different physical properties, which determine the method of possible next processing and the suitable combustion device. Wood cuttings and wood chips from coniferous trees were burned in a suitable boiler even at the high initial moisture of 58.2%. The presented gravimetric method is suitable for the research of combustible content and combustible release rate in selected solid biofuels. Graphic presentation of weight loss rate in temperature intervals of preheating and holding time at 500 °C enables to observe the volatile combustible release rate as well as oxidation rate. The volatile combustible release rate was calculated per 1 gram of dry matter and weight loss was observed in the time interval from 180 to 240 minutes i. e. the expected interval of the volatile combustible release.

Evaluating the research results, the following hypothesis was confirmed: the biomass contains high proportion of volatile combustible which was released in time interval from 260 °C to 410 °C. The rate of volatile combustible release related with the total combustible content. This fact is very important for boiler construction, because the biofuels burn with long flame and require secondary or alternatively tertiary air. Therefore the boilers for standard solid fuels (for example coal) are not suitable for biofuels in most cases.

Nineteen samples of various biofuels were examined. Statistically processed results show the differences in average values of combustible content in biofuel samples at statistical significance 0.05. Using the Scheffe test, the results were compared to each other in terms of combustible content. It allows evaluating the replacement possibility of single biofuels in practice if a particular biofuel is not available for given boiler. The research will continue to further examine the biofuels from different sources.

ACKNOWLEDGEMENTS. Supported by the Ministry of Education of the Slovak Republic, Project VEGA 1/0464/17 ‘Monitoring of the impact of ecological fuels obtained from the agricultural production and additives in hydrocarbon fuels to technical and environmental performance of internal combustion engines used in agricultural and transport technique‘.

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Productivity of various barley (*Hordeum vulgare* L.) cultivars under semi-arid conditions in southern Russia

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Abstract. Drought is a significant factor limiting crop production in arid conditions. In the dry climatic weather situation of southern Russia, ten-year laboratory trials and subsequent field experiments were laid out on various barley varieties collected across the globe during 2007–2017 period. This study was conducted to ascertain from the collection of barley cultivars of the entire world which one is best suited to stressful climatic conditions by being tolerant to drought, heat and salinity which can be adopted for barley breeding. According to the results obtained, the varieties that are tolerant to dry climatic conditions are as follows: Alga (Lithuania), Brenda, Henni (Germany), Décor (Great Britain), Furat 5 (Syria), Vakula (Ukraine), Ataman (Belarus) and Vladimir (Russia); heat resistant varieties are: Brenda (Germany), Alga (Lithuania), Furat 5 (Syria), Ataman (Belarus), Vladimir and Ratnik (Russia); Salt-resistant varieties: Alga (Lithuania), Henni (Germany) and Vladimir (Russia). The selected varieties did not show any sign of adverse weather effect resulting in stable grain productivity throughout the entire duration of this research over the years, they had large grain size and stable 1,000 grains weight. However, the yield of selected cultivars varied over the years which was about 1.1–1.4 t ha⁻¹.

Key words: salt tolerance, heat resistance, drought tolerance, barley.

INTRODUCTION

Barley (*Hordeum vulgare* L.) regarded as one of the most important cereal grain crops is cultivated all over the world. Barley is a cereal crop with good adaptation to drought stress, and it can be surveyed as a genetic model plant to illustrate drought resistance mechanisms (Baum et al., 2007; Baik & Ullrich, 2008; Arshadi et al., 2018a). An investigation of the billion dollar natural disaster in the US indicated that combined accordance of heat and drought stress was more detrimental than when either of the stresses occurred singly (Mahalingam, 2017).

Barley possesses some special properties that enable it to adapt desirably into different unfavorable climatic conditions compared to other crops, ranging from dry land conditions to arctic regions of the earth with longer winter period and reduced sunlight on different continents. The phenomenon of diminishing barley yields under poor water supply situations is well known (Zare et al., 2011; Hossain et al., 2012), therefore, drought stress reduces barley grain yield by negatively affecting the yield components which are determined at various plant development stages (Beigzadeh et al., 2013, Vadez, 2014). Several studies also illustrated that high temperature and drought have adverse effects on spring crops, but also that low temperature is equally a significant constraint of the late sown crop in sub-tropical climates (Hossain et al., 2011; Hakim et al., 2012; Hossain et al., 2012) and early sowing in temperate spring crops (Timmermans et al., 2007).

Drought stress is a significant abiotic factor that can diminish photosynthesis efficiency by reducing leaf expansion, hence, causing premature leaf senescence and lower food production. Almost, 15 million km² of land surface area is dedicated to crop production (Ramankutty et al., 2008), of which 16% is predicted to be managed by irrigation. In many parts of the world, including the western parts of Asia and southern Russia (Medvedev, 1999), plants frequently encounter drought stress due to the irregular distribution of rainfall (Siebert et al., 2005).

Numerous summarizing papers on crop breeding for drought environments have been recently published (Fleury et al., 2010; Passioura & Angus, 2010; Kosova, 2014). Thereby, parameters such as drought, salt and heat resistance are important, as well as the productivity and stability of crops in difficult climatic conditions paramount in arid regions where grain crops are largely cultivated. Drought factor is responsible for the greatest amounts of damage to agricultural products among all other environmental stresses (Ceccarelli, 2010; Arshadi et al., 2016; Arshadi et al., 2018b). A rise in the frequency of drought stress can be expected because of climate change (Ceccarelli, 2010).

Understanding the relationships between yield and yield components may assist breeders to identify key traits that are involved in crop yield under temporal drought stress conditions. Screening various barley genotypes under drought stress conditions is one of the main factors for exploring 158 genetic variations to improve stress tolerant barley varieties (Haddadin, 2015).

One important option for evaluation of genotypes in different environments is that in most cases the effect of environment is great but difficult to document (IPGRI, 1994; Zargar et al., 2017). Only the effect of genotype and the interaction between genotype and environment are important in selection of stable genotypes, both genotype effect and the interaction of genotype and environment must be examined simultaneously (Yan & Kang, 2003). Nevertheless, the study and illustration of the barley selection value cultivars for tolerance to abiotic stresses and the ability to adapt under extreme conditions become urgent and vital.

The objective of this study was to select barley cultivars that have considerable stress tolerance which can be incorporated in crop improvement research, from the available genetic pool of various countries such as Russia, Ukraine, Belorussia, Lithuania, Finland, Sweden, Denmark, Syria, Turkey, Great Britain, France and Germany based on the hottest, drought and salt-tolerant varieties with desirable yield and yield component.

MATERIALS AND METHODS

Present study was conducted during the ten-successive cropping seasons of 2007–2017 in the semi-arid conditions of the southern Russia, Astrakhan region. The research station is located at 42°58' N, 47°28' E and 130 m altitude. Samples of soil were taken randomly from different spots at 0–15 cm to record the initial characteristics of the experimental field. The soil was characterized as loamy with 1.5% of organic matter and with a pH of 7.1. Fig. 1 shows the average annually rainfall and mean annually temperature data recorded in vicinity of the experimental field.

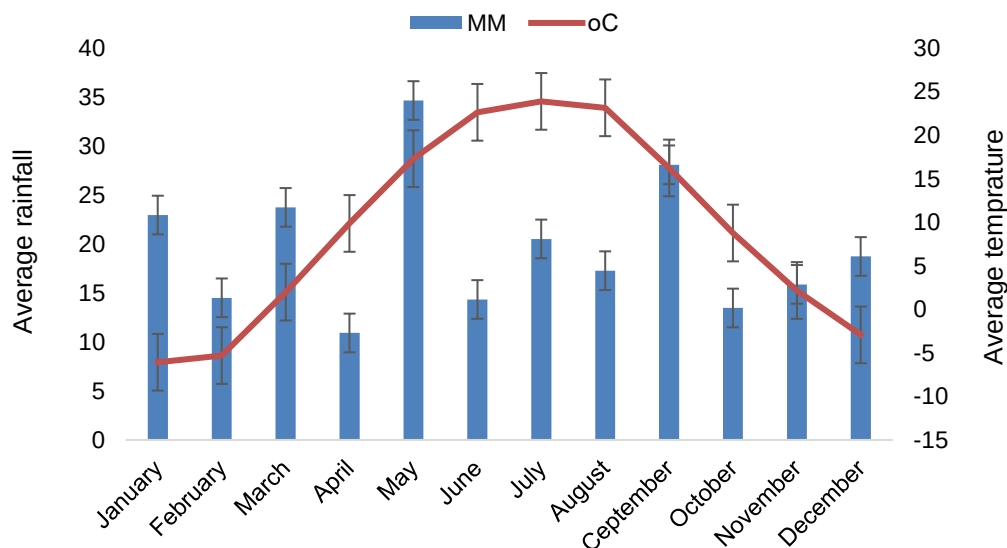


Figure 1. Average rainfall and temperature during experimental seasons.

Mentioned experiments were conducted once each year, throughout the ten-year duration, in the form of randomized complete block design with four replications. Several barley cultivars from various countries of the world (Russia, Ukraine, Belarus, Lithuania, Finland, Sweden, Denmark, Syria, Turkey, Great Britain, France and Germany) were studied and evaluated in a research that lasted for 10 agronomical years. For open field experiments, different planting dates ranging from 25 March to 5 April were set as a response to the climate changes during the 10-year period of study. In Laboratory experiments during 2007–2009, 100 barley seeds were sown per petri dish, the standard seed variety was barley Uzhniy (translated to Southern in Russian). All examined varieties were spring malting barley which were resistant to different environmental stresses.

Observations and Measurements

Under laboratory conditions, the drought resistance was determined by the percentage of seed germination in different sucrose solutions as follows: 0.3, 0.4, 0.5 and 0.6 mol with a high osmotic pressure (10, 14 and 18 atmospheres) (Shulmeyster, 1988).

The heat resistance was determined by the germination of seeds after their heating at a temperature of 58 °C. Evaluation of samples for salt tolerance was determined by the percentage of seed germination in sodium chloride solution (NaCl), for this purpose, healthy seeds were selected which were placed separately in grades into gauze bags with a label inside and treated with formalin solution (1 mL per 300 mL of water) for 3–5 minutes, then lightly dried and put into 100 seeds in a Petri dish in four replicates (Udovenko, 1988). Pre-Petri dishes and filter paper were calcined in a thermostat at 150 °C for one hour.

In each Petri dish, 6–7 mL of 10% NaCl solution was poured; seeds were germinated at 22 ± 2 °C for 6 days in thermostats. After the germination was completed, the number of sprouted seeds was determined for each variant and the percentage of germinated seeds was calculated, in salt solutions, taking as 100% the number of seeds sprouted in distilled water. Samples in the field experiment were also evaluated for drought resistance by determining the morphological and physiological parameters such as leaf drop, wax plaque, leaf color, water content of the 3rd leaf into the tube exit phase.

RESULTS AND DISCUSSION

Barley (*Hordeum vulgare*) is rather well-tolerant to drought, salinity and other dehydrative stresses. It has a very large and diverse genotype pool including several landraces adapted to arid and semiarid climates.

The drought resistance of barley samples under laboratory conditions was determined by their ability to germinate in sucrose solutions of C12H22O11. By increasing concentration of the solution and, accordingly, the osmotic pressure, seeds germination diminished significantly. The following samples showed the highest percentage of seed germination (more than 50%) were obtained at 18 atmospheres: Alga (Lithuania), Loubi (Sweden), Adora (France), Pirania (France), Arabian white (Syria) Furat 3, Furat 4, Furat 5, Furat 6 (Syria), Décor (Great Britain) Vakula (Ukraine), Ataman (Belorussia), Chill (Denmark), Mamluk, Vladimir, Yaromir and Sonet (Russia) (Table 1). Drought resistance represents a complex quantitative trait illustrated by a multitude of genes and quantitative trait loci (QTLs) which depend on the composition of a given population, plant growth stage and other factors. Yield component under drought stress conditions is influenced by both constitutive QTLs, i.e. QTLs affecting yield irrespective of environmental conditions, and drought-responsive QTLs, i.e. QTLs affecting yield only under drought situations (Collins et al., 2008; Kosova et al., 2014). Barley releases large genotypic variability as well as the effect of genotype $\times$ environment interactions by several traits (characteristics related to the flowering stage) affecting the resulting drought resistance (Kosova et al., 2014).

During the experiment, incidents of droughts were observed in all the years of research at various phases of the development of barley varieties. For the entire 10 years of study, yield results of 23 varieties exceeded the parameters of the standard cultivar Uzhniy (Southern), 0.9 t ha⁻¹. The most yielding ones were Alga (Lithuania), Loubi (Sweden) Décor (Great Britain), K-24723 (Turkey), Submedicum, Sonet, Ratnik, Pyramid (Russia), Arabian white and Furat 5 (Syria) from 1.2 to 1.4 t ha⁻¹ (Table 2). The drought resistance in the flowering and earing stages was determined by the number of grains per spike.

Table 1. The most stable barley varieties according to the results of laboratory trails in solutions of different concentrations

Origin	Variety	The percentage of sprouted seeds at a sucrose concentration			Sustainability Group
		10 _{atm}	14 _{atm}	18 _{atm}	
Russia	Standard Uzhniy	83	56	40	2
Lithuania	Alga	92	91	82	3
Sweden	Loubi	97	88	80	3
France	Adora	98	89	70	3
France	Pirania	97	90	71	3
France	Concerto	76	58	43	2
Germany	Brenda	80	53	44	2
Germany	Grace	81	57	50	2
Syria	Arabian white	94	71	69	3
Syria	Furat 3	92	89	82	3
Syria	Furat 4	95	90	76	3
Syria	Furat 5	96	87	79	3
Syria	Furat 6	100	91	74	3
Great Britain	Décor	82	74	51	2
Ukraine	Vakula	88	81	78	3
Belorussia	Ataman	97	91	78	3
Denmark	Chill	81	70	55	2
Russia	Ermak	76	70	50	2
Russia	Mamluk	81	71	54	2
Russia	Vladimir	97	91	78	3
Russia	Yaromir	80	72	56	2
Russia	Sonet	100	91	75	3
LSD _{0.05}		0.04	0.04	0.02	

The most drought-resistant are those with less variability in the number of years. In the experiments conducted, samples from these varieties were observed: Alga (Lithuania), Adora (France), Henni, Brenda (Germany), Furat 3, Furat 5, Arab white (Syria), K-24723 (Turkey), Ratnik (Russia) and series other samples (Table 2). Drought resistance in grain stage was determined by the degree of reduction in the grains mass under drought condition. The most drought-resistant can be accredited as those in which the mass of 1,000 grains shows less variability by the years. Under water deficit conditions plants may use different mechanisms to alleviate the stress. For example, Kamboj et al. (2015) compared different barley genotypes under salinity stress, and found that the pathway of abscisic acid is among the most important physiological mechanisms determining barley tolerance under stress. Barley response under water deficit conditions is correlated with changes in plant physiological and morphological parameters as different barley genotypes indicate significant differences.

In our experiments, on average, the following varieties were distinguished based on the years of study in following indicators: Alga (Lithuania), Adora (France), Henni (Germany), Décor (Great Britain), Vladimir, Ratnik and Pyramid (Russia). For the basis of results comparison obtained at the field and laboratory analyzes, we were able to identify varieties that are resistant to drought in arid conditions [(Alga (Lithuania), Brenda, Henni (Germany), Décor (Great Britain), Furat 5 (Syria), Vakula (Ukraine), Ataman (Belarus) and Vladimir (Russia)]. The isolated samples can be used for further

selection when creating drought-resistant varieties for arid conditions. As stated by Subhani et al. (2015) drought tolerance indices which provide the measure of yield losses under drought conditions in contrast to normal conditions have been used to screen the drought tolerant genotypes.

Table 2. The most drought-resistant varieties in field conditions during 2007–2017 Agronomical years

Origin	Variety	Yield (t h ⁻¹)	Weight 1,000 grains (g)	Number of grains per spike
Russia	Standard Uzhniy	0.9	30.4	19.9
Lithuania	Alga	1.3	48.4	22.1
France	Adora	1.1	45.1	23.1
France	Tabora	1.1	36.4	23.2
Germany	Henni	1.2	41.4	23.5
Germany	Brenda	1.4	31.8	21.4
Syria	New arabian 9	0.6	36.2	21.8
Syria	Arabian white	1.2	39.1	22.3
Syria	Furat 3	0.7	31.2	23.0
Syria	Furat 4	0.5	35.4	22.8
Syria	Furat 5	1.2	29.8	20.7
Syria	Furat 7	1.1	32.6	21.7
Sweden	Loubi	1.3	31.6	20.2
Finland	Jnari	1.1	33.7	22.4
Great Britain	Décor	1.3	41.9	22.8
Ukraine	Vakula	1.1	39.5	23.5
Belorussia	Ataman	1.1	39.4	23.7
Turkey	24723	1.3	36.6	23.4
Turkey	9265	1.0	32.1	20.5
Russia	Ermak	0.9	36.7	20.7
Russia	Granal	0.8	25.5	21.1
Russia	Pastbishny	1.1	36.7	19.5
Russia	Submedicum	1.2	31.2	19.4
Russia	Zernogradets, 770	1.1	27.5	19.7
Russia	Priazovsky 9	1.1	33.1	20.0
Russia	Sokol	0.9	28.6	19.1
Russia	Vladimir	1.3	40.7	21.2
Russia	Sonet	1.2	39.5	20.1
Russia	NUR	0.9	27.2	19.9
Russia	Ratnik	1.2	33.3	22.1
Russia	Piramida	1.2	44.7	21.8
LSD _{0.05}		0.04	1.4	0.9

In this regard, Lalic et al. (2017) stated that barley production, within the productive area of the world, is commonly exposed towards a number of stressful factors which significantly affect grain yield and quality, especially in malting barley. The most common biotic and abiotic stress factors in our conditions have been caused by various factors such as soil salinity, low temperatures, drought and high temperatures. Hence, water shortage and drought stress are considered as the most principal environmental factors, reducing the productivity of crops in many arid and semi-arid areas, which are

intensively influenced by climate changes (Wassmann et al., 2009). This matter is more noticeable when we find out that higher than one fourth of the earth land areas are arid and semi-arid areas (Mardeh et al., 2006).

