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MOUNTAIN FARMING IN AUSTRIA

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ABSTRACT

For decades, the mountain regions in Austria have been of regional, social, environmental and economic relevance. The European Union has ranked them among the most disadvantaged areas for agriculture. Nevertheless, in mountainous regions, 'disadvantaged' is not synonymous with marginal. On the contrary, agriculture is central to mountainous regions. In Austria the mountain area makes up 70% of national territory and 50% of the utilised agricultural area. Mountain agriculture is largely family farming. It is as diverse as the myriad mountain landscapes available, but at the same time, there are also commonalities to farming in lowland regions. With this in mind the research questions are: (i) what are the characteristics of family farming in mountain regions and (ii) how do they differ from agriculture in lowland regions? Following some definitive remarks on family farming and the classification of mountain regions, this paper outlines the situation in Austria using select key indicators from the agri-structural data of the Integrated Administration and Control System and income data, 2014. Next, certain characteristics are highlighted using select data from the survey 'Life and working conditions of female farmers in Austria' conducted in the year 2016. When analysing the agriculture in mountain and lowland regions we employed descriptive statistics. Based on the findings, we try to give some suggestions on the need for further research and on future perspectives for both mountain and lowland agriculture.

Keywords: *Agriculture, lowland regions, mountain regions, Austria.*

INTRODUCTION

Austria is a mountainous country. Mountain areas and mountain farming are of great national importance. Mountain farming in Austria has a good image and a clear backing in the population and in politics (at least at regional and rural level) (Hovorka, 2011). The mountain area makes up 70% of the national territory and 50% of the utilised agricultural area (Hovorka, 2017). Mountain farming is confronted with great natural constraints (climate, topography, etc.). In Austria farms are classified according to their constraints as groups of the register for mountain farms (Hovorka, 2017; Tamme et al., 2002). Austria also has one of the highest proportions of mountain areas within the European Union. Mountain farming has a European dimension and is, therefore, of European interest as a living, economic, recreational and biodiversity area (see also Alpine Convention, Carpathian Convention, etc.). Furthermore, this issue has also been addressed at the Rio Earth Summit 1992: focusing on the proper management of mountain resources and socio-economic development of the people deserves immediate action (United Nations, 1992, Agenda 21, Chapter 13: "Managing Fragile Ecosystems – Sustainable Mountain Development") (Hovorka, 2004). In fact, mountain farming has been looked at from many different global perspectives: (i) analysing the existence of positive externalities (Cobbing and Slee, 1993; Dax and Hovorka, 2012), (ii) assessing the impacts of climate change on biodiversity and agriculture in mountain regions (Diaz, Bradley, Ning, 2014; Huber et al., 2005), (iii) as the testing ground for different institutional solutions for property and resource management (Gurung and Banskota, n.d.; Pandit and Thapa, 2004; Turkelboom et al., 2004), (iv) evaluating the importance of incentives and policy measures (European Network for Rural Development Contact Point, 2009; Hovorka, 2014, 2017) and (v) hypothesising on the future of farming in the Alps (Flury et al., 2004; Flury et al. 2013; Frey, 2006; Schermer and Kirchengast, 2007). While official agricultural statistics give a very good image of the farm and production characteristics of mountain and lowland farming we know little about the living and working situations of female farmers in mountain and lowland regions. However, the present state-of-the-art analysis, which deals with the current situation, largely neglects the long-term attractive forces and interregional shifts in these forces. Against this background we shall provide some statistical evidence on the nature of farming in mountain and lowland regions. After clarifying the definitions (family farming, mountain and lowland regions) and methods applied we analyse select structural data from the Integrated Administration and Control System (IACS) and the farm income data (BMLFUW, 2015) of the year 2014 as well as data from the survey of female farmers in 2016 in order to answer the following research questions: (i) what is the significance of the mountain farming? (ii) What are the characteristics of mountain farming? (iii) How does this differ from farming in lowland regions? This can give us further insights into the continuing development of farming as a whole and emerging research needs.

MATERIAL AND METHODS

The analysis presented in this paper refers to the areas designated as mountainous less favoured areas according to Article 18 of Regulation (EC) No 1257/99 (European Communities, 1999). In Austria we distinguish between farms with and without constraints. The former are mountain farms and the latter farms in lowland regions. The classification is done according to the groups of the register for mountain farms (Tamme et al., 2002). To obtain a picture of farming in Austria the structural features of IACS data (BMLFUW, 2014), income data (BMLFUW, 2015) and select data from the survey of female farmers throughout Austria were analysed. The agricultural population/sample data was obtained from the Austrian IACS farm data set 2014 where farm operators had (i) the legal status of natural person or group person (two families or marriage relationship without community pastures and cooperatives), and an email address. These farms are family farms (Böhme, 2013). The agricultural population overall was 116,615 and the study population for the survey was 36,573 farms. The survey was conducted as an online-questionnaire. 2,200 questionnaires from female farmers in the rural areas and demi-town could be used after a quality test (consistency check) for the analysis of the research questions. The response rate was 7% of the net sample. The data thus collected was analysed by using Statistical Package for Social Sciences (SPSS) for reporting results and drawing conclusions.

RESULTS AND DISCUSSION

Density and farm characteristics

As shown in Table 1 there is no difference between the farming in mountain and lowlands regions regarding the legal forms marriage relationship and group holding. Significant differences were found as follows: (i) more people live in lowland regions, (ii) more farms are full-time farms, (iii) there are more farms managed by farmers in mountain regions, (iv) the proportion of ecological farms is higher, (v) farms are smaller and have less livestock units (LSU) per hectare utilised agricultural area (UAA), and (vi) the income from agriculture and forestry is lower.

Table 1. Select density and farm characteristics of mountain and lowland regions.

Density and farm characteristics			Lowland region	Mountain region
Population density in population per sqkm**			84	48
Full-time farms**			47%	53%
Legal form	Natural person*	male	49%	53%
		female	28%	26%
	Marriage relationship		17%	17%
	Group holding		6%	4%
Ecological farms**			10%	24%
Ø hectare of UAA per farm**			24	17
Ø number LSU per 100 hectare UAA**			106	62
Ø number of RGVE per 100 hectare UAA**			103	37
Income from agriculture and forestry in euro per unpaid labour**			21,934	16,531

Source: IACS data 2014; BMLFUW 2015, ** statistically highly significant ($p < 0.01$), * statistically significant ($p < 0.5$).

Activities characteristics

The production activities can be characterised in Table 3 as follows: Milk production, suckler cows and forestry are more important in mountainous than in lowland regions. The production of cash crops, pig farming, vegetables, fruit and vines have a significantly higher share as the most important area of operation in lowland regions. This is also confirmed by the structural data in the Green Report (BMLFUW, 2015) and the Farm Structure Survey (Statistik Austria, 2013).

Table 2. Select activities characteristics by female farmers of mountain and lowland regions.

Activities characteristics		Lowland region	Mountain region
Most important area of operation**	Milk production	16%	40%
	Production of cash crops	22%	2%
	DM, HoF, Heuriger etc.	9%	12%
	Suckler cows	5%	13%
	Other animals	10%	11%
	Pig farming	9%	1%
	Vegetables, fruit and vines	15%	1%
	Forestry	4%	11%
	Cattle fattening	4%	6%
	Other	7%	4%
Additional activities		52%	48%
of which	Direct marketing**	30%	22%
	Holiday on farms**	24%	10%
No off farm job of the female farmer**		60%	66%
No off farm job of the partner**		49%	43%

Source: Survey of female farmers 2016 (lowland regions n = 1,140 and mountain regions n = 1,060), Direct marketing (DM), Holiday on Farms (HoF), ** statistically highly significant ($p < 0.01$).

Furthermore, additional activities are more present in lowland regions. In detail, direct marketing and holidays on farms are significantly more attractive for female farmers in the lowland regions than in mountain ones. 33% of the female farmers work off the farm in lowland regions and 23% in mountain regions. The proportion of the partner having an off farm job is definitely higher. In lowland regions 51% of the partners work off-farm and in the mountain regions 57% of them do

Family characteristics

The family characteristics in Table 3 show that there is no significant difference between the farming in lowland and mountain regions when looking at the average number of people and children on the farm, the marital status and the transfer of the farm regulated. Definitely, compared to the lowland regions (i) there are more farms with adult children in mountain regions, and (ii) there are also fewer farms that have no children or children requiring care.

Table 3. Select family characteristics by female farmers of mountain and lowland regions.

Family characteristics		Lowland region	Mountain region
Ø number of people on the farm		4.9	5.1
Ø number of children per female farmer		2.5	2.6
of which farms with	adult children*	47%	53%
	children requiring care*	45%	43%
	childless*	8%	5%
Marital status	marital cohabitation	83%	85%
	extra-marital cohabitation	9%	7%
	single	8%	8%
Transfer of the farm regulated		29%	31%

Source: Survey of female farmers 2016 (lowland regions n = 1,140 and mountain regions n = 1,060), * statistically significant ($p < 0.5$).

Assessment characteristics

Looking at the assessment characteristics in Table 4 shows that more female farmers in mountain regions consider themselves as profession female farmers than their counterparts in lowland regions do. Also in the mountain regions more respondents would become a female farmer again. No significant difference is shown for the assessment of the female famers' image towards other professionals and the evaluation of the current situation of agriculture and forestry as well as the future of the farm.

Table 4. Select assessment characteristics of female farmers of mountain and lowland regions.

Assessment characteristics		Lowland region	Mountain region
See themselves as a professional 'female farmer'**		67%	77%
Would become a female farmer again**		69%	78%
Option of Image of female farmers towards other professionals	somewhat higher	5%	4%
	equal	30%	29%
	somewhat lower	67%	67%
Assessment of the current economic situation of agriculture and forestry	very good	0%[#]	1%[##]
	good	17%	16%
	poor	57%	58%
	very poor	26%	25%
Assessment of the future development of the farm	very good	3%	3%
	good	51%	53%
	poor	37%	37%
	very poor	9%	7%

Source: Survey of female farmers 2016 (lowland regions n = 1,140 and mountain regions n = 1,060), ** statistically highly significant ($p < 0.01$). [#]Lowland regions (0.2%), [##]mountain regions (0.5%)

CONCLUSIONS

A mixed picture of mountain and lowland agriculture emerges from the analysis. Agriculture and its related activities remain key components of the mountain rural economy and of land use in mountain areas. Mountain farming has an excellent record for using the land for milk production and forestry. The farming is less intensive (number of ecological farms, LSU). The farms are smaller. The income from agriculture and forestry per unpaid labour is lower. More female farmers consider themselves as professional female farmers and would become a female farmer again. These may also be one of the reasons more partners work off-farms and more female famers remain at home. Mountain farming does however face specific permanent handicaps which limit its capacity to adapt and rationalise its economic activity. However, natural constraints have not resulted in a state of inflexibility or disillusionment regarding the future of mountain farms. Mountain famers evaluate the future of their farms similar to lowland farmers. Given this optimism there is clearly a potential for development which could be innovative with the right change in framework conditions. Currently, in the discussion about the future of mountain farming there are two pertinent concepts, namely agricultural industrialisation and the development path of a European agricultural model of multifunctional agriculture. In view of this, there is a need for the following further research: (i) gleaning more information about future developments by looking at structural developments in the past, (ii) exploring the different approaches to sustain the agricultural production potential, (iii) the future of mountain farming for enhancing food security, alleviating poverty, and contributing to environmental and welfare issues, (iv) approaches and partners for an inclusive food value chain for the produce from mountain farms, and (v) greater clarity is needed on the extent of female farmers' involvement in mountain farming as well as on the economic and cultural factors determining the specific forms that their involvement takes.

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BIOFERTILIZER BASED ON SILICATE SOLUBILIZING BACTERIA IMPROVES PHOTOSYNTHETIC FUNCTION OF *BRASSICA JUNCEA*

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ABSTRACT

Usage of biofertilizers is one of the important components of integrated nutrient management, as they are renewable source of plant nutrients, ecologically safe compared to chemical fertilizers and cost effective. Silicate solubilizing bacteria (SSB) can play an efficient role not only in solubilizing insoluble forms of silicates but also potassium and phosphates, hence increasing soil fertility and thereby enhancing plant productivity. The aim of this study was to investigate the influence of SSB-enriched biofertilizer on the structural and functional parameters of photosynthetic apparatus of *Brassica juncea* (L.) Czern. The pure culture of SSB was isolated from the clay substrate, cultivated on Zak–Alexandrov medium and identified as *Bacillus sp.* To obtain the biofertilizer, SSB culture ($0.6 \ast 10^{8}$ cfu mL^{-1}) was mixed with steriled peat (1:1, v/w) and dried at 35–40°C. Plants were grown from seeds during two months in the pots with adding SSB-enriched biofertilizer to the mixture of clay and soil (1:10, w/w). The clay substrate plus peat without SSB was used as a control. It was found that addition of SSB-enriched biofertilizer to clay substrate significantly increased the content of total nitrogen, phosphorus and potassium in the leaves of *B. juncea*. The thickness of mesophyllic layer and the number of mesophyll cells were increased on average by 24 %. It was correlated with a sharp increase of photosynthetic pigment content and CO_2 uptake (1.5–2.0 times). We can conclude that SSB-enriched biofertilizer improves the photosynthetic function of *B. juncea*.

Keywords: *biofertilizer, silicate solubilizing bacteria, Indian mustard, photosynthesis.*

INTRODUCTION

At present, the production of a sufficient number of “ecologically clean” food products is one of the global challenges facing humanity (Dubey and NidhiShukla, 2014). To satisfy the demand in food and increase the productivity of crops some non-environmentally friendly technologies are commonly been used such as pesticides and synthetic fertilizers, which can cause health problems. An alternative to this is organic agriculture which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. This is accomplished by using new safe technologies, such as the usage of biofertilizers (Aggani, 2013; Malusà *et al*., 2016). Biofertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients, ecologically safety instead of chemical fertilizers (Raja, 2013). Different kinds of promoting plant growth microorganisms (PGPM) can be used for the production of biofertilizers. Silicate solubilizing bacteria (SSB) can play an efficient role not only in solubilizing insoluble forms of silicates but also potassium and phosphates, hence increasing soil fertility and enhancing plant productivity (Han and Lee, 2005). Phosphate and potassium are major essential macronutrients for plant growth and development and soluble P and K fertilizers are commonly applied to replace removed minerals and to optimize yield. This is especially important for the reclamation of infertile or disturbed soils that are not suitable for sustainable agriculture. Numerous studies showed the effect of SSB on the nutrient uptake from the soil, their positive influence on photosynthesis and the growth of some crops (Han and Lee, 2005; Han *et al*., 2006; Tripti *et al*., 2017). However, the structural characteristics of the photosynthetic apparatus, that provide plant productivity, practically were not investigated. The aim of this study was to investigate the influence of SSB-enriched biofertilizer (SSB-EB) on the structural and functional parameters of photosynthetic apparatus of *Brassica juncea* (L.) Czern.

MATERIAL AND METHODS

The pure culture of SSB (*Bacillus sp.*) was isolated from the clay substrate taken from the mountain territory of Middle Urals (Russia), and cultivated on Zak–Alexandrov medium in following composition (g L^{-1}): $MgSO_4 {*} 7H_2O$ – 0.15 g, NaCl – 0.15 g, $MnSO_4$ – 0.05 g, $FeSO_4 {*} 7H_2O$ – 0.05 г, Na_2SiO_3 – 2.0 g, $CaCO_3$ – 2.0 g, $Ca_3(PO_4)_2$ – 1.5 g, KNO_3 – 1.0 g, sucrose – 20 g, agar – 15 g. The isolate was identified by morphological and biochemical test followed by Bergey's manual (Bergey *et al*., 1984). To obtain the biofertilizer, SSB culture ($0.6 {*} 10^8$ cfu mL^{-1}) was mixed with purified sterile peat (1:1, v/w) and dried at 35–40°C.

Brassica juncea was grown from seeds in the pots with adding SSB-EB to the clay soil (1:10, w/w). The clay substrate plus peat without SSB were used as a control. After two months, mature leaves were used for the physiological experiments. The content of total nitrogen (N) and phosphorus (P) in *B. juncea* leaves was determined with a PD-303 UV spectrophotometer (Apel, Japan) after wet digestion with the mixture of acids H_2SO_4 and $HC1O_4$ as described earlier (Borisova *et al*., 2014). The content of potassium (K) was determined using the atomic absorption spectrometry (AAS Vario 6, "Analytik Jena", Germany) after wet digestion with 70 % HNO_3 (analytical grade). Leaf mesostructure parameters were determined in 20 replicates according to Ivanova and P'yankov (2002). Transverse slices of leaves were obtained using a freezing microtome MZ-2 (Russia). The thickness of leaf was measured by inspecting the leaf cross sections with a Meiji Techno light microscope (Japan) and an eyepiece micrometer AM 9-2 (GSZ, Russia). A computer-assisted protocol based on Simagus Mesoplant software (OOO Siamz, Russia) was used to determine the quantitative parameters of mesophyll cells. Leaves were preliminary macerated with 5% chromic acid dissolved in 1 N HCl. The pigment content was spectrophotometrically determined in 96 % ethanol according to Lichtenthaler (1987). The photosynthetic rate was measured as CO_2 uptake with infrared gas analyzer (LI-COR, USA). All data were analyzed statistically by analysis of variance using Statsoft Statistica 7.0. Data are presented as mean (n = 3–20) ± standard error (SE). As the most variables did not fit a normal distribution, the differences between mean values were calculated using nonparametric Mann–Whitney U-test at $p < 0.05$.

RESULTS AND DISCUSSION

Brassica juncea (L.) Czern., commonly known as Indian mustard, is an important agricultural crop in different parts of the world. It is used for oil production and as a condiment, has medicinal properties. Plants grow fast and produce large amount of above-ground biomass. In addition, they can accumulate large amount of heavy metals and accordingly been used for phytoremediation of disturbed ecosystems (Shekhawat *et al*., 2012; Singh and Fulekar, 2012). It was found that addition of SSB-EB to clay substrate significantly increased the content of studied biogenic elements in the leaves of *B. juncea* (Table 1). The amount of total nitrogen, phosphorus and potassium increased by 18, 20 and 25 %, respectively, as compared to the control. Such nutrients are required in large quantities and involved in almost all methabolic reactions in crop plants. It is known that nitrogen and phosphorus play an important role in the plant anabolic and catabolic processes.

Table 1. The content of total nitrogen, phosphorus and potassium in *B. juncea*, growing two months without and with adding SSB-enriched biofertilizer.

Variant	Total nitrogen, % DW	Total phosphorus, % DW	Total potassium, % DW
Control	3.20 ± 0.03	0.15 ± 0.01	3.47 ± 0.02
SSB-EB	3.78 ± 0.04*	0.18 ± 0.01*	4.33 ± 0.04*

Data present the mean ± standard error. Asterisks indicate significant differences from control (n = 3, $p < 0.05$).

On the other hand, potassium promotes root growth and increases resistance to cold and water stress. It directly connects with improvement of the quality of crop, reduces pest and disease incidence by enhancing crop resistance as well. Although K is not a constituent of any organic molecule or plant structure, such as N and P, it is involved in numerous biochemical and physiological processes and play an important role for plant growth, yield, quality and stress tolerance (Cakmak, 2005).

A similar trend of increase in NPK uptake by pepper and cucumber at co-inoculation with phosphate and potassium solubilizing bacteria was observed in the study of Han *et al.* (2006). The structure and functional activity of the photosynthetic apparatus should be investigated at different levels of its organization. Plants uptake nutrients from the soil by the roots, transporting them to the leaves. Variations in the macronutrient composition lead to the changes in the leaf mesostructure (Table 2), as an essential exhibition of the photosynthesis regulation at the morphogenetic level.

Table 2. The mesostructural parameters of *B. juncea* leaf: thickness of leaf and its epidermal and mesophyllic layers (A); characteristics of mesophyll cell (B) and chloroplast (C).

A

Variant	Leaf thickness, μm	Epidermis thickness, μm	Mesophyll thickness, μm
Control	183.1 ± 7.6	49.3 ± 2.6	133.8 ± 5.9
SSB-EB	197.6 ± 6.9	41.7 ± 2.0	155.9 ± 6.4*

B

Variant	Cell surface area (S), thousand μm^2	Cell volume (V), thousand μm^3	Number of cells per unit area, thousand sm^{-2}
Control	3.2 ± 0.2	14.5 ± 1.0	128.2 ± 2.4
SSB-EB	2.2 ± 0.1*	8.5 ± 0.5*	167.6 ± 1.5*

C

Variant	Chloroplast surface area, μm^2	Chloroplast volume, μm^3	Number of chloroplasts per unit area, million sm^{-2}
Control	68.4 ± 4.6	55.0 ± 5.6	3.6 ±0.1
SSB-EB	63.9 ± 2.5	48.6 ± 2.9	4.9 ±0.0*

Data present the mean ± standard error. Asterisks indicate significant differences from control ($n = 20$, $p < 0.05$).

The *B. juncea* leaf thickness was increased due to a significant increase of the thickness of mesophyllic layer (Table 2A). At the same time, the decrease in the mesophyll cell surface area and volume was compensated by the increase of their number per unit area – by 30 % of control (Table 2B). A similar trend was observed in chloroplasts, a decrease in the surface area and volume of these organelles was accompanied by the increase of their number per unit area, which led to growth of plastid material volume (Table 2C).

The ratio of the cell surface area to its volume (*S/V*) affects the intensity of the gas exchange. According to Fick's law for passive diffusion of gases, the time for which the concentration of CO_2 in the cell increases from zero to half the concentration in the external medium is inversely proportional to the ratio *S/V*

(Niklas, 1997). It was found that growing *B. juncea* with adding SSB-EB to clay substrate significantly increased this ratio (by 16 %). The larger the ratio of the cell surface area to the volume, the higher the diffusion rate of CO_2 from the intra-leaf space to the chloroplasts.

It is known that the content of photosynthetic pigments can significantly affect the functioning of the photosynthetic apparatus and the metabolism of the whole plant. Their amount was found increased in the leaves of *B. juncea*, grown with adding of SSB-EB (Fig. 1A).

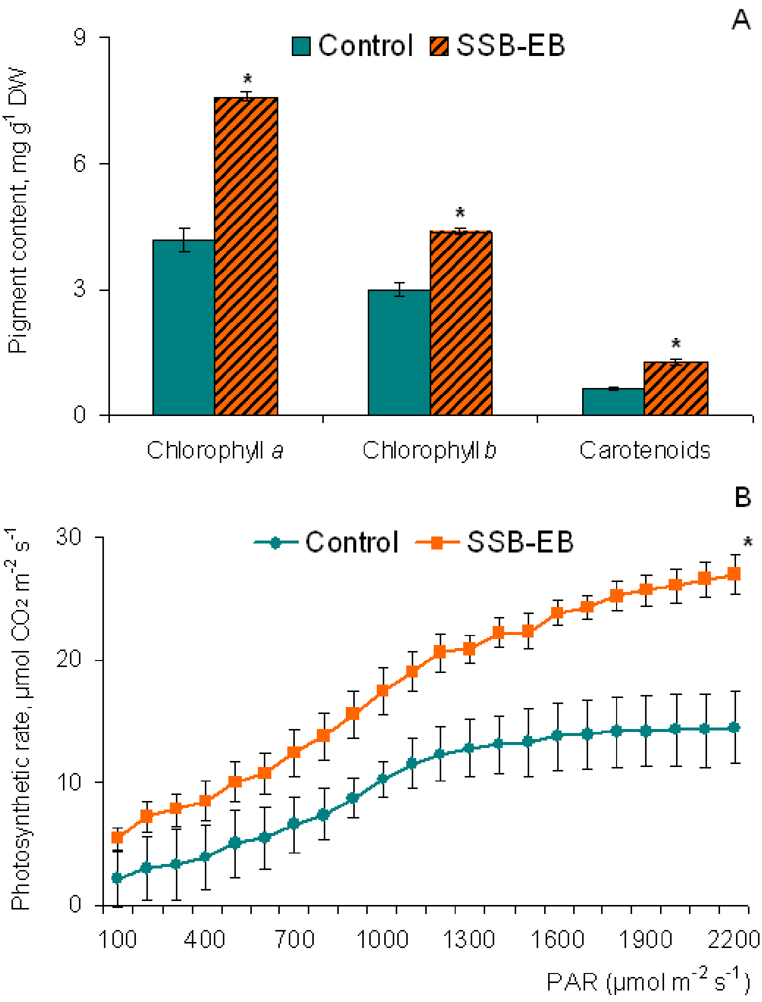

Figure 1. The content of photosynthetic pigments (A) and photosynthetic rate (B) in the leaves of *B. juncea*, growing two months without and with adding SSB-enriched biofertilizer. Data present the mean ± standard error. Asterisks indicate significant differences from control ($n = 3$, $p < 0.05$).

The content of chlorophyll *a* increased more significantly (by 82 %) than chlorophyll *b* (by 46 %). The content of carotenoids raised almost 2 times as compared to the control. The increase of chlorophyll amount in the mustard grown with adding SSB-EB can obviously be explained by an increase in the content of total nitrogen in the leaves, since it participates in the construction of these pigments. A sharp increase of photosynthetic pigment content correlated with CO_2 uptake ($p < 0.01$). The rate of photosynthesis was almost 2 times higher in experimental plants (Fig. 1B).
Photosynthesis is one of the leading plant functions, which ensures their growth, development and productivity. Changes in the structural and functional parameters of the photosynthetic apparatus are a reflection of morphogenetic regulation in plants.

CONCLUSION

The adding SSB-enriched biofertilizer to clay substrate significantly increased the thickness of mesophyllic layer, the number of mesophyll cells, the plastid material volume and the photosynthetic pigment content in the leaves of *B. juncea.* This led to enhanced CO_2 uptake by Indian mustard. Consequently, we can conclude that biofertilizer based on silicate solubilizing bacteria improved the photosynthetic function of *B. juncea*.
Changes in the studied parameters of mustard plants grown with adding of SSB-EB can be regarded as the result of increasing the available forms of macronutrients content in substrate due to the solubilization of clay silicates. This is confirmed by enlargement of the total phosphorus and potassium content in the leaves of *B. juncea.*
Hense, the bioformulation with adding SSB can be used as effective eco-friendly fertilizer for reclamation of infertile or disturbed lands, as well as for increasing the productivity of crops in organic agriculture with minimal costs.

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GREENPRENEURSHIP AT THE BASE OF THE PYRAMID: A SMALL SCALE COMMUNITY-BASED AFFORESTATION PROJECT IN INDONESIA

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ABSTRACT

Approximately 41% of the Earth’s land surface is covered by drylands that supports 36% of the world’s human population. In Yogyakarta Province, Indonesia 53% of the province is covered by drylands in which the driest regency, Gunung Kidul that occupies nearly 47% of the province, is covered by 70% agricultural dryland. Despite the phenomena of forest degradation activities for agricultural developmet by large corporation, nearly abandoned agricultural drylands such as in Gunung Kidul Regency exists all over Indonesia. Such drylands have not attracted large investors nor government to develop into agricultural activities due to economic reasons. This research explored a community based afforestation project being carried out by the support of social investors and academic researchers, virtually without involvement of the government. Among other, teak is one of only few plantations that could grow in such dryland areas and being the core competency of local farmers. The basic arrangement between the investors and farmers was mudharabah revenue sharing as an alternative to conventional pay-in-cash to land owners and smallholder farmers. The economic benefits of the program were valued by using enterprise budget method by considering the capital investment and operating expenses in which cost inflation and teakwood prices increases were incorporated. This research proved that the community-based teakwood afforestation activities along with mudharabah revenue sharing in marginal drylands could fairly improve the wealth of all stakeholders in the program.

Keywords: *afforestation, dryland agriculture, enterprise budget, mudharabah, smallholder farmers.*

INTRODUCTION

The increase in agricultural production due to population growth could lead to either agricultural expansion or agricultural intensification (Carr *et al.*, 2009). Agricultural expansion is believed to lead to deforestation and environmental degradation, whereas agricultural intensification is closely related to the development of new technology. While smallholder farmers at the base of the pyramid are blamed to be responsible for environmental degradation, large corporations enjoy the government's support to develop large scale agricultural business. Washington-Allen *et al.* (2010) stated that 41% of the Earth's land surface was covered by drylands whereas in Yogyakarta Province Indonesia, 53% of the area is covered by unproductive dryland agriculture. Due to lack of investor's interest in developing such drylands, this research explored the involvement of social investors and academicians to encourage "from the bottom up" participatory of smallholder farmers to develop teak *(Tectona grandis L.f)* plantation. Among other, teak is one of only few plantations that could grow in such dryland areas and being the core competency of local farmers. The participation of self-organized farmers being supported by social investors and academicians are a supplement to and in line with earlier research being carried out by Alemagi (2010) and Carberry *et al.* (2011). The financial benefits relation between investors and smallholder farmers had been discussed by Sugiharto and Lestari (2014) who exercised a sukuk-based revenue sharing of teak plantation project. Further, Sugiharto (2017) and Sugiharto *et al.* (2017) quantitatively identified the existence of surplus value and determined a fair distribution of mudharabah revenue sharing between the investors (Rab al-Mal) and smallholder farmers (Mudharib). Both articles demonstrated that revenue sharing gave better result being compared to conventional fully-paid arrangement, both to investors and smallholder farmers. By using enterprise budget method, this research explored the existence of surplus value of teak plantation program and determined a fair distribution of revenue sharing for agricultural development in dryland areas.

MATERIAL AND METHODS

Yogyakarta Province, Indonesia covers an area of 3,186 square kilometers in the mid-southern part of Java island with the population of approximately 3.5 million. The province is devided into five regencies namely Gunung Kidul, Bantul, Kulon Progo, Kodya Yogyakarta, and Sleman. The land of Yogyakarta Province is covered by 53% dryland agriculture, 18% irigated agricultural land, and 29% of non-agricultural land (Agricultural Office of DIY, 2010). Table 1 shows the portion of drylands in each of the regencies in Yogyakarta Province, its relation to population density and poverty rate.

Table 1. Portion of Drylands Related to Population Density

Regency	Dryland (%)	Population Density (persons/sqkm)	Poverty (%)
Gunung Kidul	70.1	463	22.05
Kulon Progo	59.7	675	23.15
Bantul	26.9	1,829	16.09
Sleman	29.0	1,934	10.70
Kodya Yogyakarta	5.8	12,164	9.75

*Sources: Agricultural Office of DIY (2010), and BAPPEDA DIY (2012).

The driest regency, Gunung Kidul, occupies nearly 47% area of Yogyakarta in which over 70% are dry agricultural land. Table 1 also demonstrates that Gunung Kidul Regency is the least populated area (463 persons /sqkm) with relatively highest (22.05%) poverty rate (Agricultural Office of DIY, 2010; BAPPEDA DIY, 2012). Based on these census data, Gunung Kidul Regency was selected as the location for experimental field research as can be seen on Figure 1.
Gunung Kidul Regency is located between 7^0 46' – 8^0 09' south latitude and 110^0 21' – 110^0 50' east longitude. An area of five hectares was selected to the south-eastern part of Gunung Kidul Regency, approximately 80 kilometers to the south-east of Kodya Yogyakarta. This research was conducted between June 2008 and December 2016 using both the primary and secondary data.
The main plantation for afforestation is teakwood that has been grown by farmers in Gunung Kidul since 1960's as the main source of long term saving out of their drylands (Pramono *et al.* 2010). Perdana (2011) stated that in 2007, the private teak forest occupied over 58,000 hectares of land in Yogyakarta Province – half of it (over 29,000 hectares) was located in Gunung Kidul Regency representing over 70% of teak tress in Yogyakarta Province.

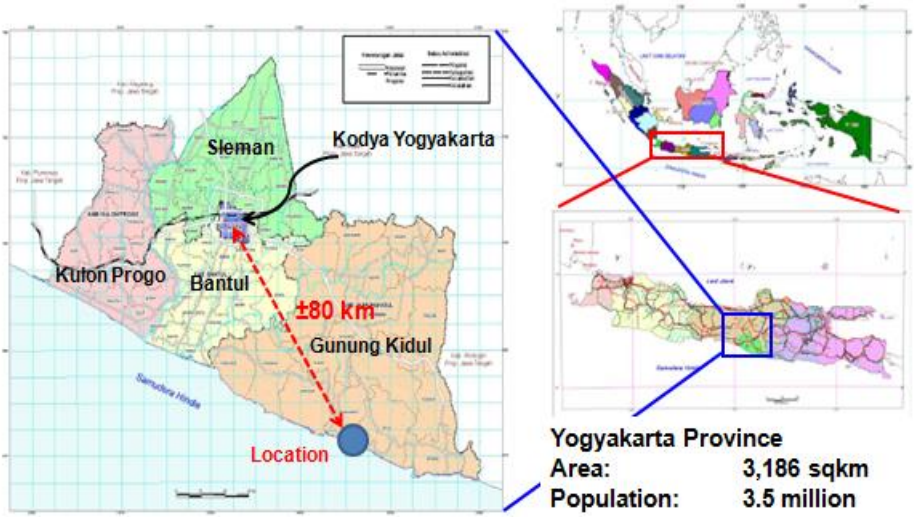

Figure 1: Research Location

For most smallholder farmers, however, teak plantation is not the main business activity since the harvesting time requires a relatively long period of time. The teak density is 1,100 trees per hectare at grid plantation spacings of 3x3 meters.
Agroforestry appraisal process was carried out by using enterprise budget method with three main components namely capital investment, operating expenses, and revenue (Godsey, 2008; Kay *et al.*, 2012) as can be seen in Figure 2. For the purpose of this research, tax had not been incorporated.

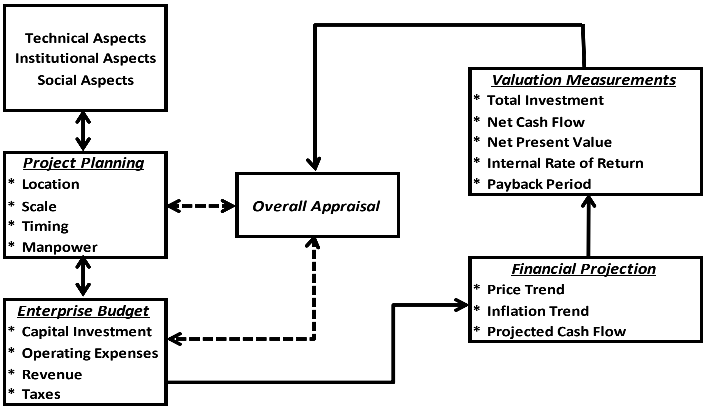

Figure 2: Enterprise Budget Framework

Several aspects of technical, institutional, and social issues were brieftly reviewed. A project planning were reviewed by considering the location, scale, timing, and manpower issues. Teak price increase of 15% per year and inflation rate of 6% per year were considered to arrive at finalcial projection (Sugiharto, 2013). The main valuation criteria being analyzed was total investment, net cash flow, net present value, internal rate of return and payback period.

RESULTS AND DISCUSSION

The capital investment for teak plantation is demonstrated in Table 2. The useful life of car, water well, small office, and chainsaw were 10, 25, 15, and 10 years respectively. The operating costs is shown in Table 3. It is assumed that teakwood will be harvested starting at year 9 at a maximum of 10% standing trees per year.

Table 2. Capital investment for Five Hectares of Teak Plantation

	Unit	Price/unit (IDR)	Useful live (years)
Car	1	150,000,000	10
Water well	1	25,000,000	25
Field office	1	25,000,000	15
Chainsaw	2	7,000,000	10

Table 3. Operating Costs for Five Hectares of Teak Plantation

	Unit	Costs /unit (IDR)
Establishment		
Field survey and socialization	/ha	1,000,000
Land clearing	/ha	2,000,000
Planting expenses	/ha	1,000,000
Planlet price	/tree	12,500
Maintenance		
Professionals: 1 person	/person /month	2,500,000
Farmers: 10 persons	/person /month	600,000
Land rental	/ha /year	250,,000
Fertilizer: 1 kg	/tree /month	1,000
Harvesting	/m3	300,000

One of the critical issues is the determination of cost of capital in which interest rate to calculate the discount rate by using capital asset pricing model *(CAPM)* was not allowed in mudharabah Islamic finance. As an alternative, the trend of gold prices in the last 10 years were used as can be seen in Figure 3 which indicated an average of 11% price increases per year.

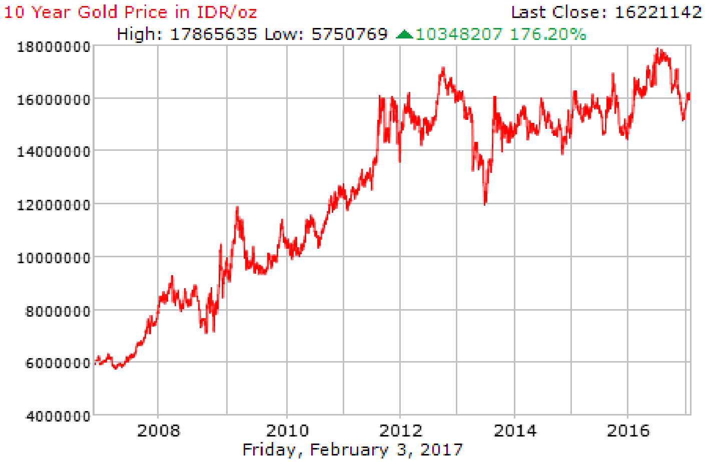

Figure 3. Gold Prices Trend

Total Investment before receiving any positive cash flow of this project is IDR 1,738 million. In an attempt to reach a fair distribution of outcome and equal risk of the project, revenue sharing instead of receiving pay-in-cash was appllied to professional salary, land rental, and farmers. With this arrangement, the total investment of investors is reduced to IDR 394 million instead of IDR 1,738 million. Assuming that there was equal investment opportunity to spend, to receive the same amount of Net Cash Flow of IDR 23,238 million, the investors (Rab al-Mal) requires 21.20% revenue sharing only. Similarly the Mudharib (professional, land rental, and farmers) requires a revenue sharing of 10.40%. Total revenue sharing to arrive the same amount of net cash flow would only be 31.60% leaving the remain 68.40% as surplus value. By considering time value of money, to arrive the same amount of net present value for both Rab al-Mal and Mudharib should receive revenue sharings of 23.12% and 29.70% respectively. Total profit sharing of 52.82% leave a surplus value of 47.18%. Figure 4 demonstrates to arrive at the same amount of net cash flow, line “AC” represents the minimum revenue share being received by mudharib whereas line “CB” represent minimum revenue share being received by Rab al-Mal. Line “AB” is the net cash flow surplus value line in which any revenue sharing along this line will result a better value to both Rab al-Mal and Mudharib. To arrive at the same amount of net present value, line “XZ” represents the minimum revenue share being received by Mudharib wheras line “ZY” represent minimum revenue share being received by Rab al-Mal. Line “XY” is the net present value surplus value line in which any revenue sharing along this line will result a better value to both Rab al-Mal and Mudharib.

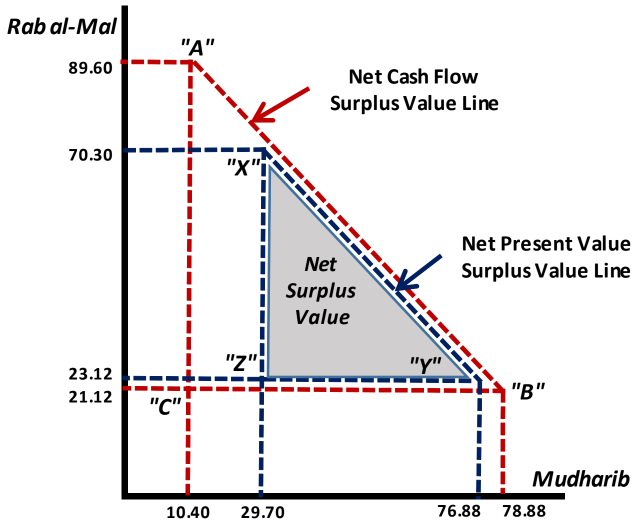

Figure 4. Determination of Profit Sharing and Surplus Value

The surplus value of 47.18% should be distributed proportionally to Rab al-Mal (20.65%) and Mudharib (26.53%) so that the final revenue sharing would be 43.77% for Rab al-Mal and 56.23% for Mudharib. Using the same amount investment of IDR 1,738 million and a revenue sharing of 43.77%, the valuation comparison between conventional and mudharabah arrangements of Rab al-Mal is demonstrated in Table 4.

