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CONTENT

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Kristin STEINFURTH, Michael VAN LAAK [1]

META-ANALYSIS OF THE YIELD RESPONSE TO PHOSPHORUS FERTILIZATION BASED ON LONG-TERM FIELD EXPERIMENTS

SUMMARY

Phosphorus (P) fertilizer recommendations in Germany and most other European countries are based on plant-available soil P contents and results from long-term field experiments. Site-specific conditions are often neglected, and consequently excessive P fertilizer rates have often been applied in the past decades. In this study, long-term field P fertilization experiments including relevant site and soil parameters were evaluated in order to analyze the yield response. The database comprises about 2000 datasets from 30 field experiments from Germany and Austria. Statistical evaluations using a classification and regression tree approach, and multiple linear regression analysis indicate that besides plant-available soil P content, soil texture and soil organic matter content have a large influence on the effectiveness of P fertilization. This study methodology can be a basis for modification and specification of existing P fertilization recommendations and thus contribute to mitigate environmental impacts of P fertilization.

Keywords: *CART, Crop yield, Fertilization, Phosphorus, Plant-available soil phosphorus.*

INTRODUCTION

In most countries, P fertilizer recommendations are based on the expected nutrient uptake by crops and the plant-available P content in the soil (Jordan-Meille *et al.*, 2012), and the procedure entails three steps: (i) Extraction of plant-available soil P, (ii) calibration of those soil test results, (iii) deducing recommended P fertilizer amounts.

In the calibration step, plant-available P contents are categorized into several classes (in Germany and many other countries five classes), which are interpreted in terms of nutrient supply and crop yields. These calibrations are mostly based on long-term fertilization trials (Kuchenbuch and Buczko, 2011; Buczko *et al.*, 2018). However, the data base used for the calibration step is mostly not accessible in the international literature, and even in countries which

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use the same extraction procedure, the boundaries of the nutrient availability classes may diverge considerably (Jordan-Meille *et al.*, 2012). Various studies have shown that P fertilization recommendations in Germany and several other European countries have been too high in the past (Ott and Rechberger, 2012; Tóth *et al.*, 2014; Withers *et al.*, 2014), and consequently, the boundaries of the P fertility classes have been too high (VDLUFA, 2015). Moreover, besides plant-available soil P contents, other factors have an influence on crop yields, for instance pH value, soil organic carbon content, clay content, weather and climate parameters. Although in Germany and other countries there is a large number of long-term field experiments dealing with the effects of P fertilization on crop yields (e.g., Baier *et al.*, 2001; Spiegel *et al.*, 2001; Merbach and Schulz, 2012), the results of those field fertilization trials have mostly not been compiled nor evaluated and analyzed as a whole in form of a meta-study. The objectives of this work were to compile a large database of long-term P fertilization experiments from Germany and Austria with special emphasis on data which have until now not been published. A meta-analysis of this data was conducted including statistical methods to evaluate the influence of various site-specific soil and environmental factors on the effectiveness of P fertilization.

MATERIAL AND METHODS

Data of 30 phosphorus fertilization trials across Germany and Austria were compiled (Buczko *et al.*, 2018). All field experiments focused on the effect of P fertilization on yields and are therefore one-factorial fertilization trials with application rates ranging between 10 and 210 kg P ha^{-1} y^{-1}, i.e., 30 to 2000 % of P export by crops. The final data base comprised well over 2000 data sets. Effects of fertilizer application rates on yields were compared by calculating the relative yield increases (YI in %) from the ratio of the yield of the fertilized treatment (y_f) and that of the zero fertilization (control) treatment (y_0):

$$YI = ((y_f / y_0) - 1) * 100$$

i.e., YI is the percentage value of the increase in crop yield of the fertilized treatment compared with the corresponding control treatment.

The studied soils have rather high soil P contents. Nevertheless, P fertilizer application rates are very high: in 50 % of the data, more than 158 % of the P was exported by harvested crops.

The fertilization trials have mostly been conducted over many years, and the duration of the experiments utilized in the present meta-analysis is in some cases longer than 20 years. The most frequent soil types were Luvisols. The crop rotations are dominated by the crops grown most commonly in Germany, i.e. winter wheat (n = 568), winter barley (n = 305), summer barley (n = 202), sugar beet (n = 200), potato (n = 197), and oilseed rape (canola) (n = 129).

The data of the field trials were analyzed with a classification and regression tree (CART) approach and multiple regression analysis. The CART methodology is based on splitting the data set into segments with a distinct factor

combination. The impact of several predictor variables on a dependent variable is analyzed by successive binary splits. The resulting trees are easy to interpret, since the successive binary splits indicate the relative importance of the predictor variables in explaining the dependent variable. In addition, multiple linear regression analyses were conducted for comparison. Both the CART and regression analyses were done using the program SPSS (version 20.0).For both the CART and regression analyses, the dependent variable was the relative yield increase (YI), and the influencing factors (predictor variables) were plant-available soil P content (soil test phosphorus, STP), clay content, organic carbon content, pH value, relative P fertilizer application rate, and crop species.

RESULTS AND DISCUSSION

The relation between STP and YI for all data points (Fig. 1) reveals highest YI for the soil P content class B and lower YI for higher P content classes. For all soil P classes, the large number of data points with negative YI, i.e., yield depressions, is striking. This is a phenomenon commonly observed in long-term fertilization trials (e.g., *Jungk et al.*, 1993; Kuchenbuch and Buczko, 2011). The yield depressions are observed here more or less equally for all soil P content classes (A: 20 %, B: 30.6 %, C: 29 %, D; 30.5%, E: 41% of datasets). High levels of plant available P (as provided by mineral fertilizer) in general reduce root density (Forde and Lorenzo, 2001), and development of mycorrhiza (Williams *et al.*, 2017). This could have a negative impact on the uptake of water and other nutrient elements, for instance the micronutrients Zn and Cu, thus reducing the yield of fertilized treatments.

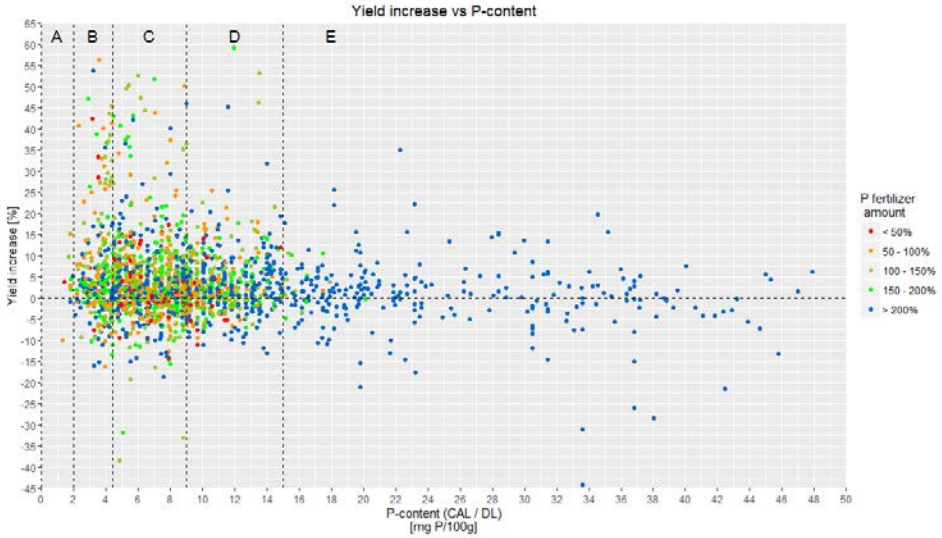

Figure 1. Rel. yield increase (YI) vs soil P content (CAL or DL); fertilizer application rate ("fertilizer amount") expressed as % of P export by harvested crop; P-content classes according to VDLUFA (1997).