Abiotic stress factors such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress pose a serious threat towards plant varieties in agriculture. The heat resistance of the samples was evaluated by the reaction of the variety of samples in both laboratory and field conditions. The reaction of the samples to sudden sharp increase in temperature accompanied by a strong wind, low relative humidity of the air, which causes the phenomenon of whitening of the top of the ear, the entire ear or the tips of the leaves was observed.

Stress factors do not usually affect plants independently, but in different combinations under field conditions and the effect of joint stress factor action does not equate the sum of separate stress factor effects (Mittler, 2002; 2006). Regarding the plant phenological phase affected by drought, different kinds of stress may occur as following: pre-flowering water deficit (regions of South America); grain-filling (post-anthesis) water deficit (Mediterranean regions); continuous water deficit (Reynolds et al., 2005).

Dry wind situation was observed during the entire duration of the research over the years, which can cause whitening and die-off at the tips of plant leaves. By the degree of whitening of the leaves, we estimated the field heat resistance of the samples. The most resistant to dry wind (5–7 points) were varieties from France (Adora), Germany (Brenda), Lithuania (Alga), Syria (Furat 5), Belarus (Ataman), Russia (Vladimir, Ratnik). It should be noted that among the samples studied, those found in the period of the most intense heat and drought folded their leaves into a tube which is seen as way of response by desert plants and some steppe grasses to reduce transpiration by 46–63%, thereby saving a considerable amount of moisture (Knezevic, 2004). Among the samples studied, such signs were different: Alga (Lithuania), Furat 5 (Syria), Ratnik (Russia). In laboratory conditions, the samples were evaluated for heat resistance by heating the seeds at a temperature of 58 °C. High heat resistance, where the energy of germination and when germination was at 100%, was shown by the following samples: Alga (Lithuania), Loubi (Sweden), Sega (Denmark), Ataman (Belarus) (Table 3).

No less important factor is salinity, which limits the productivity of crops and has a profound effect on the vital activity of plants. For the life of plants under saline conditions, of particular importance is the change in the water-osmotic regime, especially the degree of osmoregulation. Many authors are of the opinion that an increase in the osmotic potential of plant cell sap is a protective-adaptive response in conditions of salinity (reviewed in Chaves et al., 2003; Yamaguchi-Shinozaki & Shinozaki, 2006). In cultivated plants, salinization leads to changes in the stomatal apparatus, while the size of the stomata decreases, and their number per unit area increases.

The adaptation of plants to the conditions of salinization is carried out in many ways, the most important of which is osmoregulation and specialization (modification of transport processes). Therefore, to obtain salt-tolerant plant forms, it is necessary to carefully study the transport of ions, depending on the ionic composition of the medium and the genotype. Salt-resistant species have the ability to accumulate sodium ions (Na^+) in vacuoles, absorb it from the xylem and transport it to the medium. Peculiarities of potassium-sodium metabolism on plasmalemma and the accumulation of Na^+ and Cl^- ions in vacuoles of cells and in cell walls have been noted in some studies, where it was

suggested that there is a highly efficient mechanism for pumping out Na^+ ions in salt-tolerant plants.

The increased salt tolerance of plants is due, firstly, to the excretion of Na^+ and Cl^- ions from young leaves, and secondly, by the predominantly basal migration of Na^+ from the leaves and its excretion into the substrate and, thirdly, by the restriction of movement of Cl^- from the root to the stem (Zohary, 2000; Kosova, 2014).

Table 3. The most heat-resistant barley cultivars by the results of laboratory trials

Origin	Variety	Energy of germination (%)	Germination (%)
Russia	Standard uzhniy	92	93
Russia	Zernogradsky 584	90	95
Russia	Sonet	48	91
Russia	Vladimir	99	100
Russia	Priazovsky 9	74	80
Russia	Mamluk	89	100
Belorussia	Ataman	100	100
Lithuania	Alga	100	100
Sweden	Loubi	100	100
Sweden	Halikko	95	98
Denmark	Sega	100	100
Denmark	Chill	89	95
France	Adora	95	93
France	Pirania	96	100
France	Tabora	98	97
France	Concerto	70	89
Germany	Brenda	99	97
Germany	Henni	97	97
Syria	Arabian black	90	92
Syria	Arabian white	91	97
Syria	Furat 5	99	100
Syria	Furat 7	83	87
Turkey	9265	94	97
	LSD _{0.05}	0.05	0.06

It is known that high concentrations of salts directly or indirectly suppress protein synthesis, destroy the structure and inhibit the activity of enzymes of primary nitrogen assimilation. This leads to the accumulation of amino acids in plant tissues, a sharp increase in some of them – tyrosine, leucine, phenylalanine adversely affects the vital activity of plants. Along with this in the tissues of plants on salinization glycolysis and the pentose-phosphate cycle are intensified. In response to the action of salt stress in the plant, low-molecular compounds such as proline, betaine, polyamines, organic acids, sugars, and peptides are formed and accumulated (Udovenko, 1988; Zohary, 2000; Knezevic, 2004). At present, in order to increase the resistance of plants to adverse factors, a search for salt tolerance donors is necessary.

The salt tolerance of a variety is determined by the amount by which its yield diminishes in saline conditions, in comparison with the yield of this variety on a non-saline background. Therefore, the level of salt tolerance of the variety is higher, the lower its productivity decreases with salinity of the substrate. In conditions of excessive

salinity of the soil, the seed germination and the intensity of plant growth often decrease. We determined the salt tolerance of plants by germinating seeds in salt solutions. Average data for ten years of study are presented in Table 4.

Table 4. Salt-resistant of barley varieties (germination, %)

Origin	Variety	Average	Control
Russia	Standard Uzhniy	59	100
Belorussia	Ataman	97	100
Belorussia	Paletan	67	100
Ukraine	Vakula	76	100
Lithuania	Alga	100	100
Finland	Jnari	89	100
Sweden	Loubi	93	100
Sweden	Halikko	91	100
Denmark	Sega	99	100
Denmark	Chill	97	100
Great Britain	Décor	84	100
France	Adora	26	100
France	Pirania	95	100
France	Tabora	77	100
Germany	Brenda	83	100
Germany	Henni	100	100
Germany	Grace	98	100
Russia	Yaromir	85	100
Russia	Mamluk	92	100
Russia	Vladimir	100	100
Russia	Ptiazovsky 9	73	100
Russia	Zernogradsky 584	93	100
Russia	Ratnik	91	100
Russia	Sonet	75	100
Syria	Arabian white	77	100
Syria	New arabian	81	100
Syria	Furat 3	91	100
Syria	Furat 4	93	100
Syria	Furat 5	89	100
Syria	Furat 7	90	100
	LSD _{0.05}	0.04	0.08

Abiotic stress leads towards morphological, physiological, biochemical and molecular changes which negatively affect the plant growth and productivity. Drought, salinity, extreme temperatures and oxidative stress are commonly connected and can induce similar cell damage. For example, drought and salinization are primarily expressed as osmotic stress where they affect homeostasis and ion distribution inside the cell (Serrano et al., 2001). High temperatures accompanied by oxidative stress, salinity or drought may cause denaturation of functional and structure proteins. Barley is one of the most extensively cultivated cereals in the Mediterranean region, and although water stress reduces its productivity (Lopes et al., 2004) it is, among the main temperate cereals, the one that adapts best to water shortage (Sanchez-Diaz et al., 2002).

The varieties Alga (Lithuania), Henni (Germany), Vladimir (Russia), having 100% germination in salt solution turned out to be more salt tolerant. Germination at the level of 90% or higher was achieved by varietal samples from Denmark (Sega, Chill), Germany (Grace), Byelorussia (Ataman), France (Pirania) and a number of others that can be used as sources for selection for the salt tolerance of barley.

CONCLUSIONS

When creating varieties that meet modern requirements, one of the important things to look for is its genetic sources, which is especially important for soil and climatic conditions of arid territories. Our long-term studies of the collection of barley cultivars made it possible to identify the most drought-, heat- and salt-resistant samples, and the most valuable samples were identified by comparing the results of laboratory and field tests. So, the most drought-resistant, in the arid conditions of the south of Russia, were the varieties: Alga (Lithuania), Brenda, Henni (Germany), Décor (Great Britain), Furat 5 (Syria), Vakula (Ukraine), Ataman (Belarus), Vladimir (Russia). According to the heat resistance, Brenda (Germany), Alga (Lithuania), Furat 5 (Syria), Ataman (Belarus), Vladimir, Ratnik (Russia) stood out. For salt tolerance, we had varieties: Alga (Lithuania), Henni (Germany), Vladimir (Russia). All the varieties identified by us can be used by breeders as sources of resistance for the traits under study in further breeding.

ACKNOWLEDGEMENTS. This paper was financially supported by Ministry of Education and Science of the Russian Federation on the program to improve the competitiveness of RUDN University among the world's leading research and education centers during 2016–2020.

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Productivity, quality and economics of four spring wheat (*Triticum aestivum* L.) cultivars as affected by three cultivation technologies

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Abstract. Managing farming inputs in wheat production technologies is an issue of paramount importance to attain optimum profitable production. To examine how varying the farming inputs affects the nutrients uptake and productivity of spring wheat (*Triticum aestivum* L.) cultivars and to determine the economic efficiency of various cultivation technologies, three-year field experiments were laid out at the Russian Research Institute of Agriculture, during the 2015–2017 growing seasons. Experiments were conducted once a year using randomized complete block arranged in a split plot experimental design with three replications, with the cultivation technology treatments (basic, intensive and high intensive technology) as the main plots, and spring wheat cultivars (Zelata, Lubova, Liza and Ester) as the sub-main plots. The highest grain yield (10.8 t ha⁻¹), harvest index (42.9%), gluten content (39.45%) and gluten index (71.17%) observed for spring wheat cultivar Lubova with the moderate application of farming inputs as an intensive cultivation technology. Highest protein content (18.02%) was attained for both intensive and high intensive cultivation technology with the cultivar Lubova, and the highest 1,000 grains weight (46.32 g) was recorded by cultivar Lubova in basic cultivation technology. Applying moderate amount of inputs as an intensive cultivation technology resulted in highest wheat yield and net income.

Key words: spring wheat, cultivars, cultivation technology, economic efficiency.

INTRODUCTION

Wheat is one of the most important cereal crops across the globe with the widest distribution and is also the main cereal which provides proteins and energy to the most of the world population (Hurkman et al., 2013; Wan et al., 2014).

World population is forecasted to reach its maximum (~10 billion people) by the year 2050, which will raise the demand for food. The intensive farming systems developed during the Green Revolution employ a macro-level, large-scale mono-crop production that utilizes field-level, uniform input applications of chemicals. These

systems have been integral to response enhancing agricultural production demands during the past half century (Tilman et al., 2001; Weekley et al., 2012).

Advanced farming system is fundamentally based on cultivars bred for high performance under high input farming approach, which generally do not perform well under low-input agricultural technologies (Tiffany et al., 2011). In developed countries, modern cropping is fundamentally based on high input farming, which is not sustainable given resource limitations foreseen to occur in the near future (Phillips & Wolfe, 2005). As resources diminish and world population grows, high-input cropping systems become less sustainable. A paradigm shifts of research subjects and crop science objectives from high-input farming systems to those with a developed justification between yield and energy input is needed. Crop management concentrated more on nutrient economy will help reduce energy demands for agricultural production while still providing adequate amounts of high quality food as global resources decline and population is increasing (Sthapit et al., 2008).

Low-input farming relies on the improved resources management, ultimately resulting in a sustainable agroecosystem, due to low dependence on resources (Murphy et al., 2005). Low input systems have reduced usage of chemicals including fertilizers and pesticides, but not eliminating them (Abay & Bjornstad, 2009). Low-external input farming system in developed world may resemble farming in marginal environments of developing countries (Desclaux, 2005; Dawson et al., 2008; Zargar et al., 2017). Crop cultivars adapted to low-input cultivation systems are essential in both developed and developing countries.

In developed agricultural systems, the use of high yielding modern cultivars is the norm. Using off-farm inputs involving fertilizer and pesticides makes the growing conditions similar from farm to farm and region to region (Sperling et al., 2001; Dawson et al., 2008). Hence, there is concern over the rising cost of inputs and growing interest in sustainable cropping systems. Low-external-input farmers choose to limit their inputs for the reasons such as economic and environmental concerns (Murphy et al., 2005; Tiffany et al., 2011).

The need to reduce inputs in cropping systems throughout the globe is a challenge for both plant breeders and producers. Diminishing inputs can benefit producers in marginal environments developed and developing countries, and also those farmers who are seeking to lower their synthetic inputs for economic reasons (Dawson et al., 2008). The objectives of the study were to assess the effect of various cultivation technologies such as basic, intensive and high intensive cultivation technologies with varying fertilizers and pesticides use on the uptake of nutrients and yields of four spring soft wheat cultivars and on the economic efficiency.

MATERIALS AND METHODS

Site Description and Soil

Three field experiments were performed during 2015–2017 in the Russian Research Institute of Agriculture, Moscow region, Russia. The site is located at 54° 45' N, 37°38' E and 200 m altitude. In order to test the soil, the samples were randomly collected from the depth of 0–30 cm and different parts of the land recording the initial characteristics

of the experimental soil. The soil type was a loamy with 1.6% organic matter and a pH of 5.8. The experimental field was ploughed before sowing seeds, and the field was prepared by roller harrowing. Dolomitic powder 4.5 t ha⁻¹ was applied to the seedbed so as to raise the soil pH.

Climatic Condition

Growing season of 2015 and 2016 were moderate in the amount of rainfall precipitation and also with high mean daily air and soil temperatures; moisture deficiency was achieved in the middle of vegetation season (Fig. 1). Weather condition of winter 2017 was almost non typical in comparison with average for Moscow region, soil was frozen up to 36 cm; snow level was high up to 38 cm. Growing stage of spring wheat began 01.06.2017 because of low temperature condition for a long period of time.

During experimental years, meteorological data regarding temperature and rainfall was achieved from Russian Research Institute of Agriculture.

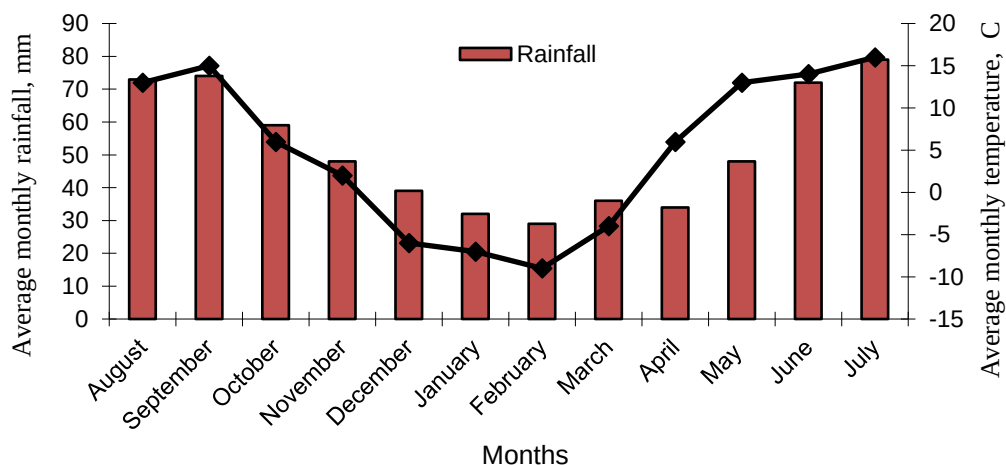


Figure 1. Average monthly rainfall and temperature during 2015–2017, Moscow region.

Experimental Design and Treatments

Field experiments were laid out once in each of the year during three-year of study. Randomized complete block arranged in a split plot experimental design with three replicates was used. Three cultivation technology treatments (basic tech, intensive tech and high intensive tech) were the main plots, and wheat varieties (Zelata, Lubova, Liza and Ester) were in the sub-main plots.

The soil was sampled from various surface layers of the fields (in depths 0 to 30 centimeters), prior to the experimentation. Sowing was done (seeder SN 16 PM) at the beginning of May maintaining plant densities of five million viable seeds per hectare.

The experiments were carried out in the field where crop rotation is adopted using different cultivation technologies and alternating crops, for instance: busy steam (vetch + oats), winter cereals, potatoes, spring cereals, and legumes. The predecessor crop to the experiment was potatoes.

Basic cropping system or (extensive farming) is an agricultural production system that uses lesser inputs of labor, fertilizers and pesticides. Basic cropping system most

commonly refers to traditional farming in areas with low productivity, but can also refer to large scale of wheat cultivation. Intensive cropping system is a production system characterized by the high use of inputs involving: labor, pesticides and fertilizers. The details of the input used in three tested technologies of the experiment are given in Table 1.