Table 4. Valuation Comparison of Rab al-Mal (Investor)

		Conventional	Mudharabah
Net Cash Flow	IDR Million	23,238	50,469
NPV @11%	IDR Million	1,861	4,934
IRR	%	18.1	20.6
Pay Back Period	Years	16.9	13.6

CONCLUSION

This research proved that dryland areas could be developed into profitable investment by the support of social investors and smallholder farmers at the based of the pyramid. Instead of pay-in-cash arrangement to Mudharib, to arrive at the same amount of financial valuation criteria, the total required revenue sharing of 52.82% is less than 100% indicating the existence of surplus value to an amount of 47.18%. This surplus value should be distributed to all stakeholders. The final revenue sharing of Rab al-Mal (investors) would be 43.77% and the final revenue sharing of Mudharib (professional and smallholder farmers) would be 56.23%. It was also resolved that with the same amount of investment, Rab al-Mal received better outcome in terms of Net Cash Flow, Net Present Value, Internal Rate of Return, and Pay Back Period through revenue sharing mudharabah arrangement compare to conventional pay-in cash arrangement.

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ECOSYSTEM SERVICE ASSESSMENT IN AGRICULTURAL WATERSHED BY USING TOPMODEL

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ABSTRACT

In Millennium Ecosystem Assessment established by the United Nations, the ecosystem services (ES) provide benefits for human life as well as the environment. There is “regulating services” among all the supporting services. As a regulatory service, forests alleviate the flood risk after heavy rain by storing rainfall temporarily into forestlands and prevent the sudden increase in river discharge. The purpose of this research is to develop a hydrological modelling to assess this service in a watershed where consists of not only forestland but also grassland. TOPMODEL is applied for the quantification. This model was invented to forecast river discharge in watersheds where the land use is uniform. However, the model has not been applied to a watershed where agricultural and forest area are mixed in Japan. This research aimed to develop TOPMODEL to apply to such complexed land use. Because the targeted watershed is consisted of two land-use types, TOPMODEL was applied in each grassland and forestland. It predicted the river discharge by combining the predicted discharge from the different types of land calculated by TOPMODEL. The result confirmed that by developing the model, it was able to assess the water discharge from the both grassland and forestland in a watershed. The developed model also showed the better reproducibility of river-discharge prediction than the conventional TOPMODEL. In addition, it clarified that the forestland stores more water than grassland into the ground. Therefore, the effect of flood control which is the regulatory service of ES was assessable through the developed model.

Keywords: *hydrological modelling, river discharge, agricultural watershed, land use.*

INTRODUCTION

The ecosystem nurtured by coexisting with a human activity, agriculture brings various services to our life. Millennium Ecosystem Assessment (MA) established by the United Nations, put ecosystem services into three groups: “provisioning”, "regulating" and "cultural" services. Regulating services includes the effect of flooding and landslide control, such as water retention function by forests, flood control by riparian forests and soil erosion control by vegetation cover. However, despite this well-known function of the ecosystem, there has been no previous case that quantitatively assessed such regulating services in Japan with a hydrological modelling.

This research aims to develop a hydrological modelling to assess the flood control effect by an ecosystem service in a watershed of agricultural area. Hydrological modelling is the model that enables us to predict the amount of river flow affected by rainfall. The research applies a hydrological modelling, "TOPMODEL". TOPMODEL is a conceptual model widely used for assessment of river discharge amount. This model takes up water flow in the size of a watershed for analysis. It is a distribution model that divides a watershed into grids and calculates the surface flow generated from each grid. However, it regards the groundwater flow as a lumped model by considering the flow as one group in a watershed. Therefore, TOPMODEL is called a semi-distributed model.

This model has been applied to watersheds where land use is uniform and, in most of cases, only forestland. There have been a few studies which applied the model to a complex land use where forestland and grassland are combined. For these reasons, this research aims to develop TOPMODEL, and to assess its applicability for prediction of water flow in a complex agricultural basin consisting of forestland and grassland in Hokkaido, Japan.

Study site

As seen in **Fig. 1**, the objective watershed is Igarashi River watershed which is a tributary of Shubuto River, located in southwestern Hokkaido, Japan. The watershed area is 6.9 km^2 and the river length is 7.3 km. It is consisted of 2.7km^2 of grassland and 4.2 km^2 of forestland, which covers 31 per cent and 69 per cent respectively. This grassland is mainly used for pasture for livestock. In a part of the upstream of the water shed, there is cropland.

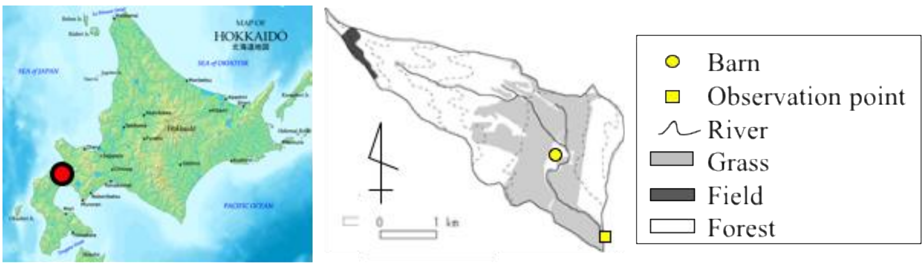

Fig.1 Igarashi River Watershed

This research took data of the river discharge and precipitation daily recorded from June 1, 1998 to October 31, 1998 by Okazawa, et al. (2002). With these data, the applicability of the improved version of TOPMODEL is analyzed. Penman-monteith method (Allen, 1998) is applied for calculation of daily evapotranspiration. Daily data of temperature, wind speed and hours of sunshine which are required to calculate daily evapotranspiration, are obtained from AMeDAS in Kuromatsunai managed by Japan Meteorological Agency.

TOPMODEL

TOPMODEL is a semi-distributed model suggested by Beven et al. (1979). This model divides soil layer into root zone, unsaturated zone and saturated zone. The semi-distributed model calculates the upper layer which contains root and unsaturated zones by each grid as a distribution type. For the lower layer, which is saturated zone, it is calculated as concentration type, thus every grid has the same value. TOPMODEL has a character that calculates the status of drying state of surface layer of basin from the topographical index (TI) induced from digital elevation model (DEM) and spatially evaluates the amount of the surface-flow.

1) Fundamental Equation of TOPMODEL

In TOPMODEL, the groundwater flow is calculated in the following equation:

$$q_i = T_0 \tan\beta_i \cdot e^{-S_i/m} \qquad (1)$$

$$T = T_0 \cdot e^{-S_i/m} \qquad (2)$$

Here, q_i is the downslope saturated subsurface flow rate at any grid i on a hillslope $[L^2T^{-1}]$. T_0 is the downslope transmissivity when the soil is just saturated $[L^2T^{-1}]$. The $\tan\beta_i$ is the surface ground gradient of grid i. S_i is the local storage deficit until the saturation [L]. m is a model parameter [L].

It is assuming that the groundwater is lateral with the ground surface gradient in **Eq. (1)** and that transmissivity has an exponential relationship with storage deficit. The following equation is assuming the groundwater flow if a steady state is established in each time step:

$$q_i = r_i \cdot a_i \qquad (3)$$

a_i is the area of the hillslope per unit contour length (m^2) [L]. r_i is a spatially homogenous recharge rate $[LT^{-1}]$. r_i is the effective recharge rate that permeates soil and flows into the groundwater. Thus, from **Eq. (1)** and **(3)**, the following equation is generated:

$$S_i = -m \ln\left(\frac{r_i \cdot a_i}{T_0 \tan\beta_i}\right) \qquad (4)$$

An expression for the watershed mean storage deficit, $\overline{S_i}$ is calculated by seeking the average of all the grids within the watershed.

$$\overline{S_i} = \frac{1}{A_T}\sum_i -m \ln\left(\frac{r_i \cdot a_i}{T_0 \tan\beta_i}\right) \qquad (5)$$

Here, A_T is the total of the entire area of the watershed. The above equation assumes to be completed in a flooding state ($S_i < 0$).

It hypothesized that r is spatially homogenous within a watershed. Then the following **Eq. (6)** is obtained by removing r through **Eq. (4)** and **(5)**. It is a prerequisite of TOPMODEL that the following equations are completed.

$$\overline{S_i} = S_i - m\left(\gamma - \ln\frac{a_i}{T_0 \tan\beta_i}\right) \qquad (6)$$

$$\gamma = \frac{1}{A_T}\sum_i \ln\frac{a}{T_0 \tan\beta_i} \qquad (7)$$

$\ln(a_i/T_0 \cdot \tan\beta_i)$ is the soil-topographic index. γ is the mean value of the index over the watershed area. A grid which has the same value between soil and the soil-topographic index, has the same character in hydrology. Then it is called hydrological similarity. The spatial mean value of T_0 , T_e is given by the following equation:

$$\ln T_e = \frac{1}{A_T}\sum_i \ln T_0 \qquad (8)$$

With this equation, Eq.(6) is organized as follow:

$$\frac{\overline{S_i} - S_i}{m} = \left(\ln\frac{a_i}{\tan\beta_i} - \lambda\right) - (\ln T_0 - \ln T_e) \qquad (9)$$

and

$$\lambda = \frac{1}{A_T}\sum_i \ln\frac{a_i}{\tan\beta_i}. \qquad (10)$$

$\ln(a_i/\tan\beta_i)$ is called Topographic Index (TI). λ is the mean Topographic Index in the watershed. TI is the index that expresses the topographic character of the catchment. It divides the watershed into the voluntary size and the watershed area that each grid has in the upper stream, $(a)[L^2]$ and the ground surface gradient of grid, $\tan\beta_i$ are obtained from the following equation:

$$TI = \ln\frac{a}{\tan\beta_i} \qquad (11)$$

After obtaining TI in every grid in the watershed, a histogram of TI is formed (**Fig. 2**).

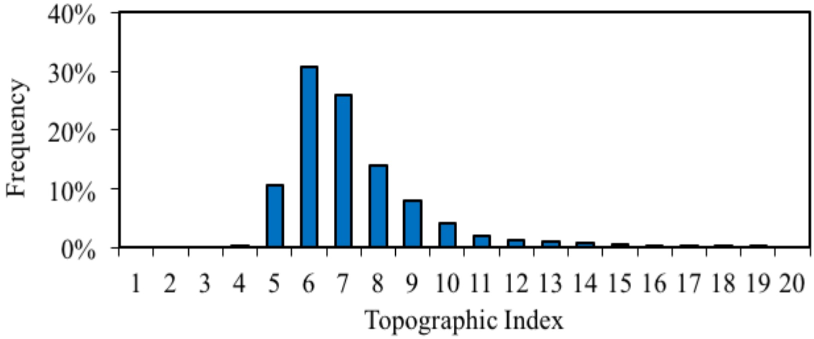

Fig. 2 Histogram of Topographic Index

2) Computational Procedure of TOPMODEL

TOPMODEL considers three storage parts, root zone, saturated zone and unsaturated zone as seen in **Fig. 3**, and calculates in each grid in the watershed.

a) Water balance equation of root zone

In root zone, the amount of water that can be stored within the root zone is calculated from the water balance of rainfall (R)[L], actual evapotranspiration amount (ET_a)[L], water available amount within root zone (SR_{max})[L] and storage deficit in root zone (SRZ)[L]. When redundant water, (EX_i)[L] is caused in root zone (SRZ<0), the redundant water is supplied to unsaturated zone and added to storage water (SUZ_i)[L]in that zone. Potential evapotranspiration (ET_0) is calculated by Penman-Monteith (PM) method, and ET_a is treated as the function of ET_0, SR_{max} and SRZ.

$$E_A = ET_0\left(1 - \frac{SRZ_t}{SR_{MAX}}\right) \qquad (12)$$

b) Water balance equation of saturated zone

The base-flow, Q_{sub}[LT^{-1}] from the whole watershed is treated as the concentrated amount per watershed. Base-flow is calculated by the following equation using the mean value of downslope transmissivity when the soil is just saturated, (T_e)[L^2T^{-1}], the mean topographic index of watershed, (λ) [-], the mean storage deficit in watershed, ($\overline{S_t}$) [L] and model parameter, (m):

$$Q_{sub} = T_e \exp(-\lambda)\exp\left(-\frac{\overline{S_i}}{m}\right) \qquad (13)$$

c) Water balance equation of unsaturated zone

Unsaturated zone is a temporary water storage zone that connects between root zone and saturated zone. It is calculated as a distribution model. The mean storage deficit amount in the watershed at the starting point of calculation, $\overline{S_t}$ is obtained from **Eq. (13)**, assuming that the initial discharge at the start is Q_0[LT^{-1}].

$$\overline{S_i} = -m \cdot \ln \frac{Q_0}{T_e \exp(-\lambda)} \qquad (14)$$

S_i in **Fig. 3** expresses the storage deficit of each grid, [L], and obtained from **Eq. (6)** and **(14)**. UZ_i is the amount of water supply from unsaturated zone to saturated zone [L], i is the number of grid. However, because a grid which has the same value as TI is regarded as hydrological similarity, a grid is calculated in each status class of TI rather than that the water amount is calculated in each grid (**Fig. 3**).

If S_i is 0 or negative, that class is regarded as saturation. Therefore, the excessive water inflow from root zone is return surface-flow (EX_i). If S_i is positive, the excessive water inflow is temporarily added to SUZ_i. UZ_i is

$$UZ_i = \frac{SUZ_i}{S_i \cdot t_d} \qquad (15)$$

where t_d is a parameter that expresses the period of retention, [LT^{-1}].

These are the concept of TOPMODEL. Operation of the model requires to determine five unknown parameters,"m", "T_e", "t_d", "SR_{max}", and "SRZ_0". This research determined the optimal value of five parameters by Monte Carlo method. Monte Carlo method is the generic term of a numerical simulation that uses random numbers.

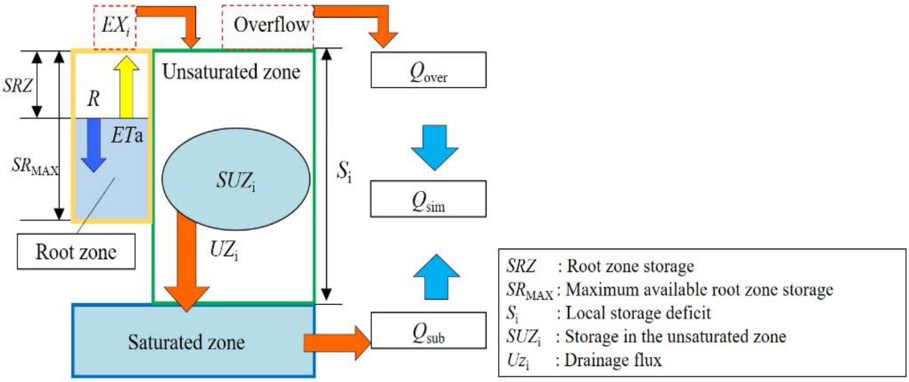

Fig. 3 Concept of TOPMODEL

TOPMODEL for Combined Forestland and Grassland

In general, TOPMODEL is applied to a simple land use at forest watershed. However, this study discusses the applicability of an improved TOPMODEL which considers the different land use of forestland and grassland, that is, a watershed with a complex land use (**Fig. 4**). The followings explain how this research calculated the complex land use.

1) Land use division

The area of forestland and grassland in the Igarashi river watershed is obtained from the 100 m mesh data of Land classification in National Land Numerical Information provided by the Ministry of Land, Infrastructure, Transport and Tourism of Japan. The ratio of land use for each forestland and grassland was 61% and 39 % respectively. From this, the mean topographic index of forestland, (λ_1) and grassland (λ_2) is calculated.

2) Computational Procedure

a) Water balance equation of saturated zone

From **Eq. (2)**, the base-flow of the whole forestland, Q_{sub1}[LT^{-1}] and the base-flow of the whole grassland, Q_{sub2}[LT^{-1}] are calculated. Then, the summation of the both values is regarded as the base-flow from the whole watershed.

$$Q_{sub} = Q_{sub1} + Q_{sub2} \qquad (16)$$

b) Water balance equation of unsaturated zone

It is necessary to gain the initial river discharge (Q_{01}, Q_{02})[LT^{-1}] from forestland and grassland to gain the storage shortage (S_{i1}, S_{i2}) of each grid of forestland and grassland. Here it assumed that the ratio of flow amount from the both of forestland and grassland is always constant, and obtained the initial value of the river discharge in the following equation.

$$Q_0 = KQ_0 + (1 - K)Q_0 \qquad (17)$$

Here, it assumed that the ratio of water outflow from the forestland to the whole watershed is $K(0<K<1)$, the ratio of grassland is $(1-K)$, and K is an unknown parameter.

c) Other calculation

When it calculates root zone, UZ_i, it divides the watershed into forestland and grassland. From this, it determines the eleven unknown parameters "m_1", "T_{e1}", "t_{d1}", "SR_{max1}", "SRZ_{01}", "m_2", "T_{e2}", "t_{d2}", "SR_{max2}", "SRZ_{02}" and "K" for the improved TOPMODEL.

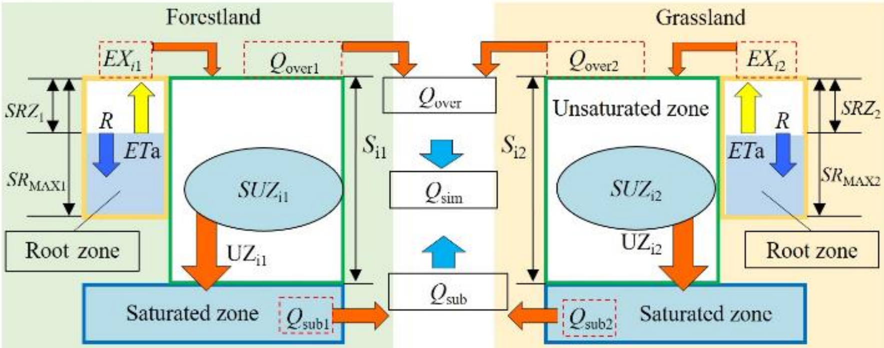

Fig. 4 Concept of Improved Version of TOPMODEL

Method of Identification and Comparison of Unknown Parameter

This research determined the optimal values of the eleven unknown parameters for the improved TOPMODEL by using Monte Carlo method. Through the method, generating random numbers for each eleven unknown parameter, "m_1", "T_{e1}", "t_{d1}", "SR_{max1}", "SRZ_{01}", "m_2", "T_{e2}", "t_{d2}", "SR_{max2}", "SRZ_{02}" and "K", 100,000 sets of combination were created. As the result of 100,000 times of calculation, it obtained the combination that accords the closest between the actual value of flow amount and the estimated value.

Evaluation Function

The compatibility of the actual value of river discharge and the estimated value is evaluated by Nash-Sutcliffe coefficient (NS value) and Root Mean Squared Error (RMSE). N stands for the total number of calculation time, $Q_{obs}(i)$ is the actual river discharge at any time step of i, $Q_{sim}(i)$ is the estimated river discharge of at any time step of i, and Q_{av} is the mean value of the actual river discharge.

NS value is calculated in **Eq. (18)**. The closer the value is to 1, the higher the accuracy of the model's estimate is.

$$NS = 1 - \frac{\sum_{i=1}^{N}[Q_{obs}(i) - Q_{sim}(i)]^2}{\sum_{i=1}^{N}[Q_{obs}(i) - Q_{av}]^2} \qquad (18)$$

RMSE is calculated in the following **Eq. (19)**. The closer to 0, the higher the reproducibility of the estimated value is.

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}[Q_{obs}(i) - Q_{sim}(i)]^2} \qquad (19)$$

RESULT AND DISCUSSION

The actual value of rainfall and river discharge in Igarashi River watershed and the predicted value generated by both of the conventional TOPMODEL and the improved TOPMODEL are shown in **Fig. 5**. Both models estimated the change of river discharge according to the change of rainfall well. However, the improved TOPMODEL showed the closer value to the actual value than the conventional one when the river dischaege decreases after it reached the peak point.

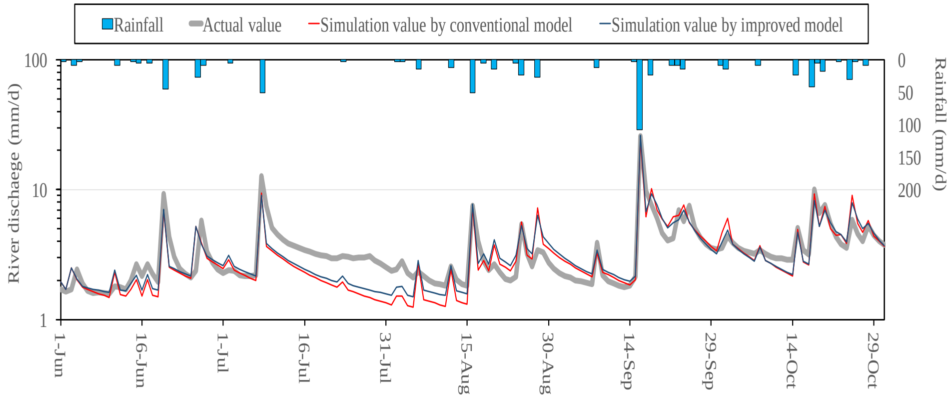

Figure 5. Comparison between the conventional and the improved TOPMODEL

The function value showed the accuracy of prediction of the both models. On one hand, the conventional TOPMODEL showed 0.823 for NS and 1.069 for RMSE. On the other hand, the improved TOPMODEL showed 0.853 for NS and 0.973 for RMSE. From these values, it confirms that the improved TOPMODEL had the higher accuracy of prediction of river flow change by rainfall than the conventional one throughout the target period.

The optimal value of unknown parameters of the conventional TOPMODEL and the improved TOPMODEL determined by the Monte Carlo method are shown in **Table 1**. The improved model showed the higher value in T_e and t_d than the conventional one. In addition, the improved model showed the two high values in forestland than grassland. As T_e expresses the downslope transmissivity when the soil is just saturated, forestland has better transmissivity than grassland. According to Ohte et al. (1989) and Ohta et al. (1989), transmissivity of forestland is generally high in Japan. This is because the transmissivity in grassland is lowered due to soils compressed by tiller machine or tractor. For this reason, it is valid that T_e is higher in forestland than grassland.

Table 1. Comparison of Unknown Parameters

Unknown parameter		m (mm)	T_e (mm/d)	t_d (mm/d)	SRZ_0 (mm)	SR_{max} (mm)	K
Conventional model		46.0	2.5×10^6	0.0104	0.52	0.62	
Improved model	Forestland	24.5	9.0×10^6	0.0206	0.42	0.85	0.43
	Grassland	81.6	6.5×10^6	0.0198	0.43	0.49	

t_d is a parameter that shows the delayed time caused when water moves from unsaturated to saturated zone. That is, this research showed that water flows slower from unsaturated to saturated zone in the improved model than the conventional one.

K showed 0.43. Although the ratio of land use for forestland is higher than grassland in the watershed, the ratio of outflow from forestland is lower than grassland. In the grassland, although the surface flow is high at the time of rainfall, the baseflow is remained within a certain limit because the ground cannot retain water regardless of the precipitation amount. On the other hand, in the forestland although the surface flow is low during rainfall because the ground stores the water, that stored water is discharged as baseflow after the rainfall. Comparing the amount of discharge per 1 km^2 for 153 days between forestland and grassland, forestland was 48.86mm and grassland 63.09mm. It means that forestland stores more water than grassland. Therefore, it clarified that forestland has the effect of flood control.

CONCLUSION

This research showed the applicability of the improved version of TOPMODEL for the case of watershed with the complex land use. Especially it has the high reproducibility when the actual river discharge is low. In addition, it showed the flood control effect in forestland by comparing with unknown parameters. In this way, the regulating service, which is one of the ecosystem services, is assessed in the complexed land-use by applying the improved TOPMODEL.

In the future study, the comparison of the improved TOPMODEL with the Soil and Water Assessment Toll (SWAT) is considered to clarify the higher accuracy of water flow prediction in the mixed land use watershed. SWAT is a model that considers the land use to evaluate the ecosystem service in watersheds consisting of agricultural land and forestland.

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STUDIES ON JOINT TOXIC EFFECTS OF A GLYPHOSATE HERBICIDE (FOZÁT 480) AND A HEAVY METAL (CADMIUM) ON CHICKEN EMBRYOS

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ABSTRACT

The aim of the study was to determine the individual and combined toxic effects of the herbicide Fozát 480 (glyphosate [isopropylamine salt] 480g/l) and cadmium sulphate ($CdSO_4$) on the development of chicken embryos. On the first day of incubation, chicken eggs were injected with 0.1 ml of cadmium sulphate solution (0.1%) and/or with 0.1 ml of Fozát 480 (2.0%). The chicken embryos were examined on the 19th day by measuring the rate of embryo mortality and body weight and by identification of different types of developmental anomalies and macroscopic malformations. The body weight data were statistically evaluated by one-way ANOVA and Dunnett tests, while the embryonic mortality and the developmental anomalies were analysed by Fisher test. Our teratogenicity study revealed, that the combined administration of cadmium sulphate and glyphosate (isopropylamine salt) containing herbicide formulation caused a significant reduction in the body weight of embryos and increased the rate of embryonic mortality. The joint toxic effect of cadmium sulphate and Fozát 480 is an additive effect compared to the individual toxicity of the test materials.

Keywords: *glyphosate (isopropylamine salt), cadmium sulphate, joint toxic effect, ecotoxicology, chicken embryo.*

INTRODUCTION

Preservation of natural values and protection of the environment are integral parts of social life and economy. Environmental pollution, exploitation of natural resources and imbalances in the dynamic equilibrium of living systems may lead to the constricting of living spaces and expose our health and safety to danger in the short run. Nowadays, one of the most important basic principles is to produce high quality safe food that indispensably requires clean environment. The European Union pays particular attention to the environmental protection and attaches great importance to environmentally sound management. In addition to the production of healthy foods, agricultural production have to minimize the environmental load while promoting the energy-efficient use of non-renewable raw materials. Unfortunately, there are still large quantities of chemicals in our environment of which biological activity is not fully known.

Because of the rapid industrial and agricultural development, humans and the surrounding environment suffers from a significant chemical load within which pesticides play an important role. Although pesticide use helps fighting off harmful organisms providing higher yield safety, all chemicals are potential poisons and present serious risk. The fauna of the cultivated areas and humans using the pesticides are primarily exposed to chemical loads. Agricultural lands provide food source, shelter and nesting place for our wild fowls. Pesticides sprayed during plant protection have not only impacts on adult fowls but also on the embryos developing within the eggs (Várnagy and Budai, 1995). The direct exposure of wild fowls' eggs can be increased, inter alia, by irregular use of the pesticides, by inappropriate spraying techniques and by side drifting of spray liquids (Várnagy and Budai, 1995). Pheasants are exposed to the effects of chemicals used in agriculture during egg laying and foraging, their reproductive period usually coincide with crop protection measures, which indicates the necessity of dealing with the harmful effects of pesticides on living organisms from ecotoxicological view of aspects (Köhler and Triebskorn, 2013). Ecotoxicological testing methods of pesticides focus on certain chemical agents mainly separately, but a fact cannot be neglected, that chemical loads present in a complex way thus joint toxic effects, interaction of simultaneously presenting chemical substances have to be considered where the components may modify the toxicity of each other (Oskarsson, 1983; Danielsson *et al*., 1984; Speijers and Speijers, 2004).

MATERIAL AND METHODS

For modelling the environmental cadmium load, 0.1% cadmium sulphate solution (Reanal-Ker Ltd., Hungary) (Safety datasheet, 2014) was used in individual and joint treatments. The herbicide Fozát 480 (480 g/l glyphosate [isopropylamine salt], Agro-Chemie, Budapest, Hungary) (Ocskó *et al.*, 2017) was used in individual and joint treatments in typical field application rate (2.0%). The studies were conducted with purebred fertile Farm hen's eggs derived from the stock farm of Goldavis Ltd. (Sármellék, Hungary). The eggs were incubated in a Ragus type hatcher (Vienna, Austria). During the incubation, the appropriate temperature (37–38°C), air humidity (65–75%) and the daily rotation of eggs were provided (Bogenfürst, 2004). The treatment of eggs (n=35/group) was performed on the day of initiation of hatching. In the individual treatments, solutions and emulsions made from test chemicals in 0.1–0.1 ml end volume were used while in the joint treatments, 0.2 ml of the chemical agents were injected into the air chambers of eggs in each combination. For the preparation of solutions and emulsions as well as in the control treatments, distilled water was used. The incubation was started immediately after the treatments. The processing was conducted two days before the expected hatching on the 19th day of incubation. Within the framework of the pathological studies, the body weight of embryos, the number of dead embryos and the macroscopic malformations were determined and recorded. In case of the body weight data of live embryos, statistical comparisons among the groups were made with one-way analysis of variances. Because the results showed significant differences, Dunnett tests were also performed. In case of the biometric processing of the embryonic mortality and malformations, exact test according to Fisher was used.

RESULTS AND DISCUSSION

The embryonic mortality in the control group treated with distilled water was 8.82% (Table 1.). The rate of embryonic mortality could be considered sporadic which made it possible to use that group as a frame of reference. Malformation occurred in one case in that group (3.33%) (Table 2.). On the effect of the injected distilled water in the control group, the average body weight of embryos was 22.61 grams (Figure 1., 2.). On the effect of the 0.1% cadmium sulphate solution the embryonic mortality significantly increased ($p<0.001$) to 85.71% in comparison to the control group (Table 1.). There was not any malformation in this group (Table 2.). Injection of cadmium sulphate decreased the body weight of embryos (avg. 21.14 g) in comparison to the control group (22.61 g) (Figure 1.) but the difference could not be proved statistically. The treatment with the herbicide Fozát 480 (2.0%) caused 40.63% embryonic mortality (Table 1.). The increase in mortality proved to be significant ($p<0.01$) in comparison to the control group. There was not any malformation in the group treated with the herbicide (Table 2.). On the effect of the injection of the herbicide a significant decrease ($p<0.05$) in body weights (avg. 20.79 g) was revealed compared to the control (22.61 g) (Figure 1.).
The combined test with $CdSO_4$ and Fozát 480 herbicide resulted in a 100% embryonic mortality (Table 1., 2., Figure 1., 3., 4.).

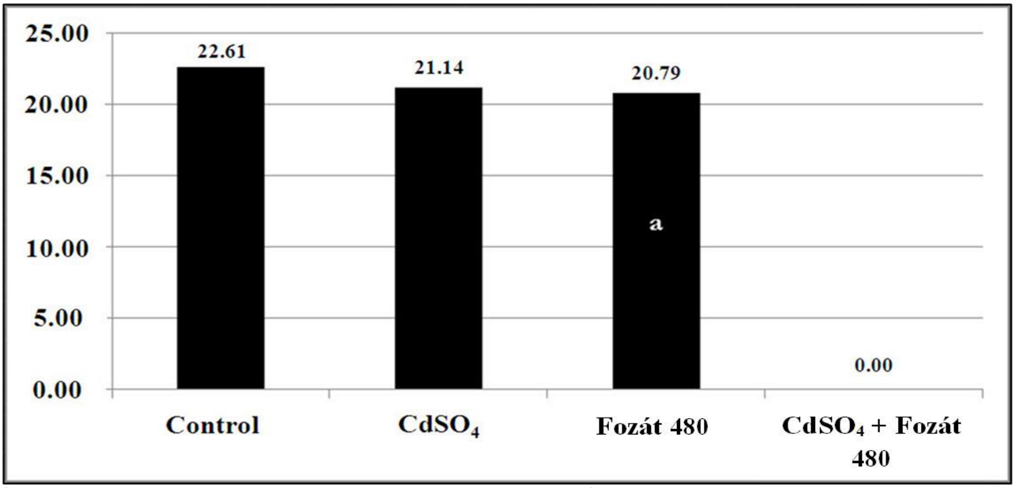
Table 1. The number and rate of dead embryos in the avian teratological test of individual and joint toxic effect of Fozát 480 (480 g/l glyphosate [isopropylamine salt]) and cadmium sulphate performed with injection treatment method.

Treated groups	**Number of dead embryos/ number of fertile eggs (pcs)**	**Rate of dead embryos (%)**
Control	3/34	8.82
Cadmium sulphate	30/35[a1]	85.71
Fozát 480	13/32[a2]	40.63
Cadmium sulphate + Fozát 480	34/34[a2]	100.00

[a]Significant difference compared to the control group ([a1] $p<0.05$, [a2] $p<0.001$).

Table 2. Number and rate of malformed embryos in the avian teratological test of individual and joint toxic effect of Fozát 480 (480 g/l glyphosate [isopropylamine salt]) and cadmium sulphate performed with injection treatment method.

Treated groups	**Number of malformed embryos/ number of alive embryos (pcs)**	**Rate of malformed embryos (%)**
Control	1/30	3.33
Cadmium sulphate	0/5	0.00
Fozát 480	0/19	0.00
Cadmium sulphate + Fozát 480	0/0	0.00



[a]Significant difference compared to the control group ([a]$p<0.05$).

Figure 1. Data of embryonic body weights in the avian teratological test of individual and joint toxic effect of Fozát 480 (480 g/l glyphosate [isopropylamine salt]) and cadmium sulphate performed with injection treatment method.

Figure 2. Chicken embryo on the 19th day of incubation

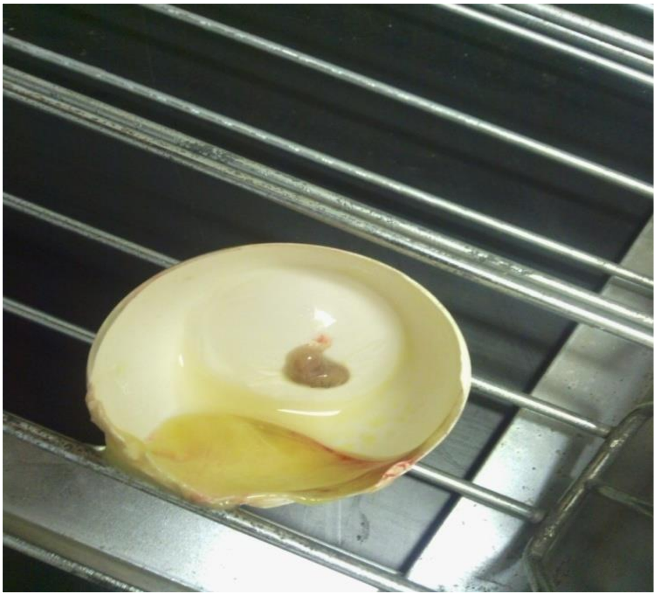


Figure 3. Mortified embryo on the 7th day of incubation

Figure 4. Mortified embryo with open chest cavity on the 18th day of incubation

Similarly to previous results (Juhász *et al*., 2006; Szabó *et al*., 2011), according to the pathological studies it was established that individual treatments with cadmium sulphate significantly increased the embryonic mortality. The herbicide Fozát 480 in individual and combined administrations significantly increased the embryonic mortality. The rate of malformations remained at a low level in all treated groups, so teratogenic effect could not be proved. Teratogenic effects of different doses

(500, 750 and 1000 mg/kg) of the herbicide Roundup (glyphosate) was investigated by Dallegrave *et al*. (2003) on pregnant rats. In their studies, it was revealed that different doses of the herbicide increased the embryonic mortality, which reached 50% at the highest dose. The frequency of embryonic skeletal malformations increased in the treated groups, which proved the teratogenic effect. The processing of results data showed a significant decrease in body weights in the treated groups compared to the control. In the group treated individually with 0.1% $CdSO_4$ the decrease in body weights was not significant, while 100% embryonic mortality was detected in the combined treated groups thus body weight data could not be measured.

CONCLUSION

Based on our avian teratological studies made with Fozát 480 and cadmium sulphate in individual and combined tests, it was established, that joint effects of both chemical agents in case of relatively low environmental cadmium load (which in itself could be less embryo toxic) the herbicide treatment with Fozát 480 in typical field rate additively increased the embryonic mortality, which represented in a significant decrease in bodyweights of embryos and in an increased embryonic mortality under the circumstances used in our experiments. According to research results, joint administration of pesticides generally increase moreover highly increase the toxicity of the chemical components multiplying the risk of their use as well. These effects are dependent on the species, time of exposition and doses so it is rather difficult to routinely forecast the expected effects (Thompson, 1996).

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A VIEW ON THE UNINFORMED CONSUMERS TOWARDS QUALITY STANDARDS IN THE CONTEXT OF THE TTIP NEGOTIATIONS

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ABSTRACT

Radio broadcasts, TV shows and online media make a significant contribution to day-to-day consumer information and have a great impact on public opinion. The present study provides an overview of the German reporting about the quality standards of the agri-food industry in the context of the negotiations towards a EU-US Transatlantic Trade and Investment Partnership (TTIP). On the basis of a guided empirical content analysis, 436 publications released via radio, TV or Internet in the period from June 2013 to December 2016 were quantitatively and qualitatively analyzed. The quantitative analysis showed that the agri-food industry was generally of minor relevance in the coverage of TTIP, but focused mainly on quality standards. The term 'quality and consumer protection standards' frequently appeared in the reportings with the topics such as genetic modification, use of hormones, antibiotics or pesticides and the 'chlorine-washed chicken'. These are not standards for official definition of the general food law. It was established that all publications lacked information about specific standards. Thus, the quantitative analysis showed a superficial view of quality standards with only symbolic characteristics. The results of the qualitative media analysis indicated a negative picture of the effects of TTIP concerning the agri-food sector and its standards. Due to the complex structure of the globalised agri-food chains, the importance of comprehensive consumer information was highlighted. Overall, German media failed to provide scientifically based information. Unfortunately, they just highlighted the possible negative changes which could be caused by TTIP.

Keywords: *agri-food industry, food safety, quality management, quality standards, Transatlantic Trade and Investment Partnership (TTIP)*

INTRODUCTION

A possible bilateral Free Trade Agreement (FDA) between the EU and the US has been debated closely since 2011. Negotiations were started in June 2013 (European Commission, 2013). Intensive discussions are being held on possible impact for European standards on consumer protection and EU quality policy. A great interest by the media and the attention of the civil society has emerged. In a very short time, countless alliances with the participation of political parties, associations, trade unions and citizens have been established who call the population to protest against the FDA. A strong presence of counter-movements could be achieved mainly by distributing its content to online media. Many of these non-governmental organizations (NGOs) work together in their protests against TTIP and thus achieve an enormous range. So far, only the sector-neutral information events of the anti-TTIP campaign movement in Germany have been analyzed, with the result that citizens are the victims of a professional disinformation campaign (Bauer, 2016). Furthermore, the general mood of TTIP in other European countries and the USA were analyzed. The findings indicated that the TTIP negotiations in the considered European countries do not play a major role (Maier, 2014). This situation has an impact on civil society. According to a study in 2015, the EU-wide acceptance to TTIP decreased by 2% compared to the year 2014 (Bluth, 2016). The reporting in the mass media influences the level of information and the opinion of the consumers significantly. Because of that a comprehensive, complete and scientifically correct presentation is indispensable for differentiated opinion formation (AGR, 2015). Although the media have an educational mandate, the consumer is offered a variety of topics that affect the opinion about the FDA negatively. The population is not properly informed and insecure. The relevance has not yet been properly researched with regard to the presentation of the quality standards of the agri-food industry within the scope of the TTIP negotiations. This study aims at filling this research gap.

MATERIAL AND METHODS

Media can be differentiated into mass media and lead media. Mass media are characterized by a wide range of information. Leading media publications are to be used for information and form opinions of the public and other mass media (Gendolla *et all*, 2009; Hasebrink *et all*, 2013). Three types of media were analyzed: radio broadcasts, TV shows and online media. The radio is considered as background medium, which is used throughout the day (Engel, Breunig, 2015). Consequently, the coverage of radio broadcasts is rather high and it has a significant impact on shaping of public opinion and information status to consumers. As a medium of information and entertainment in Germany, television is of great importance in the everyday life of consumers. TV shows of the category "educational television" were the focus of analysis. In order to establish a representative cross-section in the presentation of quality standards of the agri-food industry within the context of the TTIP reporting, the websites of TTIP-counter-movements and consumer protection organizations (Non-Government-Organizations (NGOs)) were analyzed. The overall analysis took into account 169 radio broadcasts, 144 TV shows and 123 articles from eight websites of NGOs. They have been reviewed in terms of their relevance to the research question, thus the developed category system has been applied to a total of 51 TV shows, 34 radio broadcasts and 19 contributions on the respective websites of NGOs. Because TTIP should regulate all European and US standards of the agri-food industry, mainly the standards were considered that are in the media discussions of particular relevance. The following international standards were of particular importance: DIN EN ISO Standards, International Featured Standards (IFS), Global G.A.P, GS1 Germany, QS and EQA Standards. All selected media contributions were analyzed with the focus on Germany. The analysis period was set from the beginning of the TTIP negotiations in June 2013 until 31.12.2016. Data were collected from April 2016 to January 2017. Since the media's portrayal on the quality standards of the agri-food industry as part of TTIP was a complex analysis object, empirical content analysis was combined with the model of guideline analysis. In this way, formal and content-related elements could be presented in a reduced form. For a thorough analysis of the presentation of topics in different media formats and assessing the impact of mass media on public opinion of consumers, various elements had to be considered. Apart from the content, stylistics was also the focus of the investigations. The procedure of content analysis had to be intersubjectively comprehensible and was carried out in four phases (Früh, 2015): the planning phase, the development phase, the test phase and the application phase. Two types of content analysis are distinguished: the manifest analysis and the latent analysis. In this study, the latent content analysis was performed. In order to reflect the underlying significance of a media contribution, each article was read and subjectively assessed. The guideline analysis as a useful addition to the empirical content analysis allows the consideration of complex issues, it captures the content and the function of a media report. Both methods were applied to contributions that dealt with defined keywords on quality standards as well as the impact on the agri-food industry. The eight defined keywords were „chlorine-washed chicken“, „genetic modification“, „hormone meat“, „cloning“ and the use of pesticides and antibiotics in agriculture. In addition, the “precautionary principle” was included in the analysis, which plays a key role in the TTIP negotiations and is opposed to the US aftercare principle. The precautionary principle is essential in the European agri-food industry to produce high-quality and safe products. Another keyword was "weakening of standards". The standards of the agri-food industry had to be differentiated from the standards of other industries. Accordingly, only statements were considered, which were directly related to the agri-food industry or those which are responsible for consumer protection. For the analysis a five-stage system was used. Their categories were described by variables. The categories of identification, formal design, origin, content and function were selected. Different variables have been assigned to each category. For each medium an adapted guideline sheet was compiled to answer the research questions. The content and wording of the guidelines were adapted to the respective media format.