Relative yield increases by P fertilization as a function of soil pH class show highest YI values for pH classes A and B (i.e., acid conditions with low pH values, <6), and lowest values for class E (high pH values, >7). This is probably connected with the direct correlation between STP and pH values (Pearson r = 0.41). At low pH values (< 5.5), P is strongly adsorbed (e.g., by Fe- and Al-Oxides) in soils and therefore less readily plant-available. Additionally, soil pH influences the availability of other essential plant nutrients and soil microorganisms and might therefore cause yield effects not investigated in the evaluated phosphorus experiments. YI increases with SOM content, and highest YI values are observed for SOM contents of 2.5–3 %. However, for SOM > 3 %, YI is again significantly lower (but the number of data in that group is lower than in the other groups). In general, P availability is directly correlated with SOM contents, because adsorption of P is reduced by organic anions such as citrate or malate which compete with phosphate anions for adsorption sites at Fe and Al oxide surfaces (e.g., Gerke, 2015). This may explain the higher effect of P fertilization with higher SOM contents observed for our data. The relation between P fertilization rate and YI is evaluated here in terms of relative rates, i.e., P input divided by the P export by the harvested crop. Although the YI values are on average highest for relative rates of 100–150 %, the differences among the groups are mostly not significant, and conspicuously, the YI values are relatively low for high rates of P input (> 200 % of exported P). Such a lack of stringent relation between P fertilizer amount and yield increase has been reported in previous studies (e.g., Jungk *et al.*, 1993) and suggests that in most cases the pool of plant available P in the soil is sufficient for high crop yields, and the applied fertilizer P is used mainly to maintain or even enhance this soil P pool. This is in accordance with the philosophy of "maintenance fertilization" (Jordan-Meille *et al.*, 2012), although recently this approach has been questioned (Withers *et al.*, 2014). Additionally, more important than the applied amount of fertilizer is the P content of the control plot. In cases where the soil P content of the unfertilized control is above 9 mg P / 100g, the fertilized plots only show an average yield increase of 1.1%, irrespective of the total available P (soil P + fertilizer P) in the treatment plot.

When evaluating the effect of P fertilizer type on YI, there are statistically significant differences between treatments fertilized with Superphosphate and Thomasphosphate on one hand, and Triplesuperphosphate and Hyperphosphate on the other hand. The lower effectiveness of Hyperphosphate (i.e., finely ground rock phosphate) in non-acid soils compared with Superphosphate is expected and in line with previous studies (e.g., Spiegel *et al.*, 2001; von Tucher, 2013). However, one would expect that Superphosphate and Triplesuperphosphate are similarly available, since both are produced by reaction of rock phosphate with inorganic acids (sulfuric acid and phosphoric acid). In contrast to Triplesuperphosphate, Superphosphate contains remnants of sulfate, which is a macronutrient. This could be an explanation for the higher effectiveness of Superphosphate. The relatively high effectiveness of Thomasphosphate could

possibly be caused by the high content of Ca and micronutrients (e.g., Fe, Mn, Zn), and the alkaline soil reaction induced by this fertilizer.

A comparison of the effectiveness of P fertilization among the six most common crops shows overall highest yield increase for summer barley, and lowest increases for winter wheat.

All the influencing factors mentioned above are considered in the CART and multiple regression analyses as independent variables. The first split in the CART analysis was set for the independent variable plant-available soil P content (STP) at a value of 3.34 mg P 100 g^{-1} soil (Fig. 2).

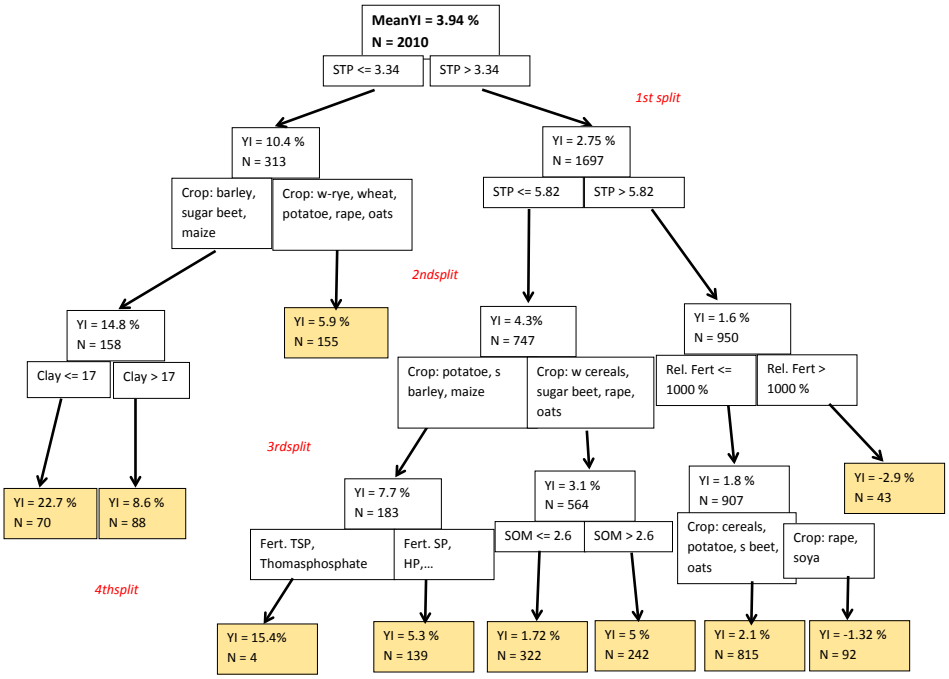

Figure 2. CART analysis of rel. yield increase. Considered independent variables are STP, pH, SOM, clay content, rel. P fertilizer amount (%), crop type and fertilizer type; not all these independent variables appear in the graph.