Table 1. Inputs used in different cultivation technologies

Cultivation Technology	Fertilizers (kg ha ⁻¹)	Crop protection details
1. Basic	Basal application N ₄₅ P ₆₀ K ₉₀	vincite forte 'seed treatment' (seed treat) (1.25 L t ⁻¹) + picus (L t ⁻¹); lintur (150 g ha ⁻¹) + dietox 1 L ha ⁻¹) + retardant (perfect) (0.3 L ha ⁻¹)
2. Intensive	Basal application N ₄₅ K ₆₀ P ₁₂₀ Top dressing, at the start of stem elongation Tillering stage N ₃₀	vincite forte (1.25 L t ⁻¹) + picus (1 L t ⁻¹); accurate extra (25 g ha ⁻¹); + dietox (1 L ha ⁻¹) + alto super (0.5 L ha ⁻¹); retardants perfect (0.3 L ha ⁻¹) (phase GS 21–22) + retardant (perfect) (0.3 L ha ⁻¹) (phase 31–32, according to the forecast)
3. High-intensive	Basal application N ₄₅ K ₉₀ P ₁₅₀ Top dressing Tillering stage N ₃₀ and through the tube N ₃₀	vincite forte (1.25 L t ⁻¹) + picus (1 L t ⁻¹); accurat extra (35 g ha ⁻¹) or aton (20 g ha ⁻¹) + danadim power (0.6 L ha ⁻¹) + alto super (0.5 L ha ⁻¹); consul (0.7 L ha ⁻¹), retardants perfect (0.3 L ha ⁻¹) (phase GS 21–22) + supress (0.3 L ha ⁻¹) (phase 31–32) + impact super (0.75 L ha ⁻¹) + vantex (60 mL ha ⁻¹)

Data Recording

Agrophysical, agrochemical and biological observations in experiments were performed during the growing season according to the accepted methods Evans (Evans, 1993).

Statistical Analysis

Pooled data were subjected to analyses by M-STAT C (Russell, 1991, while Duncan's multiple range test was used to verify the significant differences between treatments means as described by Duncan (Duncan, 1955).

RESULTS AND DISCUSSION

Quality and productivity of four spring wheat varieties

Crop yield is the main determinative factor in the selection of a specific cultivation technology. In this study, efficacy of three cultivation technologies including intensive and high intensive technology I on spring wheat varieties was assessed. Grain yield of Lubova significantly enhanced over the three years of study ($P < 0.05$) with intensive cultivation technology, with a low coefficient of variation (3.12 to 6.33%). The highest wheat yield (10.81 t ha⁻¹) was observed with the variety Lubova under intensive technology (Table 2). Higher yields may be due to increased nutrient availability and superior growing conditions (FAO, 2012), which enhance the physiological development of wheat.

Zelata (10.46 t ha⁻¹) and Liza (10.48 t ha⁻¹) varieties also performed desirably under high intensive and basic cultivation technologies (Table 2). Managing nutrition inputs in wheat production systems is important in order to achieve maximum profitable production, and minimum negative environmental impact (Mandic et al., 2014). Le Gouis et al. (2000) found that some modern wheat varieties performed well in conditions where nutrients were comparatively low.

Table 2. Interaction effects of cultivation technology and variety on wheat yield

Interaction CT x V	Wheat yield (t ha ⁻¹) 2015	Wheat yield (t ha ⁻¹) 2016	Wheat yield (t ha ⁻¹) 2017	Average wheat yield (t ha ⁻¹) (2015–2017)	+/- % to the base
CT1 x V1	8.23 d	8.17 c	8.54 c	8.31	-
CT1 x V2	8.65 d	10.25 a	9.30 b	9.75	1.44 / 17
CT1 x V3	10.80 b	10.28 a	10.36 a	10.48	2.17 / 26
CT1 x V4	8.35 d	8.18 c	8.75 c	8.42	-
CT2 x V1	9.67 c	9.92 b	9.87 b	9.82	1.40 / 16
CT2 x V2	11.10 a	10.69 a	10.66 a	10.81	2.39 / 28
CT2 x V3	8.36 d	8.42 c	8.13 d	8.30	-
CT2 x V4	9.70 c	8.64 c	9.12 bc	9.15	1.02 / 12
CT3 x V1	10.50 b	10.57 a	10.32 a	10.46	2.16 / 26
CT3 x V2	7.68 a	7.05 d	8.05 d	7.59	-
CT3 x V3	8.91 d	8.01 cd	9.38 b	8.76	1.17 / 15
CT3 x V4	9.43 c	10.40 a	9.49 b	9.88	2.29 / 30
LSD (0.05)	0.19	0.24	0.20		
CV%	3.12	6.33	5.89		

Means in columns followed by the same letter are not significantly different at $P = 0.05$; Abbreviations: V1, V2, V3, V4 = Varieties: Zelata, Lubova, Lisa and Esther, respectively; CT1, CT2 and CT3 = Basic, intensive and high intensive cultivation technology, respectively; CV = Coefficient of variation.

Most studies show mineral fertilization to have a positive effect on wheat productivity, with this effect being greatest in the case of nitrogen fertilization (Gevrek & Atasoy, 2012; Harasim et al., 2016). Duan et al. (2014) determined that the grain yield of wheat with 150 kg N ha⁻¹ increased from 51.4 to 66.6% compared with control. In our study, intensive cultivation technology, involving the increase in the fertilizers rate of NPK, resulted in a significant effect in grain yield.

Wheat is an essential part of the diet of the world population therefore its quality traits are most critical. The interaction of experimental treatments of this study significantly affected the yield contributing traits of the spring wheat. The highest nature of the wheat grain value attained in intensive technology with Zelata (818.1 g L) and Liza (818 g L). Zelata in high intensive technology also had the high (817 g L) nature of grain. The lowest grain nature value (807.5 g L) was obtained in high intensive technology for wheat variety Liza (Table 3).

The highest gluten content (39.45%) was obtained under intensive technology with the Lubova variety. The lowest gluten percentage (35.85%) was attained in high intensive technology with Lisa. The treatment CT1 x V4 (basic technology with the Ester variety) had the lowest gluten index (58.00%). The highest gluten index (71.17%) was achieved for CT2 x V2 (intensive technology with the Lubova variety) (Table 3). Plants require three major mineral macronutrients NPK and a host of other essential

micronutrients in order to develop properly (Tiffany et al., 2011) and stimulating efficacy of fertilization intensity on the growth of crops was reported earlier (Ellaminn, 2001; Fageria et al., 2008; Zargar & Pakina, 2014).

The important index to evaluate quality of wheat is grain protein (Sun et al., 2013; Ahmed & Hassan, 2015). The significant effect of fertilization on grain chemical composition was investigated earlier (Mohammed et al., 2013). The analysis of variance for protein content revealed that it was significantly influenced by level of technology applied and the variety cultivated. The highest protein content (18.02%) was obtained by both CT2 and CT3 (intensive and high technologies) with Lubova variety. Lowest protein content (16.08%) was attained by CT1 (basic technology) with Esther variety (Table 3). In this study, intensity of cultivation technology significantly affected all the investigated components of spring wheat. An increase in NPK fertilizers rate resulted in enhancement of grain protein content by an average of 18.02% for high intensive cultivation technology system (Table 3). In a similar study, Varga et al. (2003) declared that the use of an intensive production technology compared to an extensive technology significantly enhanced protein content (16.9%), gluten (59.7%), falling number (7.8%), drought resistance (138.1%).

Table 3. Interaction effects of cultivation technology and variety on grain quality traits, 1,000 grain weight and harvest index of wheat (2015–2017)

Interaction CT x V*	Nature of the grain (g L)	Gluten content (%)	Gluten index (%)	Protein content (%)	1,000 grain weight (g)	Harvest index (%)
CT1 x V1	811.2 ab	37.02 bc	68.33 a	17.04 b	45.65 ab	42.80 a
CT1 x V2	811.0 ab	38.35 ab	66.00 ab	17.31 b	46.32 a	42.77 a
CT1 x V3	814.3 ab	38.20 ab	67.33 a	17.60 b	44.3 b	41.01 b
CT1 x V4	812.7 ab	38.10 ab	58.00 b	16.08 c	43.37 c	38.59 c
CT2 x V1	818.1 a	37.00 bc	68.22 a	17.28 b	45.5 ab	41.40 b
CT2 x V2	814.0 ab	39.45 a	71.17 a	18.02 a	46.2 a	42.90 a
CT2 x V3	818.0 a	38.20 ab	58.10 b	16.80 c	45.5 ab	42.44 a
CT2 x V4	812.1 ab	37.45 abc	66.02 ab	16.76 c	44.3 b	41.00 b
CT3 x V1	817.0 a	37.55 abc	68.20 a	17.28 b	45.5 ab	41.40 b
CT3 x V2	812.0 ab	36.70 bc	68.00 a	18.02 a	46.2 a	42.80 a
CT3 x V3	807.5 b	35.85 c	69.51 a	16.80 c	45.5 ab	42.44 a
CT3 x V4	813.0 ab	37.10 bc	70.03 a	16.76 c	44.3 b	38.59 c
LSD (0.05)	8.91	2.17	8.59	1.92	2.88	0.95
CV%	2.88	3.58	7.88	4.40	4.79	6.03

Means in columns followed by the same letter are not significantly different at $P = 0.05$;
*Abbreviations: V1, V2, V3, V4 = Wheat varieties: Zelata, Lubova, Lisa and Esther, respectively; CT1, CT2 and CT3 = Basic, intensive and high intensive cultivation technology, respectively; CV = Coefficient of variation.

There was a significant difference ($P < 0.05$) between treatments in 1,000 grain weight. The highest 1,000 grain weight (46.32 g) was with basic technology using Lubova variety, which was statistically similar to that of CT2 (intensive technology) with Lubova and CT3 (high intensive technology) with Lubova (46.2 g). Hence, the variety Lubova was the best performed spring wheat cultivar under all three levels of technology having consistent and highest 1,000 grain weight. The 1,000 grain weight of variety Esther (43.37 g) in CT1 (basic technology) was the lowest. However, the grain

weight of the Esther variety significantly improved as the level of technology intensity increased from 43.37 g basic technology(43.37 g) to both intensive and high intensive technology (44.3 g) (Table 3).

Wheat yields improved greatly over the last 50 years. Phillips & Wolfe (2005) opined that much of this improvement has been due to adjusting the harvest index in favour of grain yield. The highest harvest index (42.90%) was attained in CT2 x V2 (intensive technology x Lubova variety) and was statistically similar to that CT1 x V1 (42.80%), CT1 x V2 (42.77%), CT2 x V3 (42.44%), CT3 x V2 (42.80%) and CT3 x V3 (42.44%). These findings were in agreement with those of Varga et al. (2001). The lowest (38.59%) harvest index was observed in both CT1 x V4 and CT3 x V4 (basic technology x Esther and high intensive technology x Esther).

Baking quality of wheat flour

The whole grain products consumption can be illustrated by improving their perceived attractiveness (Boz & Karaoglu, 2013). The vitality of gluten is generally assessed by its ability of enhancing the volume and improving the crumb structure of bread baked from standard flour fortified with gluten (Esteller et al., 2005). The effect of three different cultivation systems (basic, intensive and high intensive technology) on four wheat varieties such as Zelata, Lubova, Lisa and Esther were assessed for the bread quality parameters like crumb color, general score and volume output. The specific volume of bread significantly improved with intensive cultivation technology use in wheat variety Lisa (Table 4).

Table 4. Processing bread quality attributes of spring wheat grain as affected by treatments (2015–2017)

Interaction	Standard baking		
	Volume output, cm ³	Crumb color	General score
CT1 x V1	1,009.01 cd	4.81	4.60
CT1 x V2	998.10 d	4.88	4.85
CT1 x V3	1,010.00 cd	4.80	4.91
CT1 x V4	973.09 e	4.51	4.74
CT2 x V1	1,007.16 cd	4.55	4.65
CT2 x V2	1,017.21 c	4.61	4.72
CT2 x V3	1,151.19 a	4.29	4.88
CT2 x V4	962.00 e	4.38	4.89
CT3 x V1	1,014.28 c	4.48	4.64
CT3 x V2	1,037.07 b	4.44	4.75
CT3 x V3	902.50 g	4.50	4.61
CT3 x V4	924.51 f	4.47	4.80
LSD (0.05)	14.69	NS	NS
CV%	6.09	2.69	2.39

Means in columns followed by the same letter are not significantly different at P = 0.05.
*Abbreviations: V1, V2, V3, V4 = Wheat varieties: Zelata, Lubova, Lisa and Esther, respectively; CT1, CT2 and CT3 = Basic, intensive and high intensive cultivation technology, respectively; NS = Not significant; CV = Coefficient of variation.

It was concluded that intensive cultivation technology (CT2) had a greater effect than basic and high intensive technologies in increasing the volume output to 1,151.19 cm³ with variety Liza. The lowest specific volume (902.5 cm³) was obtained with wheat variety Liza when high intensive technology (CT3) was used. Color of the crumb and overall score of the bread were not significantly affected by tested three cultivation technologies and four wheat varieties (Table 4). Intensive cultivation system, specially more intensive nitrogen fertilization, had a significant effect on wheat grain composition (Johanson, 2002). Pushman & Bingham (1976) declared that intensified wheat resulted in better milling and baking quality through enhanced, grain protein content, flour water absorption and bread volume. Varga et al. (2001) also demonstrated that intensive application of fertilizers specially nitrogen significantly improved flour properties and baking quality as a consequence of bread volume.

Interrelationship between grain yield attributes and yield

Correlation coefficient of genotypic and phenotypic are important in determining the degree to which different yield contributing characters are related. Correlation coefficients of various wheat traits indicated positively and significant correlations between nature of grains with volume output ($r = 0.380$, $p < 0.05$) and crumb color ($r = 0.704$, $p < 0.01$), but negatively with harvest index ($r = 0.125$, $p < 0.05$) (Table 5). Gluten content had significantly high correlation with volume output ($r = 0.205$, $p < 0.05$) and protein content ($r = 0.150$, $p < 0.05$). The correlation of gluten index with 1,000 grains weight ($r = 0.940$, $p < 0.01$) and protein content ($r = 0.787$, $p < 0.01$) were positive and significant (Table 5).

Table 5. Correlation coefficients of wheat yield and yields components

	GY	NG	GC	GI	PC	GW	HI	VO	CC
NG	00.41								
GC	0.090	0.003							
GI	-0.009	0.044	-0.097						
PC	0.042	-0.043	0.150*	0.787**					
GW	0.039	0.085	-0.152	0.940**	0.876**				
HI	0.151	-0.125*	0.153	0.224	0.241	0.320			
VO	0.080	0.380*	0.205*	-0.074	0.108*	0.153	0.502*		
CC	0.111	0.704**	0.172	-0.111	-0.239	-0.128	-0.235	0.125	
GS	0.116	-0.289	-0.047	-0.093	0.104	-0.044	-0.009	-0.139	0.030

*and **: significant at 0.05 and 0.01 probability levels; GY: grain yield; NG: nature of the grain; GC: gluten content; GI: gluten index; PC: protein content; 1,000 grains weight; HI: harvest index; VO: volume output; CC: crumb color; GS: general score.

Protein content was the most important effective secondary trait, which had a positive and significant genotypic correlation with 1,000 grains weight ($r = 0.876$, $p < 0.01$) and volume output ($r = 0.108$, $p < 0.05$). Volume output had the highest correlation with harvest index ($r = 0.502$, $p < 0.05$) (Table 5). The yield stability depends on yield components and other factors (Kang, 1998). Parihar et al. (2016) have reported that various wheat yield and yield components could have positive and negative correlation.

Economic efficiency of tested treatments

Most of the available research studies have focused on the production of high grain yields of wheat and have neglected significance of economic efficiency. The economic efficiency of fertilization depends on the optimization of the input norms and the ratios between the nutrients, the soil fertility, the meteorological conditions, the level of the applied technologies for crops cultivation.

The highest income was achieved with intensive cultivation technology in variety Lubova, when net income was 58,955 RUB ha⁻¹ and payback 2.66 RUB RUB (Table 6).

The most profitable cultivation strategy was intensive cultivation adoption with the variety Lubova as it had the highest payback 2.66 RUB RUB, which was simpler to that obtained with basic cultivation technology using variety Liza (2.65 RUB RUB). However, the lowest profitability (1.5 RUB RUB) was observed when high intensive technology was used with a huge amount of inputs inclusive of labor, chemical fertilizers and pesticides. Jat et al. (2014) have also reported that the diversified cropping systems affected the net returns due to higher yields and differential cost of production.

Table 6. Economic efficiency of four wheat cultivars as affected by three cultivation technologies in spring wheat (average for 2015–2017)

Cultivation technology	Yield (t ha ⁻¹)	Gross income (RUB ha ⁻¹)	Cultivation cost (RUB ha ⁻¹)	Net income (RUB ha ⁻¹)	Cost: Benefit Ratio (Pay back, RUB RUB)
Variety: Zelata					
Basic Technology	8.31	62,325	21,512	40,813	1.89
Intensive Technology	9.82	73,650	22,120	51,530	2.32
High intensive Technology	10.46	78,450	22,785	55,665	2.44
Variety: Lubova					
Basic Technology	9.75	73,125	21,512	51,613	2.39
Intensive Technology	10.81	81,075	22,120	58,955	2.66
High intensive Technology	7.59	56,925	22,785	34,140	1.50
Variety: Liza					
Basic Technology	10.48	78,600	21,512	57,088	2.65
Intensive Technology	8.30	62,250	22,120	40,130	1.81
High intensive Technology	8.76	65,700	22,785	42,915	1.88
Variety: Ester					
Basic Technology	8.42	63,150	21,988	41,162	1.87
Intensive Technology	9.15	68,625	22,702	45,923	2.02
High intensive Technology	9.88	74,100	23,029	51,071	2.21

CONCLUSIONS

The ‘*intensive farming technology*’ (moderate application of farming inputs) significantly enhanced grain yield (10.8 t ha⁻¹), harvest index (42.9%), gluten content (39.45%) and gluten index (71.17%) of spring wheat cultivar Lubova. The highest protein content (18.02%) was observed with ‘*intensive and high intensive farming technologies*’ with the cultivar Lubova, while the highest 1,000 grains weight (46.32 g) was recorded with cultivar Lubova in ‘*basic cultivation technology*’.