RESULTS AND DISCUSSION

In the following, results regarding relevance, frequency of the keywords, use of subject-related information source and content of the quality standards of the agri-food industry in the context of TTIP reporting are presented and discussed.

Relevance of media reporting

As shown in Table 1, a total of 436 contributions of different media formats were considered, of which 104 articles were relevant for the formulated research question and were analyzed. This corresponded to a relevance of 23.9%. The share of relevant contributions in television was highest with 35.4%. Radio stations have published a larger number (169) of contributions, but their relevance to the research question was lower (20.1%). The least relevance of contributions was found at the NGOs (15.4%).

Table 1. Relevance of the considered analysis items and share of reporting on the agri-food industry (AFI) and quality standards (QS)

	considered	relevance	relevance [%]	AFI [%]	QS [%]	QS on AFI [%]
TV	144	51	35.4	23.9	13.0	54.4
Radio	169	34	20.1	10.7	5.0	46.7
NGOs	123	19*	15.4	text 16.9 film 69.7	11.6 23.5	68.5 33.7
	Σ 436	Σ 104*	**Ø 23.9**			

* The difference is explained by 15 unrecognized contributions from 3 NGOs. In these cases, the value of the analyzed items could not be determined by the structure of the websites, so that only the contributions of 5 other NGOs are summarized.

Decisive for the results is the period during which the study was carried out. During the data collection from April 2016 to January 2017 three rounds of negotiations, the US presidential election and the publication of TTIP leaks by *Greenpeace* took place. Given the broad basis for negotiation and the large number of contentious issues of the agreement, it was expected that the share of reporting on the agri-food industry and its standards would be low, as confirmed by the analysis results.

Frequency of keywords

The most frequent use of all the keywords under investigation was in the medium of television. It is clear from Figure 1 that it was reported most frequently on "genetic modification" (78.4%), "weakening of standards" (72.6%) and "chlorine-washed chicken" (54.9%). Most often mentioned by radio broadcasts were "genetic modification" (50.0%), "chlorine-washed chicken" (41.2%) and the "precautionary principle" (29.4%). The "weakening of standards" was most frequently reported by NGOs in the online media with 79.4%, followed by "genetic modification" (58.8%) and "precautionary principle" (29.4%). Overall, the "genetic modification" (62.4%) was most frequently mentioned in the reporting. The "weakening of standards" was the second most common statement of the study at 55.6%. The next important keyword with an average consideration of 40.9% was the topic "chlorine-washed chicken". With a share of 30.1% the "precautionary principle" was applied. About the "use of hormones" was reported in 22.2% of all coverage. The most rarely used keywords were "use of pesticides" (9.5%) and "use of antibiotics" (7.2%). Little has been reported on the topic of "cloning" (8.2%). Overall, the frequency of the examined keywords was low in all media formats. The general explanation of quality standards was of most importance in television (53.0%), whereas the radio was the rarest of 17.7%. Furthermore, an increased use of keywords in the negative presentation of TTIP compared to the neutral reporting was to be seen. An exception was the medium radio, which published most frequently neutral contributions. Neutral presentation, meaning without judgment, took place via radio broadcasts on "genetic modification" (51.6%), "weakening of standards" (16.1%), "use of antibiotics" (9.7%), "use of pesticides" (6.5%) and "cloning" (3.2%). The general term "standards" was most frequently used in TV shows with a neutral view regarding TTIP (80.0%). Finally, it should be noted that the fixed keywords of the analysis were significantly used by the media.

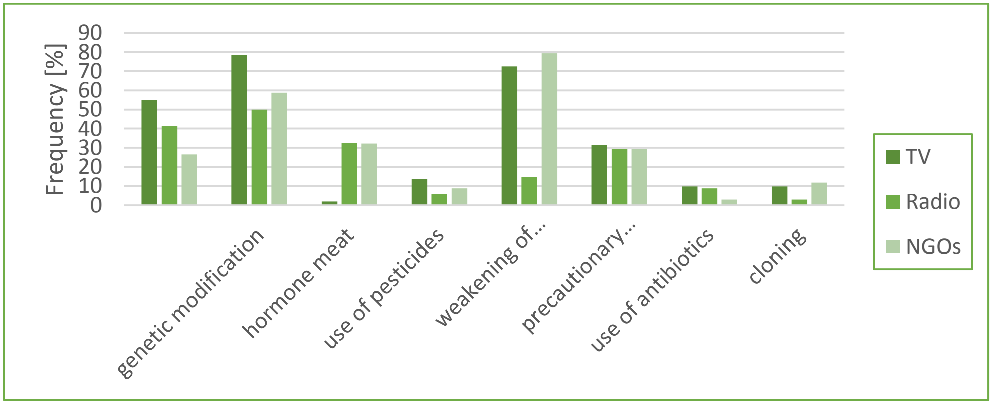

Figure 1: Selection and frequency of used keywords in the analysis items

Sources of information

The results provide an overview of the most common sources of information, which were used by media in terms of the positions and representations of TTIP. The medium of television generally used the most information sources. The most important sources of information were statements by politicians (43.4%), German representatives of industry and commerce (30.3%) and consumers (29.4%). In radio broadcasts, only the moderator or the journalist were involved in 44.1% of the contributions. The most important source of information for the radio were politicians (55.9%). Consumers, upholders of consumer protection and entrepreneurs were of secondary importance in radio shows. Moreover, in 47.1% of the contributions of NGOs, no references were mentioned. Their main sources of information were economic studies (23.5%) and statements of interested entrepreneurs (20.6%). Quotes from stakeholders of consumer protection (2.9%)

and consumers (2.9%) were the least relevant. Statements by representatives of the European Commission were found at 5.9% less frequently than those of politicians who are not participating in the negotiations (14.7%). With the exception of contributions from affected farmers and food companies, 98.3% of all analyzed items were made by persons who are not familiar with the agri-food industry. Given the fact that reports on the agri-food industry have a extensive impact on consumers as well as a high emotional effect, it has been expected that the anti-TTIP-organizations have often reported about this sector. This aims at mobilizing the population against TTIP. The analysis showed that the expectation of published films and videos, which emphasized the negative consequences of the FDA and invoked consumers to take part in demonstrations, was confirmed. The results on the publications of the NGOs illustrated the organized structure against TTIP. At the same time, it showed that NGOs, through their large presence, were able to influence consumers without having to provide detailed information. De facto, in most of the articles, no sources of information on the agri-food industry and its quality standards were used. Thus, the omission of scientific sources of information can be used as a means to achieve a desired effect on consumers.

Presentation of content

All analyzed media were verified for their content. The results showed a very low positive view on TTIP. At least, TV shows had a positive share of 5.90%. In contrast, radio broadcasts and NGOs did not show any positive depictions about TTIP. The neutral reporting predominated with 91.2% in radio shows. A prevailing negative presentation of the entire analysis period was noticed for TV stations (54.9%) and NGOs (100%). Positive presentations were achieved primarily by the fact that potential negative effects are weakened. The neutral impression was achieved by the simultaneous use of positive and negative contributions. In the case of the positive presentation, sentences were used to insure the future import ban on hormone meat, genetic modification, cloned meat or "chlorine-washed chicken", and to prevent a reduction in consumer protection standards. TTIP supporters recognized the differing perceptions of certain quality criteria between the EU and the US. It has repeatedly been explained that the EU does not have higher standards of protection in all areas, for example in the case of limiting values of contaminants and microbiological quality at raw milk products. Above all, there were different points of view and argumentative structures about the precautionary and aftercare principles. While some of the TTIP supporters require the unaltered maintenance of the precautionary principle, others argue that both the precautionary and aftercare principle are effective approaches that should be better united in the future. Concerning the proposed mutual recognition of protective standards, different statements have been made. On the one hand, the position was held that mutual recognition should not be accepted so that the different levels of protection should continue in the future and common standards have to be developed on both sides of the Atlantic. On the other hand, mutual recognition was seen positively as long as labeling requirements for the origin and production processes are mandatory. Global harmonization could affect the international standards (e.g. DIN EN ISO, IFS, Global GAP, GS1, QS, EQA). In none of the reports were detailed contents or specific quality standards of the agri-food industry mentioned, which could be altered by the agreement. In addition, it was found that the argumentation with false facts concerning the "chlorine-washed chicken" and other stereotypes prevents an impartial view of the chances of TTIP. In the negative reports, the emotions were mainly achieved through dramatizations, musical effects, metaphorical pictures and evaluative language as well as biased and generalized statements. The quality standards of the agri-food industry were without exception presented as endangered, without mentioning specific regulations. Consumer voices were involved in the media reporting, but the share in positive presentations was lower than in neutral and negative reports. Farmers were mostly negative about TTIP, while representatives of other economic sectors were in positive mood. NGOs were mainly represented in negative reports on TTIP. Negative statements were often dramatized. For example, it has been predicted that unlabelled banned goods will be imported on the European market and the competition between the manufacturers will be increased. TTIP would also contribute to a reduction of the biological diversity, the wide range of products and quality of food. Apart from that the discussion about the "chlorine-washed chicken" was used in order to bring European standards of intensive animal husbandry into a better light. It should be noted that the concept of animal welfare has always been discussed differently in the European population than in the USA. In Europe, animal welfare is part of the transformation processes to more sustainability and is related to healthy animals mean safe food. This is now recognized not only by science, but also by the industry. Of course, it is also part of the preventive idea behind the EU food law. Regardless of the medium, the European agri-food industry was seen as the loser of the TTIP agreement, as the differences in this area were fundamental and the higher-regarded European standards of protection were inevitably seen as jeopardized. By moral concerns about the methods in agriculture especially emotional aspects could be used without distinguishing between scientific and ethical foundations. The different levels of protection of the standards were critically discussed in all media, without any concrete standards being mentioned. In principle, more detailed information were provided on subjects not related to the agri-food industry than on quality standards in the agri-food sector. Their role seems to be underestimated, as in the future, the private standards should be more closely integrated into the negotiations on international trade agreements (Petersen, Lehnert, 2017).

CONCLUSIONS

The planned EU-US Transatlantic Trade and Investment Partnership (TTIP) was presented in the media by supporters as well as by critics. Consequently, the different points of view and statements were the focus of this specific analysis. It has been shown that a change in European quality standards has been recognized in the population and interest has developed. For example counter movements have been established. However, consumers should not be informed and educated, as an intensive debate on standards of the agri-food industry would automatically lead to a discussion about negative effects. As a result, both the presentation of TTIP supporters and TTIP critics kept the level of information on the agri-food industry low. In reports with a positive attitude towards TTIP, the relationship to the agri-food industry is lower, thereby avoiding the fear of consumers about the threat in this sector. An exclusion of possible negative effects is discussed. The benefits of TTIP for the EU were presented from an economic point of view or in other economic sectors. It was found that in none of the analysis items specific terms of the standards of agri-food industry were declared. The use of the term "standards" in the media does not correspond to the official definition of the European food law and was therefore just of general nature. The discussion about modification of protection standards in the food production was used as a polarizing and emotionalizing aspect in the reporting about the agri-food industry under the TTIP negotiations where the consumer can generally be assumed to be uninformed or non-expert. Indeed, there was a lack of consumer-oriented information.

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STUDY OF THE AGROECOLOGICAL TRANSITION IN EXTENSIVE AGRICULTURE IN THE SEMIARID REGION OF CÓRDOBA, ARGENTINA

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ABSTRACT

Extensive agriculture in the semi-arid region of Córdoba, Argentina, generated an intense degradation of the biophysical environment, a decrease in ecosystem self-regulation mechanisms, a considerable reduction of agrodiversity and the loss of associated environmental services. The physical-biological responses of different agroecological practices were evaluated for ten years in permanent macroplots located in three agricultural systems in order to study the transition towards more sustainable systems, which can be extrapolated to the rural area of central Córdoba. This process generated local information compatible with the farmers' technology. The results showed that *winter cover crops* allowed good weed control. Although they affected the soil water content, they did not produce significant differences in summer crop yields and they increased the level of surface organic carbon, thus mitigating the edaphic densification processes caused by no till farming in the medium term. *Crop rotation* contributed with more organic carbon to the system. *Open-pollinated maize varieties* produced a good yield of grains and biomass, even in years of marked water deficits, showing their adaptation to semi-arid conditions and their good plasticity. *Fertilization with vermicompost* in reduced doses increased grain yields and biomass production. The integrated management of these practices improved the balance and biological interdependencies that favoured biotic regulation between phytophagous and predatory arthropod populations as well as the regulation of spring weed populations.

Keywords: *permanent macroplots, agroecological practices, semi-arid environment, extensive crops.*

INTRODUCTION

In the last three decades, the extensive agricultural systems of the central region of Cordoba, Argentina, underwent transformations in their structure and function due to a process of productive simplification, which generated an intense degradation of the biophysical environment and a general decrease of the mechanisms of self-regulation. The agriculture specialized in commodity production, the adoption of transgenic materials in association with no-till farming (NT) and the use of chemical inputs contributed to this modification (Manzanal, 2017). The technological package (GMO soybean, NT and Glyphosate) favored the expansion of the agricultural frontier, replacing livestock and natural vegetation areas and traditional crops (maize, peanuts, sorghum and beans); this resulted in a drastic reduction of technological and biological diversity that caused the loss of environmental services associated with biodiversity (Alessandria, 2001). For example, the exclusive use of chemical supplies prevails as the pest control method, increases selection pressure on weeds and insects, and contributes to weakening regulatory mechanisms (Nicholls, 2006). Soybean monoculture negatively affects soil biophysical and chemical fertility due to its low residues contribution andit is considered an extractive crop because it does not generate surplus nitrogen (Zamar, 2004). Agroecology proposes process technologies with different practices that contribute to increase spatiotemporal biodiversity of agricultural ecosystems to provide ecosystem services, such as nutrient cycling, biotic regulation, genetic conservation and water regulation, which are linked to sustainability (Sarandón and Flores, 2014). Therefore, the favorable properties of the following agro-ecological practices are these:

The incorporation of *winter cover crops* (CC) provides an additional source of living cover and a significant amount of residues, improves soil organic carbon balance (Metay *et al*., 2007, Basanta *et al*., 2008), improves the soil physical condition increasing water aggregate stability, infiltration, total porosity and decreasing compaction (Villamil *et al*., 2006). In addition, grasses and legumes CC contribute to the input, recycling and availability of nutrients (Kuo and Jellum, 2000), inhibit the emergence of weeds (Kruidhof *et al*., 2009; Zamar *et al*., 2000), stimulate diversity (Ferreira *et al*., 2010) and provide food and shelter to predatory insects, parasites and parasitoids (Altieri, 1999). *Crop rotations* reduce the proliferation of pathogenic pests and microorganisms by disrupting their biological cycles when changing the plant species sequence (Altieri and Nicholls, 2012). They also modify the composition of weeds associated with each crop, reducing its effects and the development of resistance to chemical control. *Open-pollinated maize in the crop sequence* has better adaptation to the limiting conditions of the semi-arid zone, provides higher biomass volume, and decreases seed and feed supply costs for animal production (Kutka, 2011). The application of biofertilizers contributes to biological diversity and allows to restock nutrients extracted by the crops and to enhance the synergy among beneficial microorganisms ableto stimulate plant growth (Boraste *et al*., 2009). The use of *reduced doses of*

*vermicompostin extensive crops*is an incipient practice in no-till farmingthat contributes to root development and biomass production (Alessandria, 2013).
These practices were applied with the objective of evaluating the agroecological transition of extensive agricultural agroecosystems from the semi-arid zone of Córdoba, Argentina, in order to improve biophysical conditions, increase agrodiversity and reduce the incorporation of supplies. The transformations were proposed in close interaction with farmers to recover the local information and generate an adequatetechnological management and one that is compatible with the local socioeconomic conditions.

MATERIAL AND METHODS

The experience was developed in predominantly agricultural production systems in the town of Lozada (Santa Maria Department, Córdoba, Argentina). The studied farms are located in a geomorphological region of the Rafael García-Lozada basin called Central Planicie, with a complex of soils classified as typical argiustoles, with mostly silt loam texture (Zamar, 2004). The climate corresponds to that of semi-dry, semi-humid domain with water deficit, without thermal winter (Capitanelli, 1979), with an annual mean precipitation of 686 mm and

Figure 1. Map of Argentina with situation of the Province of Cordoba (left) and with designated locality of investigations (Department of Santa Maria) (right)

evapotranspiration of 850 mm annually, which determines the existence of periods with soil water deficiency (Vettorello, 2008).The experimental macroplots of 1500 square meters were established since 2005 to 2014 in three farms. A completely randomized block design (three production systems) was developed. The treatments were: consociated winter cover crop (poaceae and fabaceae), corn-soybean rotation and application of 200 kg / ha of vermicompost at the time of sowing (agrodiverse treatment) and soybean monoculture with chemical fallow without biofertilizer (control treatment).

The following biophysical variables were measured in both treatments:
Edaphic variables: bulk density (DA) at 0-5 and 10-15 cm depth (cylinder method); penetration resistance (with impact penetrometer); infiltration (single ring infiltrometer); organic carbon content and water-aggregate stability (Walker and Reuter, 1998) at depths of 0-5 and 5-20 cm. The water content was measured for the 0-100 cm profile at CC sowing, in its drying and at the time sowing of the summer crop.
Biological variables: aerial biomass of cover crop and summer crop; grain yield of crop; records of richness (R) and specific abundance of spontaneous plant species; richness of phytophagous arthropods and predators in summer crops with the vertical cloth method (Drees *et al*., 1985). We calculated an index that relates the richness of predatory and phytophagous arthropods (index: R predators / R phytophages) at different times of the summer crop cycle.

RESULTS AND DISCUSSION

The results obtained from the experiment are presented in Table 1 where we compare the average data of the initial situation (year 2005) of the biophysical conditions of the production system studied and the data of the treatments applied after ten years of experience.

Table 1. Average data of edaphic conditions in three macroplots in different farming systems

Soil dept (cm)	Organic carbon (%)	Bulk density (gr / cm3)	Water-aggregate stability (%)	Infiltration (mm/h)	Penetration resistance (Mpa)	Water Content (mm)
	Before start of the experiment (2005)					
0-5	1.08	1.23	39			
5-20	0.74	1.43	30			
				23.87	1.623	
	Status in year 2014: control					
0-5	1.39	1.47	46.6			
5-20	0.92	1.65	33.4			
				19.22	1.850	
	Water contents (mm) in 100 cm of soil depth: Cover crops sowing					152.67
	Cover crops drying					183.75
	Crop Summer Sowing					250.24
	Status in year 2014: agrodiverse treatments					
0-5	1.85([a])	1.38([a])	73.4([a])			
5-20	0.98	1.63	40.0([a])			
				24.44	1.598	
	Water contents (mm) in 100 cm of soil depth: Cover crops sowing					159.47
	Cover crops drying					101.06 ([b])
	Crop Summer Sowing					229.81

(a) Indicates significant differences with the initial situation (p<0,10); (b) Indicates significant differences with respect to the control (p<0,10).

In the agrodiverse treatment, organic carbon content and water aggregate stability were improved due to the contribution of carbon by the inclusion of grasses (maize and CC) (Basanta *et al*., 2013, Villamil *et al*., 2006). However, the values of bulk density, infiltration and penetration resistance did not show favorable changes due to the compression exerted by the continuous agricultural machinery traffic. This is common in the management of no-till practices, which produces soil densification (Schmidt and Amiotti, 2015) in extensive areas of the central region of Córdoba.
The results of the effects on the water content of the soil up to one hundred centimeters of the profile by the winter CC showed that the control treatment presented higher accumulated water content than the treatment with CC at the time of its cycle cut. This result coincides with that observed by Restovich *et al*. (2012) and Basanta *et al*. (2008) and the reason of it is that CC utilized much of the profile water for biomass production, which averaged 1255.2 kg / ha. In the first forty centimeters of the profile, there were no significant differences at the time of sowing of the summer crop between the two treatments, caused by a recharge of the profile between the drying time of the CC and the moment of the sowing of the summer crop due to the precipitation occurrence.

Table 2. Average data of the biological conditions in three macroplots designed in different agricultural systems

Final conditions after ten years of the experiment			
Control treatment (soybean monoculture)		Agrodiverse treatment	
Biomass accumulated over 10 years (Kg MS / ha summer crops + winter cover crops)			
49513.8		95617.3(*)	
Soybean (2013)	Maize (2014)	Soybean (2013)	Maize (2014)
Reproductive biomass (Kg MS / ha)			
2214.9	2955.6	3241.7(*)	6714.3
Vegetative biomass (Kg MS/ha)			
3990.2	5178.6	5712.5(*)	16376.3
Spontaneous spring-summer vegetation			
Coverage (%)	Richness	Coverage (%)	Richness
18.3	10.3	2.1(*)	2.0(*)
Arthropods in soybean crop in different phonological stages (year 2013): Richness (Predator / phytophagous index)			
R2 stage: 0.64	R6 stage: 1.27	R2 stage: 1.44(*)	R6 stage: 1.67

(*)Indicates significant differences with respect to the control (p<0,10)

The accumulated aerial biomass production of the agro diverse treatment during the experiment was significantly higher (93.11%) than in the control. Both reproductive and vegetative biomass show significant differences in favor of the agro diverse treatment. Rotation and CC increased the amount and diversity of residues, favoring the temporal distribution of their inputs and contributed to improve the biological activity of the soil. This can be attributed to the fact that

longer periods of active root growth would provide an environment that is more favorable to the development of microbial populations (Ferreras *et al*., 2010). Maize varieties produced grain yield and biomass volume similar to those of regional averages, even in years with marked water deficits, indicating their adaptation to semi-arid conditions and adequate plasticity (Kutka, 2011).
The consociation used as winter cover crop significantly reduced the spring weed community coverage and richness (Kruidhof *et al*., 2009).
In the last two years of the experiment, it was observed that soybean crop in the agrodiverse treatment maintained the best values of the predator / phytophagous richness index in relation to the control treatment during its reproductive stages (R2 and R6). The following year, when maize was introduced into the sequence, a significant drop in the abundance and richness pattern of both arthropod groups occurred in this crop; these results could be explained by a temporary disruption of the biological cycles that could affect the presence of specific pests (Altieri and Nicholls, 2012).

CONCLUSIONS

The agroecological transition process for extensive agriculture proposed in this work together with the implementation of a set of suitable practices to the semi-arid environment provided greater biodiversity and reduced the intensity of external resources´ use. The soil biophysical conditions were modified with a better water-aggregate stability and an increase of the organic carbon that contributed to progressively mitigate the processes of soil degradation due to monoculture. The summer crops yields and their contribution of residues to the soil were increased. The equilibrium and biological interdependencies of phytophagous and predatory populations were improved as well as the regulation of spring weed populations.

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FOUR NEW STRATEGIES TO GROW THE ORGANIC AGRICULTURE SECTOR

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ABSTRACT
This paper presents four new strategies for growing the organic agriculture sector. Globally there are 51 million hectares of certified organic agriculture land and a further 39 million hectares of wild culture land. For the past two decades organic agriculture has been growing at 11.9% per annum, thereby doubling the size of the sector every six years. Nevertheless, despite ten decades of advocacy for organics, only 1.1% of the world's agricultural land is certified organic. From the outset, the strategy has been to advance the sector 'one farm at a time'. This strategy has left the organics sector well short of the vision of the pioneers of organics who saw organic farming as a universal solution and a practice suited for all farmers and all agriculture. Successful exemplars of marketing strategies of converting 'one consumer at a time' remain elusive. Recent years have seen the development of new strategies for growth of the organics sector. The strategy of 'one crop at a time' has proved successful for the Dominican Republic which now produces 55% of the world's certified organic bananas. The strategy of 'one state at a time' has seen the state of Sikkim (in India) declare itself as the first Indian organic state. Meanwhile, other Indian states are working towards all-organic status, including Mizoram, Goa, Rajasthan and Meghalaya. The strategy of 'one island at a time' has seen the Pacific islands of Cicia (in Fiji) and Abaiang (in Kiribati) commit to 100% organic farming. The strategy of 'one country at a time' sees Bhutan with the stated goal of being the world's first organic nation. These new strategies rely for success on the tripartite cooperation of government, community and commerce. In the meantime, as these new strategies play out, only 11 countries report that 10% or more of their agriculture land is organic, while 111 countries report that less than 1% of their land is certified organic, which reveals great potential for new growth strategies.

Keywords: *India, Sikkim, Bhutan, Fiji, Kiribati.*

INTRODUCTION
The vision of the pioneers of organic agriculture was a global vision, not a niche vision. The Haber-Bosch process revolutionised warfare and then agriculture. The demonstration by Fritz Haber and Carl Bosch, in 1909, of a process for capturing

nitrogen from the air, unleashed the availability of cheap and unlimited explosives and the massive and unprecedented destruction of the Great War (Charles, 2005; Paull, 2009a; Smil, 2001). After WW1, this massive industrial output of nitrogenous material was quickly repurposed from military explosives to synthetic agricultural fertilizer.

When Rudolf Steiner railed against synthetic fertilisers at his Agriculture Course in 1924 it was the first rallying cry against the upcoming domination of food production by the chemical industry. Steiner called for a differentiated agriculture, one not dependant on synthetic chemicals (Paull, 2011; Steiner, 1924). It was an international project right from the outset. At Steiner's Agriculture Course of 1924 there were delegates from six countries: Germany; Poland; Austria; Switzerland; France; and Sweden (Paull, 2011). Steiner initially differentiated his agriculture as 'Anthroposophic farming'. This evolved into 'biodynamic farming' with the publication of Ehrenfried Pfeiffer's book *Bio-Dynamic Farming and Gardening* which was published in five languages: English, German, Dutch, French, and Italian. Following the first biodynamic farming conference in Britain (in 1939) and soon after that the outbreak of WW2, the concepts were quickly evolved by Lord Northbourne into 'organic farming' and presented in his 1940 book *Look to the Land* (Northbourne, 1940; Paull, 2014b; Pfeiffer, 1938).

The vision of organic agriculture was never of a niche agriculture but rather of an agriculture for all. This paper presents some new strategies for growing the sector.

MATERIALS AND METHODS

Global statistics have been published annually by the Swiss Research Institute of Organic Agriculture (FiBL), in association with various associates, beginning in the year 2000 through to 2017 (Willer & Lernoud, 2017; Willer & Yussefi, 2000). The present paper draws on the organic hectares and organic producers statistics from the FiBL reports. These statistics underestimate the extent of the organics project since they do not account for non-certified organic production (for which statistics are not available). The identification of new strategies reported here for the uptake of organics draws on the analysis of a variety of accounts including research papers, reports, and media accounts.

RESULTS AND DISCUSSION

Over the past two decades the reported area devoted to organic agriculture has been growing year on year by 11.9% which is to say it is doubling every six years (with 7.5 m ha of certified organic agriculture reported in 2000 and 50.9 m ha reported in 2017) (Willer & Lernoud, 2017; Willer & Yussefi, 2000) (ultimatecalculators.com) (Fig. 1). Nevertheless, organic agriculture now accounts for just 1.1% of global agricultural hectares. One hundred and seventy nine countries currently report organic agriculture statistics. However the "big five" (Australia, Argentina, USA, Spain, and China) account for 62% of the total while the remaining 174 countries account for 38% of the global total (Fig.2).

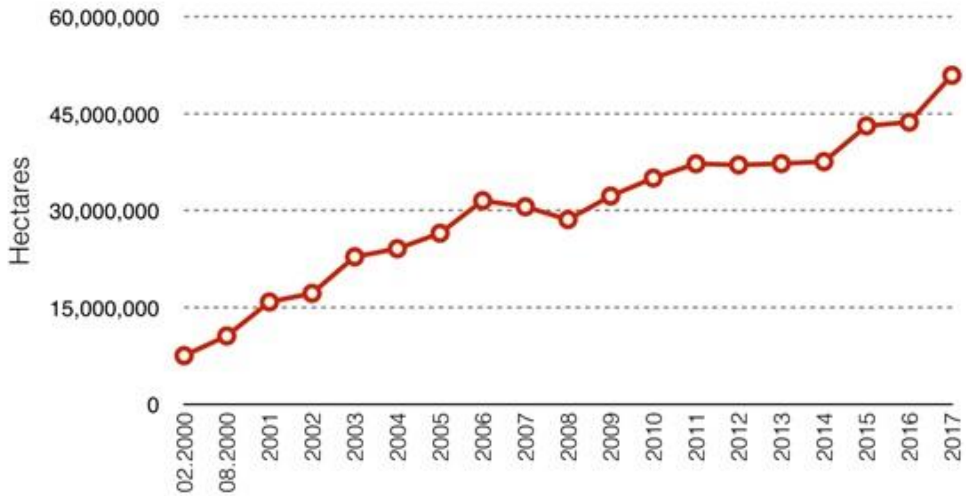

Figure 1. Certified organic agriculture hectares have grown at nearly 12% per annum for the past two decades but currently account for only 1.1% of world agriculture (Source: (Paull, 2017a).

One farm at a time

One-farm-at-a-time has been the ‘classic’ method of the uptake of organic agriculture since the time of Rudolf Steiner. The Experimental Circle was the original organic agriculture research entity. It was geographically diffuse, with members throughout continental Europe, as well as Britain, Australia, New Zealand and USA. In their application to join, each member of the Experimental Circle agreed to put Steiner’s ideas to the test and they nominated their test site, usually a farm. In Australia, for example, Ernesto Genoni nominated Dalmore Farm in Victoria (in 1928), and Ileen Macpherson nominated Demeter Farm at Dandenong (in 1936), an outer suburb of Melbourne (Paull, 2014a, 2017).

Organics advocates have followed an uptake strategy of one-farm-at-a-time since those early days. These pioneers of organics clearly had in mind an agriculture for all. There is no mention of a niche agriculture in the works of the early advocates of organics. And yet, after nine decades of advocacy what we have is a niche agriculture accounting for 1.1% of global agriculture and too close to oblivion for comfort. Even with a buffer of non-certified organic farming of unknown size, it is ‘too niche’ for complacency and is a cause for impatience.

Recently, alternative strategies for growing the organics sector have emerged which are less ad hoc and more strategic; four such strategies are described in this paper.

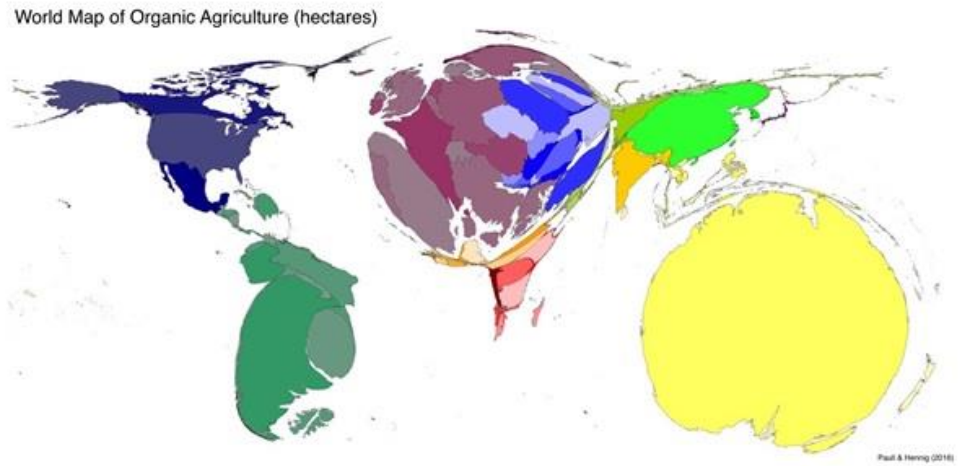

Figure 2. World map of organic agriculture: a cartogram with countries sized according to their reported organic agriculture hectares (Source: Paull & Hennig, 2016).

One-state-at-a-time

In 2003 the Indian state of Sikkim, in the foothills of the Himalayas, began the journey to 100% organic agriculture and this goal was achieved in 2016. It is the first Indian state to achieve such a goal and now other states of India are seeking to emulate this achievement.

In January 2016 India's Prime Minister, Narendra Modi, declared Sikkim as "India's first organic farming state" (Chief Minister's Office, 2016, p.23) He stated that: "Sikkim has become an example for the country. They have remained resolutely focused on their organic farming mission ever since the mission was declared in 2003 … I salute the entire farming fraternity of Sikkim who … didn't take their eye off the goal … They moved on resolutely, ignoring skepticism … Sikkim has been an example. This organic air should not be contained just within Sikkim but it has to spread all over India" (Narendra Modi in Chief Minister's Office, 2016, pp.629-30)

In 2003 the Chief Minister of Sikkim, Pawan Chamling, declared the "goal of making Sikkim entirely organic" (Chief Minister's Office, 2016, p.23). It was an ambitious objective for a "Total Organic State" and was to be backed up with policy interventions. An action plan "Going for Organic Farming in Sikkim" was prepared. Four points of the action plan were "(1) promotion of on-farm production of organic manures; (2) capacity building; (3) establishment of bio-fertilizer production units; and (4) establishment of soil testing laboratories" (Chief Minister's Office, 2016, p.392).

The Sikkim State Organic Board (SSOB) was established to oversee the implementation of the plan. A State Organic Committee … The implementation began with one hundred villages which were declared as "bio villages" and farmers were trained in organic practices. The success of the inaugural bio villages was a proof of concept for Sikkim villages and attracted other farmers. By October 2009 there were 396 bio villages. Under the Sikkim Organic Mission of 2010 farmers were provided with seeds, manure and training. In 2014 the Sikkim Agricultural, Horticultural Input and Livestock Feed Regulatory Act banned the use of synthetic fertilisers and pesticides. By 31 December 2015, 75,000 hectares of agricultural land were certified organic (Chief Minister's Office, 2016, p.23). Nahendra Modi declared: “Sikkim has paved its way into history and has set an example for the entire world that nature needs care and protection” (Chief Minister's Office, 2016, p.387)

One farmer Dhanpati Sapkota, explained the conversion experience: “Since 2003, crop yield was too low for two or three years than they used to while using chemical fertilizers. Gradually as we started using cow dung and vermicompost, micro-organisms multiplied and today our vegetables and crop yield is double than we used to while using chemicals” (Chief Minister's Office, 2016, p.386). “The youth of Sikkim have embraced organic farming as a fashionable profession” (Chief Minister's Office, 2016, p.382).

Sikkim is a success story in its conversion to organic bringing its vision progressively to fruition over a period of thirteen years (2003-2016). Six organic certifiers are involved in this project which has included farmer group certification (SOM, 2014). Sikkim now produces 6.5% of the organic production of India (80,000 tonnes of 1.24 million tonnes) (TOI, 2017b). Six products have been targeted for export: cardamom, ginger, turmeric, buckwheat, tea and cymbidium orchids (Chief Minister's Office, 2016). There are now prospects for organic tourism.

Multiple states of India have expressed aspirations of replicating Sikkim’ success including: Goa, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, Mizoram, Rajasthan, Tamil Nadu, Uttar Pradesh, and Uttarakhand (Chief Minister's Office, 2016; Mizoram Department of Agriculture, 2016; NDTV, 2016; TOI, 2003, 2014, 2015a, 2015b, 2016, 2017a).

India has 1.18 million hectares of certified organic agriculture (ranking 9th in the world), 585,200 organic producers (ranking 1st in the world) and 0.7% of its agriculture land is organic (ranking 81st in the world). The Sikkim roadmap to organics offers a path to grow these statistics in India and elsewhere.

One-country-at-a-time

Bhutan has the aspiration to be the world’s first organic country. “Bhutan could become the first country in the world to become ‘organic’ by 2020, which could have a huge promotion value for Bhutanese products” (DoA, 2006, p.17). The Constitution of the Himalayan country of Bhutan enshrines happiness as a national value (Royal Government of Bhutan, 2008). The King of Bhutan, Jigme Singye Wangchuck declared in 1972 the novel guiding maxim for Bhutan: “Gross National Happiness is more important than Gross National Product” (IIEP, 2015).

Organic farming offers employment opportunities. “Organic farming is labour intensive but also has the potential to generate higher labour income than conventional farming. The introduction of organic farming could stall urban migration by giving better paid work opportunities on the land and in rural enterprises" (DoA, 2006, p.17). Food security and biodiversity can be enhanced. "The Organic farming system has the potential to increase household food security and income while conserving biodiversity on farms" (DoA, 2006, p.17).

At the 2013 Delhi Sustainable Development Summit, Bhutan's Minister for Agriculture and Forestry, Dr Pema Gyamtsho, confirmed the aspirations for his country to be the world's first country to go 100% organic. Gyamtsho stated that: "Ours is a mountainous terrain. When we use chemicals they don't stay where we use them, they impact the water and plants. We say that we need to consider all the environment. Most of our farm practices are traditional farming, so we are largely organic anyway". He added that "we are Buddhists, too, and we believe in living in harmony with nature. Animals have the right to live, we like to to see plants happy and insects happy" (Paull, 2013a).

Attention has been paid to the language of organics. "Rangshin Sanam … is suggested as the Dzongkha term. The words 'certified organic' should be reserved for products with formal certification mainly for export" (DoA, 2006, p.31).

The National Framework for Organic Farming document recognises that there can be some resistance to the transformation to organics and that this may be especially from the agrotocracy. "The biggest challenge in conversion will be the conversion of the mind sets of the research and extension staff who have been trained according to traditional agrochemical based education … organic farming can be promoted and adapted to Bhutan and incorporated into Bhutanese agriculture as a way of life" (DoA, 2006, p.41).

A past prime minister of Bhutan, Jigmi Thinley, writes that: "Since agriculture is the foundation of all civilizations, I believe organic agriculture, as the only way of growing sustainable food, must constitute a primary thrust in any new development paradigm" (Thinley, 2014).

Thinley states that the country "must remain mindful of the reality that the ultimate well-being, happiness and the very survival of the human race together with all other sentient beings will depend on organic agriculture. The Royal Government of Bhutan on its part, will relentlessly promote and continue with its endeavour to realize the dreams we share - of bringing about a global movement to transit to organic agriculture so that crops and the earth on which they grow will become genuinely sustainable. And so that agriculture will contribute not to the degradation but rather to the resuscitation and revitalization of nature" (Thinley, 2014).

When the National Framework for Organic Farming in Bhutan was published (DoA, 2006), Bhutan did not appear in the list of countries reporting organic agriculture (Willer & Yussefi, 2006). Currently there are 6,950 ha certified organic hectares in Bhutan (Willer & Lernoud, 2017). This is an impressive rate of growth from a very modest base, nevertheless this accounts for just 1.3% of Bhutan's agriculture area.

Whether Bhutan can deliver on its bold ambition to be 100% organic and whether it can transmute its rhetoric to reality remains to be seen. The rate of growth of Bhutan's organic sector is impressive yet the actual achievement is modest, unless there is some cryptic-cache of unreported uncertified organic hectares. In the meantime Bhutan might learn the lessons from its neighbour, the Indian state of Sikkim, which has, in a comparable timeframe, achieved what Bhutan aspires to achieve.

One-crop-at-a-time

The Dominican Republic produces 55% of the world's organic bananas (FAO, 2017). In 2015 more than 240,000 tonnes of organic bananas were exported by the Dominican Republic with almost all (95%) the organic banana exports shipped to the European Union (FAO, 2017). The annual world production of bananas is 125.6 million tonnes (Calberto, Staver, & Siles, 2015) so the Dominican Republic is a minor player on the world stage of banana production accounting for less than 1% of global banana production. The Dominican Republic has a reported 163,936 hectares of certified organic hectares which is 7.0% of its total agricultural land (Willer & Lernoud, 2017). There has been a concerted effort to convert banana production to organic and this has been supported by the Banana Accompanying Measures (BAM) program with funding from the European Union (ICAD, 2017).