This indicates that plant-available soil P content is the most important variable determining yield increase by P fertilization. If the STP of the control is above 3.3 mg P 100 g-1 average YI is only 2.75% (compared to 10.4%). This result supports the latest VDLUFA recommendation (VDLUFA, 2015) to reduce the lower boundary of the P content class "C" to 3.0 mg P 100 g^{-1}. The second split is implemented according to crop species and again STP, i.e., these independent variables explain for each of the branches the largest part of the variance in YI. The blue end segments indicate the mean YI for the combination of parameters according to the respective branch of the decision tree. This can be demonstrated exemplarily for a data set from Rottenhaus (Austria) dating from

the year 1981 (Spiegel *et al.*, 2001). The plant available soil P content is 4.5 mg P 100 g^{-1} soil, i.e., at the lower margin of P content class C (VDLUFA, 1997). Clay content is 30 % and pH 5.98.

The fertilizer application rate of 172 kg P ha^{-1} y^{-1} in form of Superphosphate corresponds to 642 % of P export by the crop (26.8 kg P ha^{-1}). Nevertheless, the fertilizer application rate is, according to the CART analysis, not among the most important variables explaining the observed yield increase. For this dataset, the predicted YI is 5.3 % (Fig. 2), whereas measured YI is 4.5%. Similarly, multiple linear regression analysis suggests that plant available P content, pH value, and SOM content are the most significant variables, however with large differences among different fertilizer types.

CONCLUSIONS

This meta-analysis of a database of long-term field experiments of P fertilization covering various regions of Germany and Austria including about 2000 data sets from 30 field sites revealed that yield increase due to the effect of fresh P application is determined mainly by plant-available P in the soil, pH value, SOM, type of fertilizer, and crop type, whereas the exact amount of P fertilizer has less importance. The database will be expanded in the near future, and additional parameters will be included in the analysis, most notably soil type, precipitation, and air temperature. In a next step, the results will be utilized to refine the current P fertilizer recommendations.

Although only data from Germany and Austria are utilized in the present analysis, this approach can be extended to other countries worldwide, and the results gained in the analyses can be transferred to other environmental conditions and countries. This could contribute to more precise P fertilization recommendations, less application of P fertilizer, and diminished negative environmental impacts of P fertilization.

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INFLUENCE OF ABIOTIC AND BIOTIC FACTORS ON PRODUCTIVITY OF TRANSGENIC SOYBEAN AND MOLECULAR PROPERTIES OF DISEASE PATHOGEN

SUMMARY

For the first time in Ukraine, it has been shown that transgenic soybean cv. Grimo and Monro is affected by various pathogens, including viral diseases. Negative effect of the *Soybean mosaic virus* (SMV)-infection on the productivity and yield structure of soybean plants is proved. Also, significant role for the cultivation of transgenic soybeans (Kyiv and Poltava regions, Ukraine) is revealed for growth conditions. Cultivation of the cv. Monro in different weather and climatic conditions has shown that the growth limiting factor is moisture. In 2018, harvest of soybean cv. Monro in farm "Mriya" in Kyiv region was 3.1 t/ha, but in the farm "Mir" in Poltava region it was almost twice lower. We analyzed the coefficient of significance of deviations of the agrometeorological regime in 2018 comparing with the average perennial metadata. It was 1.14 - 2.30 and demonstrated that the conditions in 2018 are very different comparing with the average perennial parameters and close to the rare. SMV named SGK-17 (Ac No MG940988), isolated from transgenic soybean plants cv. Monro from Kyiv region, was studied in the detail. Nucleotide and amino acid sequences of the SGK-17 coat protein gene region (430 nt) were compared with sequences of SMV isolates from different countries. SGK-17 has the highest identity level (97.9% nt and 97.2% aa) with isolates from China, Poland, Iran, USA, Ukraine and is belonging to the one cluster with them. Four unique substitutions in CP gene of SGK-17 are revealed, which can be involved in its ability to infect transgenic soybean.

Keywords: transgenic soybean, soybean pathogens, sequencing, productivity, weather and climatic conditions

INTRODUCTION

Plants can be damaged by infectious pathogens such as viruses, bacteria, and fungi. They can also be damaged by noninfectious (abiotic) factors such as temperature and moisture extremes, chemical toxicity etc. Weather conditions

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influence on plants, phytopathogens and their vectors. There are two distinct mechanisms by which climate change can impact the relationship between pests and crop plants. Firstly, changes in climate have a direct impact on the biology of insects, including vectors, leading to differences in their survival, reproduction and spread. Secondly, there are the likely changes in agricultural practice that will take place as a result of climate change, and the influence of these changes on the availability of host plants for the pest species; e.g. the introduction of new crop species and plant genotypes, and changes in husbandry practice (Roos al., 2011). It was shown that factors like CO_2, elevated temperature and rainfall-related parameters influence on plant viruses, eg, CMV,PVY, PVX (Del Torro et al., 2015) TYLCV, and TuMV (Chung et al., 2015), Potato leaf roll virus and Potato yellow vein virus (Jones, 2014), BYDV (Nancarrow et al., 2014; Rua et al., 2013), BYMV (Guerret et al., 2016), and several important their vectors (Gillespie et al., 2012). Increased soil moisture and temperature in temperate regions, including northern Europe, are expected to increase the activities of zoospores and nematodes that transmit viruses (Jones 2014; Roos al., 2011). Potato mop-top virus (PMTV) and Beet necrotic yellow vein virus (BNYVV) are transmitted by the plasmodiophorids *Spongospora subterranea* and *Polymyxa betae*, respectively (Santala et al. 2010). The most important factor for this spread is probably the movement of infected plant material and soil, but climate factors may also have been involved. Bymoviruses and furoviruses (transmitted by *Polymyxa graminis*) are of great economic importance in autumn-sown cereals in large parts of Europe. In Canada increasing of PLRV incidence was detected due to increased vectors amount due to high winter temperatures (Boland et al., 2004).). In Germany, a clear relation was recently found between the number of infection days in autumn and BYDV-attack in winter barley fields (Habekuß et al., 2009). It was shown that rainfall less than the 30-year average caused a difference in the relative detection rate of BYDV-PAV and Cereal yellow dwarf virus, RPV strain in Alabama (Bowen, 2003).

In the future, the frequency of new encounters between viruses and plant species is likely to increase even more rapidly because of the major alterations in cultivated plant distributions anticipated from climate change. Climate change has been shown to cause emergence of a new viruses and strains in certain countries. So, many new PVY strains (PVYNTN, PVYNW) have appeared in the Netherlands over the last 12 years due to significant climate change (Lal et al., 2018). In Brazil and Azerbaijan the occurrence of BYDV was first reported only in 2013 (Mar et al., 2013; Mustafayev et al., 2013). Most of the scientific papers are review and dedicated to the prediction and development of a scenario of the impact of climate change on plant viruses. However, experimental works that would be directly focused on the influence of abiotic factors with SMV on genetically modificated (GM) soybean are absent.