Applying moderate amount of agricultural inputs as an ‘*intensive farming technology*’ resulted in highest spring wheat yield and net income (58,955 rub. ha⁻¹) and cost: benefit ratio (2.66 rub. rub payback). Hence, producers should be advised not to use large amounts of farming inputs as in ‘*high intensive farming technology*’, since it increases production costs and reduces the economic benefits.

ACKNOWLEDGEMENTS. This paper was financially supported by Ministry of Education and Science of the Russian Federation on the program to improve the competitiveness of RUDN University among the world’s leading research and education centers during 2016–2020.

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The effect of starter cultures on the qualitative indicators of dry fermented sausages made from poultry meat

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Abstract. Changes in physicochemical, rheological and microbiological properties occurring throughout the ripening (on days 0, 7, 14, 21, and 28) of dry fermented sausages made from poultry meat were studied. The effect of starter bacteria on the microstructure and sensory attributes of dry fermented sausages has also been determined. The results of physicochemical analysis of dry fermented sausage shows no significant difference ($P < 0.05$) between the test (inoculated) and the control sausages in the protein, fat, moisture, salt, ash and nitrite content. However, the significant difference ($P > 0.05$) between the control and inoculated batches in lowering the pH level, changing the critical shear stress, growth of viable microorganisms, accumulation of amine nitrogen during ripening was established. The results show, that inculcation of starter cultures accelerates biochemical processes during fermentation and thereby provides the necessary functional and technological properties of minced meat. Sensory profiling showed a more significant ($P < 0.05$) acidic and spicy flavour and intensity of acidic and smoked meat aroma; and increased firmness and cohesiveness in inoculated sausage. The results of microstructural analysis showed that the dry fermented sausages that ripened with the starter bacteria (*Lactobacillus curvatus*, *Staphylococcus carnosus*, *Pediococcus pentosaceus*), differ from the control sample compacted as a thin surface layer which is formed during the drying, smoking and maturation, and that indicates more uniform moisture removal.

Key words: poultry meat, dry fermented sausage, starter bacteria, ripening.

INTRODUCTION

Traditional technologies recommend the use of beef and pork as the basic raw materials in the production of fermented sausages (Marco et al., 2008). In several studies, the compositions of fermented sausages from different meat types, such as goat and sheep (Stajic et al., 2013), horse (Kovačević et al., 2016), camel (Mejri et al., 2017b), and mutton (Zaho et al., 2011) have been considered. Given that currently the poultry is one of the most commonly consumed type of meat in the world (Tsirulnichenko et al., 2017), it is important to expand the assortment of products from poultry meat.

Traditional production of dry fermented sausages includes the grinding of meat, formulation, stuffing into a casing, fermentation, ripening and drying. During the extended drying process, the product becomes dehydrated, so the dry fermented sausages can be characterized by low moisture content, a significant amount of fat and protein, and consequently being of high energy.

The importance of fermented sausages from the point of view of healthy nutrition should be noted. Numerous studies have shown that fermented products that contain lactic acid bacteria can have a positive influence on the digestion of nutrients, so their consumption has a beneficial effect on the gastrointestinal tract and prevents intoxication in the human body (de Vuyst et al., 2008; Turhan et al., 2017). Different probiotic lactic acid bacteria might have different functional properties. Kim (2014) demonstrated that fermented sausages with probiotic starter cultures have similar physicochemical and functional properties to those with commercial starter cultures. Gallego et al. (2018) obtained results confirming the potential of dry fermented sausages as natural sources of bioactive peptides that can exert certain bioactivities such as antioxidant and ACE (angiotensin converting enzyme) inhibitory activities.

However, despite all the advantages of the product, there is a significant disadvantage – production of fermented sausages is one of the most complex technologies in the meat processing industry, characterized by its duration and laboriousness (Marco et al., 2008). Therefore, the problem of intensification of fermented sausages production, and reduction of the technological cycle, is quite topical. The solution to the problem includes issues related to the acceleration of structural changes, intensifying flavour, taste and colour formation through the application of starter cultures, protein supplements, glucono-delta-lactone, fermented sugars and other components (Sawitzki et al., 2008).

To ensure safety for the consumers and the typical characteristics of a fermented sausage as a colour and a flavour, it is very important to use starter cultures. Lactic acid bacteria (LAB) suppress the growth of pathogenic and spoilage bacteria through the antimicrobial properties of their metabolites such as organic acids, hydrogen peroxide and bacteriocins (Ammor & Mayo, 2007). Antimicrobial compounds synthesized by LAB are considered to be natural preservatives (Tabanelli et al., 2012; El Adab et al., 2015).

During the maturation of fermented sausages, biochemical, and microbiological processes take place depending on the activity of dominant microorganisms in the meat. LAB and coagulase negative cocci are the most active indigenous microorganisms, first in the acidification process, and second in denitrification, lipolysis and proteolysis (Hammes et al., 1998; Lucke, 2000).

At the end of ripening, LAB are the dominant microorganisms due to their excellent adaptation to the meat environment and their faster growth rates during fermentation and sausage ripening. LAB play an important role in meat preservation and fermentation processes because they affect both the technological properties and the microbial stability of the final product (Lorenzo, 2012; Daba & Saidi, 2015; Cardinali et al., 2018).

Obtaining fermented sausages from poultry meat is much more complicated, which is due to the morphological and physicochemical composition of raw materials. Poultry meat is characterized by its significant water content, and such meat dehydrates much more slowly than necessary. Therefore, it is very difficult to process poultry meat, especially chicken broilers meat, in fermented sausages using traditional technologies.

Due to the accelerated and considerable loss of water the smoked sausages ripen more slowly, which can lead to a crumbly consistency and considerable deformation of the product.

Mechanically deboned poultry meat was used in fermented sausages by Dhillon & Maurer (1975) and Mc Mahon & Dawson (1976). They observed that in order to obtain an acceptable texture and appearance of the product, ground beef or hand-deboned ground poultry had to be included in the formula.

In connection with the foregoing, the purpose of this research is to study the influence of starter bacterial cultures on the quality and characteristics of the model dry fermented sausages as made from poultry meat.

MATERIALS AND METHODS

Sausage production and sampling procedures

Sausage formulation includes boneless chicken breast and thigh meat (red and white poultry meat), and frozen pork back fat. The bacterial mixture 'Start Star' was used as a starter culture (Germany, HOLKOF GmbH), which consists of lactic acid bacteria of the *Lactobacillus curvatus*, *Staphylococcus carnosus*, *Pediococcus pentosaceus* strains.

The raw meat and fat were frozen before grinding at temperature of -4°C . The sausage filling was cooked in a vacuum cutter (CFS/GEA Cutmaster). At first, pieces of frozen chicken thigh were added (45 kg), then chicken breast (40 kg), starter bacterial concentrate (10, 15 and 20 g per 100 kg of minced meat), salt (3 kg), sodium nitrite (0.01 kg), sugar (0.2 kg), ground black pepper (0.2 kg), complex food supplement 'Gypsy Plus' (0.9), and glucono-delta-lactone (0.5). For 1–2 minutes before the end of grinding, minced pork back fat (15 kg) was added. The mince for the control sample was prepared in a similar way, though without introducing the starter cultures.

The sausage batters were stuffed into edible collagen casing with a diameter of 45 mm and a length of 60 cm. Next was the pressing stage, in order to form linked sausages that were rectangular shape, after which the sausages were hung on the frames.

The linked sausage was cured for 24 h at 4°C (85–90% RH) and the speed of air movement was held at 0.1 m s^{-1} .

After curing, the fermented sausages were smoked in chamber at 20°C for 2 days by successive cycles in the 'smoke-air mixture and air mixture supply' mode, the total time within the smoke-air mixture supply being 7.4% of the total smoking time. The speed of the smoke-air mixture in the chamber was set to 5 m s^{-1} , ensuring a speed of passage through the product of up to 8 m s^{-1} .

After smoking, the fermented sausages were transferred into a climate chamber where they were dried in two stages. The first stage was the 5 days at 15°C , with a relative humidity of 82% and air velocity of 0.1 m s^{-1} . The second stage lasted 23 days at a temperature of 12°C and a relative humidity of 75%.

The pH, water-binding capacity, rheological properties, total amount of viable bacteria, amount of amino-ammonia nitrogen and volatile fatty acids were examined to assess the influence of starter cultures on microbiological, physicochemical and biochemical changes during ripening. For sampling, three sausages of each batch at 0 day (mix before stuffing) and after 7, 14, 21 and 28 days of ripening were taken for microbiological and physicochemical analyzes.

Microstructural analysis was carried out to study the effect of starter cultures on changes in the structure of poultry meat. At the end of the ripening three sausages from each batch were taken for sensory and physicochemical analysis.

Microbiological analysis

Microbial tests on Standard Plate Count Agar (PCA) were used to determine the total number of viable bacteria. Homogenized samples (10 g) were mixed with 90 mL of sterile double deionized water (dd water), and serial dilutions were prepared, then dilution (0.1 mL) was spread in PCA agar. They were incubated at 37 °C for 48 h, counted and expressed as log CFU g⁻¹.

Physicochemical analysis

Physicochemical analyses during the ripening period included the pH measurements, water-binding capacity, the amount of amino-ammonia nitrogen and volatile fatty acids.

The pH values were measured in homogenates prepared by blending 10 g of sausage with 40 mL of distilled water for 2 min. Measurements were taken with a digital pH meter (model 710 A+) equipped with a penetration probe. The water-binding capacity of the fermented sausages was determined as the difference between the moisture mass fraction in the samples and the quantity of moisture that were separated in the process of the heat treatment.

The amount of amino-ammonia nitrogen was determined according to a method based on the binding of amino groups and ammonia with formaldehyde in a neutral solution, followed by titration of carboxyl groups with sodium hydroxide, the amount of which is equivalent to the number of free amino groups.

The amount of volatile fatty acids was determined by a method based on the separation of volatile fatty acids accumulated in poultry meat during the hydrolysis and oxidation of lipids using steam distillation and the determination their amount by titration with a solution of potassium hydroxide. The amount of volatile fatty acids is expressed in milligrams of potassium hydroxide used for titrating volatile fatty acids isolated from 100 g of sample.

Moisture, fat, protein and ash content in sausages samples were determined according to standards recommended by the International Organization for Standardization. The percentage of moisture was calculated by the weight lost by the experimental sample (5 g) maintained in an oven (Memmert, UL 60) at 105 °C, until constant weight was achieved. Fat and protein content were analysed using Soxhlet extraction and protein determination by the Kjeldahl method, respectively.

The sodium chloride and sodium nitrite contents were determined according to the AOAC methodology (2002).

Investigations of rheological properties (critical shear stress) of fermented sausages during maturation were carried out at room temperature (20 ± 1 °C), using a texture analyser (Brookfield R/S) and a rotary viscometer.

Microstructural analysis

Changes taking place in tissues under the influence of biotechnological processing were determined via a histological study of the samples. After the sampling, the samples were fixed in a 10% water solution of neutral formalin, which were then washed with

cold water and compacted in gelatin. The blocks were cut and compacted in 20% formalin solution over 12 hours after cooling the gelatin solution with the samples. Then the blocks were washed, pieces were cut of 15×15×4 mm in dimension, and the sections were created on a freezing microtome. A section was placed on a glass slide treated with an ovalbumin and glycerol. The sections were painted with haematoxylin-eosin. Histological preparations were examined under a light microscope (Leika DM 1000).

Sensory analysis

To conduct a sensory analysis, sausages were subjected to microbiological research and their microbiological safety was proved (Solovyova, 2015).

A sensory evaluation was performed on the control and inoculated sausages at the end of the ripening process. A total of 15 experienced panelists, including staff members of Department of Food and Biotechnology and experts from food enterprises who had experience in assessing fermented meat products were chosen to perform a sensory characterization of the batches (ISO 8586–2:1996). The panellists were trained for 2 weeks according to the attributes and scale recommended by the International Organization for Standardization (2012).

The preparation of the samples for the sensory evaluation consisted of removing the casings and slicing cold sausages into equally sized pieces, each 0.5 cm thick. Single slices were placed into odourless, plastic white dishes and served at room temperature. Each sample was evaluated three times. Water and unsalted crackers were provided to cleanse the palate between samples.

The sensory quality was characterized on the basis of 16 sensory traits: five odour attributes (smoked meat aroma, rancid aroma, spicy aroma, acid aroma, mould aroma); three appearance attributes (fat distribution, colour intensity and colour homogeneity); three texture attributes (firmness, cohesiveness, fattiness); five attributes of flavour (smoked meat flavour, spicy flavour, acid flavour, rancid flavour and saltiness).

Each parameter in the sensorial analysis was evaluated by means of a scale from 1 to 9 with anchors of 1 meaning no intensity or extremely low intensity, 5 meaning regular intensity of dry-fermented sausage and 9 meaning extremely high intensity.

Statistical Analysis

The values are presented as the mean ± SEM. Probability values ≤ 0.05 were taken to indicate statistical significance. The data were analysed by One-way ANOVA using free web-based software offered by Assaad et al. (2014).

RESULTS AND DISCUSSION

Microstructural analysis

Microstructural studies can identify changes occurring in individual structural components and differentiate the characteristics of tissue and cellular structures. Proteolysis of proteins occurs during the ripening of fermented sausages under the influence of enzymes present in meat tissues or by enzymes of microbial origin from added starter cultures (Mejri et al., 2017a), which leads to certain changes in the structure of animal tissues. These changes cause the formation of a specific texture of raw sausages (Katsaras & Budras, 1992), and their intensity can be detected using microstructural studies.

In a microstructural study, it was found that the mince of the control (Fig. 1) and the test (Fig. 2) samples of the sausages was homogeneous, dense-friable, and slightly vacuolized.

The basis of the mince is a fine-grained protein mass with fat cell components uniformly distributed throughout the sample. There are isolated groups of unchanged striated muscle bundles which have retained their shape and size and which fit tightly to each other (Fig. 1, a).

Fig. 1, b shows that the muscle fibres are swollen bundles that have retained their integrity, and the boundaries between them are difficult to distinguish. There is practically no transverse and longitudinal striation of muscle fibres. Bunches of collagen fibres and smooth muscle tissue are weakly expressed in a state of colloidal mucoid swelling.

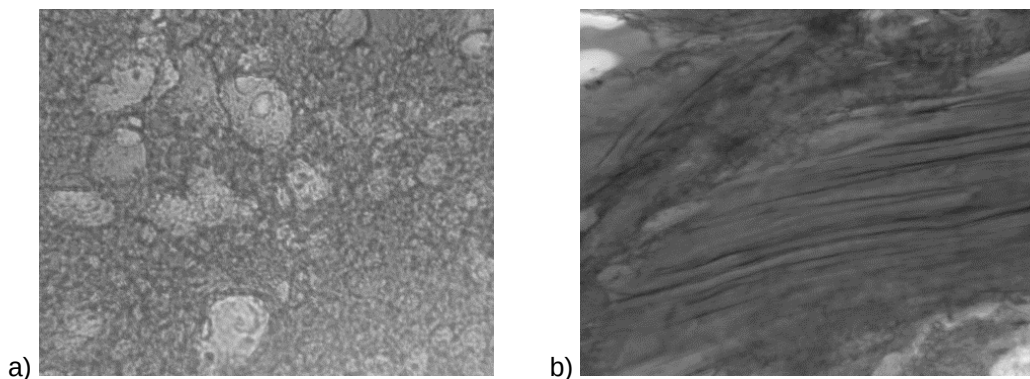


Figure 1. Microstructure of the control sample of the dry fermented sausage, magnified $\times 200$.

Individual fragments and conglomerates of fibres and bundles of transverse striated and smooth muscle with a pronounced degree of treatment are noticeable in the prototype (Fig. 2, a). This is due to the formation of a spatial framework, accompanied by the destruction of the cellular structure of the tissues, as caused by the activity of microorganisms, the development of the autolysis processes and the morphological features of muscle tissue.

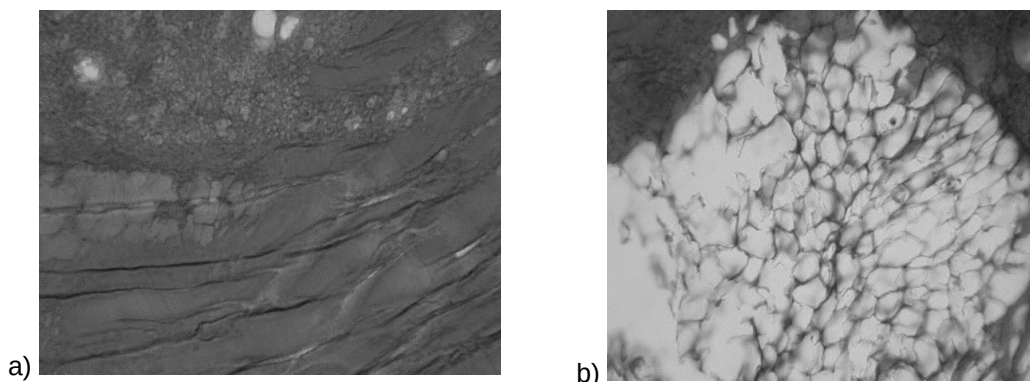


Figure 2. Microstructure of the test sample of the dry fermented sausage, magnified $\times 200$.

There are single islets from unchanged muscle bundles that have retained their shape and dimensions and fit tightly to each other (Fig. 2, b). However, unlike the control sample, bundles of smooth muscle and connective tissue fibres have a more restructured and unfolded structure.

The results of microstructural studies show that the addition of the starter cultures caused significant changes in the structure of the muscular and connective tissues, namely the loosening of the bundles into separate fibrils and their fragments. This is due to accumulation of lactic acid during the starter bacteria reproduction and their proteolytic activity. This contributes to the softening of raw materials. Aktas & Kaya (2001) determined the destructive effect of lactic acid on animal tissues. Katsaras & Budras (1992) noted a change in the structure of native muscle proteins results from different technological processes (chopping, salting, and fermentation). The salting leads to changes in the original protein structure through swelling and partial dissolution of myofibrils. The formation of a matrix of fermented sausage and its specific structure occurs during the maturation of sausages under the influence of lactic acid and due to the gradual loss of water (Katsaras & Budras, 1992).