The Dominican Republic experience is that the focus on the conversion of a particular crop to organic provides the opportunity to dominate the world market. It offers benefits for farmers by providing market opportunities by creating critical mass for certification, export, product aggregation, and knowledge sharing and cooperation. The Dominican Republic is a successful exemplar of this one-crop-at-a-time strategy for conversion to organic.

One-island-at-a-time

At least two Pacific islands have declared their intention to transition 100% to organic. The communities of the Fijian island of Cicia (3400 ha) and of the Kiribati island of Abaiang (1750 ha) have committed to organic production (AgLaw Centre & Mobium Group, 2017). These islands rely predominantly on subsistence farming. Agricultural products including coconut, banana, breadfruit, and papaya may offer organic export opportunities as fresh or processed products.

Australia is the world's largest island and with 22.69 million organic hectares accounts for 45% of the global total (Willer & Lernoud, 2017). However there continues to be little or no institutional or policy support for organics in Australia and no government or national vision for an Organic Australia (Paull, 2013b). For the immediate future we can foresee that the past and current experience of conversion one-farm-at-a-time based on individual decisions will continue into the future. The island state of Tasmania would appear to be a logical magnet for conversion to organic but currently just 4003 hectares of agriculture land is certified organic despite organics advocacy dating from 1946 (AgLaw Centre & Mobium Group, 2017; Paull, 2009b, 2010). The Falkland Islands (Malvinas) has exhibited an uneven commitment to organics. In 2009 no organic agricultural land was reported (Willer & Kilcher, 2009). In the following year, 414,474 ha were reported (accounting for 36.9% of the total agriculture) (Willer & Kilcher, 2010). This remained little changed for the following six years until most recently, in 2017 a dip to 139,041 ha was reported (12.5%) (Willer & Lernoud, 2017).

CONCLUSION

The message from Pawan Chamling in Sikkim is: "Let us work together to recreate a world that is Totally Organic" (Chief Minister's Office, 2016, p.636). As a mission statement it carries the authority of someone who has actually done it for his own state of Sikkim, India. While some satisfaction can be drawn from the global annual growth rate of 11.9% compounding over the past two decades, this must be tempered with disappointment that certified organic agriculture still only accounts for 1.1% of global agriculture hectares. The four new strategies for growth described herein, suggest that it is time to explore the unit of conversion to organics. The beginning of the supply chain is the farm and the end is the consumer. Conversion to organic has traditionally focussed on these two end points, that is converting the farm (the push factor) or converting the consumer (the pull factor). Alternative units of include the country, the state, the island, and the crop. The Indian state of Sikkim is the standout success story with the whole state now organic. This success of one-state-at-a-time conversion may, in time, be replicated in, some or all other Indian states. However there is nothing uniquely Indian in the Sikkim experience. Sikkim has created a roadmap that has lessons for not just other states of India but for other states around the globe. There is some political risk for a political party advocating this path so it needs some political courage as well as vision as we witness in Sikkim. The Caribbean country of the Dominican Republic has demonstrated how a minnow in the sphere of banana production can be a giant in the realm of organic banana production. With 55% of the global production of organic bananas, the Dominican Republic is a success story as an exemplar of the organics strategy of one-crop-at-a-time. Its experience can yield valuable lessons for others to pursue a one-crop-at-a-time strategy for conversion to organic. The country of Bhutan is pioneering a one-country-at-a-time strategy. Bhutan is strong on rhetoric and it remains to be seen if it can carry this rhetoric through to reification, and in what time frame. Similar sentiments apply to the Pacific islands of Cicia and Abaiang - will they have the will, the perseverance, and the wherewithal to carry their organic vision through to reality?

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APPLICATION OF AUGMENTED DESIGNS FOR FIELD EVALUATION OF BREAD WHEAT DOUBLED HAPLOID LINES: A PRELIMINARY REPORT

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ABSTRACT

Doubled-haploid is an effective method to produce 100% homozygous lines in a single generation accelerating the release of new varieties and reducing the corresponding expenses. However, the existing problem in cases where the adequate quantity of seeds is limited is the inability to evaluate new germplasm in replicated experiments. In his attempt to confront this problem, Petersen proposed in 1985 the evaluation of new germplasm to be based on its division in blocks and selection to be performed regarding the yield of the randomly repeated control in each block. The aim of the present study was to use the aforementioned method to evaluate preliminary 37 doubled-haploid lines (DHL). The parental varieties of the DHLs,Greek cultivars “Acheloos” and “Vergina”, were used as controls. For the purpose of the study, 35 main spikes, one form each DHL and control, were used. The length of the spikes was measured, the number of spikelets was counted, and the 1000 kernel weight and total yield were recorded. The data analysis revealed that only one line exceeded the mean number of spikelets of the controls, one exceeded the mean yield of the controls and two exceeded the mean 1000 kernel weight of the controls (one was even better than the best control). The reported results indicate the presence of valuable genetic variability among the DHL after crossing cultivars “Acheloos” x “Vergina”. Further research is needed, after DHLs multiplication, using more plants and locations to draw more reliable conclusions.

Keywords: *block, control cultivar, traits, analysis, genetic variability.*

INTRODUCTION

The main problem in evaluating segregating generations of new germplasm is the inadequate quantity of seeds to conduct replicated experiments (Fehr, 1987). For this to be faced, various approaches have been proposed. Papadakis (1935) recommended the use of the adjusting control in order to select the best genotypes. Fasoulas suggested the honeycomb method to overcome this problem (Fasoulas, 1973). This method uses small quantities of seeds, which must be sownat least one meter apart to avoid competition of the resulted plants and has replaced plot evaluation with single plant evaluation. Another interesting designto evaluate large numbers of new selections was proposed by Petersen (1985). Petersen revised the "Augmented designs" that were originally developed by Federer (1961) and Federer and Ragavarao (1975).According to the aforementioned designs,the evaluationcan be based on the division of the new germplasminto blocks and selection to be performed regarding the yield of the randomly repeated control or controls within each block (Petersen, 1985). This will enable the breeder to perform a valid statistical analysis despite the large number of new selections.

Anther culture is a well-known and effective method to produce 100% homozygous lines in a single generation accelerating the release of new varieties and reducing the corresponding expenses (Henry and de Buyser, 1990;Deyao and Xigan, 1990; Hussain *et al.*, 2012). However, as in the case of segregating generations, the main problem isstill the inadequate quantity of seeds limiting the inability to evaluate the produced doubled haploid lines in replicated experiments. Furthermore, over locations evaluation seems to be impossible resulting in a considerable delay of estimating the yield (mainly) and quality potential of the selected material. In this case, the Petersen's approach is also suitable since the parental lines can be used as the repeated controls.

The aim of the present study was to use the augmented designs to evaluate preliminary 37 doubled-haploid lines (DHL) produced after anther-culture of the F_1 generation after crossing the Hellenic commercial cultivars "Acheloos" x "Vergina".

MATERIALS AND METHODS

A. Plant material

For the purpose of the study 37 doubled haploid (DH) lines produced by anther-culture of the F_1 generation after crossing the Hellenic commercial cultivars "Acheloos" and "Vergina" were used. The parental cultivars of the F_1 generation were originally used in the cross because "Acheloos" is a high yielding potential cultivar, responding well to anther-culture and cultivar "Vergina" is a broad adapted variety, but with null-response to anther-culture (Zamani*et al.*, 1998). The 37 DH lines were described in the past by Rigas*at al*., (2008) but without and statistical evaluation due to small quantities of seeds. The DH lines and the control cultivars were sown in autumn 2015 in a field at the University Farm of Thessaloniki, Northern Greece, in a loam (L) soil (TypicXerorthent) with pH 7.8 organic matter content 13.4 g kg^{-1},N-NO_3 38 mg kg^{-1},P (Olsen) 26 mg kg^{-1} and K 156.6 mg kg^{-1} (0 to 30 cm depth). Seedbed preparation included mouldboard plough, disc harrow and cultivator. Nitrogen and P_20_5 at 80 and 40 kg ha^{-1}, respectively, were incorporated into the soil as diammonium phosphate (20-10-0) before sowing. The crop was kept free of weeds by hand hoeing when necessary.

B. Method

Despite the considerable effort to keep the plants in good farming conditions, a severe attack by birds resulted in unequal number of plants per DH line. For this it was decided 35 main spiked from each DH line to be used in the study. The following traits were evaluated: spike length, number of spikelets per spike, yield in g and 1000 kernel weight in g. The DH lines were divided in four blocks and the parental cultivars "Acheloos" and "Vergina" were used as controls. In Augmented designs the effect of each block is estimated by the formula:

$r_j = 1/c(B_j\text{-}M)$,

where c: is the number of controls, B_j: is the sum of all controls in j block and M: is the sum of all means.

The 5% generalLSD for comparing an adjusted selection yield with the mean yield of a controlis calculated by the formula

$$LSD = t_{0.5,3}\sqrt{Svc^2}$$

df= is the block number-1.

Thedifferencebetween an adjusted selection yield and a control mean is

$Svc^2 = (b-1)(c-1)/bc$,

where b: is the number of blocksand c: is the number of controls.

The LSD value of each block is estimated by adding the value r_j of each block to the general LSD value. In order a DH line to be selected it has to exceed the value of LSD + the mean value of the controls (*) or the value of LSD + the mean value of the best control (**)

Table 1.Spike length, number of spikelets per spike, yield in g and 1000 kernel weight of the 37 DH lines and the controls.

S. No.	Genotype	Number of spikelets	Spike length	Yield (g)	1000 kernel weight
A1	Acheloos	18.37	10.39	61.9	37.85
2	Vergina	19.28	12.28	32.7	24.65
3	24	19.7	13.09	27.6	19.95
4	25	17.7	8.6	31.6	33.83
5	26	18.9	8.04	38.0	38.0*
6	27	20.2	8.62	40.5	41.85**
7	52	18.6	11.4	49.9	38.28*
8	54	22.5	10.12	76.0*	32.91
9	56	23.4	9.92	58.7	25.42
10	57	21.9	10.1	40.9	29.13
11	59	22.3	9.62	44.9	30.58
B12	Acheloos	20.1	10.12	46.6	36.57
13	Vergina	14.84	11.54	31.0	33.01
14	60	23.2*	10.02	53.7	29.28
15	90	22.1	9.9	54.3	29.61
16	106	20.9	11.78	50.7	33.01
17	107	21.9	10.3	44.9	28.4
18	108	21.7	10.7	33.0	24.3
19	120	21.2	10.9	47.3	34.75
20	121	22.5	12.91	43.9	24.33
21	122	22.4	12.5	41.6	26.93
22	123	22.14	12.6	47.6	28.79
C23	Acheloos	19.26	9.48	45.9	35.57
24	Vergina	22.1	12.35	45.9	29.45
25	125	22.4	12.4	45.2	24.88
26	126	18.51	11.97	55.5	36.29
27	127	23.03	13.2	36.1	24.68
28	128	17.9	11.37	24.3	21.83
29	151	16.9	10.9	34.3	30.94
30	152	19.2	9.45	48.8	31.02
31	156	17.85	10.7	36.0	30.72
32	157	19.2	10.8	44.7	32.43
33	173	15.0	10.0	26.7	26.98
D34	Acheloos	19.25	15.25	57.4	34.48
35	Vergina	16.11	11.4	22.8	24.91
36	191	17.4	9.7	36.2	26.2
37	255	15.54	11.48	16.0	24.11
38	262	18.4	11.7	36.7	34.4
39	282	19.46	10.85	21.6	24.8
40	295	20.8	10.88	36.9	27.33
41	297	18.54	11.35	30.9	25.85
42	306	19.37	10.55	32.0	35.21
43	308	18.54	10.58	29.6	29.29
44	314	19.31	11.08	22.8	31.12
45	352	17.57	10.85	29.9	25.13

* DH line differing from the mean value of the controls,
** DH line differing from the best control.

Table 2. LSD value for each examined trait according to mean of controls and best control.

Block number	LSD + mean of controls				LSD + best control			
	No of spikelets	Spike length cm	Yield in g	1000 kernel weight g	No of spikelets	Spike length cm	Yield in g	1000 kernel weight g
A	23.92	15.45	68.61	37.03	24.5	15.75	78.53	41.08
B	22.56	14.94	60.11	40.58	23.15	15.24	70.03	44.63
C	25.77	15.03	67.13	38.30	26.36	15.33	77.05	42.35
D	22.77	17.44	61.33	35.70	23.36	17.74	71.25	39.75

RESULTS AND DISCUSSION

It is well established that crossing two cultivars, one responding well and one non-responding, results in a F_1 generation which responds well to anther-culture (Zamani*et al*., 2000). This permits to exploiting the valuable traits of the non-responding cultivar. Thus, it was possible to benefit the good response of cultivar Acheloos and produce well responding DHLs after the aforementioned cultivar to the non-responding cultivar Vergina. Indeed F_1 plants were produced after crossing the aforementioned cultivars but the quantity of seeds produced was not sufficient to conduct replicated experiments. For this, application of an approach like augmented designs as inevitable.

The analysis of the obtained data revealed that in number of spikelets per spike despite the recorded variability, only one DH line (No 60) exceeded the mean value of the controls (Table 1). The LSD values per each examine trait are presented in Table 2. The values ranged from 15 spikelets per spike in DHL No 173 to 23.2 in DHL No. 60. In spike length, the values ranged from 8.6 cm in DHL No. 25 to 13.09 cm in DHL No. 24. Again, despite this variability the recorded differences were not also significant. In yield, the lowest value was recorded in DHL No. 255 (16g) and the highest in DHL No. 54 (76g). This last line differed significantly from the mean yield of the controls. Finally, regarding the 1000 kernel weight the values ranged from 19.95g in DHL No. 24 to 41,85b in DHL No. 27. In this trait, two DHLs were significantly better than the mean value of the controls and one line (No. 27) was significantly better than the best control. Dramalis *et al*. (2006) also reported the presence of valuable variability in the offspring of DH lines. In this last study, honeycomb selection was used.

Example of estimating LSD value per examined trait: the case of yield

Control	Block number				Total yield	Mean yield
	A	B	C	D		
Acheloos	61.9	46.6	45.9	57.4	211.8	52.95
Vergina	32.7	31.0	45.9	22.8	132.4	33.1
TOTAL	94.6	77.6	91.8	80.2	344.2	86.05
				Control	mean	43.03

Block effect: r_j= 1/c (B_j – M), where B_j: sum of all means in j block and M: sum of all means
r_1 = (96.4-86.05)/2 =4.275
r_2 = (77.6-86.05)/2 = -4.225
r_3 = (91.8-86.05)/2 = 2.8
r_4 = (80.2-86.05)/2 = -3
Analysis of variance table

Source	DF	Sum of squares	Mean squares	F-value	Prob.
Varieties	1	788.05	788.045	6.59	0.0826
Replications	3	105.90	35.298	0.30	0.8284
Error	3	358.54	119.512		
Total	7				

Svc^2 = (b-1) (c-1) MSE/bc = (4-1)(2-1)119.512/8 =44.82
LSD = $t_{0.5,\,3}\sqrt{Svc^2}$= $3.182\sqrt{44.82}$ = 21.30

LSD 1st block =21.3 + 4.275 = 25.575
LSD 2nd block =21.3- 4.225 = 17.075
LSD 3rd block = 21.3+2.8 = 24.1
LSD 4th block = 21.3 – 3 = 18.3

LSD + mean of controls	LSD + best control
Block A = 25.58 + 43.03 = 68.61	Block A = 25.58 + 52.95 = 78.53
Block B = 17.075 + 43.05 = 60.11	Block B = 17.075 + 52.95 = 70.03
Block C = 24.1 + 43.03 = 67.13	Block C = 24.1 + 52.95 = 77.05
Block D = 18.3 + 43.03 = 61.33	Block D = 18.3 + 52.95 = 71.25

CONCLUSIONS

The results presented in the study support the existence of genetic variability between the doubled-haploid lines produced after anther-culture of the cross “Acheloos” x “Vergina”. The parental cultivars were used as controls and this was the main difference from the classic augmented design analysis. Application of the augmented designs was found suitable enough in distinguishing genotypes in cases where the number of seeds excludes the establishment of replicated experiments. However, the described procedure must be repeated after multiplying the doubled-haploid lines involving more plants in the experiment. This will ensure greater credibility in drawing conclusions and in selecting the most promising line.

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RECENT EVOLUTION OF THE CONSUMPTION OF ORGANIC FOOD PRODUCTS IN BELGIUM

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ABSTRACT

The consumption of organic food products in Belgium increased significantly and continuously between 2008 and 2016, growing from 1.5 % to 3.2% of the total consumers' expenses for fresh food products. Nine percent of Belgian families buy organic food products at least once per week and represent 60% of the total expenses. The total expenses per capita reached nearly 33€ in 2015. The main expenses concerned dairy products (6.72 €/capita), vegetables (5.57) and fruits (4.40). These expenses have been globally increasing. The market share of organic products considerably varies from one type of food products to another: it is the highest for meat substitutes (24.6% in 2016) and eggs (14.0%), while it reaches 3.0% for dairy products, 6.6% for vegetables and 4.3% for fruits. These percentages have been globally increasing during the last years. Vegetable products have been proportionally more sold than animal products compared to the situation in the conventional market. Supermarkets and specialized shops are the most common distribution channels, direct sales on farms and open markets remaining marginal. Families with high income and children and retired people with high income are those who spend the most on organic food products. This evolution is supported by the Walloon government through the organic farming development plan.

Keywords: *organic products, food consumption, Belgium, evolution*.

INTRODUCTION

After several decades of an ever greater use of chemical inputs in agriculture, in order to increase the production of food to satisfy an ever increasing demand, several important pollution problems appeared and organic farming began to become an alternative during the 1980's. After a slow development, the evolution became more and more rapid and today organic farming is considered as one of the solutions to get a more sustainable world (Burny et al., 2016; Petrescu et al., 2015; Petrescu-Mag et al., 2016). Organic farming has so to be placed in a broader context, taking into account rural development, environment and society (Daugbjerg and Sønderskov, 2012), the relation between agriculture and environment becoming a priority of EU and international policies (Brezuleanu et

al., 2013; Gazquez-Abad et al., 2011). However, the success of organic farming depends not only on production, but also on consumption, which is not obvious, as prices of organic products are significantly higher than the prices of conventional ones. However, in the developed and richest countries, organic products are more and more successful among consumers, because they think organic food could protect their health and the environment (Petrescu and Petrescu-Mag, 2015) or have better sensory attributes (Bryla, 2016; Tobler et al., 2011).
Below is presented and discussed the evolution of the consumption of organic products in Belgium, trying to answer questions like: what is the evolution of the market share of organic products in the total food market? What is the market share of organic products in different types of food products? What are the expenses per capita and per year for the main organic food products? What are the main distribution channels of organic food products?

MATERIALS AND METHODS

The main statistical data are extracted from the annual report “Les chiffres du bio” published by Biowallonie asbl, the association of stakeholders officially representing the organic sector in Wallonia (South of Belgium). The data concerning consumption of organic products come from a survey organised by the private company GFK, working for the Belgian institutions in charge of the promotion of agricultural products (VLAM in Flanders and APAQ-w in Wallonia). The survey is done in such a way that it meets the requirements to be statistically representative of the Belgian population, according to several social economic criteria. Documents also come from the Walloon regional government and administration.

RESULTS AND DISCUSSION

Market share of organic products in the Belgian food market

The market share of organic products in the Belgian food market continuously increased between 2008 and 2016, from 1.5 to 3.2% (Figure 1).

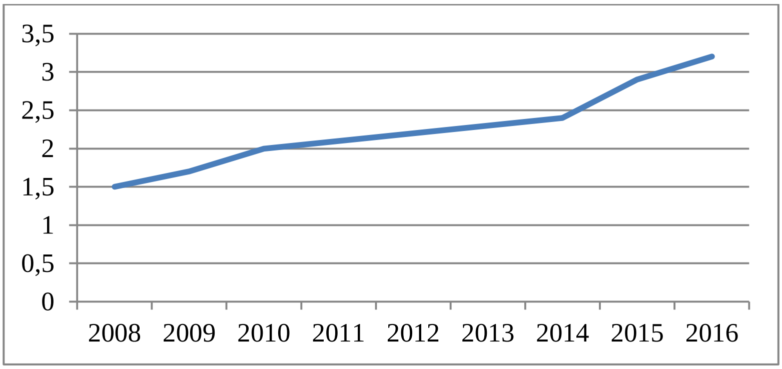

Figure 1. Evolution of the market share of organic products in the Belgian food market (%)

*Source of basic data: Biowallonie (2017)

Organic food products, though more expensive than conventional products, are more and more popular; this phenomenon even accelerated during the last two years. In the future, this share could continue to increase as it reached 8.4% in Denmark in 2015 (the highest in Europe) or 7.7% in Switzerland and 4.8% in Germany (Biowallonie, 2017). So, the target of 3.0% in 2020 defined in the Walloon development plan for organic farming is already reached.

The market share of organic products is very variable according to the type of products (Figure 2). However, it increased for all products with the exception of bread. As prices are very different from one product to another, the position of one product regarding the market share can be different from the position regarding expenses per capita. The highest market shares are observed for meat substitutes (a product which is not popular) and eggs (a cheap product), before vegetables and fruits, which are well known organic products. The market share for dairy products reached 3.0% while the expenses for dairy products are the highest, the prices per unit being higher than for other food products. Globally, the share of vegetal products is highest in the organic food market than in the conventional one.

Compared to the situation of 2010, it appears that the market shares significantly increased, with the exception of bread. For dairy products, data are available for 2014, 2015 and 2016, showing an important increase: 2.1% in 2014, 2.7% in 2015 and 3.0% in 2016. For potato, no trend could be observed during the period 2013-2016. So, it is clear that vegetables, fruits and dairy products show a strong and continuous positive trend in their demand.

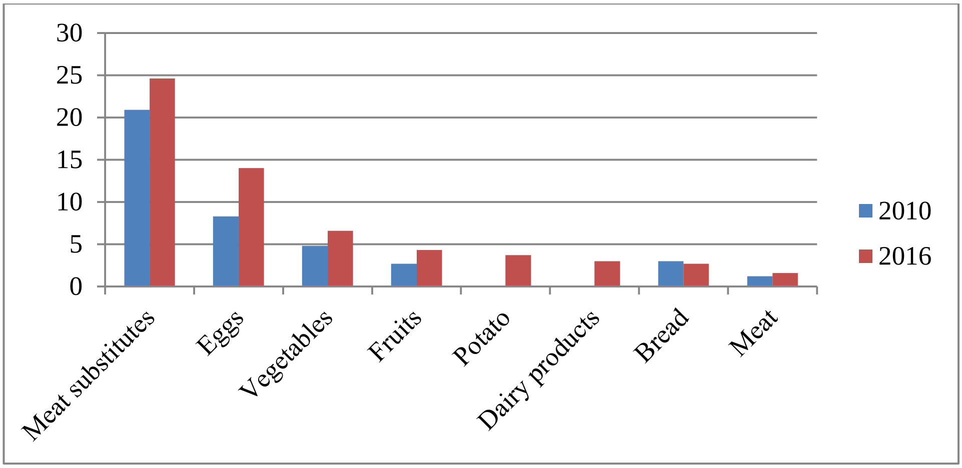

Figure 2. Market share of organic products in 2010 and 2016 (%)

*Source of basic data: Biowallonie (2017)

Expenses for organic products

Many consumers buy organic products occasionally; however, 9% of Belgian households buy organic products at least every week and represent 60% of the total expenses. The expenses per capita and per product in 2013 and 2015 are illustrated in figure 3.

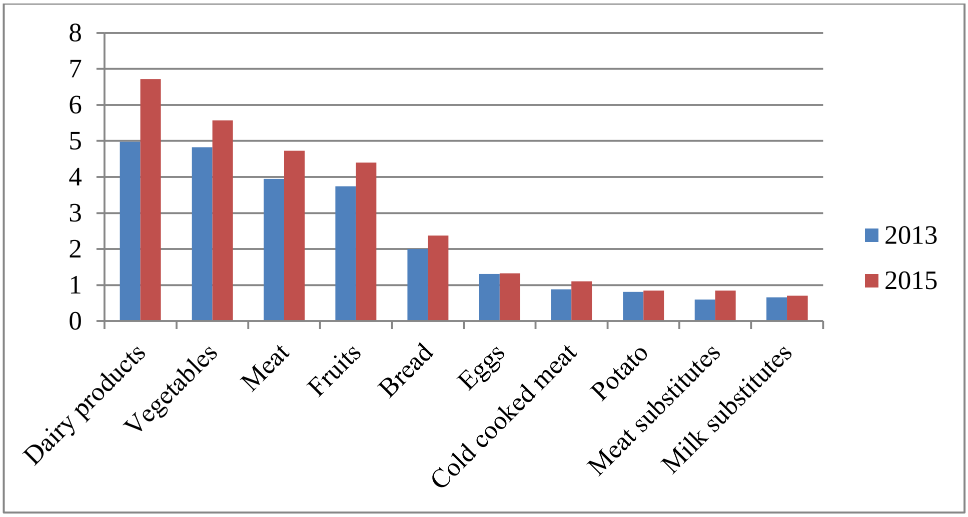

Figure 3. Expenses per capita of selected organic food products in 2013 and 2015 (€)
*Source of basic data: Biowallonie (2017)

Fresh products are the most important: dairy products, vegetables, meat and fruits, probably reflecting the fact that consumers are less confident about quality when products have been processed. Between 2013 and 2015, the expenses have increased for all types of products. Remarkably, the expenses for organic products even increased in 2014, while the total expenses for food declined for the first time since many years, due to the economic stagnation and risks of deflation. It shows that the positive trend for organic products is really strong. In 2015, the expenses for organic products reached 33 € per capita, against 27 € in 2016. Families with high income and children and retired households with high income are the main buyers of organic food products, representing half of the total expenses while they count for 39% of the population. On the opposite, households with low income and children have the lowest expenses. This shows that prices are a question of concern.

Distribution channels

Concerning distribution (Figure 4), the most common channel is the supermarkets, with nearly half of the total organic food market in 2016, which was already the case in 2008.

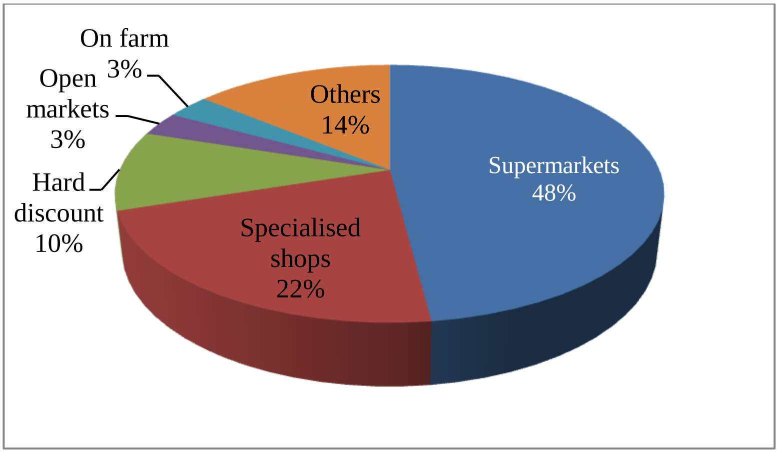

Figure 4. Distribution channels of organic food products in Belgium in 2016 (%)
*Source of basic data: Biowallonie (2017)

Hard discount shops have an increasing share, reaching 10% of the total organic food market in 2016, against 1.2% only 2008.
Shop specialised in organic products have the second position, with 22%. Direct sales are still marginal, with only 3% of organic products sold on farm and 3% on open markets, though there seems to be a move in favour of this kind of retailing. There are perhaps more initiatives in this field, but quantities remain modest compared to the significantly increasing quantities sold through supermarkets and other kinds of shops.
In Belgium, the increase of organic products consumption is parallel to the continuous increase of production, which is supported not only by the European authorities but also by the regional ones, especially in Wallonia where a specific development plan, which was integrated into the sustainable development plan, deals not only with additional financial support to farmers, but also with extension services, research, teaching, information to the public (Burny, 2016).

CONCLUSION

The market for organic products is clearly growing in Belgium, as in many countries in Europe and the world. An acceleration has even been noted in 2015 and 2016, the share of organic products reaching 3.2% of the total food market. This figure is even higher than the objective of 3% defined for 2020 in the Walloon development plan for organic farming. This trend will probably continue, though higher prices for organic products compared to conventional ones are an obstacle to their development. This evolution is parallel to the evolution of organic production, though it is still far from the strategic threshold of 20% defined by Dufumier (2012). This kind of production is also strongly supported by the public authorities. So, from both sides (production and demand), it appears that the organic sector needs a favourable economic environment in order to be prosperous, which clearly limits its development. Nevertheless, it has become now one of the serious alternatives to conventional agriculture and products.

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TRADITIONAL CULTIVATION PRACTICES MAY EFFICIENTLY PREVENT THE VIRUS SPREAD IN SUSCEPTIBLE CROPS: A CASE STUDY OF TURNIP MOSAIC VIRUS IN UKRAINE

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ABSTRACT

Turnip mosaic virus (TuMV) is a member of the largest *Potyviridae* family of plant viruses. For domesticated *Brassica* plants, TuMV is considered one of the most damaging and economically important viruses. TuMV is mainly transmitted by many aphid species non-persistently as well as mechanically from plant to plant. TuMV probably occurs worldwide and has been found in both temperate and subtropical regions of Africa, Asia, Europe, Oceania and North and South America. In Europe, TuMV was reported from the UK, Spain, Italy, Greece, Germany, The Netherlands, Czech Republic, Hungary, Bulgaria, Poland, and Russia. Despite Ukraine geographical location and wide cultivation of different *Brassica* crops for centuries, it has been only recently that the authors have registered TuMV in this country. In this study, isolates of TuMV were collected in Ukraine from naturally infected host plants, all from *Brassicaceae* family. For the first time, TuMV was shown to be widespread in agricultural and urban regions in Ukraine where it naturally infects crops, weeds and introduced species with infection rate reaching 50%. Also, we show that urban locations and concomitant weed plants are potent factors of virus epidemiology favoring extremely high virus incidence level of 89% in susceptible hosts. Importantly, we underpin the significance of trivial cultivation practices (crop rotation and eradication of diseased plants) as preventive measures for the control of damaging pathogen of brassicas, allowing for 3 times less TuMV incidence.

Keywords: *TuMV, Brassicaceae, cultivation, field, Ukraine*

INTRODUCTION

Turnip mosaic virus (TuMV) is a member of *Potyvirus* genus belonging to the largest *Potyviridae* family of plant viruses. TuMV has flexible filamentous particles ~700-750 nm long containing a single-stranded positive sense genomic RNA of about 10,000 nt (King et al., 2012).

As many potyviruses, TuMV has an extremely wide host range but infects mostly plant species from the *Brassicaceae* family and induces persistent symptoms (mosaics, mottling, chlorotic lesions, etc.). For domesticated *Brassica* plants, TuMV is considered one of the most damaging and economically important viruses (Walsh and Jenner, 2002). TuMV is mainly transmitted by many aphid species non-persistently as well as mechanically from plant to plant. TuMV probably occurs worldwide and has been found in both temperate and subtropical regions of Africa, Asia, Europe, Oceania and North and South America (Provvidenti, 1996; Ohshima et al., 2002; Schwinghamer et al., 2014). In Europe, TuMV was reported from the UK (Pallett et al., 2008), Spain (Segundo et al., 2003), Italy (Guglielmone et al., 2000, Ohshima et al., 2002), Greece (Jenner, Walsh, 1996; Tomimura et al., 2004), Germany (Tomimura et al., 2003), The Netherlands, Czech Republic (Petrzik, Lehmann, 1996), Hungary (Horvath et al., 1975), Bulgaria (Kovachevsky, 1975), Poland (Kozubek et al., 2007), and Russia (Ohshima et al., 2002; Zubareva et al., 2012). Ukraine is one of the largest European countries enjoying strategic logistic position at the doorstep of the Northern Silk Road, between the eastern EU states and Black Sea/Middle East region, where TuMV was also recently detected in Turkey (Korkmaz et al., 2008) and Iran (Farzadfar et al., 2009). Despite Ukraine's geographical location and wide cultivation of different *Brassica* crops for centuries, it's only recently that the authors have registered TuMV in our country (Shevchenko et al., 2016). In the study reported here, we include the results of TuMV screening in various ecosystems and describe the importance of preventive measures for the control of wide-spread and damaging pathogen of brassicas.

MATERIAL AND METHODS

Sampling was carried out at the end of the growing season of 2014, and was restricted to crop-producing areas in Kyiv region (Ukraine) and different locations in the city of Kyiv where Brassicaceae plants were growing/cultivated. In Kyiv, sampling locations included two botanical gardens (Botanical garden of Taras Shevchenko National University of Kyiv and Botanical garden of the National Academy of Sciences of Ukraine), the city center, Museum of Folk Architecture and Life of Ukraine (open-air location w/o agricultural activity), and private gardens where different brassica plants were regularly cultivated. Several large fields in Kyiv region used for commercial cabbage cultivation were chosen for sampling in Kyiv region. Brassica plants were visually examined; samples were collected from plants with TuMV-like symptoms typically including mosaics, mottling, vein banding and/or leaf deformation. Collected samples were tested for TuMV by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), as described previously by Clark and Adams (1977), using specific polyclonal antibodies purchased from Loewe (Germany). Briefly, 0,5 g leaf tissue was ground to a powder with a mortar and pestle in 10 mL phosphate-buffered saline, pH 7,4, containing 0,05% Tween 20, 2,0% polyvinylpyrrolidone (MW 40 000) and 0,2% bovine serum albumin. In the meantime, microtitre plates (Maxisorb, NUNC, Denmark) were coated with TuMV-specific broad-spectrum polyclonal antibodies (1:200) in carbonate buffer according to the manufacturer's instructions. Leaf extracts were then added to the plates in duplicate wells and incubated overnight at 4°C. The presence of TuMV in the samples was detected in 200 µL homogenate by TuMV-specific antibodies conjugated to alkaline phosphatase using *p*-nitrophenyl phosphate substrate (Sigma, USA). Absorbance values at 405 nm were measured using a Multiscan-334 microtitre plate reader (Labsystem, Finland). Absorbance values, measured 60 min after adding the substrate, greater than three times those of the negative controls were considered positive.

RESULTS AND DISCUSSION

A total of 54 plant samples with TuMV-like mosaic and mottling symptoms were collected in different districts of the city of Kyiv and Kyiv region. Sampling areas included both agricultural sites (two cabbage producing fields and private gardens) and urban locations where no agricultural activity was carried out (different sites in the City of Kyiv, two botanical gardens and open-air Museum of Folk Architecture and Life of Ukraine). On cabbage plants, TuMV typically induced systemic mosaics, vein banding and leaf deformation (Fig.1/2), whereas systemic mosaics and mottling were common for naturally infected radish and mustard plants.

A

Figure 1. TuMV-positive cabbage (*B. oleracea* var. capitata)


B

Figure 2. TuMV-positive cabbage (*B. oleracea* var. capitata) (A) with vein banding/clearing and mustard (*Brassica juncea*) (B) showing symptoms of systemic mottling (source: photos made by authors during the sampling)

Using ELISA, TuMV was detected in samples from cabbage, red radish, mustard, radish, white mustard, gold of pleasure, weed species (hill mustard), etc. (Table 1).

Table 1. Double-antibody enzyme-linked immunosorbent assay for the detection of *Turnip mosaic virus* by hosts (source: authors' elaboration based on the obtained results)

Plant	**No of samples**	**Positives**	**Incidence of TuMV infection (%)**
Brassica oleracea (**cabbage**) *Brassica sp.*	23	8	35
Raphanus sativus (**red radish**) *Raphanus sp.*	12	11	92
Brassica juncea (**mustard**)	4	2	50
Sinapis alba (**white mustard**)	3	3	100
Other brassicas	5	3	60
Other non-brassicas (*Asteraceae*, *Primulaceae*, *Papaveraceae*, *Malvaceae*)	7	0	0
TOTAL	**54**	**27**	**50**

TuMV has been detected in 27 samples of plants (overall 50% incidence rate in symptomatic hosts) including *B. oleracea* var. capitata, *R. sativus*, *S. alba*, *B. juncea*, *Camelina sativa*, and *Bunias orientalis* (identified as the weed host for TuMV in Ukraine). Cabbage, radish and mustard were the predominant hosts for TuMV in sampled areas which probably reflected the virus host range for the country in general.
TuMV was found in the main brassica-crop fields, private gardens and urban locations of Ukraine, with a high overall incidence of 50%. Importantly, the agricultural sites used for plant sampling were characterized with different level of incidence of TuMV infection varying from 17% and 42% for two crop fields, and to as much as 58% for private gardens (Table 2).

Table 2. Survey for *Turnip mosaic virus* by sampling sites continuously used for crop cultivation (source: authors' elaboration based on the obtained results)

Sampling site	**No of samples**	**Positives**	**Incidence of TuMV infection (%)**
Commercial cabbage producing field 1	6	1	17
Commercial cabbage producing field 2	12	5	42
Private gardens	12	7	58
Total for agricultural sites	**30**	**13**	**39**

Several sampling sites within the Kyiv city (i.e. where no agricultural activity was carried out) demonstrated even higher incidence rate of TuMV with the minimum value of 33% for symptomatic plants (Table 3). Apparently, urban sites play an important role in virus epidemiology serving as dormant 'nests' for virus populations. These results suggest that TuMV is widespread in both agricultural and urban locations but remained undetected for a long time.

Table 3. Survey for *Turnip mosaic virus* by sampling sites in urban areas (no cultivation) (source: authors' elaboration based on the obtained results)

Sampling site	**No of samples**	**Positives**	**Incidence of TuMV infection (%)**
City of Kyiv	9	3	33
Botanical garden of Taras Shevchenko National University of Kyiv	3	1	33
Botanical garden of the National Academy of Sciences of Ukraine (Kyiv)	9	8	89
Museum of Folk Architecture and Life of Ukraine (Kyiv)	3	2	67
Total for urban locations	**24**	**14**	**55**

Expectedly, different locations demonstrated high but varying level of TuMV occurrence. However, several aspects were of special interest in this regard. For the two fields used for commercial cabbage production in Kyiv region and situated in neighboring villages just 5 km apart, the TuMV incidence rate varied from 17% to 42%. This probably reflects the efficiency of the confirmed regular eradication of diseased plants in the former case (field 1) and underpins the significance of long-known simple approach – elimination of virus inocula – for the disease control.
In turn, rather high rate of TuMV infection in private gardens (58%) may be explained by both growing of infected plants and repeated cultivation of susceptible crops, as reported by the landowners. Another approach allowing to limit virus spread – crop rotation – was also missing in this case.
Obtained results clearly demonstrate that trivial measures for crop cultivation (known for decades but often thoroughly disregarded) remain highly efficient in controlling the spread of the mechanically and aphid-transmitted virus and reducing consequential damages.

CONCLUSIONS

In summary, the survey indicated high occurrence of TuMV in urban and agricultural regions in Ukraine where it naturally infects crops, weeds and introduced species with average infection rate reaching 50%. Urban locations and concomitant weed plants are potent factors of virus epidemiology favoring extremely high virus incidence level of 89% in susceptible hosts.
Wide range of infected plant species in surveyed areas obviously demonstrates lack of virus screening in Ukraine. Obtained data also suggests a just discovered long-term coexistence of the virus and the hosts in Ukraine.

Importantly, trivial cultivation practices (crop rotation and eradication of diseased plants) are shown as effective preventive measures for the control of damaging pathogen of brassicas, allowing for 3 times less TuMV incidence.

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A GEOTECHNICAL ASSESSMENT OF USABILITY OF A ROCK-SOIL MIXTURE FOR EARTH STRUCTURES

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ABSTRACT

The subject-matter of the work is a mixture of rock and soil from the Lafarge Dubie mine in Rudawa, southern Poland. The conducted tests aimed at the determination of the geotechnical characteristics of this mixture and the evaluation of its suitability for the construction of earth embankments, in particular road ones. The range of the tests comprised determination of parameters characterising physical properties, such as granulometric composition, natural moisture content, density of solid particles, optimum moisture content and maximum dry density of solid particles, as well as mechanical ones, like shear strength. The obtained results show that the tested mixture is suitable for the construction of road embankments, since as coarse-grained soil, it has a high value of the uniformity coefficient (C_u = 1913). Therefore, this is very well graded soil, which provides a good compaction when it is built into the embankment. The natural moisture content (on average w_n = 9.5%) is close to the optimum one (w_{opt} = 8.5%). The maximum dry density of solid particles (ρ_{ds} = 2.16 g·cm^{-3}) is much higher than the minimum required ($\rho_d \geq$ 1.6 g·cm^{-3}). The values of the angle of internal friction (on average ϕ = 36°) and cohesion (c = 42 kPa) indicate great shear strength, therefore this soil can be subjected to considerable mechanical stresses.