It is known that infection caused by Soybean mosaic virus (SMV) has a negative effect on the soybean productivity, structure yield, and seeds quality in Ukraine (Mishchenko et al., 2018a; Mishchenko et al., 2018b). Given the harmful

effects of the virus, new soybean varieties that are resistant to various phytopathogens, including viruses are intensively developing and introducing. However, mostly soybean varieties have complex resistance against abiotic factors and fungal or bacterial diseases. Varieties of genetically modified (GM) soybeans appeared in Ukraine that should be characterized by high productivity and resistance to diseases. Soybean varieties Grimo, Monroe, Apollon, etc already grown. Also, significant role for the cultivation of transgenic soybeans (Kyiv and Poltava regions, Ukraine) is revealed for growth conditions (abiotic factors). Therefore, the aim of our work was to investigate the possibility of infecting GM soybean by SMV, to study its effect on productivity and yield of plants, as well as to establish the molecular genetic features of the SMV isolate that has ability to affect GM plants.

MATERIAL AND METHODS

During 2017-2018 inspections of soybean plants were conducted by visual diagnostics method (Peresypkin et al., 2000). Biometrics, crop and its structure were carried out by generally accepted methods (Dospekhov, 1985).

Meteorological data (the amount of precipitation, the amount of active temperatures, and the HTC for vegetation) were provided from the agrometopost of the Ustimovka Experimental Station of Plant Production, Plant Production Institute nd. a. V. Ya. Yuryev of NAAS, Poltava region.

Analysis of weather conditions compared to the average multi-year indicators were conducted on the basis of the coefficient of deviations significance (Cs) of agrometeorological regime elements of each of the studied years from average multi-year values by the formula: (Xi-X-)/S, where Xi- element of current weather; X- - average annual value; S - mean square deviation; i- number of the year (Vyshnivskyi, 2013). Level of the coefficients of deviations significance was determined by gradation: 0-1 – conditions are close to the ordinary; 1-2 – conditions that are very different from the average perennials; 2 – conditions close to the rare (extreme).

Hydrothermal coefficient of Selyaninov (HTC) calculated using the formula: $r/ (0,1 \cdot \Sigma t>10)$, where r - total rainfall during the growing season (May-August), mm; $\Sigma t>10$ - the average daily air temperature more than 10 ° C for the same period.

Identification of the viruses was performed by DAS-ELISA using commercial antibodies against Soybean mosaic virus (Loewe, Germany). The results were recorded on Termo Labsystems Opsis MR reader (USA) with Dynex Revelation Quicklink software at wavelengths of 405/630 nm. Samples were considered positive when their absorbance values were at least three times higher those of negative controls (Crowther, 1995).

Total RNA was extracted from fresh leaves using RNeasy Plant Mini kit (Qiagen) following the manufacturer's instructions. Two step RT-PCR was performed. The reverse transcription was performed using RevertAid Reverse Transcriptase – genetically modified MMuLV RT (Thermo Scientific, USA)

according to the manufacturers' instructions. Specific oligonucleotide primers to the part of SMV CP gene (469 bp were used (Mishchenko et al., 2018b)

For sequencing DNA fragments were purified from the agarose gel using a QIAquick Gel Extraction Kit (Qiagen, Great Britain) following to the manufacturers' instructions. CP gene sequences (430 nt) of the Ukrainian SMV isolate were compared with SMV sequences in the NCBI database with the BLAST program. SMV isolates using in this study are presented in Fig. 3. Nucleotide and amino acid sequences were aligned using Clustul W in MEGA 7 (Kumar et al., 2016). Aligned CP amino acid sequences were visualized and compared using BioEdit sequence alignment editor. The percentage of the nucleotide sequences identity was presented as color blocks using the software package SDT v.1 (Sequence Demarcation Tool Version 1.1). Statistical analysis of experimental data was carried out according to the parametric criteria of the normal distribution option, the standard deviation of the mean values - according to the generally accepted method.

To calculate the dN/dS ratio, an indicator of the evolutionary direction, the CP nucleotide sequences of all SMV isolates were codon-aligned. The ratio of the rate of nonsynonymous (dN) to the rate of synonymous (dS) mutations was calculated using the Nei-Gojoboori method in the SNAP program (Korber, 2000).

Statistical analysis

Five biological repeats were conducted per each ELISA measurement. Each biological repeat contained leaves from five individual plants pooled together prior to grinding. Three technical repeats were conducted for each biological repeat. When appropriate, the technical repeats data was averaged to get the mean value for each biological repeat.

RESULTS AND DISCUSSION

Our previous studies of 30 soybean varieties in the Poltava region showed the presence of wrinkle and mosaic symptoms on leaves.

Figure 1. Symptoms caused by SMV on GM soybean varieties Monroe, Apollon and Grimo

The results of ELISA and RT-PCR showed that plants infected by *Soybean mosaic virus* (Mishchenko et al., 2017a). Among the inspected varieties, our

attention was drawn to the fact that the SMV was affecting the transgenic varieties Monroe, Apollon and Grimo. Symptoms of wrinkle and blistering, deformation and reduction of leaf size (Fig. 1). Authors have been noticed that cv. Monroe soybean seeds in 2018 were characterized by significant spotting (Fig. 2).

a b

Figure 2. SMV symptoms on GM soybean cv. Monroe in 2018: a – severe wrinkle of leaves; b – seeds spotting from SMV-infected plants, at the right – seeds from healthy plants

The next stage was to determine effect of SMV isolated from these soybean plants cv. Monroe (named as SGK-17) on the yield. The results showed harmful effect of the virus and significant yield reducing of GM-soybean plants of the Monroe variety infected by SMV SGK-17 (Table 1).

Table1. Influence of SMV infection on the yield structure and productivity of GM-soybean cv. Monroe

Variant	Branching, pcs	Stem height to 1st bean, cm	Plant height, cm	Amount of beans per plant, pcs					Total of seds, pcs	Weight of seeds from1 plant, g	Weight of 1000 grains, g
				Total	With 1 seed	With 2 seeds	With 3 seeds	With 4 seeds			
Healthy plants	3.5 ±0.5	9.6 ±1.5	110.3 ±1.8	95.0 ±4.0	47.0 ±1.0	18.0 ±2.0	18.0 ±2.0	4..0 ±0.4	153.0± 6.0	19.65 ±2.13	181 ±12
Infected plants	2.2 ±0.2	8.1 ±1.3	94.3 ±1.4	58.0 ±3.0	39.0 ±1.0	14.0 ±2.0	12.0 ±2.0	–	103.0 ±4.0	14.01 ±1.10	139 ±10

It was found that in the virus infected plants, branching was 1.6 times lower than in healthy ones. In addition, the SMV infection reduces the amount of beans per plant by 1.6 times and amount of seeds by 1.5 times. Weight of seeds from one plant is reduced by 1.4 times, the weight of 1000 grains - by 1.3 times. In healthy plants, even a small number of beans (4.0) with four seeds in the bean were noted. Infection of soybean plants by Soybean mosaic virus (SMV) leads to significant crop losses - from 8% to 50% in natural conditions and even up to 100% - in cases of epiphytosis (Liao et al., 2002). SMV infection can cause

changes in the biochemical composition of seeds, reduce the viability of seedlings (El-Amretz et al., 1987; Mishchenko et al., 2018b).