Physicochemical analysis

Such physicochemical parameters as pH, salt and moisture content can be attributed to ‘typical’ characteristics for dry fermented sausage (Montanari et al., 2018).

The pH values underwent a rapid reduction in the control and inoculated sausages (Fig. 3), but in the samples with 0.02% starter cultures, the pH decreased more quickly and reached a value of 4.95 ($P < 0.05$) for by the 14th day. When 0.015% starter cultures were added, the pH reached 4.9 ($P < 0.05$) after 21 days of ripening, whereas in the control sample over the same period, the pH reached 5.32 ($P < 0.05$). Sawitzki et al. (2008) determined a similar dependence where during the first 7 days of fermentation the pH values decreased from 5.60 to 4.97 in the inoculated salami, while in the control, pH values decreased from 5.68 to 5.34.

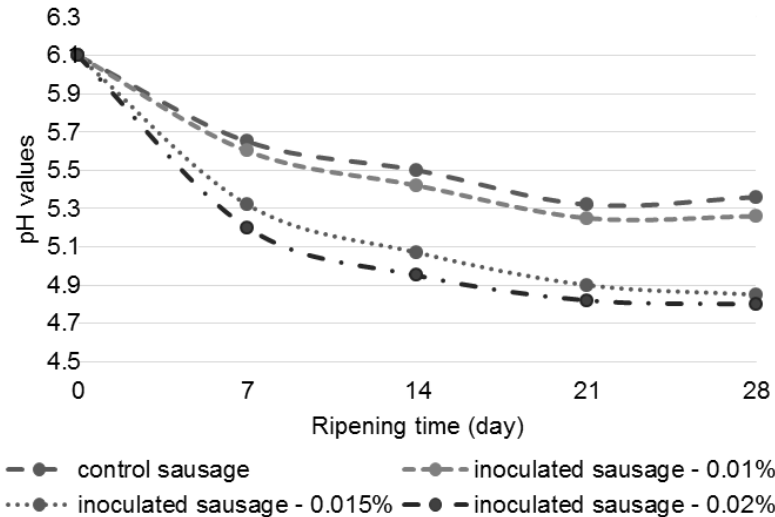


Figure 3. Dynamics of pH change during ripening.

During the ripening of fermented meat products, LAB fermented glucose to lactic acid, which was responsible for the decrease in pH (Parente et al., 2001; Drosinos et al., 2007).

The results show (Fig. 3) that by day 28 of the ripening/fermentation, the rate of decrease in pH was slowing down. The same disposition was defined in the study of fermented sausages conducted by Zdolec et al. (2008). These gradual changes in pH are explained mainly by the reduction in the number of LAB due to the exhaustion of the sugar and, secondly, to the proteolytic activity generated by microorganisms (Katsaras & Budras, 1992; Rai et al., 2010).

Water-binding capacity (WBC) affects the formation of the sausage structure. The results of the experimental studies (Fig. 4) show that large decrease in WBC was observed in samples using starter cultures, which correlates with the dynamics of pH change. Similar results were obtained by Nesterenko (2014), who found that in samples with added starter cultures, the water-binding capacity is lower than in the control sample by 2%.

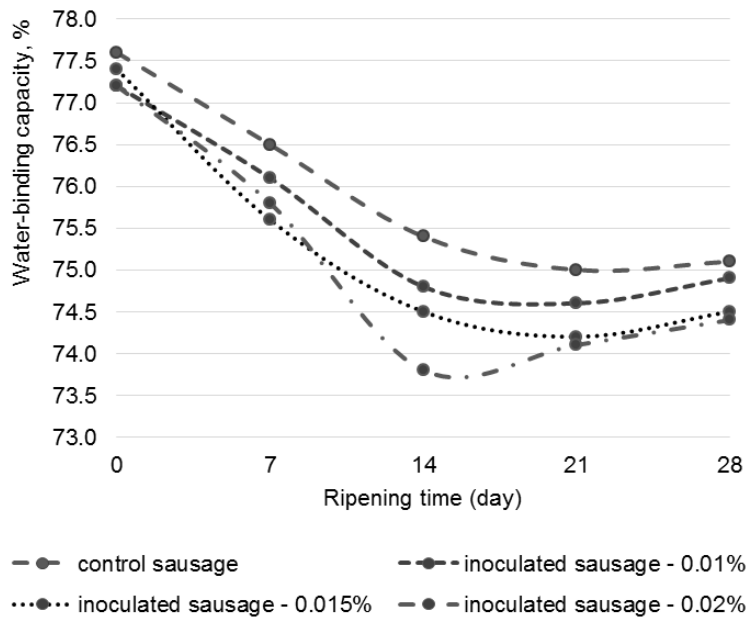


Figure 4. Dynamics of water-binding capacity change during ripening.

This effect is due to rapid glycolysis and the accumulation of acidic metabolic products of bacteria that reduce the pH value and, accordingly, affect the properties of muscle proteins (Mejri et al., 2017b). The decrease in pH causes a reduction in the water-binding capacity of the meat, accelerating the drying process aging (Työppönen et al., 2003). Mauriello et al. (2004) reported that water-binding capacity decrease is related to pH decrease. When the pH rates are close to the isoelectric point of protein, the functional properties of meat proteins are reduced and dehydration occurs.

One of the characteristics that makes sausage such an appreciated product is its texture, which is related to the mechanical properties of the product (Daros et al., 2005). Consistency of minced and finished products is best characterized by the value of the

critical shear stress (Figs 5, 6). This indicator was used for technological assessment of minced meat during the process of ripening.

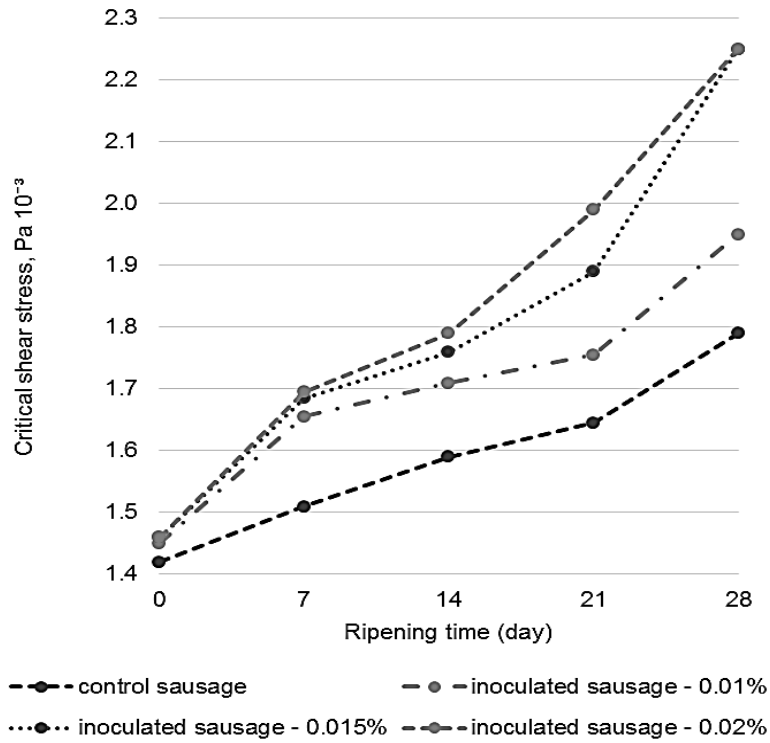


Figure 5. Dynamics of critical shear stress change during ripening.

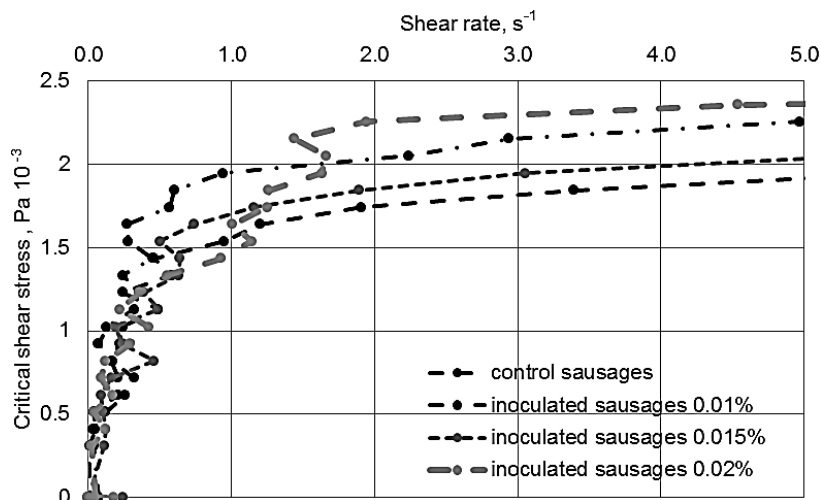


Figure 6. Example of a diagram obtained on the texture analyser (Brookfield R/S) when examining the critical shear stress of the dry fermented sausage samples after 28 days of ripening.

The data presented in Fig. 5 indicates that the compression of minced meat in a test sample with the addition of starter cultures is faster than the control. After 7 days of ripening, the critical shear stress in the test samples (1655, 1685 and 1695 Pa, respectively, ($P < 0.05$)) significantly exceeds this value in the control sample (1510 Pa ($P < 0.05$)). Structure formation for the control sample was complete by 21 days. Reducing the pH level to the value of the isoelectric point of muscle proteins leads to a decrease in the hydration properties of the meat system. Reducing the moisture content correlates with the formation of a dense structure of sausages, therefore, the shear stress increases. These results are in agreement with those found by Mejri et al. (2017b), who determined that the hardness of the control and inoculated sausages increased by 28 days of ripening. The authors attributes this to a decrease in the moisture content (drying of the sausage) and a corresponding increase in the level of fat. In addition, this was perhaps due to the accumulation of nonprotein products, namely exopolysaccharides, as a result of the lactic acid bacteria activity (Ruas-Madiedo et al., 2002), and this directly effects the structural formation of minced meat during maturation.

Thus, the use of starter cultures contributes to the formation of a dense surface layer and the monolithic structure of minced meat.

To evaluate the effect of starter culture on lipolysis and proteolysis intensity, volatile fatty acids and amine nitrogen content were determined through ripening period. Organic acids, in particular volatile fatty acids (VFA) and amino acids that accumulate in the minced meat, are associated with the formation of a specific aroma and taste of the sausage. The results of the studies (Figs 7, 8) show that during the ripening of minced meat in experimental samples, intensive accumulation of VFA and amine nitrogen can be observed.

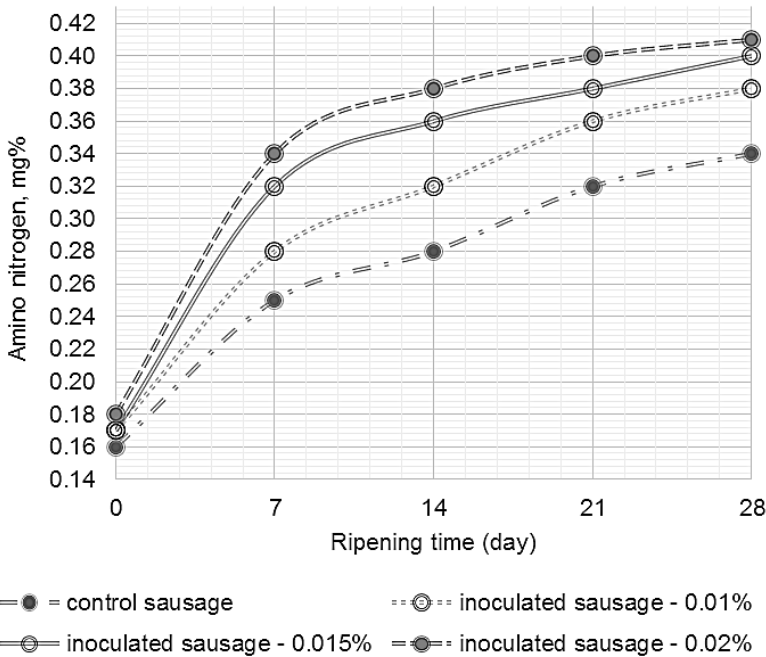


Figure 7. Dynamics of accumulation of amine nitrogen during ripening.

Thus, after 7 days of ripening, the maximum amine nitrogen content and VFA in the test samples was 4.05 and 0.34 mg in 100 g ($P < 0.05$), respectively, and in the control sample such values were achieved by 12 days in terms of the VFA content, and only by 28 days in terms of the amine nitrogen content. The dynamics of amine nitrogen and VFA accumulation indicate an acceleration of the ripening in inoculated sausages in comparison with the controls.

The hydrolysis of meat proteins generates polypeptides that can be further degraded to smaller peptides and free amino acids, where such degradation can be caused by endogenous and microbial enzymes, as reported by a number of different authors (Hughes et al., 2002; AroAro et al., 2010). El. Adab et al. (2015) demonstrated that in sausages inoculated with *S. xylosum* and *L. plantarum*, the free-acid content increased from 2,059.82 mg kg⁻¹ to 3,461.07 mg kg⁻¹ as a result of proteolysis. This difference in total free amino acid accumulation was related to the proteolytic activities of microbial enzymes. These amino acids play an important role in development of characteristic taste and flavor of the final product (Casaburi et al., 2008; Lorenzo & Franco, 2012).

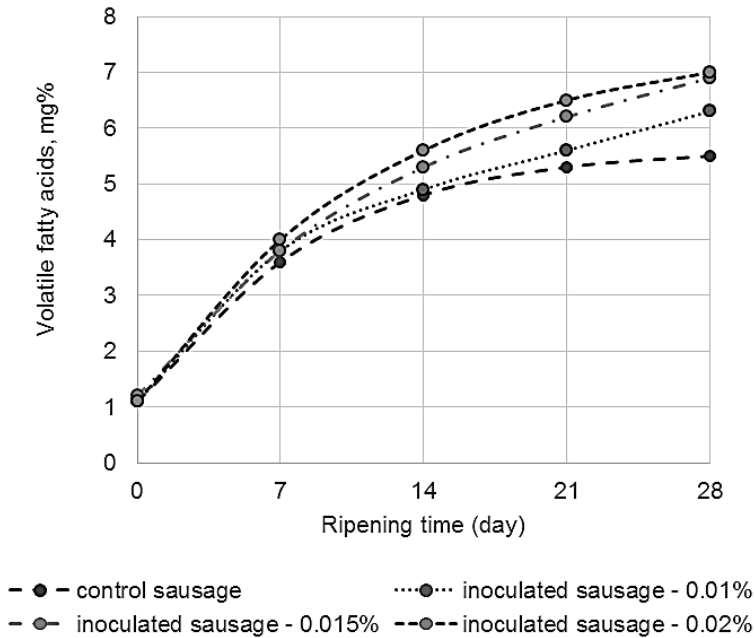


Figure 8. Dynamics of accumulation of volatile fatty acids during ripening.

The results of physicochemical analysis of dry fermented sausage (Table 1) shows that there are no significant differences ($P > 0.05$) between the test (inoculated) and the control sausages in terms of the protein, fat, moisture, salt, ash and nitrite contents. The moisture content in the sausages was between 29.8 and 31.4 ($P < 0.05$), which is typical for dry fermented sausages. Sawitzki et al. (2008) and Mejri et al. (2017b) obtained similar results, where these authors observed no significant difference ($P > 0.05$) between the inoculated and the control sausages. However, the determined that the moisture content was somewhat higher than the value reported in dry fermented Chinese-style sausage by Rai et al. (2010).

Table 1. The results of the physicochemical analysis of dry fermented sausages

Indicator	Value of indicator for samples of dry fermented sausages, %			
	Control	Test 1	Test 2	Test 3
Protein	28.7 ± 0.64 ^b	29.6 ± 0.52 ^{ab}	31.1 ± 0.42 ^a	31.2 ± 0.58 ^a
Fat	34.2 ± 0.86	33.2 ± 0.94	32.9 ± 0.74	33.5 ± 0.62
Moisture	31.4 ± 0.20 ^a	30.8 ± 0.36 ^{ab}	30.2 ± 0.22 ^{ab}	29.8 ± 0.41 ^b
Ash	4.52 ± 0.16	4.46 ± 0.16	4.44 ± 0.12	4.4 ± 0.14
Salt	3.76 ± 0.12	3.74 ± 0.08	3.8 ± 0.06	3.82 ± 0.08
Nitrite	0.003 ± 0.001	0.003 ± 0.001	0.003 ± 0.001	0.003 ± 0.001

Designation: test 1 – inoculated sausage 0.010%; test 2 – inoculated sausage 0.015%; test 3 – inoculated sausage 0.020%. Values are means ± SEM, n = 5 per treatment group. Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by one-way ANOVA and the TUKEY test.

Microbiological analysis

It is known that the maturation process of fermented products is based on the vital activity of lactic acid bacteria which gradually become dominant, suppressing the development of pathogenic bacteria (Drosinos et al., 2007; El Adab et al., 2015). However, it is not always possible to set the ripening process on the right track for fermented products, and as a result bacterial deterioration can take place (Sawitzki et al., 2008). According to Drosinos et al. (2007), Sawitzki et al. (2008) and Mejri et al. (2017b), the microbial population of lactic acid bacteria in fermented sausages is equal to or lower than 4.5 log CFU g⁻¹ at the beginning of fermentation without introduction of starting cultures. We obtained similar results at the start of fermentation where the amount of viable bacteria was 3.4 log CFU g⁻¹ in the control sample and from 3.8 to 4.1 log CFU g⁻¹ in the test samples (Fig. 9). The amount of viable bacteria by day 21 was 6.0 log CFU g⁻¹ in control sample and up to 9.2 log CFU g⁻¹ in the test samples, and which thereafter remained at the same level until the end of ripening.