Key words: *earth structures, rock-soil mixture, geotechnical properties, road embankment.*

INTRODUCTION

For the construction of road and railway embankments, it is necessary to obtain massive amounts of earth materials to form embankment bodies and load-bearing structural layers. These materials are mainly taken from native mineral deposits (natural aggregates), and, to a lesser extent, from industrial landfills – coal mining waste, steel slag, coal ash (artificial aggregates) (Kozioł and Kawalec, 2008; Pyssa, 2010; Zawisza, 2001; Zawisza, 2012). According to Kozioł (2017), the natural aggregates constitute the largest group of the extracted minerals and produced from them mineral raw materials. In Poland, in the last 25 years (1991–2015), there was a nearly 4-fold increase in the aggregate extraction – from about 63 million tonnes per year to 232 million tonnes per year (in 2011 – 333 million tonnes per year). Mostly, there are produced gravel-sand aggregates, which accounts for about 2/3 of the production. They are mainly used in civil engineering for the production of various concretes and concrete products, as well as in other sectors such as power industry, mining, agriculture and road engineering. The possibilities of using the aggregates or, in general, materials from natural mineral deposits for various applications depend on their physical and mechanical properties. In the case of using them for the construction of traffic embankments, mainly roads, it is necessary to determine their geotechnical characteristics. The paper presents the results of investigations of the geotechnical properties of the selected rock-soil mixture and evaluation of its suitability for the construction of road embankments.

MATERIALS AND METHODS

The rock-soil mixture from the Lafarge Dubie mine in Rudawa, southern Poland, was the subject-matter of the study. The tested material was delivered to the geotechnical laboratory of the Department of Hydraulic Engineering and Geotechnics UR in Krakow in two batches. The scope of the study included determination of parameters characterising the physical properties, i.e. granulometric composition, natural moisture content, density of solid particles, as well as the mechanical ones, i.e. shear strength. Due to the coarse nature of the material, the tests were performed using a medium-sized apparatus for determining compactibility and shear strength. In these cases, fractions of thicknesses greater than 63 mm were screened to meet the condition required for coarse-grained soil in accordance with the formula (Pisarczyk, 2004):

$$\frac{D}{d_{\max}} \geq (4 \div 6)$$

where:
D – side or diameter of the sample,
d_{max} – diameter of the maximum grain.

It should be emphasized that according to Pisarczyk (1977) the parameters determined for the material finer than 60 mm do not differ from the parameters specified for the soil of the full granulation by more than 1–2%, hence the results obtained can be considered representative for the whole material. The granulometric composition was determined by a combined method, i.e. sieve and areometric analyses. Part of the sieve analysis on the samples weighing several dozen kilograms was made dry with manual cleaning thick crumbs from fine fractions, and then the sieve analysis with washing with water was performed on fractions finer than 16 mm. For fractions finer than 0.063 mm, the areometric analysis was done. Grain size distribution is a leading property having a significant impact on a number of basic geotechnical parameters of the soil. It was determined for the first batch of the materials and for the material averaged out from both supplied samples. The density of solid particles was determined for the grains finer than 0.063 mm by means of a volumetric flask in distilled water, for the materials as given above. The natural moisture content was determined by a dryer method at 105°C for the materials as described above. The optimum moisture content and the maximum dry density of solid particles were determined in a Proctor medium-sized apparatus with a cylinder volume of 9.8 dm^3 (h = 20 cm, d = 25 cm), on a material averaged out from both batches with a particle size finer than 63 mm, using standard compaction energy E_z = 0.59 $J \cdot cm^{-3}$. The values of the parameters characterising the shear strength, i.e. the angle of internal friction and cohesion were determined in a medium-sized shear apparatus in a box of dimensions of 30 x 30 x 20 cm, with intermediate frames forming a shearing zone 3.0 cm thick, on two samples of the material averaged out from both batches with a particle size less than 63 mm. Using the intermediate frames enables zone shearing, limiting the impact of wedging and intermeshing grains on the value of cohesion (so called apparent cohesion). The tests were performed on the material with the grains finer than 63 mm at the optimum moisture content and the degree of compaction I_S = 0.95.

RESULTS AND DISCUSSION

The values of the geotechnical parameters of the tested material are presented in the Table 1. The granulometric composition of the material provided in the first batch was dominated by coarser fractions – gravel (about 47%) and cobbles (about 25%), a total of over 71%. The finer fraction content was as follows: sand – about 8%, silt – above 17% and clay below 4%. According to the standard PN-EN ISO 14688-2:2006, this material can be classified as a clayey gravel with a large amount of cobbles (Fig. 1). Effective grain sizes were equal: d_{10} – 0.015 mm, d_{60} – 35.0 mm, which gives the uniformity coefficient C_u = 2333.3. It means, that this soil is very well graded (multi-fractional).

Table 1. Basic geotechnical parameters of the rock-soil mixture

Parameter	Value	
	Sample number:	
	1	2
Fraction content [%]: cobbles (> 63 mm) gravel (2–63 mm) sand (0.063–2 mm) silt (0.002–0.063 mm) clay (< 0.002 mm)	 24.6 46.49 7.84 17.48 3.59	 14.03 51.48 14.64 16.5 3.35
Name of the soil acc. to PN-EN ISO 14688-2:2006	clGr+Co	clGr+Co
Uniformity coefficient [–]	2333.3	1913.0
Coefficient of curvature [–]	23.33	3.42
Density of solid particles [$g \cdot cm^{-3}$] (for d < 0.063 mm)	2.75	2.77
Natural moisture content [%]	7.31	10.66
Maximum dry density of solid particles [$g \cdot cm^{-3}$]	2.165	
Optimum moisture content [%]	8.5	
Angle of internal friction [°] at I_S = 0.97	36.5	35.6

Cohesion [kPa] at $I_S = 0.97$	40.1	43.3

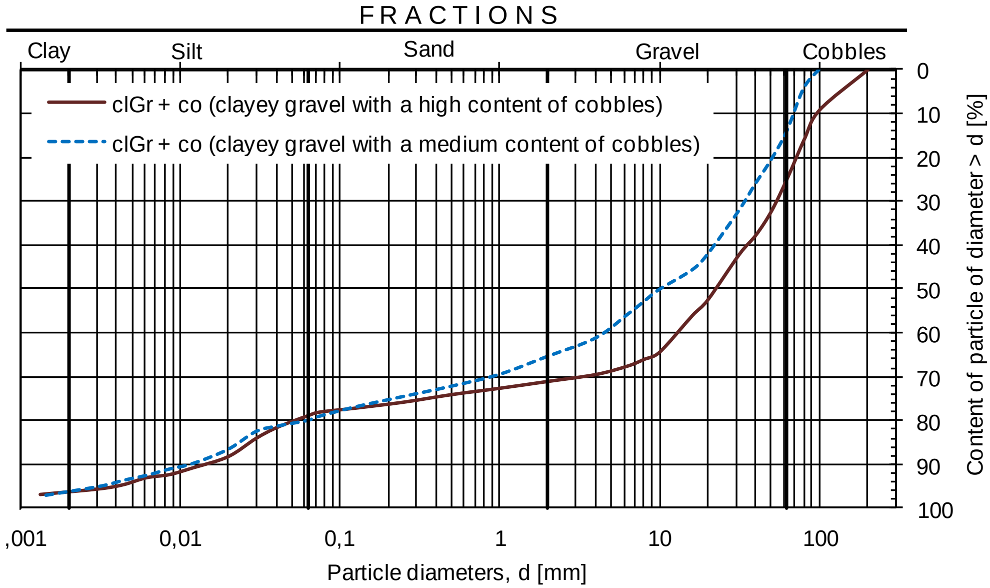

Figure 1. Granulation curve for the rock-soil mixture

In the sample averaged out from both batches of the delivered material, the content of the gravel fraction was over 51%, cobbles over 14% – a total of more than 65%. The content of the sand fraction was about 15%, silt more than 16%, and clay over 3%. This material can also be classified as a very well graded clayey gravel with a medium content of cobbles (Fig. 1), but with a greater content of finer fractions (sand and silt – by about 5%), and smaller content of coarser fractions (gravel and cobbles – by about 6%).

It follows from the above that the studied soil is coarse-grained, clayish, because the dominant fractions are cobbles and gravel (on average about 70%) and the content of clay fraction is slightly higher than 2% (on average 3.5%).

The natural moisture content determined for the sample delivered in the first batch was 7.31%, and in the second – 9.24 and 12.07%. It follows that the moisture content of the tested material was in the range of 7.3 to 12.1%. The differences of the moisture content values occurring in the individual batches of the material result from the different locations of collection points and atmospheric conditions. The average value was 9.5% and was typical for that type of soil.

The density of solid particles, determined for the clay-silt fraction ($d < 0.063$ mm) on the samples from both batches, was relatively high and equalled 2.75–2.77 $g \cdot cm^{-3}$.

The optimum moisture content and the maximum dry density of solid particles were respectively: $w_{opt} = 8.5\%$, $\rho_{ds} = 2.165\ g \cdot cm^{-3}$ (Fig. 2), and these values should be accepted as reliable in earthwork design.

The values of the angle of internal friction and cohesion were equal respectively: sample 1 – ϕ = 36.5°, c = 40.1 kPa; sample 2 – ϕ = 35.6°, c = 43.3 kPa (Fig. 3). The average values of these parameters equalled: ϕ = 36°, c = 42 kPa. These are relatively high values and indicate a great shear strength of the tested materials.

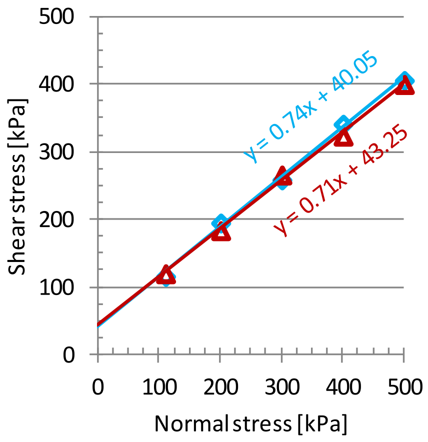

Fig. 2. Shear stress versus normal stress

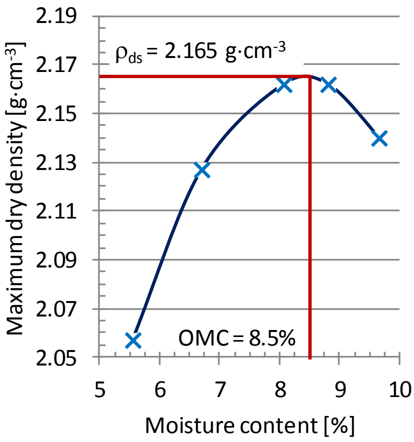

Fig. 3. Maximum dry density versus moisture content

Assessment of the tested soil for erecting road embankments

The usability of the tested rock-soil mixture for the construction of road embankments was determined on the basis of the PN-S-02205:1998 standard, which provide the requirements that should be met by the soil used to make the earth embankments. They relate to the granulation and parameters characterising the frost-heave, compactibility, consistency, organic matter content and bearing capacity. In terms of grain size distribution, the rock-soil mixture corresponds to clayey gravels with the average content of cobbles. The above cited standard describes such soils as usable for bottom layers of embankments below the freezing zone. The standard also allows this type of soil to be used for the top layers of embankments in the ground freezing zone, provided that they are improved with the binder, e.g. cement, lime or active ashes. The values of the individual geotechnical parameters of the tested mixtures and the relevant standard requirements are summarized in the Table 2. The rock-soil mixture meets most of the required criteria.

Based on the results of the study, it can be concluded that the mixture in question is suitable for the construction of road embankments because:

As the coarse-grained soil, it has a high value of the uniformity coefficient (C_u = 2333.3). Thus, it is a very well graded soil (multi-fractional), which forecasts good compactibility of the material when incorporated into the embankment.

Natural moisture content, slightly variable in different batches of the material (on average w_n = 9.5%) is close to the optimum one (w_{opt} = 8.5%), which allows it to be incorporated into the embankment without additional treatment, e.g. drying or wetting. This material has a high value of the maximum dry density of solid particles (ρ_{ds} = 2.165 g·cm^{-3}), which is significantly greater than the minimum required ($\rho_{ds} \geq$ 1.60 g·cm^{-3}). The values of the angle of internal friction (ϕ = 36°) and cohesion (c = 42 kPa) indicate great shear strength, therefore, this material can be subjected to considerable mechanical stresses. Hence, we may envisage the appropriate bearing capacity and stability of the embankments formed from this material. However, due to the excessive content of fine particles, the mixture has been classified as questionable in terms of frost heave. Given the above, the rock-soil mixture can be used for lower layers of road embankments below the freezing zone, if built in dry areas or places protected from groundwater and surface water.

Table 2. Values of geotechnical parameters of the rock-soil mixture comparing to the Polish standard requirements for road embankments

Parameter	Standard PN-S-02205:1998 requirements	Rock-soil mixture
Maximum dimension of grains, [mm]	200	lack of grains coarser than 200 mm
Uniformity coefficient, C_u	≥ 3	1913
Maximum dry density of solid particles [g·cm^{-3}]	≥ 1.6	2.165
Content of grains [%]: ≤ 0.075 mm ≤ 0.02 mm	 < 15 < 3	 21.0 13.5
Frost susceptibility group	depending on the group of soils	questionable

CONCLUSION

On the basis of the laboratory tests and analysis of the results, it can be concluded that the rock-soil mixture from the Lafarge Dubie mine in Rudawa is the coarse-grained soil that can be used as a construction material for earth structures. It is characterised by favourable geotechnical parameters. As the multi-fractional soil with the great value of the maximum dry density of solid particles, it has good compactibility. With the high degree of compaction (I_S = 0.95), it has a high shear strength. Therefore, it can be used for the construction of various types of traffic embankments – road, rail and technological ones (access roads) – for the bottom layers of the embankments below the freezing zone. In order to maintain the stability conditions, it is necessary to assure the degree of compaction suitable for the given road class at the proper soil moisture content and inclination of the embankment slope. Due to the variability of the material from different mine exploitation fragments, every time, it is indispensable to determine the geotechnical parameters necessary to assess the usability of the collected soil for the intended application. Test methods for geotechnical parameters should be appropriate for the nature of the soil, which is coarse. Therefore, research should be conducted with the use of medium-sized equipment and the results verified under conditions of construction on experimental embankments, using compacting equipment fitting to the grain size.

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USE OF LOCAL COMPOSTED WINERY WASTE FOR LETTUCE PRODUCTION IN LEBANON

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ABSTRACT

Recently, the Lebanese wine sector has been witnessing a non-precedent growth producing huge amounts of winery wastes referred to as grape marc. The effect of using grape marc compost on lettuce (*Lactuca sativa* L.) production was investigated in an open-field experiment in Central Bekaa. Seedlings of the Romaine variety were planted in different substrates: S1: 100% soil or control, S2: 75%soil + 25% grape marc compost, S3: 25%soil + 75% grape marc compost and S4: 100% grape marc compost. Root growth, leaf growth and leaf characteristics were compared among the different mixtures. Tests showed that the grape marc compost contained acceptable values of nitrogen, phosphorus, potassium, organic matter and a perfect germination index ranking between 0.8 and 1 for the direct and diluted solutions. Results showed that at early stages of growth, the best results were obtained from plants grown in the substrate S4. However, at later stages of growth, grape marc compost with a percentage higher than 50% (S4) in the mixture induced lower averages of leaf number, length and width of largest leaf and leaf weight and those higher than 25% (S3 and S4) increased dry matter and total soluble solids content due to its low water holding capacity causing a water stress on plants. Root growth was proportional to increased percentages of grape marc compost. Finally, composted grape marc provided the highest benefit to plants when added to soil in quantities of 25% by volume allowing the best yield increase (47%) compared to control.

Keywords: *Lactuca sativa*, grape marc compost, water holding capacity, plant growth.

INTRODUCTION

Winemaking industries are an important part of the economy in many parts of the world. Grape processing for winemaking results in large amounts of solid wastes

normally referred to as grape marc or pomace formed by pressed skins (about 70-80%), stems (10%), seeds (8%), stalks (2.5-7.5%) and pulp (57%) (Jiang *et al.*, 2010). The grape marc is the main by-product of the wine industry. It is a cheap product widely distributed in the Mediterranean area (Scettrini and Jelmini, 2004). Grape marc is rich in macro and micro-nutrients, nitrogen, potassium and phosphorus and is valued for its organic matter content (Eleonora *et al.,* 2014). It can be used as a crop fertilizer (Pinamonti *et al.,* 1997), however its direct application of grape marc to soil can have negative effects on the environment (Deng *et al*., 2011), thus it is applied in a composted form (Gazeau, 2012).
The main aspects studied for grape wastes are related to their physical, chemical and microbiological characteristics developed during composting (Ferrer *et al.*, 2001) which vary depending on the grape variety, season, harvest method and pressing method during processing (White, 2008). The use of composted grape marc has shown many advantages; its application to soil increased nutrients, organic matter content and microbial biomass (Arvanitoyannis *et al.*, 2006). It was successfully used in horticulture due to its physico-chemical properties (Ferrer *et al.,* 2001) like pH, C/N ratio and moisture content (Stafne and Carroll, 2008). It also improved soil properties thus could be used as soil conditioner. It does not cause problems of high salinity compared to many other types of organic compost, however it has a high drainage (58%) which can cause problems of plant mortality due to drying out of roots (Scettrini and Jelmini, 2004). Literature has reflected a positive influence of using composted winery wastes on many crops like tomato, melon (D'Addabo *et al.,* 2000), corn (Ferrer *et al.,* 2001), mushroom (Pardo *et al.*, 2007), onion (Stafne and Carroll, 2008), and on ornamental plants (Carmona *et al.*, 2012). Also, grape marc compost could partially substitute peat without causing any loss in yield and in the nutritional status of lettuce seedlings (Bustamante *et al.*, 2008) and when mixed with peat it produced good results for lettuce seedlings production (Carmona *et al.,* 2003). The production of grape marc compost is widespread in Lebanon where large amounts of wastes are produced from winemaking. There is little knowledge about the potential use of locally available winery wastes in vegetable production in Lebanon, consequently this experiment investigated the effect of grape marc compost application to soil on lettuce crop which is a valuable crop widely grown under greenhouse and open-field conditions in Lebanon.

MATERIALS AND METHODS

The experiment was carried out in spring 2014 in an open field located in Central Bekaa-Lebanon. Soil tests indicated that it was a silt-clay, rich in calcium, poor in organic matter and with acceptable values of nitrogen, potassium and phosphorus and nematode-free. Moreover, grape marc compost was provided by a local winery. Physical and chemical properties of this by-product were tested and they revealed that it was alkaline (pH=7.1), rich in organic matter (81.6%), nitrogen (3.2%) and potassium (3.08%) with a C/N ratio of 13.9. Micronutrients and heavy metals content in this compost were within the acceptable levels of application to

agricultural soils. A phytotoxicity test was performed on the composted grape marc using germination and root elongation tests on *Lepidium sativum* seeds (Zucconi *et al.,* 1981). Results for the three rates of dilution (0X, 3X and 10X) registered a germination index ranking between 0.8 and 1.0. Consequently, the substrate could be used with or without dilution.
Lettuce seedlings of the variety Dark Green Romaine (*Lactuca sativa* L. var. *longifolia*) were transplanted in 5 April into tunnels that were dug in the experimental field and filled by 4 different types of substrates: S1: 100% soil, S2: 75% soil + 25% composted grape marc, S3: 25% soil + 75% composted grape marc, and S4: 100% composted grape marc. The experimental design was a Randomized Complete Block Design (RCBD) with 3 replications per treatment and 50 plants per replication. Irrigation was done at an interval of 5 days using GR drip system after crop water requirements were deduced from cropwat 8.0 software.

Data recording

Data was collected 20 days after transplanting (date 1: rosette stage), 40 days after transplanting (date 2: head formation stage) and 63 days after transplanting (date 3: at harvest) on 5 plants chosen randomly from each plot. Investigated parameters were: length of main root, root neck diameter, number of secondary roots, number of leaves, length and width of leaves, weight of heads and root weight. Further tests were performed at the date of harvest such as Total Soluble Solids (TSS) content that was determined on the liquid extract of plant leaves using an Atago N1 refractometer. In addition, dry matter (DM) content was determined on lettuce heads oven dried at 70◦C for 48 h. Dried heads were then ground to a fine powder using a mill with 0.5mm for measuring the nitrogen (N) content (micro-Kjeldhal digestion procedure), phosphorus (P) content (Vanadate-Molybdate-Yellow method), potassium (K) content (flame photometer) and Zn, Cu, Pb, Cd, Fe and Mn content (atomic absorption spectroscopy).

Statistical analysis

Data analysis was done using Sigma Stat 3.2 Software. Multiple mean comparisons were done using the ANOVA test and Tukey tests as ranking tests depending on data distribution.

RESULTS AND DISCUSSION

Plant growth was affected differently by the substrate type. Results showed that at the rosette stage (Table 1) plant growth was positively affected by an increased proportion of composted grape marc in the substrate. The highest averages were reached in the substrate S4 for all measured parameters. In general, a better elongation of main roots was combined with a higher percentage of grape marc compost in the substrate. This was translated by a significant difference in average length of main roots that was of 13.06 cm, 13.46cm, 14.14 cm and 15.12 cm in S1, S2, S3 and S4 respectively. Moreover, average values of the majority of parameters

were significantly higher in S3 compared to S2 except the number of secondary roots and weight of heads that did not differ significantly between both substrates.
At the stage of head formation (Table 2) root parameters such as length of main root, number of secondary roots and total weight of roots recorded the highest averages in the substrate S4. The average root neck diameter did not differ significantly between the substrates S2, S3, and S4 while it was significantly higher in those three substrates compared to S1. At this developmental stage, root growth would be proportional to the proportion of compost in the growing medium. On the other hand, average number of leaves, average width of largest leaf, average length of the largest leaf and average weight of heads did not differ significantly between the treatments S2 and S3, however they were significantly higher in both substrates compared to S1 and S4 with the lowest values recorded for S1.
At harvest (Table 3), in the substrate S4 mature plants had developed the longest main roots (average length of main root in S4: 27.62cm compared to S1: 19.26cm, S2: 20.23cm and S3: 24.94cm), the heaviest root system (average weight of roots in S4: 47.09g compared to S1: 43.18g, S2: 42.92g, and S3:45.6g) and most ramified root system with the highest number of secondary roots (S4: 175.6 compared to S1: 149.8, S2: 150.4 and S3: 162.6). The average root neck diameter was almost the same in all substrate types.
Leaf development (average leaf number, average leaf length and average leaf width) was the best in S2. Also at this stage, there was no significant difference in average weight of heads in S2 and S3; however both substrates allowed the formation of heavier heads when compared to S1 and S4. Consequently, in S2 yields were improved by 47% and in S3 by 28% compared to control.

Table 1. Means and standard deviations of roots and heads parameters measured at the rosette stage

Substrate	Length of main root (cm)	Root neck diameter (cm)	Number of secondary roots	Number of leaves	Width of largest leaf (cm)	Length of largest leaf (cm)	Weight of rosette leaves (g)	Weight of roots (g)
S1	13.06±0.3d	2.0±0.2d	70.6±3.65bc	24±1.58d	7.76±0.11d	14.72±0.25d	210.6±9.73bc	18.59±0.27d
S2	13.46±0.3c	2.48±0.15c	83.6±2.41abc	26.4±2.07c	8.28±0.08c	15.56±0.11c	251± 2.53abc	20.49±0.31c
S3	14.14±0.2b	2.7± 0.1b	87.6±1.14ab	29.42±1.14b	8.82±0.08b	16±0.10b	281.4±2.37ab	21.48±0.25b
S4	15.12±0.4a	3.0±0.16a	95±3.54a	32.4±1.14a	9.58±0.15a	16.5± 0.16a	297.6± 2.13a	23.43±0.16a
Test 5%	Annova	Annova	Tukey test	Annova	Annova	Annova	Tukey test	Annova

Numbers followed by different letters are significantly different

Table 2. Means and standard deviations of roots and heads parameters measured at stage of head formation

Substrate	Length of main root (cm)	Root neck diameter (cm)	Number of secondary roots	Number of leaves	Width of largest leaf (cm)	Length of largest leaf (cm)	Weight of heads (g)	Weight of roots (g)
S1	15.34±0.18d	2.26±0.11b	105±6.17d	37.8±1.09c	11.3±0.52b	20.62±0.26c	433.37± 7.23b	30.39±1.29d
S2	16.9±0.19c	2.64±0.11a	128.1±3.21c	46±0.71a	12.7±0.4a	23.84±0.45a	678.04±96.04a	33.42±0.78c
S3	20.96±0.69b	2.56±0.11a	137.8±3.42b	52.6±1.14a	13.16±0.15a	24.46±0.79a	662.92± 12.4a	37.34±0.76b
S4	24.94±0.27a	2.62±0.31a	163.8±3.77a	40.2±0.84b	11.6±0.16b	21.96±0.24b	441.64±21.87b	42.66±1.19a
Test 5%	Annova	Annova	Annova	Annova	Annova	Annova	Annova	Annova

Numbers followed by different letters are significantly different

Table 3. Means and standard deviations of roots and heads parameters measured at the harvest stage

Substrate	Length of main root (cm)	Root neck diameter (cm)	Number of secondary roots	Number of leaves	Width of largest leaf (cm)	Length of largest leaf (cm)	Weight of heads (g)	Weight of roots (g)
S1	19.26±0.35d	2.64± 0.055	149.8±2.49c	74.8±1.3bc	13.18±0.84c	27.1±0.32c	676.2±7.59b	43.18±0.55c
S2	20.23±0.08c	2.94±0.089	150.4±2.07c	88.8±1.9a	16.7±0.34a	33.82±0.45a	994.78±21.11a	42.92±0.22c
S3	24.94±0.52b	2.8±0.16	162.6±6.35b	79.8± 0.8b	14.88±0.15b	29.28±0.28b	865.1± 15.55a	45.6±0.49b
S4	27.62±0.59a	2.62±0.31	175.6±3.78a	59.8± 4.0c	12.04±0.49d	23.46±0.35d	605.06±10.19b	47.09±0.51a
Test 5%	Annova	Annova	Tukey test	Annova	Annova	Annova	Tukey test	Annova

Numbers followed by different letters are significantly different

The effect of grape marc compost differed between growth stages. It was less evident at the rosette stage, however at later stages differences in all parameters related to head formation were more obvious where all substrates containing grape marc compost induced the formation of heavier heads with more abundant, longer and wider leaves compared to soil (Figure 1). At the stage of head formation, the substrates S2 and S3 marked the formation of best lettuce heads, while at harvest the heaviest and best developed heads were obtained in S2. Therefore, a rate of 25% of grape marc compost in the substrate was enough to induce amelioration of harvested lettuce heads.

It seemed that the use of grape marc compost has negatively affected water availability to plants because of the low water holding capacity of this substrate which caused water-stress conditions. The best crop performance that was obtained in S2 could be due to the better maintenance of internal balance by plants and the improved utilization of water and nutrients. When soil moisture deficit increases the rate of water absorption decreases, thus water deficit reduces leaf area and cell size in the whole vegetative part (Ramalan and Nwokeocha 2000). Grape marc compost has helped plant roots to explore a larger area of soil, thus it enhanced water and nutrient absorption. Lettuce appreciates a soil rich enough in organic matter (Weill and Duvall, 2009), but it requires also a well-structured, well-drained and well-aerated to provide a good growth of roots (Collin and Lizot, 2003).

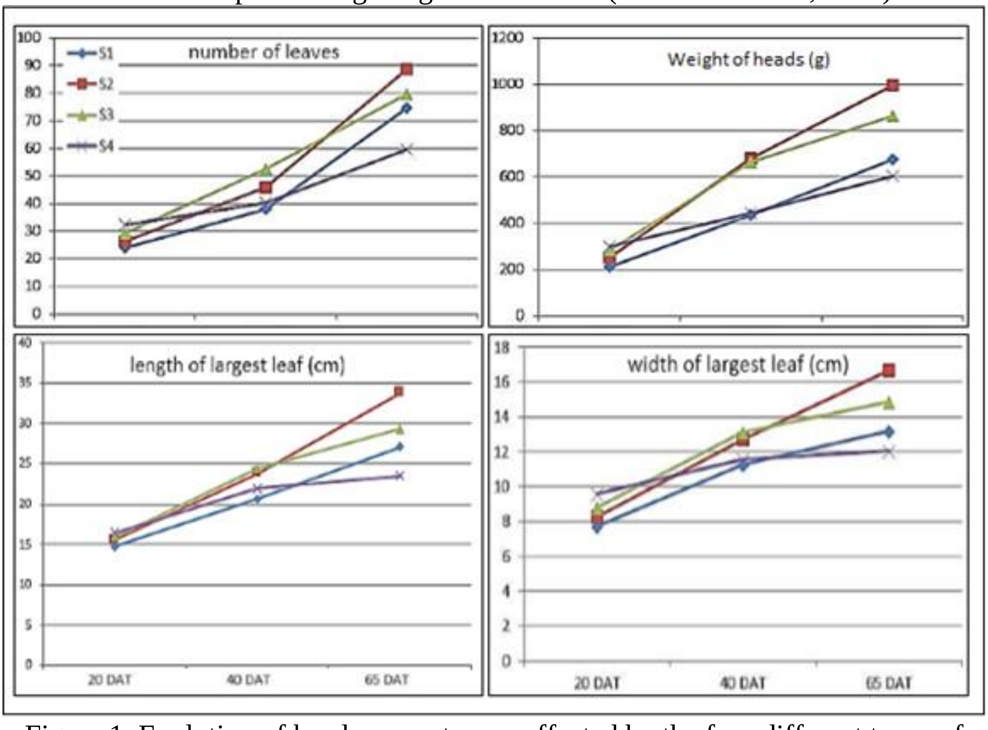

Figure 1. Evolution of head parameters as affected by the four different types of substrates

Adding grape marc compost has enhanced the nutritional importance by increasing macronutrients, dry matter and total soluble solids in lettuce leaves. N, P, K content in lettuce plants was higher in S2, S3 and S4 compared to S1 (Table 4). Nitrogen content was very close in S3 and S4 with 3.66 $mg.Kg^{-1}$ and S4: 3.67 $mg.Kg^{-1}$ respectively. Same tendency was observed for phosphorus and potassium. The high drainage of grape marc compost has caused a water stress on lettuce plants, therefore the accumulation of assimilates was greater in plant leaves. There was amelioration in DM and TSS content correlated with an increase of compost percentage. Superiority of dry matter accumulation and sugar content was for the substrate S4.

Table 4. Macronutrients, dry matter and total soluble solids content in harvested lettuce

Substrate	N ($mg.Kg^{-1}$)	P ($mg.Kg^{-1}$)	K($mg.Kg^{-1}$)	D.M (%)	TSS (◦Brix)
S1	1.93	0.357	3.01	5.2	4.4
S2	3.30	0.720	4.6	5.3	4.5
S3	3.66	0.729	4.8	5.8	5.3
S4	3.67	0.793	4.9	6.1	5.7

N: Nitrogen, P: Phosphorus, K: Potassium, D.M: Dry matter, TSS: Total Soluble Solids

Pb and Cd content in lettuce plants remained within the normal ranges indicated by Ross (1994) after addition of composted grape compost to soil. On the other hand, there was an increase in Zn, Cu, Fe, and Mn content in lettuce plants with increased rate of composted grape marc in the substrate (Table 5).

Table 5. Microelements and heavy metals content in harvested lettuce

Substrate	Zn (µg/g)	Cu (µg/g)	Pb (µg/g)	Cd (µg/g)	Fe(µg/g)	Mn(µg/g)
S1	38.68	4.95	8.50	0.425	106.38	10.00
S2	75.35	8.55	9.30	0.500	141.35	47.75
S3	76.03	8.28	9.50	0.525	150.95	58.50
S4	113.85	8.18	9.60	0.525	326.63	158.25

CONCLUSIONS

Composted winery wastes would play a positive role in Lebanese agricultural production. Those widely available wastes could be used in large quantities as a fertilizer providing nutrients to crops and could safely alternate chemical fertilizers in local cultivations. Grape marc compost also confers adequate characteristics for being used as a soil conditioner due to its richness in organic matter and could improve the physical characteristics of heavy soils by improving water drainage. It could provide the highest benefit to plant when added to soil in quantities of 25% by volume. On the economical level, using composted grape marc has a significant advantage shown by the reduction of production costs compared to conventional substrates.

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BIODIGESTER BALES: METHOD FOR THE ECOLOGICAL MANAGEMENT OF ORGANIC RESIDUES

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ABSTRACT

The environmental and public health problems by inadequate management of organic waste continue to worsen in many parts of the world. The high rates of waste generation associated with deficiencies in collection and treatment services are a source of negative impacts; the inadequate disposition of the material bound to an incorrect separation causes proliferation of pest species, bad smells, toxic gases formation, fumes and dust that contribute to the contamination of the ecosystems. The cause of these problems that alter the quality of the environment and the health of the people is certainly of anthropic origin, because in nature, the recycling of nutrients is an essential function for life. In view of this scenario, it is proposed the implementation of an ecological method, alternative to traditional compost, which has been proven effective by developing it in higher education institutions and university. Among the benefits offered is the management of tons of organic material in small spaces, the sustainable use of waste derived from food consumed in schools, among others. Studies for the analysis and evaluation of the physicochemical and nutritional quality of the organic fertilizer obtained in different climatic conditions, from biodigester bales assembled in Medellín, Colombia and Texcoco, Mexico, showed favorable amounts of nutrients that benefit the growth of seedlings planted in the same bale or when applied as fertilizer in gardens and orchards. Therefore, the development of this proposal also benefits the urban and ecological agriculture areas.

Key words: *biodigester bale, organic fertilizer, sustainability, school.*

INTRODUCTION

The question of whether organic waste is an environmental public health or natural resource problem is easy to answer if analyzed from a comparative framework between how nature handles and recycles nutrients cyclically, unlike linear and determinists methods that humanity implements, which endanger the quality of ecosystems and thus the well-being of people. Humanity has undoubtedly walked against the laws of nature, since the management of organic matter as polluting waste, deteriorates the environment and causes disease, while in soils they are a natural resource of nutrients and biodiversity.

In ecosystems such as forests, recycling of nutrients is an essential function for life, is generated through biogeochemical cycles and ecological processes of decomposition. Emulating those behaviors and strategies that are carried out in the upper mantle of the Earth, where decomposing organisms transform matter, mineralize and synthesize it by enriching the soil with nutrients and life, is possible through the method of Biodigester Bales; which is a microecosystem that is built in a handmade way, works outdoors and imitates the soil ecology, essentially in the organic horizon, where biodegradable matter accumulates and transforms into organic fertilizer, a stable and mature solid product similar to Humus; dark brown with water, air and living organisms (Ossa, 2016a).

The Biodigester Bale is a method for the biological digestion of all types of organic waste, for example, those that are generated in kitchens, such as fruit peels, vegetables, decayed or cooked foods residues or wastes. Also those produced in green areas such as leaves, seeds, fruits or branches, pruning of gardens or lawns and animal droppings (Ossa, 2016b). It is an autonomous transformation mechanism that digests organic waste through a fermentative decomposition process, without infrastructure and without any contamination. They allow disposal, treatment and use of biodegradable waste at the source, processing large quantities of organic material in confined spaces. In bales of one cubic meter for example it is possible to store between 500 and 600 kg of organic waste.

With the Biodigester Bale as organic waste is recycled and managed without generating bad odors, proliferation of flies, pollutant gases or high amounts of leachate (Cano *et al*., 2015). Biodigester Bales are cleaned and beautified environments, they also can be used as a landscape proposal for green areas, as planters or spaces conducive to the construction of organic gardens. Thus, Biodigester Bales are multipurpose by becoming also atmospheric carbon fixing systems due to the photosynthesis performed by plants that, by natural or induced ecological succession, develop on it. The process of digestion of the organic matter in the Bale takes about six months, after this period it is possible to obtain the organic fertilizer as the final product of biodegradation (Ossa, 2016c). Other studies that compares the substrates obtained from the Biodigester Bale method and composting prove the quality and maturity stage when they are submitted to fermentation processes (Posada, 2015).

Some communities that have been responsible for the organic waste they generate and recycle them by Biodigester Bales, show the benefits of this mechanism by providing healthy environments and protecting public health. The method of Colombian origin that has been applied in institutional, communal, neighborhood, industrial, residential, recreational and even investigative environments has proven to be ecologically efficient and economically viable for the integrated management of organic waste. In addition, these actions are related to the state of knowledge generated by the Ecopedagogy, considered as a new paradigm that focuses on life.

It is education for a responsible action towards and by the environment, linked to space and time, where the relations between the human being and the environment take place (Antunez and Gadotti, 2005). The purpose of this study is to carry out a comparative analysis based on the experience of the University of Antioquia, Medellin-Colombia and the Official High School Number 100 (EPO 100), Texcoco-Mexico, in order to demonstrate the socioecological importance of recycling and properly managing organic waste in educational institutions of higher education and university level, through the technique of Biodigester Bale. As a comparison mechanism, a qualitative analysis was implemented in the laboratory to evaluate the quality and the physicochemical and nutritional characteristics of organic fertilizer obtained from the assembled biodigesters in both contexts.

MATERIALS AND METHODS

The Biodigester at the University of Antioquia was assembled in the green zone between block 20 and 21 of the Faculty of Engineering, through the classroom project conducted with the students of Introduction to Sanitary Engineering of the semester 2015-2. Approximately 200 kg of kitchen waste and 400 kg of vegetable waste were used as well as a metal frame about one meter wide, one meter long and a half meter high (Ossa, 2016a). The images 1, 2 and 3 shows (Biodigester Bale setting-up) the Biodigester Bales assembled at the University of Antioquia during its construction, decomposition and extraction of the organic fertilizer sample, respectively.

Fig. 1. Construction process

Fig. 2. Decomposition process.

Fig. 3. Organic fertilizer extraction

The process at EPO 100 began at the end of 2016 through a theoretical workshop on training and awareness-raising, developed at the institution by the environmental engineer Catalina Ossa, under Erasmo Velázquez´s coordination, with a participatory environmental education proposal for students and teachers to learn the ecological management of organic waste with Biodigester Bales. This gave rise to a process of integral management of the organic residues from kitchen that are generated there, through the treatment at the bale setting and was used as organic fertilizer for the school's garden crops (Velázquez C.& Victorino, 2016)..
The first bale was made as a prototype for participants to learn how to build them. Currently, two bales are made per week of 1 cubic meter each, using pine wood mold, recycling about a ton of organic waste per week with students work. All this, through the cross-linking of specific thematic contents in subjects of natural and social sciences areas, where the teachers of these academic disciplines integrate the technique of the "Biodigester Bales" as part of their educational praxis, which is included in their didactic lesson planning (Velázquez, 2016). For this, aspects of the technical theory of the curriculum are taken up, in which "... society and culture are considered as an external network of the school and the curriculum, as a context characterized by social needs and objectives Which education must respond by discovering those needs and developing programs to achieve the goals and objectives of society "(Kemmis, 1998: 112)

Figure 4. Students and teacher´s participation in the "Biodigester bales" elaboration process at EPO100.

At the end of the decomposition process of the organic residues in the bundles or bales assembled at the University of Antioquia and in the EPO 100, a sample of organic fertilizer of 250 and 500 grams respectively was taken, which were sent to the Soil Department laboratory in the Chapingo Autonomous University (UACh) located in Texcoco and to the laboratory of the Interdisciplinary Group of Molecular Studies (GIEM) located in Medellín, respectively, in order to know its quality by means of a qualitative analysis of its physicochemical properties and the macro and micronutrients content, from the evaluation of the following parameters:
• Ph: Potentiometric in relation. Sample: Water 1: 5
• Electrical Conductivity (CE): Suspension conductivity bridge. Sample: Water, 1: 5.
• Organic matter: Calcination
• Apparent density: Probe method.

• Cation Exchange Capacity (ICC): Ammonium acetate 1.0 N Ph 7.0 and determined by steam entrainment.
• Nitrogen (N): Digested with diacid sample and determined by steam entrainment.
• Phosphorus (P): Digested with diacid sample and determined by photocolorimetry by reducing reduction with molybdo-Vanadate.
• Potassium, Sodium (K, Na): Digested with diacid sample and determined by flame emission spectrophotometry
• Calcium, Magnesium, Zinc (Ca, Mg, Zn): Digested with diacid sample and determined by atomic absorption spectrophotometry.
• Carbon / nitrogen ratio (C / N): Determined by calculation.