The yield of transgenic soybean cv. Monroe in 2017 -2018, grown in farm 'Mir' (Poltava region) was 1.3 - 1.4 t / ha while in the farm Mriya (Kyiv region), where there was more precipitation, the yield of soybean Monroe ranged from 2.18 t / ha. up to 3.0 t / ha. But the harvest in 2016, more moisture-proof and susceptible for soybean growing, was 2.6 and 2.8 t / ha, respectively. Weather in 2016 in the Poltava region was considered very dry because the hydrothermal coefficient of Selyaninov (HTC) was 0.99. HTC in 2017 was only 0.53 that means that 2017 was very dry year. Therefore, in most of the farms in the region, soybean production was at a level of 1.3 t / ha. In case of damage by SMV infection, soybean yield decreased in both farms of Kyiv and Poltava regions on 35.0 – 65.7%, respectively. More significant yield reduction (in 2.6 times) in case of viral infection was noted in the Poltava region under conditions of very dry climate (in 2017 HTC=0,53; in 2018 HTC= 0,5). (Mishchenko et al., 2018b).

We have calculated coefficient of deviations significance (Cs) for the period 1955-2018 for all months (Table 2).

Table 2. Coefficient of deviations significance of the agrometeorological regime indicators of the current year from the average perennials, 2018

Indicator	April	May	June	July	August	September
Air temperature	1,84	1,14	1,34	1,34	2,96	2,30
Precipitation amount	-0,82	-0,65	-0,65	-0,48	-1,55	0,30

The results of the table indicate that during the entire period of soybean growth in the Poltava region in 2018, the conditions for its cultivation were far from ordinary, namely: from April to July they were so much different from the average perennials. In August and September they approached the rare ones. Yield reducing of transgenic soybean cv. Monroe in the farm 'Mir' in the Poltava region was almost twice, in comparison with farm "Mriya" in Kyiv region, where the conditions of cultivation were close to ordinary and yield was 3.0 t / ha. Such yield decreasing can be explained by influence of such important abiotic factor as the coefficient of the significance of deviations of the agrometeorological regime indicators.

Climate change affects the level of damage from plant diseases, because the environment significantly affects not only plants, but also pathogens and their vectors. The appearance, prevalence, harmfulness of plant virus diseases, as well as the subsequent co-evolution of plants and their pathogens, which leads to changes in the species composition of viruses in a certain region, the appearance of differences in the properties of viral isolates, the emergence of epiphytothies directly depend on changes associated with global warming (increase in temperature, change of precipitation, increase of CO2 and ozone, drought, etc)

(Bowen,et al., 2003; Nancarrow et al., 2014;Mishchenko et al., 2017b). It is worth noting that the SMV is transmitted by vector - aphids. In this regard, climate change can indirectly affect the intensity and prevalence of SMVs through their reproductive / survival effects of its vectors. Thus, in the conditions of mild winter, there is a high migration rate of aphids in the spring and a high incidence of plant damage.

The severity of viral diseases is determined in large part by the amount of inoculum and the time of infection. Infection of plants at an early developmental stage usually leads to more severe disease symptoms, e.g., SMV (Ren et al. 1997). For some viruses, higher temperatures also cause more severe symptoms development. Insects such as aphids are expected to have increased survival with milder winter temperatures, and higher spring and summer temperatures will increase their development and reproductive rates and lead to more severe disease. Mild winters increase the survival of weeds that are the reservoirs of viruses. Increases in frequency and intensity of summer storms with high winds, rain, and hail will increase wounding of plants and result in increased transmission of viruses by mechanical means. It is also known that, at elevated air temperatures, the symptoms of viral diseases are often masked, sometimes not noticeable, which significantly complicates the visual diagnosis and control of these pathogens (Svensson, 2010; Jones and Barbetti, 2012).

Molecular investigation of the SMV isolate SGK-17 shown that the highest level of the nucleotide sequences identity (97.9%) and amino acid sequences (97.2%) it has with Ukrainian isolates, Iranian, Chinese isolates, American isolate VA2 and Polish isolate M (Fig 3).

To explore the evolutionary forces acting on the SMV CP gene, the dN/dS values were calculated for all of the SMV CP sequences included to our study. This ratio indicates the amount of nonsynonymous to synonymous mutations. dN/dS ratio for isolate SGK-17 compared to all other isolates was 0,0536, The global dN/dS ratio for all of the sequences taken to the study was 0.014 ($p < 0.01$). This indicates about higher nucleotide diversity of the isolate SGK-17 compared to SMV isolates from other countries.

SGK–17 has four amino acid substituions in the 430 nt region of coat protein gene: at the position 1 (Ser→Trp), at the position 2 (Lys→Phe), at the position 3 (Gly→Arg), and at the position 4 (Lys→Asn) (Fig. 4).

It should be noted that we revealed the same aa substitution for all SMV isolates from GM soybean which we were studied earlier (Mishchenko et al., 2018a). It was the aa Ser→Trp substitution at the position 1. It is known that only few single-amino-acid changes near the C terminus of the CP of certain SMV strains led to the impossibility to seed transmission (Jossey et al., 2013). It was also found several single nucleotide variations (SNVs) in different regions of genome of seed-transmitted SMV (Jo et al., 2017; Mishchenko et al., 2018c). So, such substitution can be involved to the ability of these SMV isolates infect GM soybean.

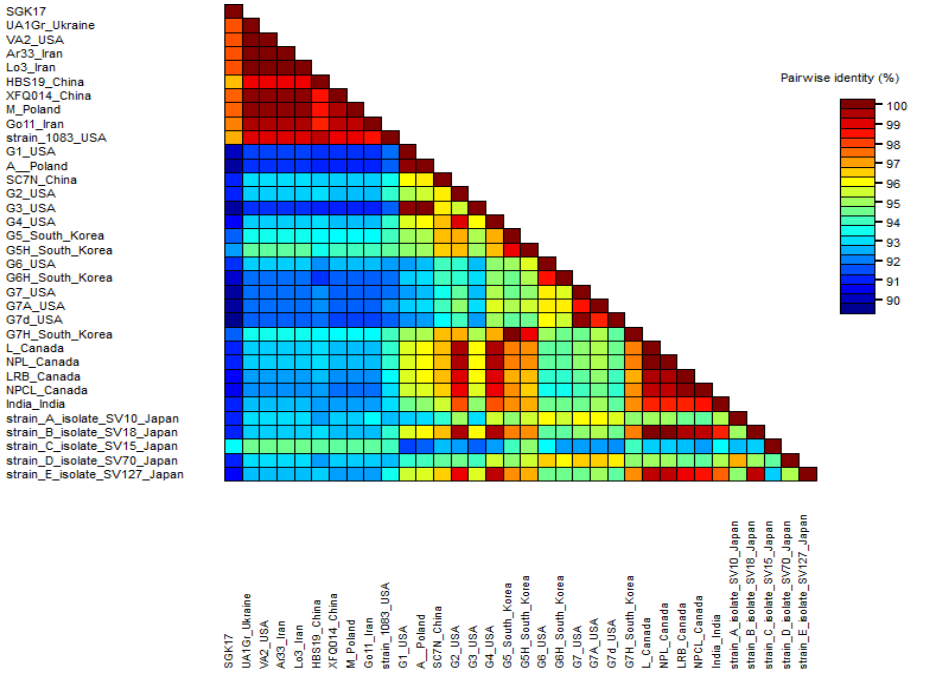

Figure 3. Graphical representation of pairwise nucleotide identity of SMV isolates (percentage of identity is represented on the scale)