The results of the studies indicate a high survival rate of the microorganisms included in the starter cultures, the metabolism of which ensures the microbiological safety of dry fermented sausages. Gao et al. (2014) demonstrated that lactic acid bacteria improve the safety, stability and shelf life of meat products.

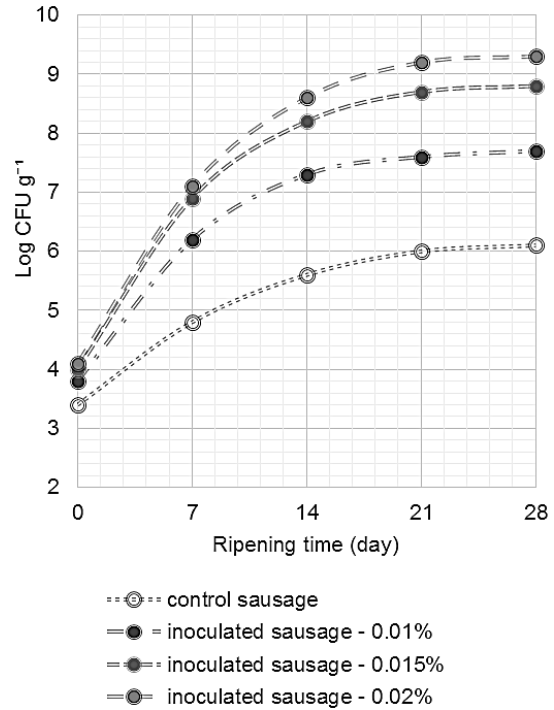


Figure 9. Growth of microbial population during ripening.

Sensory analysis

The four types of sausages were subjected to a sensory evaluation of 16 characteristics as carried out by 15 experienced panellists. Fig. 10 shows the results of the descriptive sensory analysis of the sausages at the end of the ripening period.

The complex studies concerning the importance of sensory attributes and their perception by consumers have shown that flavour is always the most important attribute of food products, followed by texture and appearance (Jaworsk & Hoffmann, 2008).

Among the 16 descriptors considered in the test, significant differences were obtained in the traits characterizing the flavour, texture and appearance of the sausages.

Regarding the flavour, the addition of commercial starter bacteria significantly affected the acidic and spicy flavours and saltiness. The highest values of acidic and spicy tastes were obtained from the batch containing 0.02% bacterial concentrate. The lowest value corresponded to the control sausages. On the other hand, we observed slightly higher scores for rancid flavour and smoked meat flavour intensity in the control batch, but the differences were not felt to be significant. Furthermore, the saltiness of the sausages was significantly reduced by the addition of starter bacteria.

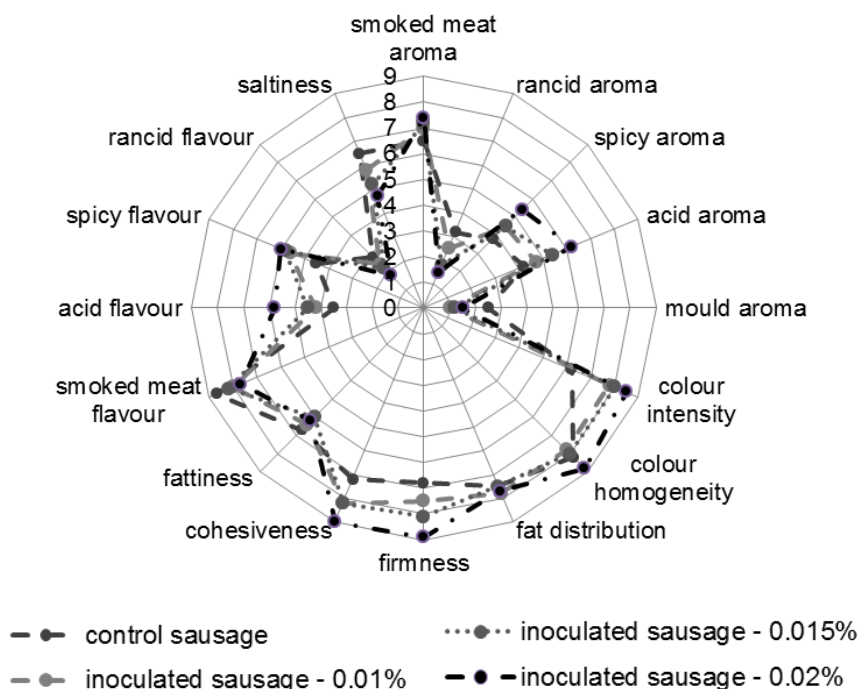


Figure 10. Sensory evaluation of control and test (inoculated) sausages.

The highest acid taste score in test batches agreed with those reported by Lorenzo et al. (2016), who also observed that non-inoculated sausages gave the lowest acid taste. Our results are consistent with the data obtained for pH values, where, as commented above, test sausages gave significantly lower pH values than the control sausages.

The extent of the rancid flavour in the control samples agreed with the results reported by Cenci-Goga et al. (2012), who found a more pronounced rancid taste in

salami made without the addition of starter cultures. The results of the sensory analyses confirm the antioxidant effect of the starter cultures in controlling lipid oxidation, which positively influence the sensory properties of sausages.

During the analysis of the aroma of inoculated sausages, a positive correlation between the amount of starter bacteria and the acid, spicy and smoked meat aroma intensities was established.

Starter bacteria noticeably influenced the texture of the sausages compared with the control sample. Inoculated sausages presented greater firmness and cohesiveness; they had a better texture, which is in agreement with the instrumental rheological analysis. No significant difference was found between any of the samples in terms of fattiness.

The test sausages presented higher colour intensity and colour homogeneity than the control sample. The most acceptable appearance was observed in sausages containing 0.02% starter bacteria. These findings were in agreement with the results obtained by other authors, who found a more intense red colour in inoculated than in control sausages (Andrade et al., 2010; Essid & Hassouna, 2013). The colour formation is related with the nitrate reductase activity of the starters used in this study, which contained *L. curvatus*, *S. carnosus*, *P. pentosaceus* with its high nitrate reductase activity.

CONCLUSIONS

This study demonstrated that the introduction of starter cultures accelerates biochemical processes during fermentation/ripening and thereby provides the necessary functional and technological properties of minced meat in the production of dry fermented sausages from poultry meat. This was confirmed by the results of physical and chemical studies, which showed a greater decrease in pH, WBC, accumulation of amine nitrogen, and increased critical shear stress in the test (inoculated) samples compared to the control sample. Microstructural and microbiological studies also confirm the effectiveness of using the starter bacteria in the production of dry fermented sausages from poultry meat. The results of intensive proteolysis and lipolysis, which develop due to the enzymatic activity of microorganisms, is responsible for the formation of the specific taste and aroma of the sausages, as determined during the sensory analysis.

ACKNOWLEDGEMENTS. The work was supported by Act 211 Government of the Russian Federation, contract №02.A03.21.0011. The authors thank the managers and employees of the meat processing complex Sitno for their assistance in the production of sausages and their research, as well as the reviewers for valuable comments in preparing this article.

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Analysis and forecast of performance characteristics of combine harvesters

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Abstract. This article presents results of an experimental research of qualitative indicators of the modern combine harvesters (Case IH Axil Flow 8230, MasseyFergusonMFT7, JohnDeereS680i, ClaasLexion760, NewHolland CR9.80) used for winter wheat harvesting. Based on the results obtained, determination was made regarding the productivity of combine harvesters on the field, fuel consumption, and field conditions influence the grain loss and grain damage caused by a harvester. When conducting the experimental research of a combine’s performance on the field, a study of the effectiveness of the combine JohnDeereS680i was made on different modes. A program ‘Machine Unit’, designed by the authors, was used for the determination of productivity, fuel consumption and quality indicator for harvesting.

Key words: combine harvester performance, fuel consumption, chaff in a grain tank, post-harvest losses, loss of grain, grain damages, plant residues.

INTRODUCTION

Due to the growing population and the simultaneous increase in food demands, the role of mechanization in agriculture is essential (Hafezalkotob et al., 2018). Agribusiness entails a large number of inherent risks associated with natural and biological phenomena (Mimra et al., 2017). Therefore, for the purpose of business development, producers of agricultural products focus all the material and physical resources on determining the ways to increase the gross harvesting of products, and improve its quality indicators compared to previous periods as well as products of competing companies. However, it is not enough to grow the crops with high level biological

parameters (as for winter wheat: the yield, weight of 1,000 grains, grain unit, glassiness, gluten, mass fraction of protein, germinative power and germination of seeds (Kirpa, 2010). In addition, it should be stated that grain loss is an inevitable part of the working process of a combine harvester, which is influenced by a wide range of parameters (Liang et al., 2017). Usually, the simultaneous minimization of grain losses and the operation time of combine harvester requires the optimal selection of the construction and operating parameters for the straw walker unit, whereas the phenomenon of grain separation and its determinants depending on these parameters are not yet well understood (Myhan & Jachimczyk, 2016). Consequently the challenge to gather a biological harvest of the plant in the most efficient way still.

The introduction of new technologies into the agricultural production requires constant upgrades of the machines (Liang et al., 2017; Hafezalkotob et al., 2018). Due to the reduction of the existing combine harvester park, physical depreciation of the machines, their obsolescence, increase in number of the broken machines, as well as an increase in the average load on the machine, it is important to choose the combine harvester that meets the conditions in the sector best (Maslacq et al., 2016). Upgrading the machines brings long-term positive results in technical and economic areas (Mimra et al., 2017), so when choosing a combine harvester one needs to analyse both the technical characteristics and the results of field trials.

A combine harvester needs to be a high-tech one in order to deliver high productivity with minimal crop losses, damage and minimal expenses on maintenance and repairs of a machine (Kavka et al., 2016; Špokas et al., 2016). If a combine harvester suffers physical and moral obsolescence, it is inappropriate from an economic standpoint to use such a combine (Mimra & Kavka, 2017).

The main requirements for crop harvesting include optimal agronomic conditions, while ensuring minimum loss of products and appropriate quality of grain, as studied for example by Kehayov et al. (2004) and by the Tymchuk et al. (2015a). It is necessary to exclude losses resulted from the mass standstill, losses caused by combine harvester passing and other losses associated with it caused due to the mechanical damage of grain (Huang et al., 2017). These losses are the result of varying realization degrees of the seed biological potential all over the total field area (various conditions for YPF consumption, level of humidity and nutrients volume). Additionally, losses increase with non-compliant adjustments of the combine harvester, which may not always provide an effective work of the harvester, with a late service maintenance and a late replacement of units, which are responsible for threshing, adjustment of gaps on a threshing device of the combine within an acceptable error margin (Tymchuk et al., 2015b).

Such a loss of winter wheat causes weeding of the field with sprouted grain and an increase in the pests and diseases population, which inevitably leads to additional costs required for alleviating relevant phenomena. In addition, minimum harvesting time needs to be guaranteed for crops gathering with a special consideration of weather conditions at the selected harvesting time. Humid weather not only stops a process of harvesting, it also leads to an active spread of diseases which causes the bud darkening, and an increase of a contaminated grains share and their germination within a year (Cherenkov et al., 2011). Thereby, it decreases purchase price for the produce.

Based on the results of the field research executed by Kirpa (2010), it was found that depending on timing and duration of a harvesting process, as well as harvesting quality assurance (combine adjustments, combine setting modes, control of work performed by the equipment), losses may reach up to 16–18% of a biological harvest (Kirpa, 2010). One of the ways to increase the gross harvesting is to reduce losses by securing high food, feed and seed qualities during gathering, transporting, post-gathering processing and storing (Huang et al., 2017). This can be attained by reducing the duration of the crop gathering to a minimum threshold, not exceeding a five-day period (Demko, 2011). The reason for this is that after the fifth day there is a significant decline in glassiness quality and a reduction in weight of 1,000 grains (Demko, 2011). A 10–20 days delay results in a significant reduction of the mass fraction of protein and a gluten quality as described by Cherenkov et al. (2011).

When harvesting, it is important to ensure effective distribution of plant residues on the field combined with a minimal fuel consumption and a maximum level of the combine harvester productivity (Gürsoy et al., 2015). Seed germination and spread of rodents and diseases depends on a stubble height (Kumhála et al., 2005), a grinding degree of the straw remains (Kviz et al., 2015) and an equality of distribution of plant residues on the field surface (Buryakov & Skoblikov et al., 2017). A grinding device of the combine harvester should provide a high-quality straw grinding – 90% of all pieces should be shorter than 80 mm (Kumhála et al., 2002). Quality of the distribution work is characterized by the residues distribution heterogeneity at a high work speed, which is a result of increase in quantity of material being delivered to a combine harvester. The more material gets to the harvester per time unit; the worse the equality of distribution of the residues becomes (Kviz et al., 2015). This is caused by the inconsistencies in using mass and engine power of the combine harvester, area of threshing cylinder and a cleaning system (Makarenko, 2014). Fuel consumption is a very important parameter as it directly correlates with the economy of agricultural machines use (Vasylieva & Pugach, 2017).

Therefore, a profitable farming system is expected to minimize the fuel consumption by the machines used in agriculture (Gürsoy et al., 2015), because cost of fuel cover 19–30% of total costs during the harvesting (Mimra & Kavka, 2017). As such, further research of modes and parameters of work in the actual field conditions is necessary.

For the efficient operations in agriculture, it is advisable to conduct a study of the performance effectiveness of a combine and to identify the relevant risks while developing a business plan (Kavka et al., 2016). The strategy for harvesting is significantly influenced by climatic zones and the terrain. Thus, a research of factors of work effectiveness of combine harvesters should be conducted in each region. Reliable data is required for developing a harvesting strategy (Špokas et al., 2016).

The goal of this study was to define qualitative factors and to analyse performance characteristics of combine harvesters during gathering early grain crops (wheat), to determine the productivity of harvesters and the actual fuel consumption for specific soil and climatic conditions. The research also aimed to evaluate the study methodology of technical factors in a production environment, using a combine John Deere S680i with a John Deere 630f reaper.

Table 1. Technical Characteristics of the Studied Combine Harvesters

	Option	J D S680i	C IH 8230	N H CR9.80	C L 760	M F MF T7
Engine	The number of cylinders, units	6	6	6	6	7
	Engine, cm ³	13,500	12,900	12,900	12,500	9,800
	Nominal power, HP	473	476	489	431	375
	Maximum power, HP	547	516	530	461	451
Rotor	The location of a rotor	Longitudinal	Longitudinal	Longitudinal	Longitudinal	Lateral
	Number of rotors, units	1	1	2	2	1
	Diameter, mm	762	762	559	445	762
	Length, mm	3,124	2,623	2,638	4,200	2,235
Separation	Rotation frequency, RPM	380–1,000	220–1,180	200–1,050	360–1,050	336–900
	The frequency of rotation of a gear, RPM	210–550			166–483	180–480
	The main metal area under the drum, m ²	1.1			1.06	0.61
	The area of the threshing and separation, m ²	1.54	2.98	3.06	4.45	3.89
	The total area of a cleaning system, m ²		6.5	6.54	5.1	4.99
	Fan speed, RPM	620–1,350	300–1,150	200–1,050	640–1,660	1,250
	Threshing system	EvenMax – Active returning of threshing returns with an additional beater	Three- rubbing drums mechanism	Roto-Tresh- double	Non- autonomous, full cycle	Two-stage
Bunker	The capacity of the grain tank, m ³	14,100	12,330	12,500	11,000	13,743
	Upload speed, L sec ⁻¹	135	113	126	130	141
Fuel tank	Capacitance, L	1,250	1,000	1,000	1,150	870
Price (euro)		290,000	255,000	280,000	280,000	267,000

MATERIALS AND METHODS

Harvesting agricultural crops is one of the most responsible and energy-intensive production processes of the crop production. In the general cost structure of crop production in Ukraine, harvesting requires 31–50% of energy and 45–60% of labour costs (Makarenko, 2014). Given a steady increase in cost of equipment and petroleum, oils and lubricants, an important task is to ensure the minimum cost of the harvesting process. In addition, it is important to preserve accumulated energy during the whole period of vegetation. The other factor for consideration is ensuring a minimum loss during threshing and minimal injury of the grain. Loss of grain during threshing and separation, damage of grain, fuel consumption, and combination of productivity are considered to be the basic criteria for the evaluation of the combine harvester performance in the field. All of the above criteria are essential and closely related to work conditions of harvesting (Špokas et al., 2016). This study was conducted in the field, using combine harvesters Case IH Axil Flow 8230-2, MasseyFergusonMFT7, JohnDeereS680i, ClaasLexion760, NewHolland CR9.80, used in the ‘Palmira LLC’, a cluster of groups of companies ‘Kernel’ in the Privitne village (Poltava region, Ukraine) from July to August 2016. Technical characteristics of the studied combine harvesters can be found in Table 1.

When analysing the performance of combine harvesters, the following criteria and methods of evaluation of the quality of the studied machines were used (Table 2):

Table 2. Criteria and Methods of Evaluation of the Quality of the Studied Machines

1. Crop that is being harvested	– Winter wheat
2. Range of a crop productivity	– 6–9 t ha ⁻¹
3. Cut height of a stubble	– max 12 cm
4. Fuel consumption	– Chronographic measurement
5. Productivity	– Chronographic measurement
6. Quantity of broken grains	– Laboratory analysis
7. Loss after passage of a combine	– Mobile laboratory
8. Distribution across the width of the reaper	– Even, with a full coverage of the width of the reaper

Chronographic Measurement

Study of the main technical and economic indicators of the combine performance was conducted by the means of time chronograph and timekeeping methods to meet the requirements of the application methods of timekeeping according to the norm GOST 24055-88 (GOST 24055-88(1988): Methods of operational and technological evaluation. General; Moscow, USSR, 1988).