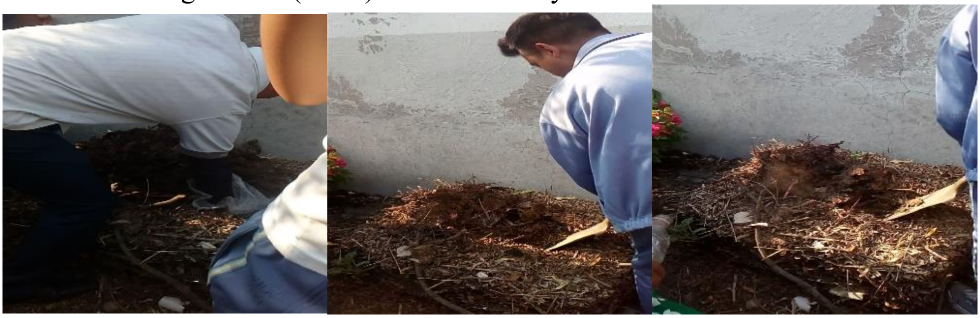

Figure 5. Obtaining the sample for the laboratory analysis of the substrate of the biodigester Bale in EPO 100.

By means of a validation carried out according to the optimum and quality ranges required by Colombian Technical Standard (NTC) 5167 for commercial organic fertilizers, analysis and discussion of the results obtained in the laboratory of both cases was made.

RESULTS AND DISCUSSION

The qualitative analysis of the laboratory to evaluate the quality of the samples of organic fertilizer obtained in each of the Biodigester Bales assembled in the University of Antioquia and in the EPO 100, showed the data recorded in table 1, where the physicochemical parameters and the respective macro and micronutrient content as well as the optimum quality value required by Colombian Technical Standard (NTC) 5167 for commercial organic fertilizers.

Table 1. Physicochemical parameters and nutrient content of organic fertilizer samples.

Parameter	**Result of organic fertilizer UdeA**	**Result of organic fertilizer EPO 100**	**Units**	**Optimal value**
pH	7.12	7.96		>4 y <9
Electrical coductivity (CE)	0.076	1.06	dS/m	Smaller than 3
Cation Exchange Capacity (ICC)	51.8	47.1	meq/100g	Mínimun 30. Optimal >67
Density	0.42	0.85	g/cm3	Max. 0,6
Total oxidizable organic carbon	21.8	15.54	%	Minimum 15%
Nitrogen (N)	1.6	1.05	%	Between 2% y 3%
Potassium (K)	0.6	0.85	%	> 1%
Phosphorus (P)	0.47	0.21	%	> 1%
Magnesium (Mg)	0.38	0.22	%	> 1%
Sodium (Na)	N.R	0.41	%	> 1%
Calcium (Ca)	4.38	0.47	%	> 1%
Zinc (Zn)	N.R	57	mg/kg	> 1%
Relation C/N	13.7	14.8		Smaller than 20

*N. R: No Record.

The physicochemical parameters have the characteristic to determine and to reflect the properties and constituents of the organic fertilizer; they are used as an indicator that establishes the quality and state of maturity of the organic matter transformed by numerical quantities (Ossa, 2016a). The physicochemical and nutritional characteristics of the samples evaluated vary according to the environmental conditions of each site, the type of material originally used for the assembly of the biodigesters, the digestion time of the material and the organisms involved in the degradation. According to the data recorded, both samples of organic fertilizer showed favorable conditions and important amounts of nutrients. The pH is an indicator to demonstrate the process of digestion of organic matter, in both samples were registered values between a range of 6 and 8, very close to neutrality, which means that the material has stabilized, acquiring a large presence of humic compounds. In the case of electrical conductivity, both samples reported values lower than 3dS / m, which is characteristic of organic fertilizers with great potential for seed germination and plant development. The density is considered a determinant of the quality of the fertilizer, it represents the capacity of absorption, aeration and structure of the material. According to NTC 5167/2004, the maximum allowable value for tradable organic fertilizers is 0.6 g/cm^3, of the analyzed samples only the fertilizer produced in the biodigester bundle of EPO 100 does not comply with this parameter, which means that this material, although of very good quality, could not be marketed according to the referenced standard. The (ICC) and the carbon-nitrogen ratio are directly related, represent the state of maturity of the organic fertilizer. In both samples analyzed the results are within the optimum value, which means that they are at suitable maturity level for use as substrate for soils, organic crops or as a source of nutrients for plants. In addition, according to the registered macro and micronutrients content, both samples are an organic fertilizer of very good quality, due to the variety of elements that can contribute as carbon, nitrogen, phosphorus, potassium, calcium, magnesium, zinc, sodium, which improve the productivity of crops, fertility and biodiversity in the soil, garden or garden, and plant health. According to NTC 5167/2004 a high quality organic fertilizer must contain higher amounts of macronutrients such as carbon and nitrogen and at least 1% of each micronutrient. In the particular case of the organic fertilizer sample of the U. de A., the carbon and nitrogen contents were 21.8% and 1.6%, respectively. No sodium and zinc contents were recorded; the potassium, phosphorus and magnesium were in amounts less than 1%, unlike calcium which represented a percentage of 4.78. In the organic fertilizer sample of EPO 100, the amount of macronutrients such as carbon and nitrogen was 15.54% and 1.05% respectively; Although phosphorous, potassium, calcium, magnesium, zinc and sodium contents were recorded, all showed less than 1%. Both studies show that the substrate of the bales can be used for the soil to benefit plants and trees, providing nutritional inputs that favor its development. In turn, the germination of seeds until their growth in seedlings within the Biodigester Bales turns out to be a timely alternative in areas where the soil is eroded or contaminated.

CONCLUSIONS

Biodigester Bales allow to treat all types of biodegradable waste; it is an efficient producer of fermented organic fertilizer that represents a macro content and important micronutrients, perhaps not in the amounts that stipulate the norm, but to supply the demand of the plants and improve the quality and fertility of soils. To obtain specific amounts of nutrients in the organic fertilizer, it will be necessary to determine the characteristics of the initial material that is available and used in the process of assembling the Biodigester Bale, which represent the final quality of the substrate. The evaluated samples showed favorable physicochemical and nutritional conditions, which means that the organic fertilizer obtained through the method of the Biodigester Bale can be used to improve the nutrient source of the soil, its biology and quality or as a substrate for seedlings, nurseries, orchards or gardens. It reduces costs in the acquisition of industrial fertilizers and definitely favors the sustainable management of organic waste that can be applied successfully in the areas of urban and ecological agriculture. The implementation and follow-up of this method allows to generate an own experience that provides relations between the individual and nature; is reflected the link with the environment and the development of a social conscience for the benefit of people and the environment. Educational institutions are the way to promote these ecological practices, respect for the environment begins at home and also at school. The awareness of young people about these settings and the way they learn to treat them will help them become responsible adults. For this, teachers play a key role in the development of these activities and to achieve them, it is possible to link the contents of the subjects with the ecological question, thus achieving a curricular environmental awareness.

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STATE AND PROSPECTS OF SUNFLOWER PRODUCTION IN UKRAINE

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is among the three most significant oilseed plants in the world (along with, soybean and rapeseed) and one of the two most produced oil crops in the European Union, together with rapeseed. Over the past decade, Ukraine has maintained its position as leading producer and exporter of sunflower seeds and ranks first for sunflower oil consumption globally. A recent United States Department of Agriculture report in 2017 suggest that, Ukraine presently (2015−2016) ranks first in sunflower production globally with a 29.3 % share of total world sunflower output of 40.57 million metric tons. Respectively, Russia and the European Union are currently ranked second and third, with a share of 22.6 % and 18.9 %, followed by Argentina and China that produced 6.7 % each. The main goal in sunflower breeding is to create hybrids with high genetic potential for seed yield above 5 t/ha, but environmental factors seems to limit current sunflower yields to the production range of 1.5–3.0 t/ha. In this study, however, we report new sunflower varieties and hybrids in Ukraine that yield even slightly above 3 t/ha. Thus, a unique sunflower production technology for the forest-steppe of Ukraine for instance now provides sunflower seed yields of 2.9-3.5 t/ha. This became possible after the introduction of new high-yielding varieties and hybrids, and the improvement of sunflower cultivation technologies for specific natural and climatic zones. Hence, further increases in global sunflower seeds output mainly from Ukraine are expected without expansions in limited agricultural lands.

Keywords: *sunflower,varieties and hybrids, yield, environmental factors, Ukraine.*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is among the three most significant oilseed plants in the world (along with, soybean and rapeseed) and one of the two most produced oil crops in the European Union, together with rapeseed (United States Department of Agriculture - USDA, 2017). A recent USDA report in 2017 suggest that, Ukraine presently (2015−2016) ranks first in sunflower production globally with a 29.3 % share of total world sunflower output of 40.57 million metric tons

(USDA, 2017). Respectively, Russia and the European Union are currently ranked second and third, with a share of 22.6 % and 18.9 %, followed by Argentina and China that produced 6.7 % each.

It is indicated that, due to the genetic variability of cultivated sunflowers, it is possible to develop hybrids with a genetic potential for seed yields of over 6 t/ha and seed oil content above 55% (Škorić et al., 2007; Škorić, 2012). Even more recently, Jocić et al., (2015) similarly stated that, the main goal in sunflower breeding is to create hybrids with high genetic potential for seed yield above 5 t/ha and oil content in seed of over 50%. In spite of this well documented potential, environmental factors seems to limit current sunflower yields to the production range of 1.5–3.0 t/ha (Kaya, 2015). Just recently, Kaya (2015) advised that Breeders should pay meticulous attention to eliminating or minimizing extreme environmental factors to ensure that a minimum of 4.0 t/ha sunflower yields are reached.

The present study therefore investigates the range of sunflower seed yield and oil content of improved hybrids under the climatic condition of the Forest steppe of Ukraine. We also ascertained the dynamics of sunflower seed production (output) along with two major oilseeds (Soybean and rapeseed) in Ukraine for the past 16 years (2000‒2016).

MATERIALS AND METHODS

The object of the research is sunflower (*Helianthus annuus* L.), namely the cultivated area, yield and elements of cultivation technology in the forest-steppe of Ukraine for the period from 2000 to 2016. Secondary data and reports were obtained from the State Statistics Service of Ukraine.

The experimental part of the work was conducted at the training and practical center of Sumy National Agrarian University (Ukraine) for two years (2015 and 2016). Experiments were laid on black soil, characteristic for coarse-medium loam. Five hybrids were sown at 3 plant densities (50, 60 and 70 thousand plants/ha) with 6 rows in each plot and 70 cm between rows. Harvesting was done manually at maturity by harvesting two inner rows in each plot.

Hydrothermal coefficient (HTC) (G. T. Selyaninov) were determined by the formula: HTC=$\sum K / \sum T \times 10$, where ∑K is the amount of precipitation, mm, for a period with an average daily air temperature above 10 °C; ∑T is the sum of the temperatures, °C, for the period with the average daily air temperature above 10 °C. The oil content of the seeds was determined using the device Spinlock Magnetic Resonance Solutions. Data were subjected to ANOVA at 5% level of probability with the statistical software STATISTICA (version 8).

RESULTS AND DISCUSSIONS

The significant potential of sunflower seeds production in Ukraine is evidenced by the positive dynamics of total harvest of sunflower seeds over the past sixteen years (Figure 1). The main component for increasing production is the increase in sunflower seed yield. Thus, the maximum gross indicator of 13626.9 thousand tons was obtained in 2016. The main areas of sunflower production currently are Kirovogradskaya (11.5%), Kharkivska (11.4%), Dnipropetrovsk (9.3%), Zaporizhzhia (7.6%), Donetsk (7.3%), Mykolayivska 7.2%). It should be noted that, there has been an increase in the share of sunflower seed production in the Central and Northern regions of Ukraine, in particular in Poltava (7.0%), Vinnytsa (5.3%), Cherkasy (4.9%), Sumy (4.2%) and Chernihiv (3.0%) (http://www.ukrstat.gov.ua/).

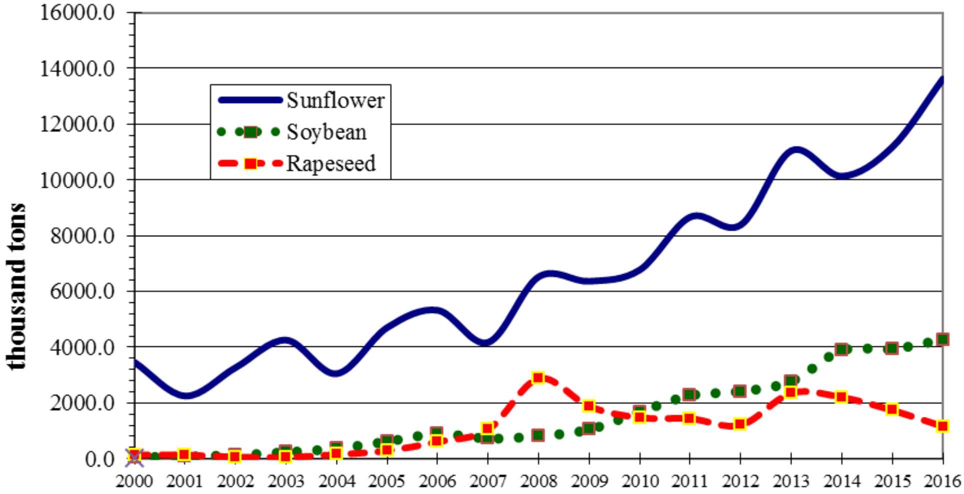

Figure1. Dynamics of seed production of major oilseeds in Ukraine from 2000-2016
(Adapted from State Statistics Service of Ukraine, http://www.ukrstat.gov.ua/)

The second place among oilseeds produced in Ukraine is soybean. According to the State Statistics Service of Ukraine, in 2016, the soybean area cultivated was 1.87 million hectares compared to 60.6 thousand in 2000. During this period, the productivity of the crop increased from 1.1 to 2.4 t/ha. In recent years, there has been a tendency to reduce production of rapeseed, due to the decline in world oil prices, as well as cheap raw materials for energy production. A similar situation is observed in Ukraine, its area is limited, and the production of seeds and oils from it is not always effective from an economic point of view. The main reason for this condition is the possibility of death due to unfavorable conditions and low yield of the seeds of this crop. Sunflower is a typical crop of the steppe and forest-steppe zone of Ukraine. The success of its cultivation is largely determined by the changing environmental conditions, that is, weather and climate. Observations by the metrological network of Ukraine testify to the fact that regional climate change, especially temperature rise, has already affected a number of meteorological characteristics. The average annual air temperature has increased, the terms of formation and duration of snow cover have changed, the heat supply of the growing season gradually increased, and the number and intensity of adverse meteorological phenomena (drought, heavy rain, etc.) has increased (Melnik et al., 2015). For example, in the Forest-steppe of Ukraine we considered the main meteorological parameters for the period from 2000 to 2016 (Table 1).

Table 1.Total temperature, total precipitation (rainfall) and hydrothermal coefficients (HTC) in the Forest-steppe of Ukraine (April-August) from 2000−2016

Year	Total temperature, ° C	Totalprecipitation, mm	HTC	Moisture for the year
2000	2528.2	269.2	1.06	normal
2001	2567.5	319.3	1.24	normal
2002	2653.1	292.6	1.10	normal
2003	2419.7	258.9	1.07	normal
2004	2311.2	351.9	1.52	wet
2005	2740.7	202.8	0.74	dry
2006	2511.5	365.7	1.46	wet
2007	2608.9	198.7	0.76	dry
2008	2626.1	304.5	1.16	normal
2009	2559.6	319.2	1.25	normal
2010	3132.2	171.4	0.55	dry
2011	2839.4	253.5	0.89	dry
2012	3090.7	218.8	0.71	dry
2013	2956.4	213.1	0.72	dry
2014	2899.8	284.1	0.98	dry
2015	2777.6	306.4	1.10	normal
2016	2865.6	461.7	1.59	wet
Average 2000−2016	2710.8	281.9	1.05	normal
Average perennial	2425.0	294.0	1.21	normal
Deviation of the parameters	285.8	-12.1		

Thus, according to the results of the analysis of meteorological conditions for 16 years, it has been established that for the period of the growing (farming) season (April-August), there was an average of 281.9 mm of rainfall, with fluctuations from 171.4 to 461.7 mm. In this region, the average temperature during this period amounted to 2710.8 ° C with fluctuations ranging from 2311.0 °C to 3090.7 °C. Increase in the heat supply of the growing season was noted at 285.8 ° C and decrease in the amount of precipitation by 12.1 mm. Based on HTC which for the past 16 years has decreased from 1.21 to 1.05, it was established that the conditions of this region correspond to the conditions of the Steppe zone of Ukraine. The temperature and moisture differed for the investigated years. From the data given (Table 1), dry conditions (HTC to 1.0) were the growing periods 2005, 2007, 2010, 2011, 2012, 2013, 2014, normal moisture (HTC 1.0-1.3) - 2000, 2001, 2002, 2003 , 2008, 2009, 2015 and wet conditions (HTC above 1.3) were only in 2004, 2006 and 2016. The influence of moisture conditions on yield is clearly shown (Figure 2).

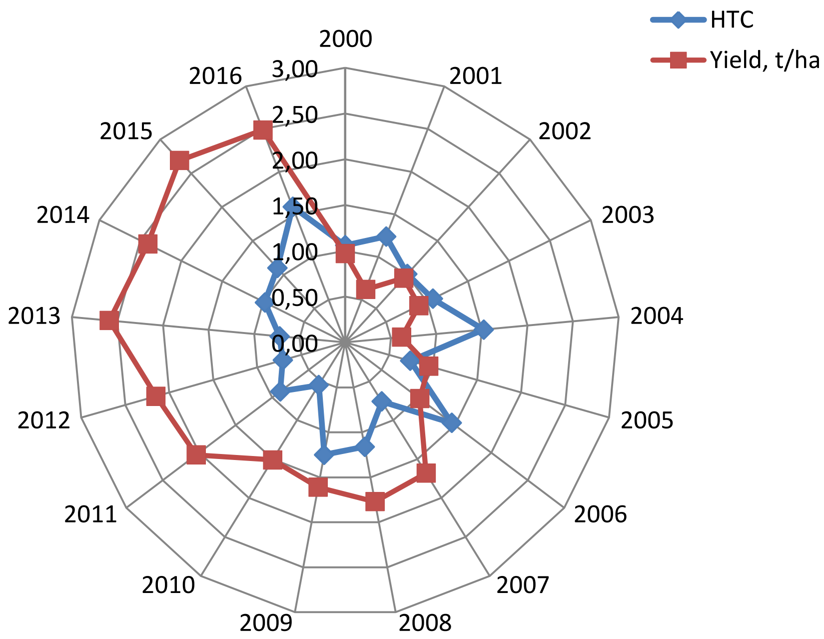

Figure 2. Dynamics ofaverage yield of sunflower and HTC under the conditions of the Forest-steppe of Ukraine (for the period of 2000-2016)

It should be noted that, there was positive dynamics in the increase in average yields of sunflower seeds in the forest-steppe of Ukraine in recent years from 0.62 t/ha to 2.69 t/ha. The highest average yield of seeds was obtained in dry and normal moisture years, while the lowest were in years with excessive moisture. The revealed facts prove that under the conditions of low temperatures and excessive moisture, the growing season of plants is prolonged, and harvesting falls in October, which causes massive damage to plants by diseases. According to results of research conducted in 2010-2015 at the Department of Crop Production in Sumy National Agrarian University in Ukraine, a regional technology for sunflower cultivation, which involves the selection of adapted hybrids and optimization elements was developed. According to the Ukrainian State Register for Plant Varieties (SRPV), in 2017, the agricultural producer presently have 740 varieties and hybrids of sunflower of which 664 are linoleic, 51 - high oleic, 22 - confectionery and palmitic – 3. An important segment of the market in Ukraine is occupied by high-performance adapted hybrids of domestic breeding: the Institute of Plant Cultivation V. I. Yuryev NAANU (52), Institute of Oilseeds of NAANU (23), Selection-Genetic Institute of NAANU (11), VNIS Ltd. (11), Ukrainian Seed Ltd. (11) and a number of other institutions.
The share of hybrids of sunflower from foreign selection is growing, in particular, the Institute of Agriculture and Horticulture, Novi Sad, Serbia (70), Euralis Semans, France (66), Syngenta, Switzerland (49), Maisadur Semans, France (41),

Kossad Semans ES , France (33), Limagrain Europe, France (28), Pioneer, Austria (26), ASPIRIA SIDS ESA, Luxembourg (19), Dow AgroSciences, Austria (15) (SRPV, 2017). The ecological testing of the modern sunflower seeds by us for this natural-climatic zone recommends these hybrids: Gaychur, Zlatson(Institute of Plant Science named after V.I. Guryev, Ukraine); Consul, Coral, Rehion (Institute of Oilseeds, Ukraine); PR63A86, PR63A90, PR64A71, PR64H32 (Pioneer, Austria), LG55.50 (Limagrain Europe, France); Condi, Tutti, Tehnika brio (Syngenta, Switzerland); Artic, Balistic, (Euralis, France). When these hybrids were harvested, more than 3.5 t/ha of seeds and 1.5 t/ha of oil were obtained.

It is known that sunflower is quite sensitive to the application of organic and mineral fertilizers. For the formation of 100 kg of seeds, it requires nitrogen – 4−6 kg, phosphorus – 2−5 kg, potassium – 10−12 kg, magnesium 1.6−3.0 kg, sulphur 2.6−6 kg, calcium 6−10 kg. Sunflower requires much microelement, particularly boron 9−16 g, manganese 10−17 g, zinc 14−23 g (Hugger, 1992). Under the influence of fertilizers, the assimilation surface increases and photosynthesis increases. It is established that the highest yield was collected using a complete mineral fertilizer in a dose of $N_{45\text{-}60}P_{60\text{-}90}K_{45\text{-}60}$. The following schemes of fertilization are offered: application of organic fertilizers at a dose of 20−40 t/ha provides an increase in yields of 0.34−0.51 t/ha; application of complete mineral fertilizer $N_{30}P_{30}K_{30}$ under cultivation contributes to the increase in yield by 0.52 t/ha; application in a 4-leaf phase in a dose of $N_{10}P_{10}K_{10}$ increases the yield by 0.34 t/ha. To realize the biological potential of modern high-yield sunflower hybrids, it is important to provide plants with trace elements for a sufficient level of nutrition with nitrogen, phosphorus and potassium. Given the proven role of most of them in the formation of the cryoprotective ability of plant organism and complex tolerance regarding the abiotic and biotic adverse environmental factors, it is relevant to study their effectiveness for the modern changes in climatic conditions. A particularly important issue nowadays is the study of mechanisms for reducing the negative impact ofcrop productivity under stressful conditions, which are typical for the last 2016 and 2017. These last two years in the Forest-steppe of Ukraine are characterized by sharp fluctuations of the temperature regime from 5−10 °C to 29−32 °C, excessive rainfall (10−15 mm per day) and dry periods (no rainfall for the month). Our preliminary research confirmed the effect of foliar application of fertilizers and plant growth regulators on productivity of sunflower plants, but the effectiveness of this agro-activity depends on the current weather conditions and requires further investigation. Spraying of crops before flowering (Sol Bor + Basfoliar 6-12-6; Wuxal Bio Aminoplant + Wuxal Boron; Spectrum Askorist + Spectrum B + Mo) resulted in a yield increase of 7.5−9.3%. These preparations contain: N, P_2O_5, K_2O, MgO, Mn, Cu, Fe, B, Zn, Mo, K, Amino acids, Seaweed extract of *Ascophyllum nodosum*. They were applied based on recommended concentrations (2.0−3.0 l/ha) by manufacturers. One of the most important elements of ecologically safe technologies is the use of microbiological preparations (agents) that improve plant nutrition, and increase resistance to harmful organisms. Such preparations are based on nitrogen-fixing, phosphorus-forming and antagonist bacteria. The positive effect of bacterial preparations on the development of agricultural plants is manifested in the secured higher yields of seeds. Our earlier research proved the feasibility of application of *Paenibacillus polymyxa KB,* which provides an increase of 11.6-16.1% and *Achromobacter album 1122* by 8.8-12.7% yield of sunflower seeds in the cultivars and hybrids (Melnyk, 2011). Plant density determines the level of provision of plants with moisture and nutrients, hence only properly formed plant density will ensure high sunflower yields. We have established that for Rehion and Tutti hybrids, optimal conditions are created for plant stand density at 60 thousand plants/ha. Harvested seeds in these hybrids generated 3.71 and 3.84 t/ha respectively. The maximum yields in the hybrids Zlatson (3.78 t/ha), ES Artic (3.74 t/ha) and PR64H32 (3.51 t/ha) were obtained for plant densities 70 thousand plants/ha (Figure 3).

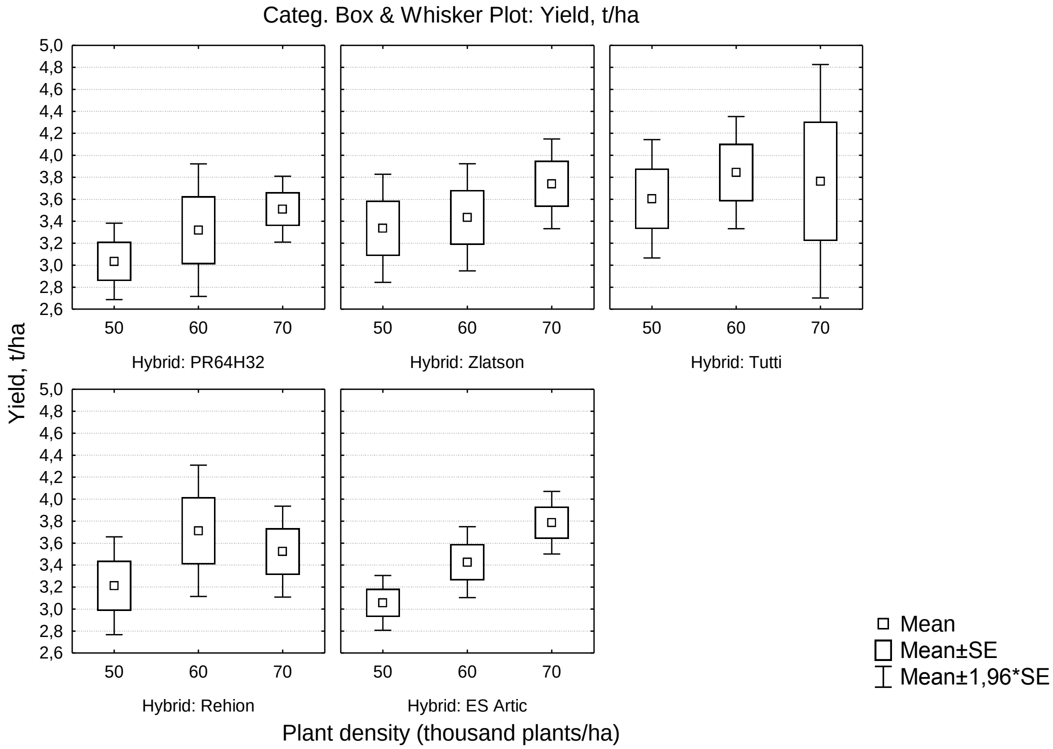

Figure 3. Yield of modern sunflower hybrids depending on the plant density (Average for 2015-2016)

On average, the studied hybrids ensured the formation of yields at the following level: Tutti (3.73 t/ha); Zlatson (3.59 t/ha); ES Artic (3.56 t/ha); Rehion (3.49 t/ha) and PR64H32 (3.37 t/ha). Under the two years (2015-2016) conditions,the oil contents for these sunflower seeds were as follows: Tutti – 47.2%; Zlatson - 46.3%; ES Artic - 47.3%; Rehion – 47.4% and PR64H32 – 48.6%.

CONCLUSION

According to the results of the analysis, trends in the meteorological parameters caused the expansion of the range of area under sunflower cultivation in Ukraine, which contributed to the increase in total national production of oilseeds. Having all the opportunities (natural, climatic, logistical and human), Ukraine will increase its presence in the world market of oilseeds. Over the past 16 years, Ukraine has increased its annual production of sunflower seeds from 3.4 million tons to 13.6 million tons. It should be noted that the increase in output over this period is more than 4 times. The main prerequisites for sustainable high sunflower harvest in Ukraine are the use of modern high-yielding hybrids and the development of regional cultivation technologies for specific climatic zones which are currently being employed. Hence, further increases in global sunflower seeds output mainly from Ukraine are expected without expansions in limited agricultural lands.

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GENETIC DIVERSITY IN SLOVAK SPOTTED BREED

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ABSTRACT

The objective of the study was to evaluate inbreeding and genetic diversity in Slovak Spotted cattle. Reference population contained genealogic information on 36949 animals (129 sires and 36820 cows) that were used in the analyses. Pedigree completeness indexes in the first three generations were on the level of 100 %, in the 5th generation it was 60 %. Since 1970, inbreeding trend was positive with significant increasing in 1990. Average relationship was 0.8 %, inbreeding rate 0.36 % and ΔF = 0.094 %. In the reference population 43 % animals was inbred, 68 % of sires and 33 % cows, with also 67 % purebred cows, as well. Total genetic diversity loss in the reference population and population of cows was the same, closely under 1%, in purebred cows 1.19 % and sires even due to higher inbreeding level 1.78 %. Genetic diversity loss was more influenced by the genetic drift 0.80% in the reference population, 1.47% in sire group, than by effective number of founder unequal contributions. F statistic showed fines superiority of heterozygosity by sire lines subpopulations, in the whole sire group (F_{IS} = - 0.12) and their minimal differentiation (F_{ST} = 0,098). Obtained results showed that inbreedization process started in this population. Monitoring and better genetic management are important from the point of its further sustainable development.

Keywords: *diversity, inbreeding, pedigree analysis, Slovak spotted, sustainable agriculture.*

INTRODUCTION

The Slovak Spotted cattle is an important dual-purpose breed has a long tradition in Slovakia mainly due to its excellent dairy as well as beef production. This autochthonous breed belongs to the Simmental cattle group. The Simmental cattle is one of the oldest and most widespread breed in the world. It is characterized by fast growth development, excellent milk production and the ability to easy adapt to climatic conditions. Mainly for these reason it is necessary to assess its current state of genetic diversity, propose measures that will prevent losses of variability and ensure the sustainable diversity of this breed for future generation. The Slovak Spotted breed were officially accepted as autochthonous breed in 1958. Purebred

Slovak Spotted breed reach a maximum population size in 1975. After 1990 size of breed has decreased significantly due to transformation processes in agriculture and exploitation of Holstein sires for crossbreeding (Kadlečík et al., 2013).

Genetic variation is vital for the populations to adapt to varying environments and to respond to artificial selection. Any conservation and development strategy should start from assessing the state of variation in the population (Toro et al., 2011). Breeding strategies should be oriented to minimize the inbreeding level and its increase. Uncontrolled inbreeding can lead to inbreeding depression which is accompanied by deterioration of performance including fitness traits. Therefore, the control of inbreeding level is an important tool in population management as well as a precondition to maintain genetic diversity (Pavlík et al., 2013). Pedigree analysis is very effective method in evaluation of inbreeding level (Kadlečík et al., 2011; Danchin - Burge et al., 2012; Pavlík et al., 2012), loss of genetic diversity (Hazuchová et al., 2013; Kadlečík et al., 2016), and genetic management of small endangered populations (Mészáros et al., 2015). The aim of the study was to evaluate level of inbreeding and genetic diversity in Slovak Spotted cattle.

MATERIALS AND METHODS

The genetic diversity analysis of Slovak Spotted cattle was performed using genealogical data obtained from SBS, a. s. of the Slovak Republic. The pedigree file (RodS) consisted of 109,686 individuals (105,229 dams and 4,457 sires). The reference population (RP) of Slovak Spotted cattle covered living animals, sires in insemination (129) and dams included in performance testing (36,949). The database of individuals was prepared by SAS v9.3 software and recoded in CFC software (Sargolzaei et al., 2006). The current state of diversity and trends of inbreeding were estimated by using Endog v4.8 software (Gutiérrez and Goyache, 2005).

The pedigree completeness was evaluated based on the equivalent complete generations of ancestors and pedigree completeness index described by MacCluer et al. (1983). The equivalent complete generations is computed as the sum over all known ancestors of the terms computed as sum of $(1/2)^n$ where n is the number of generations separating the individual to each known ancestor (Maignel et al., 1996). The pedigree completeness index was estimated as follows:

$$PCI = \frac{2C_{sire}C_{dam}}{C_{sire} + C_{dam}},$$

where C_{sire} and C_{dam} are contributions from the paternal and maternal lines, and $C=\frac{1}{d}\sum_{i=1}^{d} gi$; where g_i is the proportion of known ancestors in generation i; and d is the number of generations that are taken into account.

The loss of diversity was expressed through parameters based on the probability of identity by descent (inbreeding coefficient of an animal F, individual increase in inbreeding ΔF_i, average relatedness AR), and Wright's F-statistic. A measure of inbreeding of an individual (F) is defined as the probability that both alleles in one locus are derived from the same ancestor or are identical by descent (IBD). The increase in inbreeding (ΔF) is calculated according to Gutiérrez et al. (2009) as follow:

$$\Delta F = \frac{(F_t - F_{t-1})}{(1 - F_{t-1})},$$

where F_t and F_{t-1} are the average inbreeding at the i^{th} generation and $1 - F_{n-1}$ is increase of inbreeding in last generation. The individual increase in inbreeding is computed as:

$$\Delta F_i = 1 - \sqrt[t-1]{1 - F_i},$$

where F_i is the individual coefficient of inbreeding and t is the equivalent complete generations (Maignel et al., 1996). The average relatedness coefficient (*AR*) of each individual is defined as the probability that an allele randomly chosen from the whole population in the pedigree belongs to a given animal. *AR* can be interpreted as the representation of the animal in the whole pedigree regardless of the knowledge of its own pedigree. *AR* is an alternative or complement to *F* and can be used to predict the long-term inbreeding of a population because it takes into account the percentage of the complete pedigree originating from a founder at population level Gutiérrez et al. (2009).

The genealogical information of Slovak Spotted population was also used to infer its genetic structure based on F-statistic according to Wright (1978). Caballero and Toro (2002) formalized the pedigree tools necessary for the analysis of genetic differentiation in subdivided populations starting with the average pairwise coancestry coefficient (f_{ij}) between individuals of two populations, i and j, of a given metapopulation including all N_i x N_j pairs. For a given subpopulation i, the average coancestry, the average selfcoancestry of the N_i individuals and the average coefficient of inbreeding would be, f_{ii}, s_i, $F_i = 2s_i - 1$ respectively. From these parameters and the corresponding mean value for the entire metapopulation Caballero and Toro (2002) obtained Wright's (1978) F-statistic as:

$$F_{IS} = \frac{\tilde{F} - \tilde{f}}{1 - \tilde{f}}\ ,\ F_{ST} = \frac{\tilde{f} - \bar{f}}{1 - \bar{f}} = \frac{\bar{D}}{1 - \bar{f}}\ ,\text{and}\ F_{IT} = \frac{\tilde{F} - \bar{f}}{1 - \bar{f}}\ ,$$

where $\tilde{f}$ and $\tilde{F}$ are the mean coancestry and the inbreeding coefficient for the entire population respectively, and $\bar{f}$ the average coancestry for the subpopulation so that $(1 - F_{ST}) = (1 - F_{IS})(1 - F_{ST})$. In our study the subpopulations were defined according to sire lines. The line of sire was considered as separate subpopulation only if it contained minimally 5 bulls, regardless number of their offspring. Based on this, overall 32 subpopulations were found in pedigree file.

RESULTS AND DISCUSSION

In the reference population of Slovak Spotted cattle the equivalent complete generations of ancestors reached average value 4.53 that indicated good level of pedigree completeness comparable also with previous studies (Mc Parland et al., 2007; Sölkner et al., 1998). The average MacCluer's index of pedigree completeness was higher than 80% in the first five generation, hence presented results have good reliability. The reference population had hundred percent completed pedigrees in the 1st generation and had decreasing tendency with increasing generations (Fig. 1).

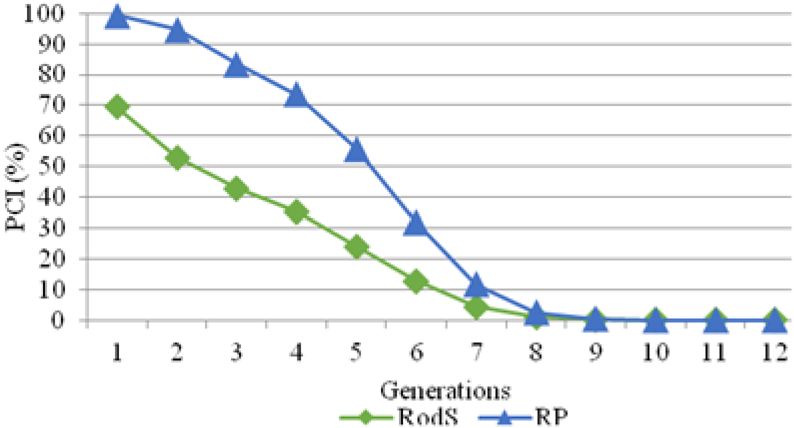

Figure 1. Pedigree completeness index by generations

Generally, the average relationship was 0.8 %, inbreeding rate 0.36 % and ΔF = 0.094 %. The average value of inbreeding coefficient was 0.14% in pedigree file and 0.76% in group of sires. The average ΔF_i among generations was highest in the group of sires (0.19%), whereas the average relatedness was relative balanced regardless gender of animals. In the future, it is therefore possible to expect the increase of inbreeding with the same intensity in population of sires as well as in dams. Compared to our results, in Irish population of Simmental cattle was found much higher level of inbreeding (around 1.35%). This value presented by Mc Parland et al. (2007) is probably overestimated due to low completeness of available pedigree information. Similarly, higher value of average *F* was reported for French population of Simmental cattle (Danchin - Burge et al., 2012). Baumung and Sölkner (2003) indicated that if the value of equivalent complete generations of ancestors is close to 5 or higher the effect of the pedigree data on the resulting value of inbreeding coefficient is only non-significant. The obtained values of all parameters derived from the probability of identity by descent indicated higher level of diversity within Slovak Spotted cattle compared to Slovak Pinzgau cattle (F_i = 0.57%, ΔF_i = 0.25%, *AR* = 1.17%) or Slovak Holstein population (F_i = 1.92%, ΔF_i = 0.48%, *AR* = 2.38%) (Kadlečík et al., 2011; Pavlík et al., 2012). The proportion of inbred animals in reference population of Slovak Spotted cattle (42.77%) was comparable with Irish Simmental cattle (< 50%), while the average inbreeding coefficient of inbred animals was lower (0.85% vs. 2.21%) (Mc Parland et al., 2007). If we taking into account pedigree file of Slovak Spotted cattle than the proportion of inbred animals was 15.95% with F_i = 0.88 %. Within inbred animals the highest level of inbreeding was found in sires (1.11%). Observed average value of inbreeding coefficient should correspond to its real value. The level of inbreeding was generally low (< 1 %). The trend of inbreeding increase in reference population and pedigree file by birth year (± 2 years) is shown on Figure

2. The average value of inbreeding coefficient in inbred animals decreased, whereas the proportion of inbred animals and level of inbreeding in metapopulation had increasing tendency probably due to common ancestors in more distant generations in pedigree. In last decade, the higher increase of inbreeding was found in population of sires compared to dams. The average value of inbreeding coefficient in group of sires increased by 0.3% compared to the previous period (1991-2000). One of the reason can be mainly the utilization of older ancestors that can be found in a large part of the pedigree file in insemination process.

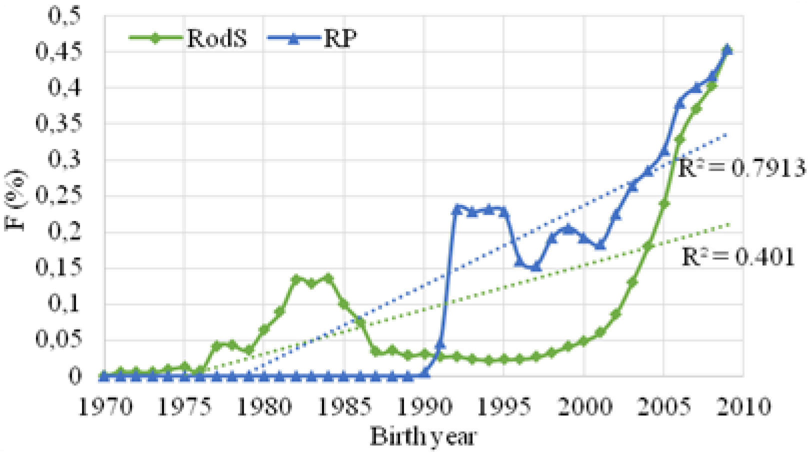

Figure 2. Trend of inbreeding in reference population and pedigree file by year of birth.