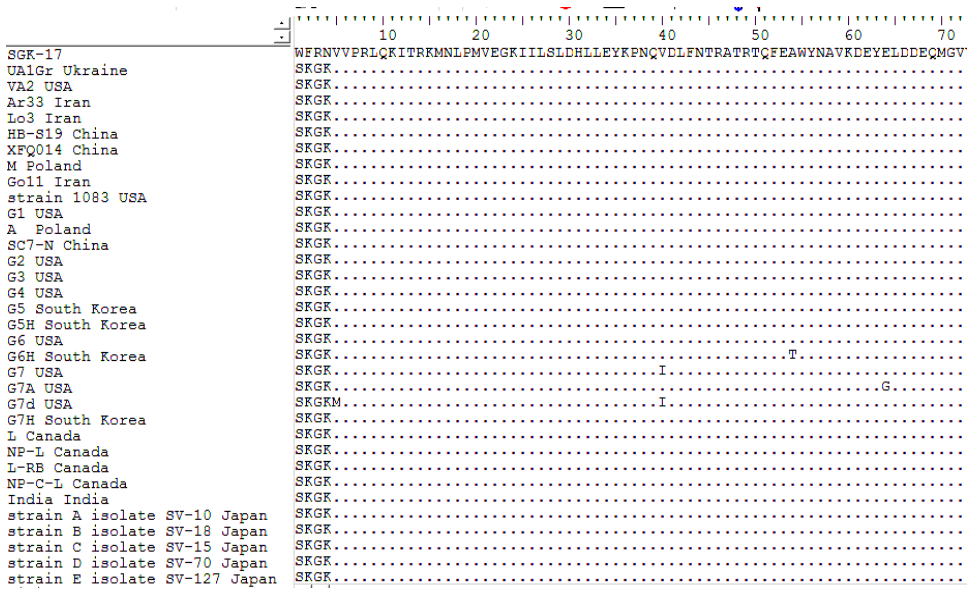

Figure 4. Comparison of the amino acid sequences of the CP fragment of isolate SGK-17 with other isolates / strains of the SMV. Numbers above represent the positions of amino acids. With dots "." indicated identical amino acids, with letters - amino acids substitutions

CONCLUSIONS

Thus, it has been shown that transgenic soybean varieties are affected by Soybean mosaic virus. It has been found that the SMV significantly reduces the yield and productivity of soybean plants. It has been established that, despite genetic modifications, the productivity of SMV-infected plants is significantly reduced. Molecular investigation of the SMV isolate SGK-17 revealed aa substitutions which can be involved to the ability of the virus infect GM soybean varieties. It was shown that HTC and so coefficient of deviations significance of the agrometeorological regime indicators play an important role in soybean productivity. Weather indicators in 2018 were significant differ from average perennials and were close to the rare conditions, that in complex with viral infection led to reducing of soybean yield.

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SINGLE-PLANT SELECTION AT ULTRA-LOW DENSITY OF FIRST GENERATION LINES OF THREE BEAN CULTIVARS UNDER WATER STRESS

SUMMARY

Nil-competition (ultra-low plant density) has been asserted to highlight individual genotypes of high yielding potential. This was tested preliminary on three determinate type bean varieties (*Phaseolus vulgaris* L.), two genetically non-uniform and with unstable yields Greek cultivars, Iro and Pirgetos and a "Great northern" type imported variety. Single-plant selection under ultra-low density (interplant distance of 100 cm) was performed in a honeycomb design experiment established during 2017 in the main farm of the University of Western Macedonia in Florina. Eighteen high yielding plants were selected and seed of each constituted a separate first generation line. In 2018, progeny evaluation was conducted in two R21 honeycomb design trials under normal and deficit irrigation treatments respectively. Compared to the original variety Iro, four of the high yielding progeny lines had higher yield plant^{-1} (by 20 to 39%) under water deficit with two being significantly different, where for the variety Pyrgetos only one first generation sister line significantly outperformed the original cultivar by 28%. Water stress affected significantly total chlorophyll content measured at 10 day intervals from start of flowering until physiological maturity with the best performing progeny lines showing higher chlorophyll concentrations especially during the seed filling stage. Significant differences between progeny lines and the original varieties were also shown on CO_2 assimilation rate under water deficit especially within the genotype Iro. Further research is needed so that any existing variation is beneficially exploited.

Keywords: Ultra-low plant density, Water stress, Chlorophyll concentration, CO_2 assimilation rate.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most important food legume, representing near 50% of grain legumes for human consumption and a significant

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source of high quality and low cost protein (Beede *et al*., 2013). Common bean is the most significant among the other pulses in Greece with increased cultivated areas in recent years (Kargiotidou *et al.,* 2019). Modern agriculture depends by far on uniform crop varieties in order to meet a growing demand for food by the world's population, and in most cases several landraces have progressively been replaced by elite cultivars satisfying the farmers and consumer's needs (Mavromatis *et al.,* 2007). The existence of genetic heterogeneity in Greek genotypes is offered for plant selection with methods of classical improvement and main criterion plant yield (Papadopoulos *et al.,* 2007). An intracultivar single-plant selection under ultra-low density has been extensively used to exclude plant-to-plant interference for resources as nil-competition boosts phenotypic expression and erases the masking effects induced by the negative relationship between yielding and competitive ability (Tokatlidis, 2015). This is making selection of desirable genotypes within a narrow gene pool and divergent environments applicable (Vlahostergios *et al*., 2018).

Climate change is inflicting a high impact on agriculture by altering the spatial and temporal distribution of rainfall, which limits water availability (Crimmins et al., 2011). Water deficit is a major limiting factor for crop productivity worldwide resulting in significant seed yield reductions across 60% of global bean production areas (Soureshjani *et al*., 2018). Reduced water availability results in lower water potential of plant tissues which decreases stomatal closure, leading to a reduction of CO_2 availability and, consequently, lower photosynthesis and transpiration rates (Teran and Singh, 2002; Bota *et al.,* 2004). Chlorophyll content of common bean is also reduced as a result of the degradation caused by drought conditions (Beede *et al*., 2013), and is directly related to biomass accumulation. These responses depend on the intensity of the stress, the plant genotype, and the plant developmental stage at stress incidence, among other factors (Beebe *et al.,* 2013).