The observation was carried out for each item of separate technological operations. The following devices were used:

- A mechanical stopwatch according to GOST 5072-79 (GOST 5072-79 (1979): Mechanical second moments. Technical conditions; Moscow, USSR, 1979), 3.0 accuracy class;
- A measuring roulette with a 50 m length according to GOST 7502-98 (GOST 7502-98 (98): Measuring metal tapes. Specifications; Moscow, Russia, 2006), 3.0 accuracy class;

- A measuring ruler with the length of 3.5 m as per GOST 427-2009 (GOST 427-2009 (2009): Linear measuring metal. Specifications; Kiev, Ukraine, 2009);
- A graduated measuring probe, with the length of 1 m according to GOST 427-2009.

Time was monitored using a stopwatch with a precision of up to a second; the average length of field parcels was measured with an accuracy of 10 m; a width of coverage by a machine – accuracy of up to 1 cm; the level of fuel in the fuel tanks – up to 1 mm.

Time keeping was carried out with consistent tracking and noting of time spent on all sorts of actions according to the mechanized technological method of harvesting winter wheat along with the measurement of the amount of work performed and the actual cost of fuel.

Time recording was held consecutively, starting with the preparation of a machine unit for work (technical maintenance). It combined the elements of technological process, its useful work in working mode, spending time on parking the unit in a working stroke for various reasons (technological, technical and organizational), on idling and turning around.

Data obtained from this research was included in an observations tracker. In the beginning of a work shift, time was recorded in the tracker. The beginning of a technological operation element was the end of the previous element. With a combination of several elements of operation, the longest of them was determined. The rest of the items were mentioned in the tracker as ancillary. In cases where the execution of some elements of technological operation took more time than per norm, the reason was noted in the tracker.

During the work of the machine, the length of time used for the operational element was measured and noted. If during the shift, one had to move from one area to another, both duration and distance of the relocation were noted. When evaluating the observations tracker, a determination was made regarding an average width of coverage by a machine unit, average working speed of the unit with a load, productivity of the unit per an hour of genuine work, and fuel consumption per 1 ha.

The average working width of coverage of the machine unit (B_p) was determined using the Eq. 1:

$$B_p = \frac{C}{n}, \text{ m}, \quad (1)$$

where C is the width of the parcel area under cultivation during the observation, (m); n – the number of passes made by the machine unit.

The average operating speed of a machine unit (V_{cp}) was calculated using the Eq. 2:

$$V_{cp} = \frac{l_{cp} \cdot n}{1,000 \cdot T_p} \text{ km h}^{-1}, \quad (2)$$

where l_{cp} – the average length of parcels of the cultivated area (m); n – number of passes made by the machine unit; T_p – working time spent throughout a period of observation, (h).

Machine performance (W ; $ha\ h^{-1}$) was calculated using the Eq. 3:

$$W = \frac{A}{T},\ ha\ h^{-1}, \quad (3)$$

where A – harvested area, (ha); T – harvest time, (hrs).

Research of Losses by Combine Harvesters

As a result, performed experimental methods were defined by technical standards related to the research of agricultural machinery and its subsequent quality testing, namely, by the technical standards OST 70.8.1-81(1982) (OST 70.8.1-81:Testing of agricultural machinery. Grain-harvesting machines. Program and test methodology; Moscow, USSR, 1981).

Determination of a Grain Yield

The yield of grain was determined by the results of weighing of grain selected for sampling for quality of machine work, including all types of losses, but excluding the addition of debris.

Equipment used during the determination:

- A sample collector (a truck, sacks 4 x 3, 5 x 4, 2 x 1.5 m);
- A spring dynamometer of a general purpose as for GOST 13837-79(1982) (GOST 13837-79: General-purpose dynamometers. Specifications; Moscow, USSR, 1982) with an increment of 1 kg within a measurements range of 0–100 and 0–200 kg;
- A moisture measurer of grain;
- A stopwatch;
- An electronic scale;
- A seeds divisor;
- A weighing bottle;
- A drying container;
- A collapsible boards;
- A mobile laboratory;
- A putty knife;
- Standard sacks and bags, size 20 x 30 cm.

Preparation for Selecting and Sampling

For sampling at 60 meters from the edge of the field, an area of the sowing plot was determined. The length of the parcel matched the length of the field rut– 1,020 m, of a rectangular shape. The width of the area allowed making selection of the samples from all combine harvesters that are being compared.

Before selecting a sample, a combine was set up at the optimal mode in accordance with the requirements of the test. The details of the mode parameters were noted in the notebook.

With every repetition of the experiment by the combine, the following thrashing products were selected for analysis: grain from a bunker; straw; chaff.

While unloading the grain, a sample with an average weight of 2–2.5 kg was selected within 5–6 rounds and placed in a bag for the analysis. Samples of straw and chaff were collected and placed in weighing bottle for analysis of humidity.

Straw, chaff and grain, collected from the site, were weighed on a scale with accuracy of up to 1 kg, and were labelled.

Sample analysis

When analysing grain in accordance with GOST 13586.3-83 (2009) (GOST 13586.3-83: Grain. Acceptance rules and sampling methods; Moscow, Russia, 2006), there are two bulk samples. The analysis was carried out according to GOST 13586.3-83 (2009).

A sample was divided into the following fractions: main grain, grain in ears and rinds, crushed grain, and an adulterant.

All fractions were weighed. Their percentage content was calculated with accuracy of up to hundredth percent share by the formula (4):

$$\Delta q = \frac{q_i \cdot 100}{Q} \quad (4)$$

where Δq_i – is the main content of grain or other factions; q_i – mass fraction in weight, (g); Q – mass of weight, (g).

The content of crushed and broken grain is determined in the percentage of grain in the grain mass in a sample.

The weight of 1,000 grains was determined according to GOST 10842-89 (89) (GOST 10842-89: Cereals, pulses and oilseeds. Method for determination of 1,000 kernels or seeds weight; Moscow, Russia, 2009), and results were recorded in the notebook. For each indicator of the grain quality, an average value of three experimental recurrences in each mode was calculated and recorded in the notebook.

Determination of Quantity of Grain Lost

Losses in the process of harvesting were determined using the trays (Fig. 1) which were placed under a combine harvester. Mass, obtained after the passage of a harvester, was sorted out using a mobile laboratory (Fig. 2) and was weighed using the scales (Fig. 3). The results were noted in the notebook.



Figure 1. Determination of losses after the passage of a combine on an area of 1 m².



Figure 2. Air blowing plant mass using the mobile laboratory

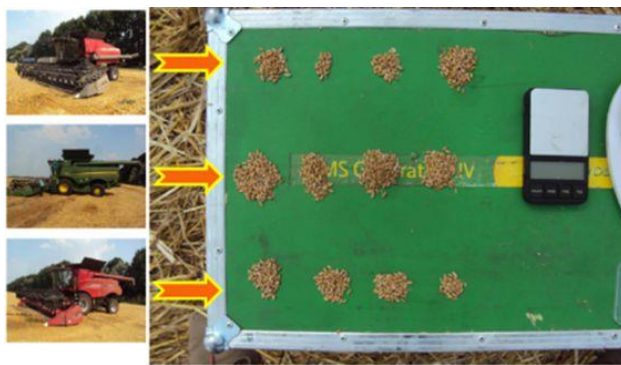


Figure 3. Determination of the quantity of grain losses after the passage of combine harvester.

The Determination of Broken Grain

After the sample weighing analysis for the detection of the micro damages, two samples of 100 pieces each were taken out of the whole grains. Each sample was placed in a paper bag, which had an indication of all the source data (the combine brand, date of collecting a research sample, number of an experiment and recurrence, etc.). Thus, every average sample had four selections for the test, totalling 400 grains.

Grains were inspected with a magnifying glass. Grains with a pushed out embryo, a defective embryo and an embryo with a damaged shell were separated.

Micro damages were calculated with an accuracy up to the tenth of a percent share. Losses through the gaps of a harvester were monitored thrice. At the end of the experiment, the grain was thrown on a shield, collected and weighed up to 1 g.

Modelling of Parameters and Operating Modes of the Combine Harvester

In the course of conducting an experimental research of the harvesters' performance on the field, a research was conducted related to work of combine harvesters on various modes of productivity, fuel consumption the indicator of the quality of collection. The research was based on the use of the programme 'Machine Unit', which was developed by the authors 'team under the scientific supervision of the professor I. Melnyk (Melnyk et al., 2015).

The first experiment was aimed at determining the dependency of the change in productivity and fuel consumption, when the working width of the reaper of combine harvesters is changed at the same speed.

In this situation a tool, which allows gathering data for processing, analysis and decision-making is crucial. Specifically, the attention should be given to a technique, which is used for obtaining information. The results of computational experiments should correspond to the results of chronographic observations in the production environment. The mathematical model and a computer program 'Machine Unit' was implemented in the Microsoft Excel namely to serve this purpose. The structure of the program is shown in the scientific study Melnyk et al. (2015).

The input data for calculation were technical characteristics of the combine harvester (a working width of a reaper, an operating speed, a bandwidth, an operating weight, an engine power, fuel consumption, a technical service system, the kinematic length of a machine, and the coefficient of machines reliability).

The outcomes of computational experiments were a study of performance, operating speed, coefficient of working moves and fuel consumption. Based on the theoretical calculation and the experimental research results of the above-mentioned indicators, it was determined that the difference in the results was within 2.5–4%.

RESULTS AND DISCUSSION

Purchase and maintenance of the agricultural producing equipment are the two most significant expenses in the agricultural production (Buckmaster, 2003). Therefore, it is important to choose the most optimal combine harvester. Under the real conditions, a combine should demonstrate the highest productivity with the lowest fuel consumption, minimal losses and grain damage (Vasylieva & Pugach, 2017).

A combine harvester is a machine that requires precise working settings and ensuring the optimum working speed (Beneš et al., 2015). Therefore, before the test, all of the machines had to be set respectively to the wheat type and working conditions.

The results of the productivity research according to chronographic data obtained in this research are listed in a Table 3. Performance results were received without taking into consideration the time needed for unloading a combine and a downtime related to a lack of transport, so only the time results related to actual work of a combine were taken into account.

Table 3. Performance Results According to Chronographic Data

A Combine Harvester + Reaper	Area, hectares	Actual working width of reapers, m	Speed of moving, km h ⁻¹	Productivity of main time, hectares per hour	Rotations of the engine, RPM	Quantity of fuel, L	Yield, kg ha ⁻¹	Humidity content, %
John Deere S680i +	17.15	9.14	5.2	5.6	2,050	319	7.07	12.8
John Deere 630f	9.29	9.14	5.0		2,050	191	7.36	10.7
Average value:	26.44		5.1			510	7.22	
CASE IH Axil Flow 8230 +	16.77	9.14	3.5	3.7	1,950	369	6.90	15
CASE IH 3020 Flex	9.41	9.14	4.0		1,950	235	7.20	12.5
Average value:	26.18		3.8			604	7.05	
CASE IH Axil Flow 8230 +	5.95	9.14	3.5	3.7	2,100	124	5.30	14.1
CASE IH 3020 Flex	4.72	9.14	4.0		2,100	104	7.20	10.8
Average value:	10.67		3.8			228	6.25	
Massey Ferguson MF T7 +	18.70	9.14	4.0	3.6	2,120	317	7.10	14.4
Massey Ferguson 8200	9.46	9.14	3.8		2,120	200	7.40	13.3
Average value:	28.16		3.9			517	7.25	
ClaasLexion 760 +	5.66	9.14	4.0	3.5	2,000	110	N/A	
ClaasCerio 930	4.68	9.14	4.5		2,000	92	N/A	
Average value:	10.34		4.3			202		
New Holland CR9.80 +	6.37	9.14	4.0	3.3	2,100	189.4	6.40	
New Holland 740CF30'DD	5.75	9.14	5.2		2,100	125	5.80	
Average value:	12.12		4.6			314.4	6.10	

A significant advantage of the John Deere S680i + John Deere 630f combine is obvious: the highest performance with the greatest speed, compared to other combine harvesters, combined with the lowest consumption of fuel and the best productivity. It should be noted that the price of the John Deere S680i is the highest of the samples presented.

It should be noted that the lowest productivity, 42% less than John Deere S680i + John Deere 630f, was demonstrated by the New Holland CR9.80 + New Holland 740CF30'DD combine. The yields harvested by the New Holland CR9.80 + New Holland 740CF30'DD were on average 15% lower than those of the John Deere S680i + John Deere 630f combine and its price is slightly lower than that of John Deere S680i.

The results of fuel consumption, according to chronographic data are listed in a Table 4.

Table 4. Fuel Consumption Results According to Chronographic Data

Combine Harvester + Reaper	Speed, km h ⁻¹	Cultivated area, hectares	Rotations of the engine, RPM	Quantity of used fuel, L	Fuel consumption, L ha ⁻¹	Productivity, kg h ⁻¹
Massey Ferguson MF T7 +	4.0	18.70	2,120	317	16.95	7.10
Massey Ferguson 8200	3.8	9.46	2,120	200	21.14	7.40
Average value:	3.9	28.16			19.05	7.25
Claas Lexion 760 +	4.0	5.66	2,000	110	19.43	N/A
Claas Cerio 930	4.5	4.68	2,000	92	19.66	N/A
Average value:	4.3	10.34			19.55	
John Deere S680i +	5.2	17.15	2,050	319	18.60	7.07
John Deere 630f	5.0	9.29	2,050	191	20.56	7.36
Average value:	5.1	26.44			19.58	7.22
CASE IH Axil Flow 8230 +	3.5	16.77	1,950	369	22.0	6.90
CASE IH 3020 Flex	4.0	9.41	1,950	235	24.97	7.20
Average value:	3.8	26.18		604	23.49	7.05
CASE IH Axil Flow 8230 +	3.5	5.95	2,100	124	20.84	5.30
CASE IH 3020 Flex	4.0	4.72	2,100	104	22.03	7.20
Average value:	3.80	10.67		228	21.44	6.25
New Holland CR9.80 +	4.0	6.37	2,100	189.4	29.73	6.40
New Holland 740CF30'DD	5.2	5.75	2,100	125	21.74	5.80
Average value:	4.6	12.12		314.4	25.74	6.10

According to the data obtained, the Massey Ferguson MF T7 + Massey Ferguson 8200 combine showed the best results. It consumed the least amount of fuel, covered the biggest area, and gathered maximum amount of the harvest. According to the research results (Table 2), the Massey Ferguson MF T7 + Massey Ferguson 8200 combine performance was 35% lower comparing to the John Deere S680i + John Deere 630f. That significantly affects the length of the harvest. This is the result of the low speed and the capacity of the combine. At the same time it has a fairly low price.

Wheat quality is characterized by attributes related to the genetic traits, physiological performance and its physical state. These factors can be negatively

impacted if the harvesting is delayed (Siddique & Wright, 2003). The use of combine harvesters in actual practice shows that nowadays harvesters do not deliver a high quality threshing. This is confirmed by the existing losses and high level of grain damage (Rozwadowski et al., 2018).

The results of detecting the adulterant content in a grain tank of a combine harvester are listed in a Table 5.

The speed of the machine, the density of the sowing and the cutting height determine the feed rate and affect the quality. The reel height and the rotational speed must allow the achievement of the efficient and smooth pushing of the crop into the header without causing shatter losses from affecting ears and stalks (Baerdemaeker & Saeys, 2013), however there are modern methods of the quality assurance collection today (Lenaerts et al., 2012). For example, Baerdemaeker & Saeys (2013) developed a multispectral sensor to measure the purity and quality of the harvested grain, or Lenaerts et al. (2012), who investigated the potential of LiDaR sensors for measuring the quality of the ejected straw. However, the sensors do not ensure high quality of measuring. Therefore, the analysis of the selected samples was conducted.

An indicator for assessing the quality of the threshing mechanisms of a combine harvester is determined by the amount of damaged seeds in the bunker, the quantity of grated grain and adulterants. The CASE IH Axil Flow 8230 + CASE IH 3020 Flex combine has the least amount of wastes in the bunker and the smallest amount of damaged seeds, with the largest grain adulterant volume and the highest grain yield. At the same time it has the lowest price. Analysing the quality of the threshing mill it was found that the Claas Lexion 760 + Claas Cerio 930 has a grain weight of 2.27 g, while the CASE IH Axil Flow 8230 + CASE IH 3020 Flex – 0.06 g; the grain adulterant volumes are 1.9 times larger, with 10% less underdeveloped seeds compared to the CASE IH Axil Flow 8230 + CASE IH 3020 Flex combine. All other indicators differ just slightly.

For loss results obtained after a passage of a combine, see a Table 6.

Due to a lack of accurate data about the yield condition on each of the individual investigated area, for the purposes of calculations we used an average value retrieved from the combine harvester on-board computers, thus, the average yield value is considered to be: 6,770 kg ha⁻¹. The smallest losses are 0.34% or 23 kg ha⁻¹ produced by the New Holland CR9.80 + New Holland 740CF30'DD combine, the largest are 1.37% or 93 kg ha⁻¹ – by the John Deere S680i + John Deere 630f combine.

Nowadays, a very small amount of organic fertilizers is applied in to the soil on the territory of Ukraine, which is considered to be disastrous (Melnyk et al., 2017). According to the research conducted by the Northeast Institute of Agriculture (Kornus, 2013), it is proved that even if all organic residues from the animal husbandry, including individual farming households, would be applied, the application standard will be only 0.6 t ha⁻¹. This digit is too low. Such a number of organic fertilizers can neither saturate the soil with necessary nutrients nor promote its structural transformation or impregnate it with biologically active organisms. As concluded in a study published by Stupak (2016), as well as in a study executed by Baumann et al. (2011), even though the soil erosion degradation has become a problem in a Soviet era already, it still remains a problem nowadays. Therefore, it is important to investigate the issue of the soil quality as well as the issue of leaving the plant residues in the field (NAAS, 2015).