As we can see on the Figure 3 the highest increase of average relatedness in both pedigree and reference populations was found in period 2001 - 2010. The cows and heifers revealed higher average relatedness (0.65%) compared to bulls (0.57%). Total genetic diversity loss in the reference population and population of cows was the same, closely under 1%, in purebred cows 1.19 % and sires even due to higher inbreeding level 1.78 %. Genetic diversity loss was more influenced by the genetic drift 0.80% in the reference population, 1.47% in sire group, than by effective number of founder unequal contributions.

Genetic differences among subpopulations derived from sire lines was tested by using Wright's F-statistic. The average coefficient of coancestry in metapopulation defined based on the sire lines was 0.0125. The observed negative value of Wright's F_{IS} index (-0.118) signalized sufficient proportion of heterozygote animals within each subpopulation. The F_{ST} index reflects amount of differences in allele frequencies among subpopulations. Its value close to zero (F_{ST}=0.098) indicated only slight level of genetic differentiation within them. All subpopulations of each defined metapopulation have almost the same allele frequency (the differences between the subpopulations are small) as the whole population, although each subpopulation has a significant amount of heterozygotes. Based on F_{ST} values we were able to detect three main genetic clusters reflecting the genetic distances among subpopulations defined by sire lines or in other words the genetic background of most frequently used ancestors (Fig. 4).

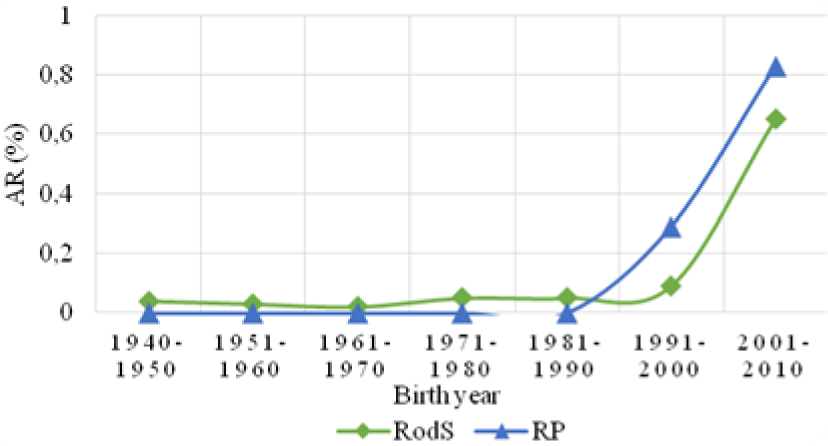

Figure 3. Average relatedness in pedigree and reference populations by birth year.

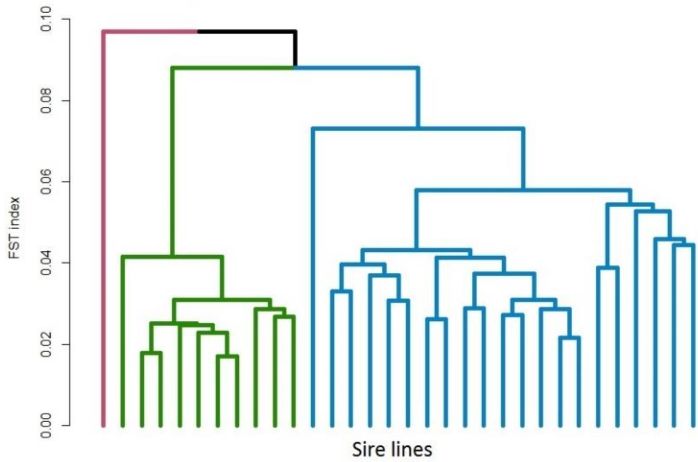

Figure 4. Genetic structure of Slovak Spotted population based on F-statistic.

CONCLUSIONS

The low obtained values of parameters derived from the probability of identity by descent point up to the fact that the breeder's effort was aimed to prepare the breeding strategy to minimize mating of inbred individuals. Compared to other breeds, these results suggest a wider genetic basis of the Slovak Spotted cattle. However, the average value of relatedness coefficient in reference as well as in the whole pedigree file was higher than inbreeding coefficient. Therefore it could be assumed that the number of inbred individuals will increase in the next generation, while continuing to practice the system of selection techniques. Mating programs are widely used already, and these can be easily modified to consider inbreeding avoidance in the next generation. Therefore long-term maintenance of genetic diversity will depend on methods minimizing mating of inbred parents. Our results may be used in genetic management of breeding practices of Slovak Spotted cattle.

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THE COMPETITIVENESS OF AGRICULTURAL AND FOOD PRODUCTS OF MOLDOVA ON THE FOREIGN MARKETS: ASPECTS AND TENDENCIES

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ABSTRACT

Competitiveness has become a key issue on international markets since it can be considered as the major source of export development. A country that utilizes the best its resources within its agricultural sector may benefit from comparative advantage on international agricultural markets. The paper analyzes the agricultural and food trade of Moldova from different aspects of inter and intra industry trade. The aim is to estimate the trend and extent of inter and intra-industry trade in agricultural and food products of Moldova. The time series from 2001 to 2015 are examined. Moldova's share on agri-food exports is still large, but various changes occurred in the total value and the geographical structure of trade flows during the analyzed period. The analysis of competitiveness was through estimation of intra - industrial and inter- industrial trade indices (Relative Trade Advantages (RTA), Grubel-Lloyd (GL)). The obtained results indicate which agri-food products were competitive, which had relative advantages and a potential to increase its competitiveness. The level of intra-industry trade in agricultural and food products for Moldova is quite high (78%). The results of the intra-industrial trade level indicated a decrease in European Union (EU) countries and increase in relation with Commonwealth of Independent States (CIS) countries. For most commodity groups the GL values presented average high variability over time, fact which reflected the structural changes in Moldavian agri-food trade.

Key words: *agriculture, food, intra-industry, inter-industry, trade.*

INTRODUCTION

Competitiveness is a key issue in international markets and a major source for a country's export development. When one country can utilize in the best way its scarce resources in the agricultural sector, it can have a significant comparative advantage on foreign agricultural markets (Yercan, 2006). For Moldova and other countries in transition, many changes during the transformation process to a market economy in the agricultural and food trade environment had occurred. One of the reasons was due to the level of economic and social development, but an important part of the transformation process was due to trade liberalization (Bonjec, 2007; Levkovich, 2007). In this paper we focus on the agri-food sector of Moldova. The main objective is to assess the competitiveness of the local agri-food products on foreign markets. The paper investigates the agricultural and food trade of Moldova from different aspects of inter and intra industry trade. The aim is to estimate the trend and extent of inter and intra-industry trade in agricultural and food products of Moldova. The time series from 2001 to 2015 are examined.

MATERIALS AND METHODS

For the analysis indicators of inter and intra industry trade were used. For the appreciation of the country's comparative advantage (or a particular sector) Bela Balassa (1965) elaborated the method that reveals the "Revealed Comparative Advantages" (RCA). This method is based on the assumption that the implicit comparative advantages find their reflection directly in the trade flows. According to Balassa, comparative advantages are manifested in relatively high shares of a particular product/sector in the structure of exports. In the same time the relative limitations are reflected through low shares of a product/sector. The RCA index or Balassa index is an indicator that characterizes the ratio of a commodity i in the total amount of country's exports and the share of this commodity in the total amount of world's exports. This index is based on observed trade patterns. This index is defined as:

$$B = (X_{ij}/X_{it})/(X_{nj}/X_{nt}) \qquad (1)$$

Where:

X – export; i – a country; j – a commodity; t – a set of commodities; n - a set of countries.

If B>1, then a comparative advantage is revealed. The standard deviation of this index across products can be used as measure of the comparative importance of inter-industry specialization or intra-industry trade. An alternative specialization of revealed comparative advantage was developed by Vollrath (1991) and was called Relative Trade Advantage (RTA). The RTA index is calculated as the difference between relative export advantage (RXA) or Balassa index and relative import advantage (RMA):

$$RTA = RXA – RMA \qquad (2)$$

Where, $RXA = B = (X_{ij}/X_{it})/(X_{nj}/X_{nt})$; (3)

$RMA = (M_{ij}/M_{it})/(M_{nj}/M_{nt})$; (4)

M – import.

The positive value of RTA indicates comparative trade advantages, while negative value indicates comparative trade disadvantages. When RTA is greater than zero, then a comparative advantage is revealed, which means that a sector of the country is relatively more competitive in terms of trade.

The most common index used to measure the intra-industry trade is the Grubel-Lloyd (1975) index. To calculate the intra-industry trade level (IIT) for an industry *i* will be:

$$GLIIT_i = \frac{(X_i+M_i)-|X_i-M_i|}{(X_i+M_i)} \times 100 \qquad (5)$$

or, it can be written as:

$$GLIIT_i = (1 - \frac{|X_i-M_i|}{X_i+M_i}) \times 100, \qquad (6)$$

where $GLIIT_i$ is the Grubel Lloyd index of intra-industry trade i, X_i and M_i represents the values of exports and imports in industry i.

GL index can take values from 0 to 100. When the GLIT value is zero it indicates that there is no IIT (because exports or imports are zero). When the value is equal to zero, then all trade is IIT (exports equals' imports).

This paper is based on secondary data from the National Bureau of Statistics (NBS). The research focuses on Moldova's intra-industry and inter-industry trade in agricultural and food commodities, analyzing time series from 2001 to 2015

In the present paper the 24 chapters, according to international nomenclature for the classification of products Harmonized Sections (HS) 2012 in two digits for agricultural and food products were analyzed.

RESULTS AND DISCUSSION

A central place in Moldova's economy belongs to the agri-food sector. According to the data from National Bureau of Statistics the share of the agricultural sector in the Gross Domestic Product (GDP), which together with food processing industry is about 35%, while agricultural products and foodstuffs are country's main export articles having a share of 40% in total exports. As well, about one third of population is employed in this sector.

Agri-food products are main exports commodities having a share of 45% in total exports. Unfortunately, their share diminished during the analyzed period with 18%, fact which affected the agri-food trade balance. Analyzing the trends in Moldova's foreign trade, during the covered time series period an increasing in value of both exports and imports is observed. Exports increased with 11% and imports with 13%, the overall trade balance remaining negative.

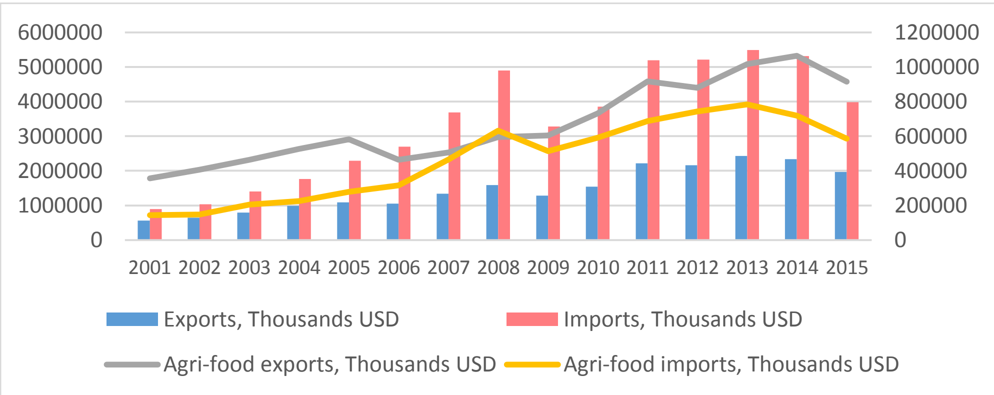

Figure 1. Dynamics of Moldova's trade flows, 2001-2015

*Source: Based on data from National Bureau of Statistics (2017).

Summarizing the evolution of agri-food trade flows, also an increasing trend is noticed in agri-food exports from 356857 thousands USD in 2001 to 914488 thousands USD in 2015. In 2006-2007 a slight decrease in agri-food exports was noticed, as result of Russia's first interdiction on wines, which is one of the main trade partners for Moldova.

The geographical structure also experienced many changes during the analyzed period. Most of agri-food exports until 2006 were oriented to CIS markets. After 2007 a large increase of trade flows with EU countries was accentuated, and a decrease of dependence from CIS markets. This was particularly due to various facilities from EU as through General System of Preferences (GSP+) in 2006 and Autonomous Trade Preferences (ATP) in 2008. Preferences were given for alcoholic drinks, sugar and some agricultural products and others. In 2014 Moldova signed a Deep and Comprehensive Free Trade Agreement (DCFTA) with EU that contributes to more trade liberalization with EU.

The EU market is highly competitive and imposes barriers to enter in terms of quality and food safety, thus a boost in the competitiveness of the exported Moldavian agri-food products is desired, and the full use of the actual potential to increase the trade relations with EU.

Besides strong trade relations with Romania among EU countries as neighboring country, also important partners are Italy, UK, Germany, Poland, France, Greece and Austria. The share of agri-food export to these countries is 85%.

Competitiveness of Moldova's agricultural and food products was assessed in the long run based on the RTA index results, in relation with both EU and CIS countries (Table 1).

According to the results (Table 1), we can delimitate three trends in the competitiveness of the agricultural and food products of Moldova. Within the EU countries competitive advantages are observed among the following aggregations: dairy produce, products of animal origin, live trees, edible vegetables, cereals, oil seeds, preparations of meat, beverages. Total disadvantages are characterized for the following aggregations: fish, coffee, tea, animal or vegetable fats, cocoa, vegetable planting materials and preparation of cereals. In the analysis some aggregations register positive values in some years and negatives in others. Thus the switching values observed denote a possibility for the increase in competitiveness of these agricultural and food products. Switching values are typical for live animals, meat, edible fruit and nuts, sugars, preparation of vegetables and tobacco.

Table 1. Competitiveness of Moldova's agricultural and food products with EU and CIS countries, based on RTA index.

HS code	2001-2007		2008-2015		2001-2015	
	EU	CIS	EU	CIS	EU	CIS
01 Live animals	51,83	-79,80	3386,77	-32,90	1830,47	-54,79
02 Meat and edible meat offal	19,85	-4885,02	2513,24	-8,62	1349,66	-2284,27
03 Fish and fish products	-0,57	-0,20	-0,94	1,75	-0,77	0,84
04 Dairy, eggs; honey etc	-0,59	7,57	3,26	-0,02	1,47	3,53
05 Products of animal origin	-0,10	-17,67	0,90	-150,87	0,43	-88,71
06 Live trees, cut flowers	1,49	-25,55	3,87	-12,75	2,76	-18,72
07 Edible vegetables etc	0,03	-14,09	5,82	-7,30	3,12	-10,47
08 Edible fruit and nuts	-0,43	-1,66	0,05	-9,83	-0,18	-6,02
09 Coffee, tea, mate	-0,34	6,85	-0,30	4,89	-0,32	5,81
10 Cereals	-4,57	0,21	-0,38	1,68	-2,33	0,99
11 Milling products; malt; starches;	-0,45	7,50	-1,20	15,63	-0,85	11,84
12 Oil seeds, seeds etc	-0,38	-1,57	0,08	-2,18	-0,13	-1,89
13 Lac; gums, resins	0,19	-17,43	3,77	-43,35	2,10	-31,25
14 Vegetable planting materials	-2,08	-0,19	-4,60	-35,83	-3,43	-19,20
15 Animal or vegetable fats, etc.	-0,36	0,26	-1,38	31,90	-0,90	17,13
16 Preparations of meat, of fish	193,75	-0,32	254,63	1,28	226,22	0,53
17 Sugars and sugar confectionery	-1,46	7,64	-0,51	3,50	-0,95	5,43
18 Cocoa and cocoa preparations	-1,96	3,48	-1,17	2,07	-1,54	2,73
19 Preparations of cereals, flour	-0,88	1,12	-0,76	5,44	-0,81	3,42
20 Preparations of vegetables	-0,18	-0,20	0,05	-0,30	-0,06	-0,25
21 Miscellaneous edible preparations	-0,35	9,11	2,01	3,38	0,91	6,05
22 Beverages, spirits and vinegar	4,90	-0,26	1,74	-0,27	3,21	-0,26
23 Residues food industry etc	0,21	0,32	1,27	6,91	0,78	3,83
24 Tobacco etc	1,65	0,19	0,90	0,55	1,25	0,38

*Source: Authors calculations based on data from NBS (2017).

In relation with CIS countries a trade advantage is present in the case of the aggregations milling products, animal or vegetable fats, preparations of meat, cocoa, and tobacco. A low level of competitiveness is characteristic for the aggregations live animals, fish, dairy produce, products of animal origin, coffee, tea, cereals, sugars and preparations of vegetables. The switching values in the results of the RTA index are regard the following aggregations: meat, live trees, edible vegetables, edible fruits, oil seeds, preparations of meat, preparations of cereals, and beverages. Total disadvantages that are common for both CIS and EU countries are regard the following aggregations: HS 09, HS03 and HS14. These are mainly exotic imports products that are not produced in Moldova. Among switching values common for both EU and CIS countries is HS02 (meat and edible meat offal), whose competitiveness could be improved. Among the most discussed issues related to world trade trends in the economic literature belongs to intra-industry trade (IIT). It can be explained as the simultaneous flows of imports and exports of commodities within the same industry group. It emphasizes trade in products that are similar but slightly differentiated based on imperfect competition or close substitutes goods demanded in different countries by consumers with distinct preferences. According to the results (Table 2) the level of intra-industry trade in agricultural and food products for Moldova is quite high. From total trade in this sector, 78% belongs to the intra-industry type.

Table 2. Intra-industrial trade with agricultural and food products between Moldova and all trade partners.

HS code	2001-2007	2008-2015	2001-2015
01 Live animals	72.3	66.9	69.4
02 Meat and edible meat offal	50.1	52.5	51.3
03 Fish and fish products	3.9	0.58	2.1
04 Dairy, eggs; honey etc	69.3	46.09	56.9
05 Products of animal origin	8.1	17.9	13.3
06 Live trees, cut flowers	37.6	25.5	31.2
07 Edible vegetables etc	62.2	52.4	57.02
08 Edible fruit and nuts	39.7	51.3	45.9
09 Coffee, tea, mate	13.7	9.02	11.2
10 Cereals	43.6	30.4	36.5
11 Milling products; malt; starches;	6.8	9.02	7.9
12 Oil seeds, seeds etc	67.3	34.06	49.5
13 Lac; gums, resins	1.3	4.4	3.01
14 Vegetable plaiting materials	67.5	39.1	52.3
15 Animal or vegetable fats etc	44.2	53.4	49.1
16 Preparations of meat, of fish	52.5	4.9	27.1
17 Sugars and sugar confectionery	76.7	71.8	74.1
18 Cocoa and cocoa preparations	24.8	28.4	26.8
19 Preparations of cereals, flour	46.5	45.1	45.8
20 Preparations of vegetables	35.3	57.2	47.04
21 Miscellaneous edible preparations	18.7	17.7	18.2
22 Beverages, spirits and vinegar	24.9	45.7	36.02
23 Residues food industry etc	74.2	82.5	78.7
24 Tobacco etc	54.6	43.6	48.7
01-15 Agricultural products	82.5	79.6	81.0
16-24 Food products	58.3	96.6	78.8
01-24 Total agricultural and food products	67.6	87.6	78.3

*Source: Authors calculations based on data from NBS (2017).

For some commodity groups the GL values present high variability over time, fact which reflects the structural changes in Moldavian agri-food trade. High and average magnitudes of intra-industry trade presents the following commodities: live animals, meat, dairy, eggs, honey, edible vegetable, edible fruit and nuts, oil seeds, vegetable planting materials, animal or vegetable fats, sugars, preparations of cereals, preparations of vegetables, residues food industry, tobacco. Low levels of intra-industry trade exhibits commodities as fish, products of animal origin, coffee, tea, mate, milling products, malt, starches. The commodities with low levels are mostly imported, mainly exotic products that are not produced in Moldova.

During the analyzed time series, the GL index tends to decrease with EU countries and increase in relation with CIS countries (Figure 2). The downward trend for EU countries, regarding exports can be explained as the lack of competitiveness for Moldavian agricultural and food products which makes it difficult to access those markets. The upward trend in relation to CIS countries might be caused by the increase of imports from those countries, mainly Ukraine.

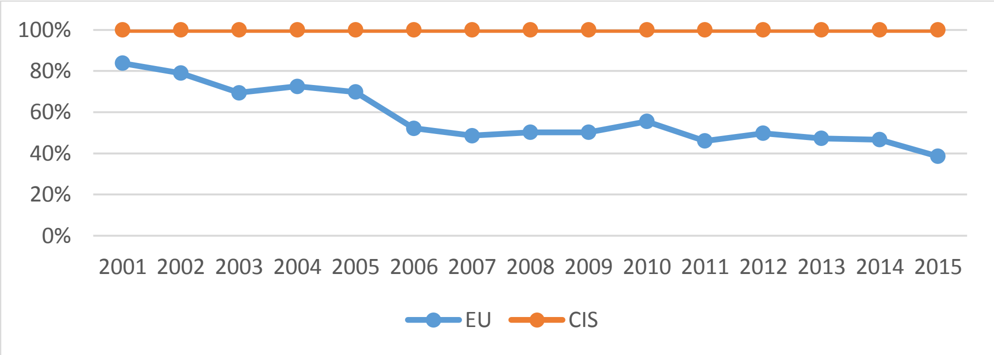

Figure 2. Intra-industry trade with agricultural and food products in relation to EU and CIS countries (2001-2015).

*Source: Authors calculations.

CONCLUSIONS

Moldova's agri-food trade flows experiences important changes during the investigated period. Both Moldova's exports and imports flows increased considerably, while agri-food imports increased faster than exports.

The agricultural and food trade of Moldova is specializing continuously and its competitive advantages are not enough stable. Thus, besides the agricultural and food products that have a clear defined advantage, were identified a list of aggregations that have switching values with a possibility of increase in their competitiveness. Trade advantages (according to RTA results) are different for EU and CIS countries. Comparative advantages with EU countries were identified for some commodities as: dairy produce, live trees and other plants, edible vegetables, cereals, oil seeds, beverages etc. In relations with CIS countries of comparative advantages benefit mostly commodities as: animal or vegetable fats and oils, preparations of meat, tobacco and others. Negative values for both CIS and EU countries are identified in the case of coffee, tea, fish and crustaceans, vegetable planting materials.

The level of intra-industry trade in agricultural and food products for Moldova is quite high (78%). The results of the intra-industrial level (GL) indicate a decrease with EU countries and increase in relation with CIS countries. The downward trend for EU countries, regarding exports can be explained as the lack of competitiveness for Moldavian agricultural and food products which make it difficult to access those markets. For most commodity groups the GL values present average high variability over time, fact which reflects the structural changes in Moldavian agri-food trade.

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DETECTION OF HONEY BEE VIRUSES ON THE TERRITORY OF UKRAINE

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ABSTRACT

A survey of honey bee viruses on the territory of Ukraine has been conducted for the first time in 2016-2017 years. The samples of adult bees, affected combs and mites were collected from apiaries in two regions (Kyiv and Cherkasy). Detailed studying of the bee samples revealed following morphological changes: deformed wings, saccular brood, blackened pistil, changing of the body color. Virus-like particles were observed under TEM after purification of bee samples. Symptomless samples were also analyzed for the presence of bee viruses by RT-PCR. According to results of RT-PCR, we concluded that the following viruses circulate on the territory of Ukraine: Acute Bee Paralysis Virus (ABPV), Kashmir Bee Virus (KBV), Israeli Acute Paralysis Virus (IAPV), Black Queen Cell Virus (BQCV), Sacbrood Bee Virus (SBV) Chronic bee paralysis virus (CBPV). Taken together, these data indicate that bee virus infections occur in bee populations on the territory of Ukraine. Despite the lack of symptoms viruses are often present in colonies and environmental factors might result disease outbreaks that lead to the activation of viral replication in bees.

Key words: *bee virus, bee colony, mites, vector.*

INTRODUCTION

Pollination of flowering plants is an important ecosystem service provided by wild insect pollinators and managed honey bees. Hence, losses and declines of pollinating insect species threaten human food security and are major concerns not only for apiculture or agriculture but for human society in general. Over the last 15 years, dramatic colony losses have been reported regularly from different regions all over the world. The current scientific consensus is that no single factor is causing honey bee colony death. However, frequently virus infections are among the key players in colony losses and therefore viruses are rightly considered a major threat to the health of honey bees, both at individual and at the colony level (Gisder, 2015). The most commonly observed and best known honey bee viruses are 30-nm isometric particles containing a single-stranded positive RNA. These viruses include Israeli acute paralysis virus, Kashmir bee virus, Acute bee paralysis virus, Black queen cell virus, Deformed wing virus, Sacbrood virus, Slow bee paralysis virus and the recently discovered Lake Sinai viruses, which are a phylogenetically unique, globally distributed group of viruses, including LSV1-7 and other variants (Brutscher, 2016). According to epidemiological data, the distribution of these viruses in honey bee colonies appear to be worldwide (Ahmad, 2016), most likely resulting from intensive exchanges of honey bee stocks throughout the world. RNA viruses are readily detected in bee samples obtained from colonies of varying health and viral presence, and abundance varies by season and geographic location (McMenamin, 2015). Viruses of the honey bee typically infect the larvae, but the symptoms are often most obvious in adult bees. The transmission of the viruses usually occurs horizontally (e.g. through the bee feces, royal jelly, the beekeeper), but the transmission of the main bee viruses occurs vertically (from the queen to the brood). Many viruses are also transmitted by mites Varroa. *Varroa destructor* has greatly contributed to increase the incidence of viral diseases (Moore, 2014). Varroa, in fact, is a passive carrier of bee viruses that are transmitted to the bees through the mite’s saliva. Varroa mites feed on hemolymph, which allows viruses transfer directly into the open circulatory system that reaches every cell in the insect body. In addition, mites weaken the immune system of the bees, which can lead to the reactivation of latent viral infections already present in the body of the bees. Honey bee viruses are not limited to honey bees. They also have been found in other non-Apis bee species, other colony inhabitants like small hive beetle, and in pollen and nectar (Bailey and Gibbs, 1964; Genersch et al., 2006; Philip, 2014).

Identification of bee virus is difficult due to the small size of viral particles. Specific laboratory methods are required for accurate diagnosis. However, symptoms of some viral diseases are more visible, especially with overt infection. A lack of symptoms does not guarantee the absence of a virus. Viruses can remain in a latent form within the host, acting as a reservoir of infection, complicating diagnosis and control, and causing outbreaks under particular conditions (Yue, 2006).

Despite the great losses of bee colonies and observation of virus like disasters, specific bee viruses have not been tested on the territory of Ukraine. These viruses are usually undervalued by Ukrainian beekeepers: they can cause serious economic losses alone and if associated with other bee diseases. The aim of present work was to identify honey bee viruses in apiaries of Ukraine.

MATERIAL AND METHODS

Sample collection

This study was conducted during the spring and summer of 2016. The first step included the monitoring of bee colonies for symptoms of viral infection with subsequent sampling and processing. The samples of bees and affected combs were obtained from two regions in Ukraine: Kyiv and Cherkasy. Healthy bees with no clinical signs of disease were collected at the same time. Additional samples of

Varroa destructor mites were collected, as these species serve as vectors for viruses. Each sample consisted of approximately 100 bees or mites, representing one location or apiary. A part of the samples was purified for preliminary identification through electron microscopy. The remaining part was stored at −20 °C until used for RT-PCR studies.

Virus isolation and purification

Adult bees were crushed in liquid nitrogen and homogenized in 700 µl phosphate buffer (pH 7.0) supplemented with 0.02% diethyldithiocarbamate. The resulting suspension was clarified with carbon tetrachloride and centrifuged for 15′ at 800× g and 4 h at 100,000× g (4 °C). The resulting pellet was resuspended in PBS.

Transmission electron microscopy

To estimate the presence of virus like particles in investigated samples transmission electron microscopy was employed. For this purpose formvar films were placed on the 400-mesh copper grids and were dipped into a sample for 2 min and negatively stained in 2% uranyl acetate. The preparations were dried and viewed under an electron microscope at an instrumental magnification of 90.000.

RNA isolation and PCR

The primers were chosen to target the conservative genome region of seven viruses (ABPV, KBV, IAPV, BQCV, DWV, SBV, CBPV. Five pairs of oligonucleotide primers were selected in accordance with data from the literature (Tab.1) (Miranda 2010, Ahmad M. Mouhanna 2016, Khaliunaa Tsevegmid 2016).

Table 1- Sequence of primers used in the study to target different region of honey bee virus genome

AKI*	F-5’- GGCGAGCCACTATGTGCTAT
	R-5’- ATCTTCAGCCCACTT
DWV	F-5’-CTTACTCTGCCGTCGCCCA
	R-5’-CCGTTAGGAACTCATTATCGCG
BQCV	F-5’-AGTGGCGGAGATGTATGC
	R-5’-GGAGGTGAAGTGGCTATATC
SBV	F-5’- ACCAACCGATTCCTCAGTAG
	R-5’- TCTTCGTCCACTCTCATCAC
CBPV	5’- ACTCCCGTCGTTGTGTTCTC
	5’- GGCGATTGGTATTTGTTTGG

*AKI primer for *Acute Bee Paralysis Virus* (ABPV), *Kashmir Bee Virus* (KBV), *Israeli Acute Paralysis Virus* (IAPV)

RNA Extraction

Individual samples were homogenized in 700 µl sterile tubes with TE reagent according to the manufacturer's instructions in PureLink RNA mini kit, USA. Next step were homogenization in TE buffer and adding 700 µl of lysis buffer and centrifugation at 8000 rpm speed for 2 min. After the samples were mixed with 10 µl mercaptoethanol and 200 µl of lysis buffer. Obtained solution was centrifuged again and the middle fraction was selected. The samples were transferred to a column for RNA isolation and centrifuged at approximately 11,000 rpm. The sediment was drained from the column and washed according to the instructions of sample buffer bottle label. Total RNA was dissolved in 50 µL of sterile water and was stored at −80 °C until further analysis. The quantity and purity of RNA in each sample was measured by agarose gel electrophoresis.

PCR assays

AKI

Synthesis of cDNA was performed with 3 µg of RNA samples from using an oligo-primer and reverse transcriptase. PCR was conducted with forward and reverse primers for IAPV, ABPV, KBV, BQCV, DWV. Amplification viruses (IAPV, ABPV, KBV) were conducted under the following conditions: 50°C 30 min reverse transcription, 2 min denaturation at 94°C, in 35 cycles which included: denaturation at 94°C for 30 sec, 30 sec annealing at 50°C, extension at 68°C for 45 sec.

BQCV

The PCR volume was 25 µl, containing 2µl template cDNA, 5µl of 5X Reaction Buffer, 0.75 µl of dNTPs Mix (10 mM), 0.75µl of each forward and reverse primers (10µM) and 0.5µl of Taq polymerase. The conditions of the reaction were the next: incubation for 2 min at 95°C, 20 sec denaturation at 98°C., annealing at 57°C for 20 sec, 30 sec extension at 72°C and 2 min for a final step at 72°C.

DWV

RT-PCR was completed as follows: 30 min at 50 ° (reverse transcription) and denaturation 15 min at 95°C, followed by 40 cycles of amplification for 30 sec at 94°C, 45 sec at 55°C, and 45 sec at 72°C.

SBV

Temperature profile for SBV RT-PCR: 30 min at 50°C (reverse transcription) and denaturation for 5 min at 95°C, followed by 40 cycles of amplification 20 sec at 95°C, 20 sec at 55 °C, and 1 minute at 72°C.

CBPV

The thermal cycling conditions were next: 5 min at 94°C (denaturation of the template and activation of the enzyme), followed by 35 cycles consisting of denaturation at 94°C for 15 sec, 30 sec annealing at 55°C and 30 sec to 2 min extension at 68°C (depending on the primer pair), completed by a final 10 min extension at 68°C.

RESULTS AND DISCUSSION

In recent years, Ukrainian beekeepers reported several observation cases of virus-like disorders; however, the causative agent has not been identified. The samples of adult honey bees originating from the affected honey bee colonies and symptomless colonies, from different apiaries in Kyiv and Cherkasy regions, were collected and tested for the presence of seven honey bee viruses. Detailed studying of the bee samples revealed following morphological changes: deformed wings, saccular brood, blackened queen bee, changing of the body color. Affected combs with symptoms of chilled brood, spotty brood pattern, dead larvae in cells were also observed (Fig.1).

Fig.1. Larvae with characteristic lesions in honeycombs

Electron microscopy revealed the presence of spherical virus-like particles about 30 nm in diameter in mite sample (Fig. 2). No significant differences in virion size and morphology were observed among the virus particles. Observed virions had a diameter and morphology compatible with that of the genus *Dicistroviridae:* ABPV, KBV, IAPV, BQCV, and *Iflaviridae*: DWV, SBV, CBPV.

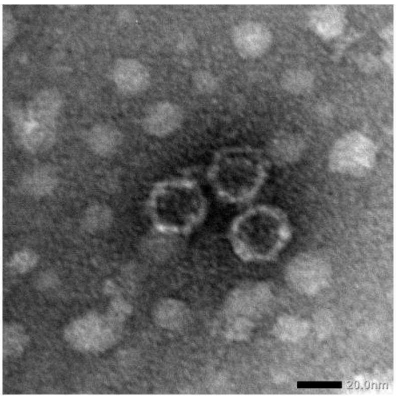

Fig.2. Electron micrograph of virus-like particles, observed in purified mites sample.

For exact diagnosis collected samples were analyzed for the presence of viruses with RT-PCR using specific primers. The following viruses were identified in samples of mites: BQCV, SBV (Fig. 3.). ABPV, KBV, IAPV, BQCV were detected in adult bees (Fig. 4).

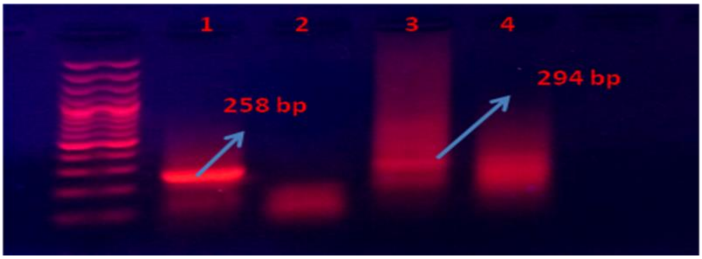

Fig. 3. RT-PCR results of bee and mites samples for SBV and BQCV: 1, 2-SBV; 3,4-BQCV; 1,3- samples bees; 2,4- sample bee

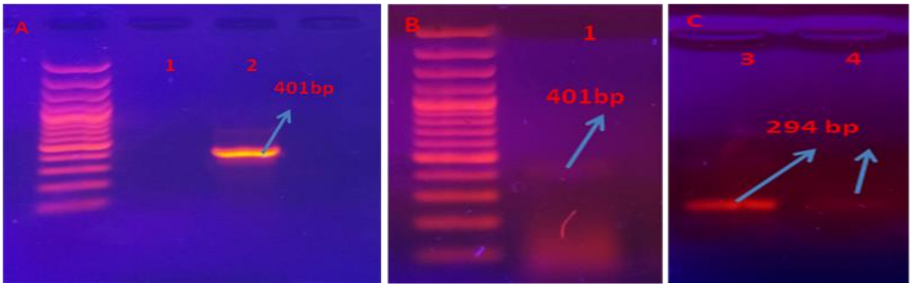

Fig. 4. RT-PCR samples results mites and bees: A, B- with primers for ABPV, KBV, IAPV; C- with primers for BQCV; 2- mites; 1, 3, 4-samples bees

RT-PCR analysis revealed the presence of two viruses: *Black Queen Cell Virus (BQCV)* and *Sacbrood bee virus (SBV)* in samples from affected combs.

Our investigations finally confirmed the circulation of specific honey bee viruses in Ukrainian apiaries. These results can be regarded as a base for establishing epidemiological surveillance of honey bee diseases in other regions of the country. The next step of our research will include sequencing of the samples for further comparison with foreign isolates and determination of phylogenetic relationships of Ukrainian isolates. Identified *Acute Bee Paralysis Virus*, *Kashmir Bee Virus*, *Israeli Acute Paralysis Virus*, *Black Queen Cell Virus*, *Sacbrood Bee Virus* are the most significant viruses as they can cause great economic losses and reduction in biodiversity in natural ecosystems. Thus, diagnosis of virus infections is the key component for surveillance, control and monitoring of honey bee diseases.

CONCLUSIONS

Beekeeping in Ukraine is a major economic activity. Ukraine is the top honey-producing country in Europe and has the largest number of managed honey bee colonies, however, the presence of honey bee viruses has never been identified in

Ukrainian apiaries. We report here the first survey of bee viruses distribution in Ukrainian apiaries. As a result of our work we estimated the presence of BQCV, CBPV, DWV, IAPV and SBV, ABPV and KBV. ABPV, KBV, IAPV, BQCV, SBV were the most prevalent in the tested samples. Obtained data is in accordance with results reported after identification of bee viruses in other countries. Honey bee colonies can suffer from multiple virus infections without showing obvious pathological symptoms, thereby confounding diagnoses. Therefore, rapid and accurate diagnosis of virus infection is a critical component of honey bee disease surveillance and control programs.

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EFFECT OF NITROGEN FERTILIZATION IN *TRITICALE* (*X TRITICOSECALE* WITTM.), CULTIVATED AFTER DIFFERENT PREDECESSORS. NITROGEN UPTAKE AND EFFICIENCY

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ABSTRACT

The study is conducted at the experimental base of Department of Plant production at Trakia University, Stara Zagora. Triticale is grown after five predecessors and different nitrogen fertilization rates. The predecessors are wintering peas, spring peas, sunflower, common wheat and triticale. Nitrogen fertilization rates are 0 (N_0), 40 (N_{40}), 80 (N_{80}), 120 (N_{120}) kg ha^{-1} after legumes and 0 (N_0), 60 (N_{60}), 120 (N_{120}), 180 (N_{180}) kg ha^{-1} after the other predecessors. The influence of the predecessors and nitrogen fertilization on the nitrogen uptake, assimilated nitrogen from fertilization, nitrogen utilization and nitrogen required for 100 kg produce of triticale is established. Nitrogen assimilation from fertilization increases with the increase of the nitrogen fertilizer rates. With the obtained yield from triticale an average of 96.53 kg ha^{-1} N is extracted from the soil with the grain, 18.97 kg ha^{-1} N with the straw or a total of 115.50 kg ha^{-1} N. When growing triticale after leguminous predecessors the utilization of nitrogen is 35.39% and after predecessors sunflower, wheat and triticale - 28.76%. Nitrogen required for 100 kg yield of grain of triticale is 1.9 kg of nitrogen when growing after legume predecessors and 2.8 kg of nitrogen after the other predecessors.

Keywords: *triticale, nitrogen uptake, utilization of nitrogen, fertilizer consumption*

INTRODUCTION

The influence of optimized nitrogen fertilization on productivity and quality of triticale has been found by many researchers (Kolev et al., 2011; Kirchev et al., 2012; Kirchev et al., 2014; Gerdzhikova, 2014; Madic et al., 2015). According to Papastylianon et al., 1981; Badaruddin and Meyer, 1994; Gibson et al., 2007, the increase in nitrogen levels leads to an increase of nitrogen content in the triticale grain, but changes in protein content are greater under the influence of the predecessor than fertilizer norms. In addition to the nitrogen, imported with fertilization, triticale also absorbs soil nitrogen from the predecessors (Nance et al., 2007). Triticale has the greater ability to accumulate nitrogen compared to wheat (Paponov et al., 1999). Effective use of nitrogen is an important indicator of the environmental impact on the production of triticale and other energy crops (Lewandowski and Schmidt, 2006). The aim of this study is to determine the impact of predecessors and nitrogen fertilization on the nitrogen uptake, assimilated nitrogen from fertilization, utilization of nitrogen and fertilizer consumption (nitrogen required for 100 kg of produce).

MATERIALS AND METHODS

The survey is conducted in the area of the town Stara Zagora, located in the region of South Central Bulgaria. The study is carried out with Triticale Rogen variety. Cultivation is performed according to the conventional technology of cropping. The soils are Gleic Chromic Luvisols, neutral, and well reserved with K_2O, moderate with P_2O_5 and low with Nitrogen. Triticale is grown after 5 predecessors: Wintering peas (*Pisum arvense* L.), Spring peas (*Pisum sativum* L.), Sunflower (*Helianthus annuus* L.), Wheat (*Tristicum aestivum* L.) and Triticale (× *Triticosecale* Wittm.). Four levels of nitrogen fertilization are applied according to the predecessors as follows: 0 (N_0), 40 (N_{40}), 80 (N_{80}), 120 (N_{120}) kg ha^{-1} after legumes and 0 (N_0), 60 (N_{60}), 120 (N_{120}), 180 (N_{180}) kg ha^{-1} after the other predecessors. The data used in the present study are for the period 2010-2012 - a part of a multi-year experience with triticale. Different metrics are calculated using the following formulas:

NU = Y*NC/1000

ANF = NU fertilized variant – NU non-fertilized variant

UN = ANF/NR%

FC = NR/Y*100

NU – nitrogen uptake, Y – yield (grain, straw), NC – nitrogen content, ANF – assimilated nitrogen from fertilization, UN – utilization of nitrogen, NR – nitrogen fertilizer rate, FC - fertilizer consumption.