The necessity to tackle this challenge has led to breeding and developing new varieties adapted to a continuously changing environment either exploiting intraspecific variability or by transferring genes from closely related wild species adapted to low irrigation (Martinez *et al.,* 2007). Extensive evidence exists to show that genetic resources for drought tolerance have potential for breeder programs (Andrade *et al.,* 2016; Farooq *et al.,* 2017). However most bean genetic diversity and bean populations are underutilized because of the difficulties that exist with the evaluation of physiological drought response dynamics in many cultivars.

The main objective of the present study was to evaluate high-yielding first generation lines of three different bean varieties selected at ultra-low density under water deficit conditions during anthesis and seed-filling growth stage. The range of variation in agronomic and physiological parameters that could exist may be utilized for identifying and developing improved genotypes which could perform better under adverse conditions.

MATERIAL AND METHODS

Plant material and experimentation

Three common bean determinate type genotypes, two Greek cultivars Iro and Pirgetos developed by the Hellenic Industrial and Fodder Crops Institute and an imported one Great-northern type constituted the source material. During 2017, single-plant selection was performed under ultra-low density (interplant distance of 100 cm) in a honeycomb design experiment in the main farm of the University of Western Macedonia in Florina as described in previous work (Papathanasiou *et al*., 2018). Eighteen high yielding plants were selected and seed of each constituted a separate first generation line. The selected first generation lines were coded hereafter GNTY1 to GNTY6, IR1 to IR6 and PIR1 to PIR6 according to the original genotype. During the 2018 season, approximately 50 plants per first generation line were assessed in two R21 honeycomb design trials under normal and deficit irrigation treatments respectively using the original genotypes as controls. The experiments were sown on 9th of May in the experimental farm of the University of W. Macedonia in Florina Greece (40°46´ N, 21°22´E, 707 m asl), in a sandy loam soil with pH 6.3, organic matter content 14.0 g kg^{-1}, N-NO_3 100 mg kg^{-1}, P (Olsen) 50.3 mg kg^{-1} and K 308 mg kg^{-1} and water holding capacity 21.8% (0 to 30 cm depth). The ultra-low density of 1.2 plants/m^2 was used i.e. single-plant hills were spaced 100 x 100 cm apart. Two or three seeds were sown in each hill and later thinned to obtain single-plant hills. A total of 400 Kg/ha 0-20-0 and 200 Kg/ha 11-15-15 fertilizers were applied at planting, while additional N (50 g per plant of a 27-0-0 fertilizer) was top-dressed when plants had reached the appropriate developmental stage. Complete weed control was obtained by tilling and hand.

Irrigation treatments

The normal irrigation received a full irrigation treatment, while deficit irrigation was 50% of the normal to simulate drought stress. A drip-irrigation water supply system of 4 L h^{-1} was established along every row, with emitters spaced at 40 cm intervals. Irrigation scheduling was based on bean evapotranspiration (ET_c) and was applied when the crop evapotranspiration rate ET_c - P (rainfall) reached 30 mm. Soil water content at this level was approximately 70% of field capacity, which is considered adequate for plant growth during all stages. The ET_c was calculated from climatic parameters measured daily from a meteorological station located adjacent to the experimental site and was used to calculate the reference evapotranspiration rate (ET_o) using the Penman–Monteith method (Allen *et al*., 1998). The ET_c, which is the product of ET_o and the crop coefficient (K_c), was calculated using values for bean K_c adjusted to Greek conditions (K_{cini} = 0.35, K_{cropd} = 0.70, K_{cmid} = 1.10, and K_{cend} = 0.30) for growth stages of 15/40/75/95 d after emergence.

Chlorophyll and gas-exchange measurements

Total chlorophyll content was measured with a hand-held dual-wavelength meter (SPAD 502, Chlorophyll meter, Minolta Ltd., Japan) at five 10-day intervals from start of flowering until physiological maturity (SPAD1 to SPAD5)

in six plants of each genotype in normal and deficit irrigation conditions. A portable photosynthesis system that measures CO_2 uptake (LI-6400 XT, Li-Cor, USA) equipped with a square (6.25 cm^2) chamber was used for determinations of CO_2 assimilation rate (A), transpiration rate (E) and stomatal conductance to water vapour (g_s) during the seed filling period. Leaf gas exchange was measured in the middle leaflet of a fully expanded trifoliate leaf close to the top of the plants. Measurements were performed on the same six plants of each genotype that chlorophyll measurements were taken from 09:00-12:00 in the morning to avoid high vapor-pressure deficit and photoinhibition at midday.

Harvest and statistical analysis

Plants were harvested individually and seed yield was measured at the physiological maturity stage and recorded at a per-plant basis for both normal and deficit irrigation treatments. Comparison of means was conducted by Least Significance Difference Test (LSD) after analysis of Variance (ANOVA), for completely random design.

RESULTS AND DISCUSSION

Mean yield and coefficient of variation (CV%) for seed yield $plant^{-1}$ (g) at ultra-low density under normal and deficit irrigation for the first generation lines compared to the three original genotypes (GNTY, IR and PIR) are presented in Table 1. For the genotype GNTY the six high-yielding first generation lines performed equally with the original genotype under the deficit irrigation treatment with no significant differences in yield $plant^{-1}$ whereas under normal irrigation the control showed higher values than all of its progenies. Compared to the original variety Iro, almost all progeny lines had significantly higher yields under normal irrigation and four of them showed higher yield $plant^{-1}$ (by 20 to 39%) under water deficit with two being significantly different. The first generation sister lines IR1 and IR6 yielded on average 121,2 and 117 g $plant^{-1}$ and showed a CV of 45,5 and 50% respectively. The respective values of the mother genotype IR-control under the same irrigation conditions were 86,8 g $plant^{-1}$ and CV of 55,6%. Similarly for the variety Pirgetos all progeny lines yielded higher than the control under normal water regime but only one first generation sister line significantly outperformed the original cultivar by 28% under the deficit irrigation. The line PIR5 showed mean yield $plant^{-1}$ 120,4 g and CV 52,6% compared to the PIR-control which yielded 94 g with a similar CV of 52,6%. This is in agreement with other studies where under adverse conditions such as high temperatures and increased biotic stress first generation sister lines of bean and/or other legumes such as lentils, outperformed the original genotypes under ultra-low density (Papadopoulos *et al.*, 2004; Vlahostergios *et al.*, 2018). The CV values under the ultra-low density for seed yield $plant^{-1}$ revealed a moderate spatial heterogeneity under deficit irrigation for all the genotypes tested. This is desirable because phenotypic screening and breeding for high yield is expected to ultimately select for potentially tolerant to water stress genotypes (Tokatlidis, 2015).