Table 5. Results of Adulterants Detection in a Grain Tank of a Combine Harvester

Combine Harvester + Reaper	The sample record number	The weight of the sample, g	Mineral adulterant, g	Organic adulterant, g	Damaged seeds, g	Underdeveloped seeds, g	Waste adulterant, g	Grain admixture, g
CASE IH Axil Flow 8230 +	1.1	100	0.02	0.42	0.20	1.66	0.44	1.86
CASE IH 3020 Flex	1.2	100	0.02	0.36	0.28	0.80	0.38	1.08
	1.3	100	0.08	0.42	0.30	0.92	0.50	1.22
Average value:			0.12	1.20	0.26	3.38	0.44	1.39
Massey Ferguson MF T7 +	2.1	100	0.02	0.26	0.28	0.20	0.28	0.48
Massey Ferguson 8200	2.2	100	0.02	1.08	0.34	0.30	1.10	0.64
	2.3	100	0.02	0.38	0.26	0.90	0.40	1.16
Average value:			0.06	1.72	0.29	1.40	0.59	0.76
John Deere S680i +	3.1	100	0.02	0.44	0.54	0.52	0.46	1.06
John Deere 630f	3.2	100	0.02	0.34	0.24	0.86	0.36	1.10
	3.3	100	0.02	1.26	0.42	0.64	1.28	1.06
Average value:			0.06	2.04	0.40	2.02	0.70	1.07
New Holland CR9.80 +	4.1	100	0.08	0.02	1.28	0.56	0.10	1.84
New Holland 740CF30'DD	4.2	100	0.06	0.02	0.96	0.80	0.08	1.76
Average value:			0.14	0.04	1.12	1.36	0.09	1.80
Claas Lexion 760 +	5.1	100	0.08	0.04	1.74	0.94	0.12	2.68
Claas Cerio 930	5.2	100	0.02	0.04	2.80	2.80	0.06	5.60
Average value:			0.10	0.08	2.27	3.74	0.09	4.14
CASE IH Axil Flow 8230 +	6.1	100	0.04	0.04	0.10	0.80	0.08	0.90
CASE IH 3020 Flex	6.2	100	0.02	0.04	0.02	3.36	0.06	3.38
Average value:			0.06	0.08	0.06	4.16	0.07	2.14

Table 6. Loss Results Obtained After a Passage of a Combine Harvester

Combine Harvester + Reaper	The area of the land, hectares	The number of studied areas	Productivity of a sample plot, kg ha ⁻¹	The weight of the grains (on an area of 1 m ²)	Loss, kg ha ⁻¹	Loss, %
CASE IH Axil Flow 8230 + CASE IH 3020 Flex	17.3	1.1	6,770	6.00	60.00	0.89
	17.3	1.2	6,770	3.30	33.00	0.49
	9.3	1.3	6,770	4.30	43.00	0.64
	The average by the areas of:			4.53	45.33	0.67
Ferguson MF T7 + Massey Ferguson 8200	17.3	2.1	6,770	3.10	31.00	0.46
	17.3	2.2	6,770	5.30	53.00	0.78
	9.3	2.3	6,770	6.20	62.00	0.92
	The average by the areas of:			4.87	48.67	0.72
John Deere S680i + John Deere 630f	17.3	3.1	6,770	10.20	102.00	1.51
	17.3	3.2	6,770	8.20	82.00	1.21
	9.3	3.3	6,770	9.50	95.00	1.40
	The average by the areas of:			9.30	93.00	1.37
New Holland CR9.80 + New Holland 740CF30'DD	6.0	4.1	6,770	0.80	16.00	0.24
	5.0	4.2	6,770	1.50	30.00	0.44
	The average by the areas of:			1.15	23.00	0.34
Claas Lexion 760 + Claas Cerio 930	6.0	5.1	6,770	0.80	16.00	0.24
	5.0	5.2	6,770	6.40	128.00	1.89
	The average by the areas of:			3.60	72.00	1.06
CASE IH Axil Flow 8230 + CASE IH 3020 Flex	6.0	6.1	6,770	0.40	8.00	0.12
	5.0	6.2	6,770	3.70	74.00	1.09
	The average by the areas of:			2.05	41.00	0.61

Accordingly, the plant residues must be grinded and evenly distributed all over the surface of the soil. It was found that a rotary combine has a special technological process of threshing which allows it to smash the straw more intensely compared to a drum combine (Kumhála et al., 2005). This improves quality of the next cultivation, eliminates problems related to clogging of working parts of the combine, as well as provides an even saturation of the soil with organic fertilizers, which as a result allows to create the optimal conditions for the growth and development of the future crops. In addition, with the No-Till approach the even distribution of plant residues provides an elongation of the moisture in the soil (João et al., 2016). Therefore, the performance of a straw spreader was examined within the study of qualitative indicators of combine harvesters as well.

Grinding and Distribution of Crop Residues

In the course of studies of the work of combine harvesters, we measured the quality of the shredder. The results are presented in the Table 7.

Table 7. Grinding and Distribution of Crop Residues

Combine harvesters	Quality of the shredder combine harvester	
	Distribution of crop residues over the entire width of the reaper	Ejection of plant mass onto standing plants left along the edges of the passage
New Holland CR9.80 + New Holland 740CF-30'DD	+	+
ClaasLexion 760 + ClaasCerio 930	-	+
Case IH Axil Flow 8230 + Case IH 3020 Flex	+	+
Massey Ferguson MF T7 + Massey Ferguson 8200	-	±
John Deere S680i + John Deere 630f	+	+

The results of the study of the combine harvester New Holland CR9.80 with a reaper New Holland 740CF-30' DD demonstrated a good quality and equal distribution of crop residues over the entire width of the reaper. It was noted that no plant masses were thrown on the standing plants, left on the edges of the passage.

According to the results of the study of the combine harvester Claas Lexion 760 with a reaper Claas Cerio 930, an uneven and incomplete distribution of crop residues on the working width of the reaper was noted. It was noted that no plant masses were thrown on the standing plants, left on the edges of the passage.

The results of the study of a combine harvester Case IH Axil Flow 8230 with a reaper Case IH 3020 Flex demonstrated a high quality and an equal distribution of crop residues along the entire width of the reaper. It was noted that no plant masses were thrown on the standing plants, left on the edges of the passage.

The results of the study of a combine harvester Case IH Axil Flow 8230 with a reaper Case IH 3020 Flex demonstrated a high-quality and an equal distribution of crop residues along the entire width of the reaper. It was noted that no plant masses were thrown on the standing plants, left on the edges of the passage.

On the Quality indicators of the grinder of a combine harvester is affected by:

- threshing system;
- design features of the structure of the chopper;
- quality settings of the chopper.

Modelling Operating Modes of the Combine Harvester with the Definition of its Operational and Qualitative Performance Indicators

Ismail et al. (2009) noted that the cost of harvesting makes up about 35% of the total crop production costs, and there is a need for development of reliable methods for selecting optimal machines for harvesting in specific natural areas. For the analysis in the chapter "Results and Discussion" only John Deere S680 + John Deere 630f was chosen, because we managed to conduct more in-depth investigations only with this machine. Based on the results of the calculation, certain dependencies were noted:

- Productivity of a combine – on the width of the working grip (Fig. 4),
- Fuel consumption by a combine harvester – on the width of the working grip (Fig. 5),

- Productivity of a combine harvester – on the actual operating speed (Fig. 6),
- Fuel consumption – on the actual operating speed of a combine harvester (Fig. 7),
- The number of damaged seeds caused by the moving combine with an actual operating speed (Fig. 8),
- The number of losses caused by the combine moving with an actual operating speed (Fig. 9).

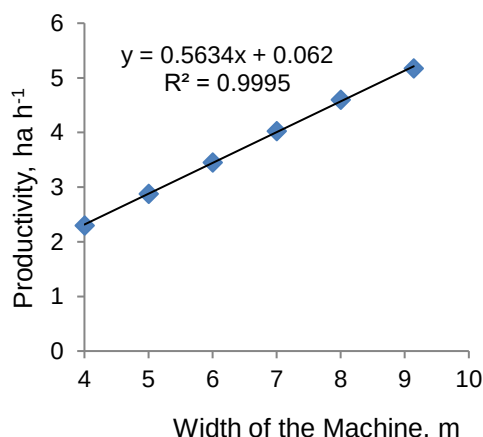


Figure 4. Dependence between the Work Productivity and the Working Width of the Grip of John Deere S680i with a John Deere 630f Reaper.

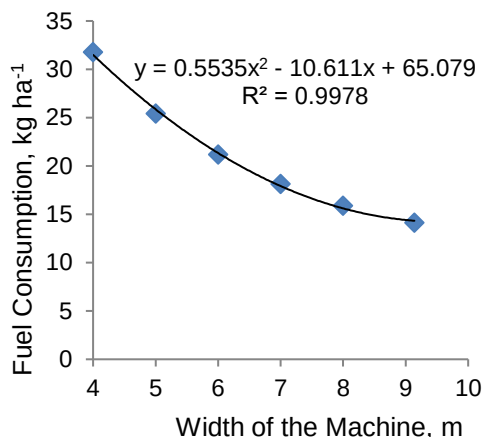


Figure 5. Dependence between the Work Fuel Consumption and the Working Width of the Grip of John Deere S680i with a John Deere 630f Reaper.

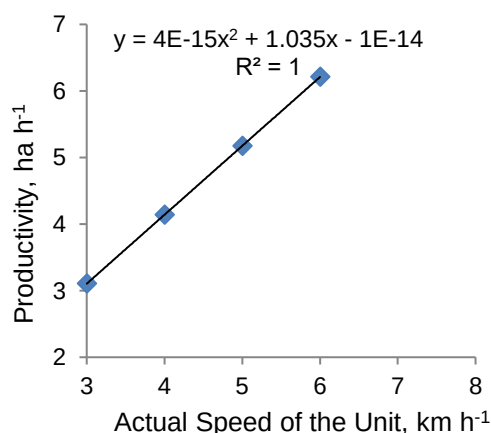


Figure 6. Dependence between Productivity and the Actual Operating Speed of the John Deere S680i Harvester with the John Deere 630f Reaper.

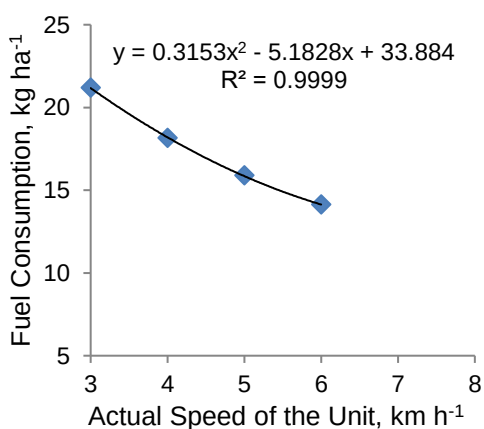


Figure 7. Dependence between Fuel Consumption and the Actual Operating Speed of the John Deere S680i Harvester with the John Deere 630f Reaper.

According to the analysis of the chart for work productivity dependence on the working width of the grip, it can be concluded that with the increase in the width of the grip, productivity performance of the machine increases as well.

To save the time and to guarantee the high productivity and quality of harvesting, it is desirable to predict their operating parameters using a mathematical model. The working parameters of the combine were analysed on the basis of the characteristics of the actual fieldwork and the mathematical model of losses. The reduction of the threshed grain quality was established in accordance with the classical empirical equations of rotary threshing.

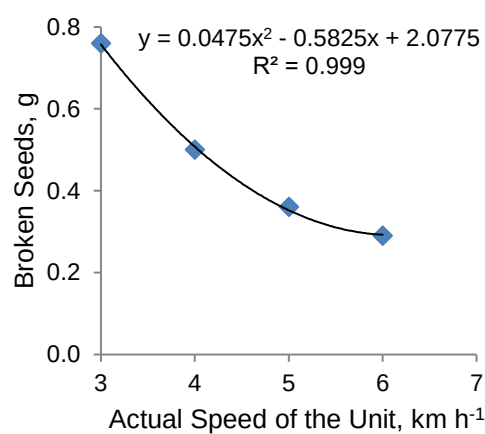


Figure 8. Dependence between the Quantity of Damaged Seeds and the Actual Operating

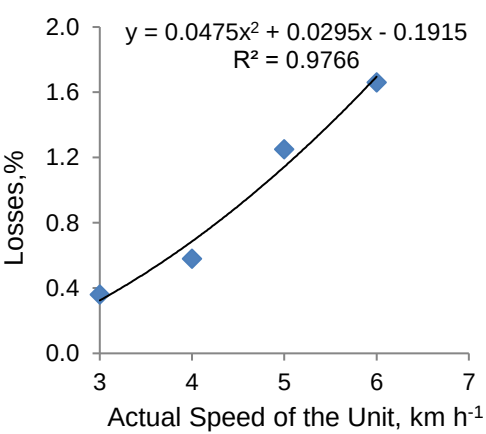


Figure 9. Dependence between the Volume of Losses and the Actual Operating Speed of the John Deere S680i Harvester with the John Deere 630f Reaper.

Upon analysing quantitative indicators, it was found that when using a constructive width of the grip of 9.1 m and a speed of 5 km h⁻¹, the productivity of a combine is 5.2 ha h⁻¹. However, when using a 4 m width of the grip, the productivity of combine harvesters drops by 56% to 2.3 hectares per hour. The change of the reaper width also significantly affects the fuel consumption. As such, using the JohnDeereS680i combine harvester with a JohnDeere630f reaper at an optimal 9 m reaper width results in the fuel consumption at the level of 14.1 kg ha⁻¹. In case the width of the reaper is reduced to 4 m, it leads to the overconsumption of fuel, which is 31.8 kg ha⁻¹, that is 57% higher than the norm.

The second experiment was focused on determining the dependencies between the productivity and the fuel consumption change during the change of the working speed of a combine with the constant width of the reapers.

Our results show that productivity of a combine harvester increases along with its working speed. This reflects a linear dependence between the parameters. As such, an increase in speed from 3 to 6 km h⁻¹ resulted in an increase in productivity by 3.1 hectares per hour, meaning an increase in productivity by 49%.

Based on our results, it was found that a change in working speed of a harvester significantly affects its fuel consumption, particularly an increase in speed results in a

decrease in cost per hectare. This happens due to a more effective method of the harvester loading. So, when the actual speed of the unit increases from 3 to 6 km h⁻¹, the fuel consumption decreases down to 7.07 kg ha⁻¹ or by 33%.

According to the analysis conducted on the quality of work performed by a combine with different speed levels, it was determined that an increase in the actual speed of the unit from 3 to 6 km h⁻¹ leads to a decrease in the number of damaged seeds by 0.47 g, meaning decrease of damaged seeds by 62%.

During the analysis of the quality of work performed by a combine harvester, it was found that an increase in the speed of the machine from 3 to 6 km h⁻¹ led to an increase in a number of seeds lost by 1.3%.

Thus, to ensure the effective operations of enterprises, the management has to choose an effective combine harvester for their business purposes in accordance with their main requirements. In addition, the enterprise needs to be provided with an additional tool for analysing technical and economic indicators of the equipment operation. This will optimize technical and economic indicators of the enterprise as well as the quality of the technological operations.

CONCLUSIONS

For the purposes of ensuring financial efficiency of the profitable farming system, it is necessary to select the fleet of machines that meets the requirements of the enterprise activities. For this sake, it is meaningful to conduct the analysis of the equipment with the use of the 'Machine Unit' software in order to determine optimal equipment for the existing operational conditions. At the second stage, technical and economic indicators as well as indicators of quality of the selected units under an actual production conditions must be determined.

During the research conducted in the Sumy region (Ukraine) that took place from July to August 2016 the following outcomes were obtained:

- i) The volume of grain loss after the passage of a combine harvester on a 1 m² area was the least in case of the John Deere S680i + John Deere 630f use. It had the highest productivity and speed, and the lowest fuel consumption, compared to other combine harvesters;
- ii) The Massey Ferguson MF T7 + Massey Ferguson 8200 had the lowest fuel consumption according to the chronographic data;
- iii) CASE IH Axil Flow 8230 + IH CASE 3020 Flex had the lowest amount of adulterants in the grain tank and the smallest number of damaged seeds;
- iv) New Holland CR 9.80 + New Holland 740CF30 ' DD had the lowest grain loss after the harvester passage;
- v) New Holland CR9.80 + New Holland 740CF-30'DD, Case IH Axil Flow 8230 + Case IH 3020 Flex, John Deere S680i + John Deere 630f provided the even distribution of plant residues on the field surface and the absence of throwing the plant mass on the standing plants at the edges of the passage.

The John Deere S680i combine, used by the enterprise, had the least volume of grain loss after its passage on 1 m² area, however, it cedes to the other equipment in terms of the fuel consumption, presence of adulterants in the grain tank and the volume of grain loss caused by the passage of a harvester.

Basing on the results of the experiment, it was determined that the difference between the results of the theoretical estimation and the experimental research of technical and operational indicators is within 2.5–4%. Therefore, the estimation results match the practical ones.

The economic calculations of the process will help farmers to choose the optimal equipment for their actual needs and will assist in making management decisions.

ACKNOWLEDGEMENTS. The sincere gratitude should be expressed to the Professor Ivan Melnyk for the help and counselling. Furthermore, this research was supported by the Internal Grant Agency of the Faculty of Tropical AgriSciences [20185010]. Moreover, the first author would like to acknowledge International Credit Mobility: Cooperation between Czech Republic and Ukraine (KA107-034537). Finally, we would like to thank Czech Development Agency, which allowed this cooperation to start.

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