RESULTS AND DISCUSSION

Nitrogen content in grain and straw dry matter of triticale

Higher nitrogen content in the grain dry matter of triticale viewed by predecessors, is obtained after predecessor sunflower, average for the period - 24.14 g in 1000 g dry matter (DM) and lower after spring peas - 21.07 g in 1000 g DM. On average, for the three-year period after legume predecessors nitrogen content is lower by 3.71% in the non-fertilized variant compared to cereals and sunflower (Table 1). Nitrogen fertilization leads to increase in the nitrogen content in the DM of triticale grain. The highest values are obtained at fertilizer rate of 180 kg ha^{-1}, applied after non-leguminous predecessors. Nitrogen in the fertilized variants is 9% more than non-fertilized ones on average for the trial period. According to Papastylianon et al. (1981); Badaruddin and Meyer (1994); Gibson et al. (2007), the percentage of nitrogen content in triticale grain increases by increasing nitrogen rates.

Higher nitrogen content after cereals and sunflower compared to leguminous predecessors and with an increase in the applied nitrogen fertilizer rates is also found in triticale straw. On average, for the trial period nitrogen content in grain DM of triticale is 371.82% higher than nitrogen content in straw DM.

Nitrogen uptake with grain+straw yield of triticale

To form a unit of produce plants export from the soil various macro- and trace elements. From the macroelements, nitrogen is important. On average, for the trial period after leguminous predecessors of the non-fertilized variants, nitrogen uptake is 68.14 kg ha^{-1}, and after cereals and sunflower 69.43 kg ha^{-1} - by 1.86% more (Table 1). Introducing increasing rates of nitrogen fertilizer results in increase nitrogen uptake from the soil with grain yield of triticale after all predecessors. After leguminous predecessors, on average for the period export compared to the non-fertilized variant increased by 11.73%, 37.91% and 52.45%, respectively, with mineral nitrogen rates 40, 80 and 120 kg ha^{-1}. On average, after cereals and sunflower, nitrogen uptake is higher than after legumes and increased by 19,18%, 45,95% and 66,95%, respectively, compared to the non-fertilized variant with fertilization at 60, 120 and 180 kg ha^{-1} of nitrogen, respectively. On average for the study period, nitrogen uptake, average from all fertilized variants is 40.06% higher than the average of the non-fertilized variants. Nitrogen uptakes with triticale straw on average, for the three-year period, ranged from 11.41 kg ha^{-1} in the non-fertilized variant to 22.49 kg ha^{-1} for N_{120} after leguminous predecessors and from 15.04 kg ha^{-1} to 23.27 for N_{180} after cereals and sunflower. When comparing nitrogen-free variants between the two groups of predecessors, nitrogen uptake is 24.14% lower after leguminous. After all predecessors export increases by increasing nitrogen fertilization rates. The average value of all non-fertilized variants ix exceeded by that of the fertilized ones by 39.59% on average for the trial period. The amount of nitrogen extracted with grain+straw of triticale in plants fertilized with the highest nitrogen fertilization rate of 180 kg ha^{-1} on average after cereals and sunflower reached 139.25 kg ha-1 and exceeded nitrogen uptake of the non-fertilized variant by 64.87%. N60 and N120 fertilization increases nitrogen uptake compared to the non-fertilized variant by 19.66% and 40.00%, respectively. After leguminous predecessors, nitrogen uptake is lower, both in the non-fertilization variant and the respective fertilization rates compared to non-leguminous predecessors. Here again nitrogen uptake increases with the increase of the nitrogen fertilization rate with biggest amount of nutrients with the main and additional produce being exported as a result of the highest rate of N120 - 126.37 kg ha-1 exceeding export in the non-fertilized variant by 58.86%. At fertilization rates of N40 and N80 this increase is 13.74% and 40.04%, respectively, compared to N0.

Table 1. Yield of grain and straw, kg ha^{-1}, nitrogen content in grain and straw, g/1000 g DM, nitrogen uptake with grain, straw, grain+straw, kg ha^{-1} average for the three-year period

Predecessor	Nitrogen fertilization, kg ha^{-1}	Yield of grain, kg ha^{-1}	Yield of straw, kg ha^{-1}	Nitrogen content in grain, g/1000 g DM	Nitrogen content in straw, g/1000 g DM	Nitrogen uptake with grain, kg ha^{-1}	Nitrogen uptake with straw, kg ha^{-1}	Nitrogen uptake with grain+ straw, kg ha^{-1}
1. Wintering peas	0	3280.4	2897.5	21.75	4.15	71.29	11.95	83.24
	40	3553.8	3546.7	22.27	4.89	79.05	17.45	96.50
	80	4162.5	3934.2	23.46	4.38	97.63	17.23	114.86
	120	4550.8	4310.8	23.11	4.18	105.17	18.05	123.22
	0-120	3886.9	3672.3	22.65	4.40	88.29	16.17	104.46
	40-120	4089.0	3930.6	22.95	4.48	93.95	17.58	111.53
2. Spring peas	0	3295.4	2889.2	19.73	3.81	64.99	10.87	75.86
	40	3572.1	3541.7	20.54	3.20	73.21	11.25	84.46
	80	4181.3	4013.3	21.60	4.41	90.30	17.65	107.95
	120	4581.3	4347.5	22.41	6.18	102.60	26.92	129.52
	0-120	3907.5	3697.9	21.07	4.40	82.78	16.67	99.45
	40-120	4111.5	3967.5	21.52	4.60	88.70	18.61	107.31
3. Sunflower	0	3200.4	2815.0	22.65	5.77	72.46	16.32	88.78
	60	3613.8	3453.3	23.68	6.23	85.51	21.84	107.34
	120	4273.8	3983.3	24.52	4.63	104.74	18.49	123.23
	180	4708.8	4259.2	25.70	4.96	120.95	21.14	142.08
	0-180	3949.2	3627.7	24.14	5.40	95.92	19.45	115.36
	60-180	4198.8	3898.6	24.63	5.27	103.73	20.49	124.22
4. Wheat	0	3252.1	2855.8	20.29	6.11	65.93	17.76	83.70
	60	3646.3	3518.3	21.81	5.95	79.48	21.01	100.48
	120	4341.7	3973.3	23.54	4.35	102.10	17.31	119.41
	180	4760.4	4415.0	23.29	6.85	110.72	30.25	140.96
	0-180	4000.1	3690.6	22.23	5.82	89.56	21.58	111.14
	60-180	4249.4	3968.9	22.88	5.72	97.43	22.86	120.28
5. Triticale	0	3220.0	2836.7	21.69	3.85	69.92	11.05	80.96
	60	3621.3	3486.7	22.99	3.50	83.26	12.19	95.45
	120	4319.2	3765.8	22.50	4.00	97.15	15.03	112.18
	180	4734.2	4324.2	24.52	4.34	116.07	18.73	134.79
	0-180	3973.7	3603.4	22.92	3.92	91.60	14.25	105.85
	60-180	4224.9	3858.9	23.34	3.95	98.83	15.32	114.14
6. Average leguminous predecessors (1-2)	0	3287.9	2893.3	20.74	3.98	68.14	11.41	79.55
	40	3562.9	3544.2	21.40	4.04	76.13	14.35	90.48
	80	4171.9	3973.8	22.53	4.39	93.97	17.44	111.40
	120	4566.0	4329.2	22.76	5.18	103.88	22.49	126.37
	0-120	3897.2	3685.1	21.86	4.40	85.53	16.42	101.95
	40-120	4100.3	3949.0	22.23	4.54	91.33	18.09	109.42
7. Average non-leguminous predecessors (3-5)	0	3224.2	2835.8	21.54	5.24	69.43	15.04	84.48
	60	3627.1	3486.1	22.83	5.23	82.75	18.35	101.09
	120	4311.5	3907.5	23.52	4.33	101.33	16.94	118.27
	180	4734.4	4332.8	24.50	5.38	115.91	23.37	139.28
	0-180	3974.3	3640.6	23.10	5.05	92.36	18.43	110.78
	60-180	4224.4	3908.8	23.62	4.98	100.00	19.55	119.55
	x-0	3249.7	2858.8	21.20	4.70	68.92	13.59	82.51
	x-40-180	4174.7	3924.9	23.10	4.80	96.53	18.97	115.50
	x-0-180	3935.7	3662.8	22.48	4.72	88.94	17.42	106.37

Assimilated nitrogen from fertilization, exported by grain+straw yield of triticale: During the first year of the trial higher assimilation of the exported nitrogen from triticale with the grain+straw yield in the fertilized variants is established on average after predecessors wintering and spring peas - 37.71 kg ha^{-1} on average of the three fertilization rates (Table 2). In the second and third year assimilated nitrogen from fertilization is more on average after non-leguminous predecessors by 31.74% and 23.01%, respectively. On average, for the years of the trial, assimilated nitrogen from fertilization is 14.83% more after cereals and sunflower. Assimilated nitrogen from fertilization increases with increasing the fertilization rates. After leguminous predecessors the values are 10.93; 31.85 and 46.82 kg ha^{-1}, respectively, at N_{40}, N_{80} and N_{120} respectively, and after cereals and sunflower - 16.61; 33.79 and 54.80 kg ha^{-1}, respectively, at N_{60}, N_{120} and N_{180}, respectively, on average for the trial period. On average, from the three fertilization rates nitrogen assimilated from fertilization ranges from 28.29 kg ha^{-1} after wintering peas to 36.59 kg ha^{-1} after wheat on average for the three-year period. **Utilization of nitrogen:** Nitrogen utilization rate depends on the nitrogen assimilated from fertilization. In the first year it ranged from 19.27% after triticale to 50.46% after wintering peas (Table 2). On average, after legumes, the utilization rate of nitrogen is 45.00% and is 63.58% higher compared to the average after the cereal predecessors and the sunflower. In the second year, nitrogen has a slightly higher utilization rate after the group of the non-leguminous predecessors. In the last year, as in 2010, the nitrogen utilization rate is the highest after spring and wintering peas. This tendency is maintained and is on average for the three-year trial period - 35.44% after spring peas and 35.33% after wintering peas and is the lowest after triticale - 26.69%. On average, during the study period, nitrogen utilized from fertilization is 31.41% on average from all fertilization rates after the five predecessors. It is the highest after legumes at fertilization with 80 kg ha^{-1} nitrogen - 39.82%. Of the three fertilization rates on average after legumes, nitrogen utilization 23.05% higher compared to the average after cereal predecessors and sunflower. **Fertilizer consumption (nitrogen required for 100 kg of produce):** The nitrogen required for 100 kg of triticale produce does not differ significantly during the three years of the trial. Lower values were obtained in the first year, and higher - in the last one. Viewed by predecessors, fertilizer consumption is lower after both peas and higher after non-leguminous predecessors - sunflower, wheat and triticale. For the formation of 100 kg of triticale produce after wintering and spring peas and fertilization rate of 40 kg ha^{-1}, between 1,1 and 1,2 kg of nitrogen are needed and at rates of 80 and 120 kg ha^{-1} - from 1,8 to 2,1 kg, and from 2.5 to 2.8 kg nitrogen, respectively. On average, for the three-year trial period after leguminous predecessors 1.1 kg of nitrogen is required at fertilization rate of 40 kg ha^{-1} (Table 3). With increase of nitrogen rates, the amount of nitrogen required to obtain 100 kg of produce is also increased: 1.9 kg and 2.6 kg, respectively, for fertilization with 80 and 120 kg ha^{-1} nitrogen on average for the period. After cereal predecessors and sunflower to obtain a yield of 100 kg produce, 1.7; 2.8 and 3.8 kg of nitrogen are required with fertilization with 60, 120 and 180 kg ha^{-1} nitrogen, respectively, on average during the trial period. The highest is fertilizer consumption at nitrogen fertilization rate of 180 kg ha^{-1} in 2012, when 4.0 kg of nitrogen was needed after cereal precursors and sunflower to form 100 kg of triticale produce.

Table 2. Assimilated nitrogen with grain+straw yield of triticale, kg ha^{-1} and utilization of nitrogen, %

Predecessor	Nitrogen fertilization, kg ha^{-1}	2010	2011	2012	Average	2010	2011	2012	Average
		Assimilated nitrogen, kg ha^{-1}				Utilization of nitrogen, %			
1. Wintering peas	0								
	40	23.20	6.04	10.54	13.26	58.00	15.09	26.35	33.15
	80	38.27	31.33	25.25	31.62	47.84	39.16	31.56	39.52
	120	54.66	30.10	35.19	39.98	45.55	25.08	29.32	33.32
	(40-120)	38.71	22.49	23.66	28.29	50.46	26.45	29.08	35.33
2. Spring peas	0								
	40	7.17	10.08	8.55	8.60	17.92	25.20	21.37	21.50
	80	35.69	34.73	25.86	32.09	44.61	43.41	32.33	40.11
	120	67.27	47.51	46.20	53.66	56.06	39.59	38.50	44.72
	(40-120)	36.71	30.77	26.87	31.45	39.53	36.07	30.73	35.44
3. Sunflower	0								
	60	25.49	12.61	17.60	18.57	42.48	21.02	29.33	30.94
	120	33.82	37.44	32.09	34.45	28.19	31.20	26.74	28.71
	180	53.81	54.37	51.73	53.30	29.90	30.21	28.74	29.61
	(60-180)	37.71	34.81	33.81	35.44	33.52	27.48	28.27	29.76
4. Wheat	0								
	60	17.08	17.56	15.72	16.79	28.47	29.26	26.20	27.98
	120	33.50	40.49	33.14	35.71	27.92	33.74	27.62	29.76
	180	59.13	59.64	53.03	57.27	32.85	33.13	29.46	31.81
	(60-180)	36.57	39.23	33.97	36.59	29.75	32.04	27.76	29.85
5. Triticale	0								
	60	7.80	21.03	14.63	14.49	13.00	35.05	24.38	24.14
	120	21.49	45.34	26.81	31.22	17.91	37.79	22.34	26.01
	180	48.41	62.57	50.51	53.83	26.89	34.76	28.06	29.90
	(60-180)	25.90	42.98	30.65	33.18	19.27	35.87	24.93	26.69

6. Average leguminous predecessors (1-2)	0								
	40	15.18	8.06	9.54	10.93	37.96	20.15	23.86	27.32
	80	36.98	33.03	25.55	31.85	46.23	41.28	31.94	39.82
	120	60.97	38.81	40.69	46.82	50.81	32.34	33.91	39.02
	(40-120)	37.71	26.63	25.26	29.87	45.00	31.26	29.90	35.39
7. Average non-leguminous predecessors (3-5)	0								
	60	16.79	17.07	15.98	16.61	27.98	28.45	26.64	27.69
	120	29.61	41.09	30.68	33.79	24.67	34.24	25.57	28.16
	180	53.78	58.86	51.76	54.80	29.88	32.70	28.75	30.44
	(60-180)	33.39	39.01	32.81	35.07	27.51	31.80	26.99	28.76
	x-40-180	35.12	34.06	29.79	32.99	34.50	31.58	28.15	31.41

On average, for the period for all fertilization rates after leguminous predecessors to produce 100 kg of triticale yield, 1.9 kg of nitrogen is needed - 31.3% less than 2.8 kg of nitrogen after the other predecessors.These results confirm the positive effect of leguminous predecessors on soil fertility and its nitrogen enrichment through nitrogen fixation and determine their role on the productivity of the subsequent crops in the crop rotation.

Table 3. Fertilizer consumption (nitrogen required for 100 kg of produce), kg

Predecessor	Nitrogen fertilization, kg ha^{-1}	2010		2011		2012		Average 2010 - 2012	
		Grain yield, kg ha^{-1}	kg N for 100 kg grain	Grain yield, kg ha^{-1}	kg N for 100 kg grain	Grain yield, kg ha^{-1}	kg N for 100 kg grain	Grain yield, kg ha^{-1}	kg N for 100 kg grain
1. Wintering peas	0	3436.3		3281.3		3123.8		3280.4	
	40	3760.0	1.1	3506.3	1.1	3395.0	1.2	3553.8	1.1
	80	4383.8	1.8	4282.5	1.9	3821.3	2.1	4162.5	1.9
	120	4828.8	2.5	4585.0	2.6	4238.8	2.8	4550.8	2.6
	(40-120)	4324.2	1.8	4124.6	1.9	3818.3	2.0	4089.0	1.9

2. Spring peas	0	3441.3		3317.5		3127.5		3295.4	
	40	3773.8	1.1	3545.0	1.1	3397.5	1.2	3572.1	1.1
	80	4426.3	1.8	4285.0	1.9	3832.5	2.1	4181.3	1.9
	120	4853.8	2.5	4615.0	2.6	4275.0	2.8	4581.3	2.6
	(40-120)	4351.3	1.8	4148.3	1.9	3835.0	2.0	4111.5	1.9
3. Sunflower	0	3347.5		3252.5		3001.3		3200.4	
	60	3800.0	1.6	3575.0	1.7	3466.3	1.7	3613.8	1.7
	120	4500.0	2.7	4355.0	2.8	3966.3	3.0	4273.8	2.8
	180	5017.5	3.6	4631.3	3.9	4477.5	4.0	4708.8	3.8
	(60-180)	4439.2	2.6	4157.1	2.8	3970.0	2.9	4198.8	2.8
4. Wheat	0	3388.8		3287.5		3080.0		3252.1	
	60	3848.8	1.6	3607.5	1.7	3482.5	1.7	3646.3	1.6
	120	4642.5	2.6	4380.0	2.7	4002.5	3.0	4341.7	2.8
	180	5043.8	3.6	4730.0	3.8	4507.5	4.0	4760.4	3.8
	(60-180)	4511.7	2.6	4239.2	2.7	3997.5	2.9	4249.4	2.7
5. Triticale	0	3375.0		3210.0		3075.0		3220.0	
	60	3805.0	1.6	3575.0	1.8	3483.8	1.7	3621.3	1.7
	120	4645.0	2.6	4341.3	2.8	3971.3	3.0	4319.2	2.8
	180	5045.0	3.6	4651.3	3.9	4506.3	4.0	4734.2	3.8
	(60-180)	4498.3	2.6	4189.2	2.8	3987.1	2.9	4224.9	2.8
6. Average leguminous predecessors (1-2)	0	3438.8		3299.4		3125.6		3287.9	
	40	3766.9	1.1	3525.6	1.1	3396.3	1.2	3562.9	1.1
	80	4405.0	1.8	4283.8	1.9	3826.9	2.1	4171.9	1.9
	120	4841.3	2.5	4600.0	2.6	4256.9	2.8	4566.0	2.6
	(40-120)	4337.7	1.8	4136.5	1.9	3826.7	2.0	4100.3	1.9
7. Average non-leguminous predecessors (3-5)	0	3370.4		3250.0		3052.1		3224.2	
	60	3817.9	1.6	3585.8	1.7	3477.5	1.7	3627.1	1.7
	120	4595.8	2.6	4358.8	2.8	3980.0	3.0	4311.5	2.8
	180	5035.4	3.6	4670.8	3.9	4497.1	4.0	4734.4	3.8
	(60-180)	4483.1	2.6	4205.1	2.8	3984.9	2.9	4224.4	2.8
%6/7			69.2		67.9		69.0		68.7

CONCLUSIONS

For the production of 100 kg grain yield, an average of 1.9 kg nitrogen is needed for growing triticale after a leguminous predecessor and 2.8 kg nitrogen for growing after sunflower, wheat and triticale. After leguminous predecessors, nitrogen fertilizer consumption is 31.3% less compared to cultivation after other predecessors. With the obtained yield from triticale an average of 96.53 kg ha^{-1} N is extracted from the soil with the grain, 18.97 kg ha^{-1} N with the straw or a total of 115.50 kg ha^{-1} N (average for fertilization cultivation). The difference in the amount of nitrogen extracted from the soil in triticale cultivation without fertilization and with fertilization is 40% on average. When growing triticale after leguminous predecessors the utilization of nitrogen is 35.39% on average, and after the predecessors sunflower, wheat and triticale - 28.76%. After leguminous predecessors the utilization rate of nitrogen is 18.7% better.

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EVALUATION OF THE COMPOSITION OF WINTER WHEAT GRAINS GROVN IN LATVIA AND NORWAY

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ABSTRACT

Wheat is one of the major grains in the diet of the world's population and therefore it may have a great impact on human health. The yield and chemical composition of grains can vary greatly due to genetic and environmental factors. The aim of the present study was to assess how different climatic conditions affect the chemical composition of wheat grains of the same genotipe. The field trials were established in Stende (Latvia) and at Apelsvoll (Norway). Wheat varieties used were 'Edvins', 'Ellvis' and 'Skagen' In the studied samples the content of protein, amino acids, 1000 grain weight, hectolitre weight and falling number were determined. The protein content in the grains ranged from 8.9% to 11.8% in organic fields and from 10.0 to 13.1% in conventional fields and differed between the varieties. The protein content in the samples of the same variety differed significantly due to environmental conditions. The difference in protein content was greater in the organic fields. The 1000 kernel weight in the wheat grain samples varied from 39.9 to 49.2 and depended on the variety and the growing place. The ratio of essential amino acids to the total content of amino acids (in %) ranged from 30.8 to 33.3% in the conventional growing system and from 30.5 to 32.9% in the organic growing system. Significant difference between the varieties was not detected.

Key words: *organic and conventional farming, amino acids, protein, quality, environment.*

INTRODUCTION

Cereals is one of the basic component of healthy diet. Latvian consumers prefer traditional cereals – wheat, rye, but Norwegians – wheat, barley and oats. Longer and warmer growing seasons in Northern part of Europe may widen utilization of spring cereal genetic resources. Moreover, this may lead to more diverse cereal use in human diet and to expand the number of cultivars suitable for breeding

conditions. The chemical composition and nutrition value of grain are influenced by genetic and environmental factors (Arendt, Zannini, 2013). According to many authors, the choice of variety is a crucial factor in efficient organic farming. Modern cultivars of wheat do not satisfy all the requirements and demands of organic agriculture. Therefore, more attention should be given to the breeding of specific cultivars adapted to the agronomic conditions on organic farms and complying with the philosophy of organic farming (Lammerts van Beuren, 2002)
The survival of the wheat plants during winter and early spring time depends on local weather conditions, genotype, physiology, and growing technology. It is closely connected with obtained grain yield and quality. Evaluation of the quality of wheat includes the functional properties of wheat flour for bread making and the nutritional composition of wheat flour. Protein composition and contents play a critical role in bread quality and are governed by a combination of genetic and environmental factors (Hussain et al., 2009). Cereals are important sources of protein for human nutrition but have low quality due to limitations in the amounts of essential amino acids, notably lysine. These deficiencies result from the low levels of these amino acids in the prolamin storage proteins and hence are exacerbated when high levels of nitrogen fertiliser are used to increase yield and total protein content (Shewry, 2007). The main task of this research was to evaluate the grain quality and chemical composition of winter wheat varieties grown under different climatic conditions

MATERIALS AND METHODS

Field Experiments Latvian and Norwegian winter wheat varieties were tested in the field experiments at two locations - in the Stende, Latvia (N 57.1°, E 22.3) and in the Apelsvoll, Norway (N 60.7°, E 10.9°) one growing season. The varieties – 'Edvins', 'Ellvis' and 'Skagen' were tested under conventional (CS) as well as organic (OS) growing conditions. Each field experiment was carried out using a block design with four replicates. Plot size was 10 m^2 in the Latvian trials, and 12 m^2 in the Norwegian trials. In the Table 1. summarized information about soil characterization, fertilization, sowing rate and time.

Table 1. Soil and sowing characterization at different growing places.

	Stende (LV)		Apelsvoll (NOR)	
Indicator	CS	OS	CS	OS
Soil characterization	Podzolic sandy loam	Sandy loam	Morenic loam	Morenic loam
pH KCl	5.6–6.0	6.6–6.8	6.3	5.8
Humus content, %	2.0	3.4–3.8	5.2	6.0
P_2O_5 mg kg^{-1}	169–232	208	68	71
K_2O mg kg^{-1}	140–177	124	64	130
Fertilization	N:P:K+S 15-15-15+S 500 kg ha^{-1}	–	N:P:K 22-2-10; 330 kg ha^{-1} N:P:K 27-0-0 150 kg ha^{-1}	Organic hen manure 120 kg N ha^{-1}
Sowing time	22.09.2015.	19.09.2015.	24.09.2015	24.09.2015
Sowing rate, gerninable seed m^{-2}	450	450	450	450

Climate data In September 2015 meteorological conditions was suitable for winter cereals during the sowing time and further development. After a lot more rain than normal in September, the soil conditions in the conventional field were difficult. Plant establishment was not optimal before winter, although October was warmer than normal. Soil conditions, and plant establishment was much better in the organic field. The winter and early spring was mild with small overwintering problems.
Sum of the monthly precipitation at the different locations were calculated for the period Mart to September during the year 2016. Table 2 show significant differences in precipitation between locations. April and Mai 2016 were warmer than normal, and with normal precipitation. The experimental fields were established in the first part of May under near optimal conditions. For a little period after sowing, there were less precipitation than normal, but in June, July and August there were nearly normal precipitation and somewhat higher temperature than normal. This gave good conditions for plant growth. September was warmer and drier than normal, and gave good conditions for harvesting the grain.

Table 2**.** The sum of monthly precipitation and temperatures at different locations.

Growing place	Marth	April	May	June	July	August	September
	Precipitation						
Apelsvoll	42.2	74.7	66.1	29.5	59.4	109.1	21.4
Stende	14.6	38.4	46.9	92.5	91.7	59.5	-
	Temperature						
Apelsvoll	1.3	3.9	10.7	15.1	15.5	14.2	13.6
Stende	1.4	6.1	13.6	15.5	17.1	15.2	-

Physical and chemical grain analyses. Representative samples from each replicate of the trials were analyzed for test weight, crude protein content (%) and volume weight (gL^{-1}) of whole kernels were determined by near infrared transmission (NIT) using Foss InfratecTM 1241 Grain Analyzer (FOSS Tecator AB, Höganes, Sweden). Thousand grain weight (TGW) was calculated by using Opto-Agri12 Seed Counter (Opto Machines, Riom, France) in Norwey. The test weight from representative samples from each replicate of the Latvian trials analyses combining seed counter Contador and ISTA method. Then the grain samples from each plot of the Norwegian trials were made available for amino acid analysis in Latvia.
Determination of amino acid (AA)s. Dried, defatted barley samples were treated with constant boiling 6N hydrochloric acid in the oven at around 110°C for 23 h using the Waters AccQ Tag chemistry package. Hydrolyzate was diluted with 0.1% formic acid. Amino acids were detected using reversed-phase HPLC/MS (Waters Alliance 2695, Waters 3100, column XTerra MS C18 5 μm, 1x100 mm). Mobile phase (90% acetonitrile: 10% deionized water) 0.5 mL/min, column temperature at 40^0C was used. The identity and quantitative analysis of the amino acids were assessed by comparison with the retention times and peak areas of the standard amino acid mixture.

Statistical analysis Descriptive statistics were used to characterize barley varieties yield, 1000 grain weight, volume weight, protein content in each of experimental management system and at each location. An analyses of variance (ANOVA) was performed to assess whether growing location and management system influenced yield and quality parameters

RESULTS AND DISCUSSION

The yield and quality of tested winter wheat varieties grown in Stende and at Apelsvoll under conventional and organic farming systems assumed in the Table 3. showed difference among the growing places.

Table 3. The quality traits of wheat varieties in the organic and conventional systems.

Variety	Growing place	Yield, t ha^{-1}	Protein, %	Moisture, %	TGW, %	Volume weight kghL^{-1}	Falling number
		Conventional system					
'Edvins'	Stende	8.1 ±0.2	12.7 ± 0.6	13.8 ± 0.8	39.9 ± 1.5	75.4 ± 1.0	388 ± 9
	Apelsvoll	2.4 ± 0.9	13.1 ±1.5	23.3 ± 5.5	45.8 ± 2.1	80.5 ±1.8	272 ± 47
'Ellvis'	Stende	10.6 ±0.2	11.2 ± 0.4	13.6 ± 0.5	37.5 ± 0.8	75.6 ± 1.1	380 ± 7
	Apelsvoll	3.63 ± 0.9	10.0 ±0.4	24.7 ± 4.7	40.0 ± 2.7	79.7 ± 1.7	328 ± 19
'Skagen'	Stende	11.2 ± 3.2	11.3 ± 0.4	13.3 ± 0.8	47.1 ± 13.2	77.8 ± 0.3	407 ± 15
	Apelsvoll	2.93 ± 0.9	11.9 ± 0.6	25.4 ± 7.3	44.2 ± 3.6	80.4 ±1.8	298 ± 54
		Organic system					
'Edvins'	Stende	3.9 ± 0.4	11.8 ±0.7	12.4 ± 0.6	44.0 ± 1.2	79.3 ± 1.7	291 ±25
	Apelsvoll	4.1 ± 0.9	9.9 ± 0.3	15.8 ± 0.9	49.2 ± 0.8	83.4 ± 0.9	286 ± 32
'Ellvis'	Stende	5.4 ± 1.6	9.9 ±0.2	12.6 ± 0.7	34.8 ± 12.4	76.4 ±0.7	376 ± 7
	Apelsvoll	5.3 ± 0.9	8.9 ± 0.6	15.5 ± 1.1	41.6 ± 1.7	81.4 ± 0.9	334 ± 22
'Skagen'	Stende	4.7 ± 0.5	11.4 ±0.3	13.7 ± 0.4	47.1 ± 0.1	77.8 ± 0.4	371 ± 9
	Apelsvoll	5.1 ± 0.9	9.8 ± 0.4	16.6 ± 0.9	49.0 ±0.9	83.5 ± 0.2	328 ± 32

The content of protein in samples of wheat grain depending of varieties ranged from 9.9% to 12.7% grown at Stende and from 8.9 to 13.1% grown at Apelsvoll. The protein content in samples of same variety differed significantly by environmental conditions ($p<0.05$). Growing conditions, environment and fertilizer use have a significant effect on the protein content of wheat which varies from 7 to 20% in a single variety (Arendt, Zannini, 2013). The falling number of grains, which characterized functional properties of wheat flour for bread making ranging from 291 to 407 at Stende and from 272 to 334 at Apelsvoll.

The results of a study on the quality of wheat (*Triticum aestivum L.*) grown in a 21 year agrosystem comparison between organic and conventional farming in central Europe show that the 71% lower addition of plant-available nitrogen and the reduced input of other means of production to the organic field plots led to 14% lower wheat yields. However, nutritional value (protein content, amino acid composition and mineral and trace element contents) and baking quality were not affected by the farming systems (Mader et.al., 2007). The protein content of winter wheat grain grown in organic and conventional systems showed in figure 1.

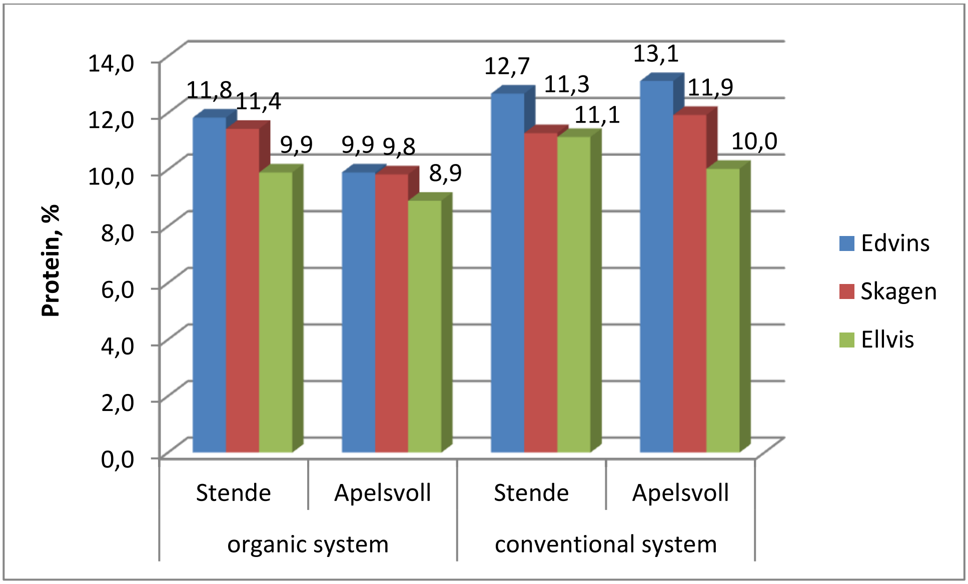

Figure 1. Variation of yield and protein content in the wheat varieties.

The protein content of grains grown in organic system at Stende determinated significantly higher ($p<0.05$) in all varieties – 'Skagen' 11.4 ± 0.3 %; 'Edvins' 11.8 ± 0.7 %; 'Ellvis' 9.9 ± 0.2 % in comparison with the samples of Apelsvoll 9.8 ± 0.4 %; 9.9 ± 0.3 % and 8.9 ± 0.6 % respectively. The diference in protein content of samples from conventional system was smaller and was not significant. Environmental interactions during grain filling influence final grain weight, protein and starch contents (Altenbach et al., 2003).

Protein quality strongly depended on its amino acid composition, especially on the amount of essential amino acids, its level in total amino acids. Therefore composition of amino acids in grains of wheat varieties was determined. The results showed in the Table 4.

Wheat grains are characterized by high glutamic acid and proline content and relatively low content of basic amino acids. For all studied varieties and at both locations, glutamic acid content in wheat grains ranged from 26.4 to 34.0 g kg^{-1} in CS and from 22.6 to 31.7 g kg^{-1} in OS. The proline content ranged from 9.9 to 13.8 g kg^{-1} in CS and from 7.8 to 13.4 g kg^{-1} in OS. Total amino acid content ranged from 86.1 to 108.9 g kg^{-1} in the conventional growing system and from 72.8 to 100.2 g kg^{-1} in the organic system depending on varieties and growing places. This variation was due to specific wheat genotipe and climatic conditions at the growing place.

Protein nutritional quality is determined by its contents of essential amino acids, those which cannot be syntesized by animals and must be provided in the diet. Ten amino acids are strictly essential: lysine, isoleucine, leucine, phenilalanine, tyrosine, threonine, tryptophan, valine, histidine, methionine variety (Arendt, Zannini, 2013).

Table 4. The composition of amino acids of winter wheat grain growing at different places.

	'Skagen'		'Ellvis'		'Edvins'	
	Stende	Apelsvoll	Stende	Apelsvoll	Stende	Apelsvoll
	CS / OS	CS / OS	CS / OS	CS / OS	CS / OS	CS / OS
Asp	3.4 / 3.9	3.8 / 3.6	3.3 / 3.4	3.4 / 3.4	4.2 / 3.0	3.5 / 2.9
Glu	28.9 / 31.7	31.3 / 27.0	30.3 / 26.6	26.4 / 22.6	30.5 / 29.9	34.0 / 27.8
Ser	4.5 / 5.2	5.1 / 4.2	4.9 / 4.6	4.4 / 3.7	4.9 / 5.4	5.9 / 4.8
Gly	4.3 / 4.6	4.4 / 4.2	4.7 / 4.3	3.8 / 3.3	4.2 / 5.4	5.2 / 4.1
His*	2.2 / 2.4	2.4 / 2.1	2.3 / 2.2	2.1 / 1.8	2.2 / 2.5	2.8 / 2.2
Arg	4.2 / 4.6	4.4 / 3.9	4.4 / 4.2	3.8 / 3.4	4.3 / 4.8	4.9 / 4.1
Thr	2.6 / 2.8	3.0 / 2.5	2.7 / 2.5	2.5 / 2.2	2.6 / 3.1	3.3 / 2.6
Ala	3.2 / 3.7	3.5 / 3.1	3.4 / 3.5	3.1 / 2.8	3.5 / 3.8	4.0 / 3.4
Pro	10.7 / 12.1	11.7 / 9.2	12.0 / 10.6	9.9 / 7.8	11.2 / 13.4	13.8 / 10.5
Tyr*	1.7 / 1.8	1.9 / 1.4	1.8 / 1.5	1.5 / 1.3	1.9 / 1.8	2.1 / 1.5
Val*	4.2 / 4.3	4.5 / 4.1	4.3 / 3.5	4.0 /3.5	4.1 / 4.4	4.9 / 4.6
Met*	1.6 / 1.7	1.7 / 1.5	1.7 / 1.5	1.3 / 1.3	1.7 / 1.9	1.7 / 1.7
Cys*	1.7 / 1.8	1.8 / 1.7	1.5 / 1.7	1.6 / 1.3	1.9 / 2.1	2.1 / 1.8
Iso*	3.2 / 3.3	3.4 / 3.0	3.4 / 2.9	3.1 / 2.6	3.1 / 3.4	4.0 / 3.3
Leu*	6.8 / 7.3	7.4 / 6.2	7.1 / 6.6	6.3 / 5.4	7.0 / 7.8	8.2 / 7.0
Phe*	4.7 / 4.9	5.2 / 4.3	5.1 / 4.4	4.6 / 3.8	4.7 / 5.0	5.6 / 4.9
Lys*	2.7 / 2.7	2.7 / 2.4	2.8 / 2.7	2.4 / 2.3	2.7 / 2.9	3.0 / 2.6
TAA**	**90.8 / 98.8**	**98.0 / 84.0**	**95.7 / 86.9**	**86.1 / 72.8**	**94.6 / 100.2**	**108.9 / 89.9**
EAA*	**28.9 / 30.1**	**30.9 / 26.9**	**29.9 / 27.2**	**28.7 / 23.05**	**29.2 / 31.7**	**34.4 / 29.6**
EAA/TAA	**31.8 / 30.5**	**31.5 / 32.0**	**31.2 / 31.3**	**33.3 / 31.7**	**30.8 / 31.6**	**31.6 / 32.9**

*Essential amino acids (EAA)
**Total amino acids

There were not observed significant diference among growing places in connection of essential amino acids. The amount of essential amino acids was lower in samples of variety 'Ellvis'. With the aim to evaluate the quality of wheat grain protein – the ratio of essential amino acid to total (EAA/TAA in %) was calculated. This ratio ranged from 30.8 to 33.3% in conventional growing system and from 30.5 to 32.9% in organic system. There was no significant difference between growing places same as between varieties.

The composition of essential amino acids of winter wheat varieties grown at Stende and at Apelsvoll are shown in Fig. 2.

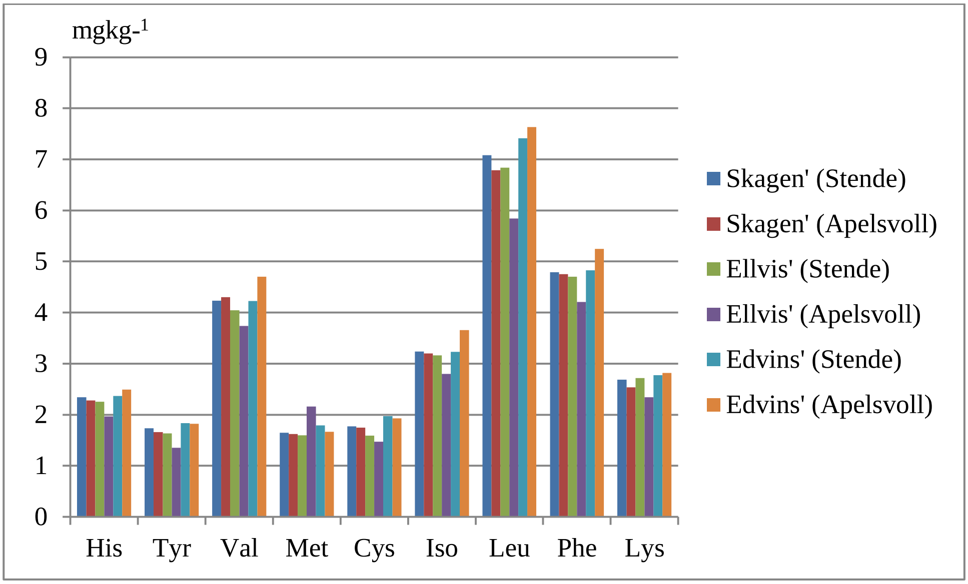

Figure 2. Amount of essential amino acids in different winter wheat varieties

As it is showed in figure 2, grains of varieties ‘Skagen’ and ‘Edvins” has higher level of histidine, valine, Isoleucine, leucine, phenilalanine and lysine than variety ‘Ellvis’. Composition of amino acids in the grains of variety ‘Ellvis” significantly differed between growing places.

CONCLUSION

The content of protein in samples of winter wheat grain depending of varieties ranged from 8.9% to 13.1%. The protein content in samples of same variety differed significantly by environmental conditions ($p<0.05$). There were not observed significant diference among growing places in connection of essential amino acids. The amount of essential amino acids was lower in samples of variety ‘Ellvis’.

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- ACKNOWLEDGMENTS

If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

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