Physiological parameters such as mean chlorophyll content, assimilation rate A, stomatal conductance to water vapour g_s and transpiration rate E under normal and deficit irrigation are shown in Table 2 for all genotypes evaluated. Reduction in water supply was associated with decreased chlorophyll content (SPAD) during the seed filling stage. The high-yielding progenies IR1, IR5 and IR6 had significantly higher values than the IR-control during the late seed-feeling stage (SPAD 5). Similar results were observed for the line PIR5 with significant differences only during the early seed-filling stage (SPAD4). Chlorophyll content has been proposed as a good indicator of green color and the stay green characteristic under water stress is a commonly observed phenomenon (Fotonat *et al.,* 2007).

Table 1. Mean yield and coefficient of variation (CV%) for grain yield plant^{-1} (g) at ultra-low density under normal and deficit irrigation for the first generation lines originating from the three genotypes (GNTY, IR and PIR) and the control.

	Normal Irrigation		**Deficit Irrigation**	
First generation lines	**Yield g plant^{-1}**	**CV%**	**Yield g plant^{-1}**	**CV%**
GNTY1	147,6*	45,2	91,6	55,6
GNTY2	158,3	44,2	82,8	66,7
GNTY3	149,9	55,6	90,0	83,3
GNTY4	152,6	43,3	92,1	62,5
GNTY5	153,4	44,4	91,0	58,8
GNTY6	164,3	44,2	98,4	62,5
GNTY-Control	**176,7**	**37,6**	**90,6**	**58,8**
IR1	161,8**	40,5	121,2**	45,5
IR2	147,3	51,0	100,5	43,5
IR3	158,3*	48,8	112,7	62,5
IR4	152,5*	40,7	82,4	58,8
IR5	177,4**	48,3	104,6	55,6
IR6	178,0**	50,0	117,0*	50,0
IR-Control	**115,4**	**56,5**	**86,8**	**55,6**
PIR1	176,7	34,7	96,0	71,4
PIR2	158,5**	46,3	84,6	58,8
PIR3	153,7*	56,2	81,8	62,5
PIR4	144,0	51,8	91,3	52,6
PIR5	168,0**	44,4	120,4*	52,6
PIR6	141,9	44,2	97,9	55,6
PIR-Control	**117,0**	**50,3**	**94**	**52,6**

*, ** Denotes significant superiority to the mother landrace (t test for independent means and different standard deviations at the levels $P<0.05$ and $P<0.01$ accordingly)

Compared to the original variety Iro the first generation sister lines IR1 and IR6 showed significantly lower reduction in A, g_s and E. The higher stomatal

conductance of these two progenies under the water deficit conditions led to an increased CO_2 availability which had a direct positive effect on photosynthesis compared to the IR-control. Similar results have been reported by Soureshjani *et al*. (2018). Although the higher yielded progeny PIR5 had a better physiological response than the PIR-control no significant differences were observed.

Table 2. Mean chlorophyll content (SPAD 4 and 5) during early and late seed-filling stage at two intervals of 10 days, assimilation rate A (□mol CO_2 m^{-2} s^{-1}), stomatal conductance to water vapour g_s (mol of H_2O m^{-2} s^{-1}) and transpiration rate E (mol of H_2O m^{-2} s^{-1}) at ultra-low density under normal and deficit irrigation for the first generation lines originating from the three genotypes (GNTY, IR and PIR) and the control.

	Normal Irrigation					**Deficit Irrigation**				
First generation lines	**SPAD 4**	**SPAD 5**	**A**	**g_s**	**E**	**SPAD4**	**SPAD5**	**A**	**g_s**	**E**
GNTY1	39,22*	32,98*	13,78	0,360*	3,91	35,60	28,22	9,20	0,200	2,60
GNTY2	45,67	37,57	15,74	0,592	4,84	33,52*	25,35*	8,33	0,123*	2,16*
GNTY3	41,82	33,97*	14,92	0,482	4,35	35,87	26,92	7,47	0,138	2,26*
GNTY4	43,73	38,92	14,33	0,488	4,11	41,53	30,82	8,91	0,125*	2,38*
GNTY5	40,23*	34,47	16,85	0,513	5,62	38,98	23,93*	8,35	0,126*	2,19*
GNTY6	40,98*	31,43	14,22	0,460	5,04	39,35	26,78*	9,75	0,193	2,45
GNTY-Control	**44,68**	**37,40**	**16,07**	**0,462**	**4,92**	**39,48**	**31,70**	**10,49**	**0,218**	**3,34**
IR1	39,32	34,50	16,20*	0,432	4,76	38,85	31,58*	12,56*	0,230*	2,53
IR2	41,37	33,88	11,84	0,410	3,85	35,68	29,63	8,92	0,147	2,34
IR3	40,10	32,87	14,22	0,443	4,31	38,03	28,33	12,36*	0,188	2,81
IR4	43,12*	40,07	14,10	0,478	3,94	32,83	25,52	7,85	0,130	2,35
IR5	43,75*	37,50	19,36*	0,497	5,14	43,88*	30,93*	12,01	0,208	2,76
IR6	42,02	37,10	18,18*	0,525*	5,31	40,45	31,43*	12,81*	0,237*	3,29*
IR-Control	**39,15**	**32,43**	**11,59**	**0,390**	**4,36**	**36,87**	**26,02**	**8,90**	**0,138**	**2,33**
PIR1	45,10*	38,83*	18,99*	0,502	5,16	34,75	28,55	10,70	0,153	2,41
PIR2	42,57*	36,27*	13,88	0,478	4,14	36,47	29,18	8,18	0,230	3,10
PIR3	41,92*	34,63	15,22	0,480	4,27	32,15*	27,08	8,69	0,182	2,49
PIR4	40,70*	34,37	13,57	0,488	4,00	37,45	31,32	10,17	0,203	2,84
PIR5	44,18*	36,25*	15,10	0,392	4,26	42,82*	30,13	11,88	0,213	2,74
PIR6	40,70*	32,28	13,85	0,475	4,81	40,95	28,63	11,30	0,173	2,91
PIR-Control	**37,12**	**32,05**	**13,96**	**0,600**	**4,57**	**37,58**	**28,43**	**11,02**	**0,155**	**2,58**

*, ** Denotes significant superiority to the mother landrace (t test for independent means and different standard deviations at the levels $P<0.05$ and $P<0.01$ accordingly)

CONCLUSIONS

The results of this study demonstrate that there is intracultivar variation on seed yield under deficit irrigation during a thesis and seed filling stage within first generation sister lines. Also physiological traits were related to deficit irrigation tolerance which could assist in the identification of mechanisms underlying these adaptation processes and in the selection of improved genotypes of common bean. Further research is underway to confirm the results of the present study and to exploit further any existing variation.

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RISK ANALYSIS IN THE PEASANT FRAMEWORK: EMPIRICAL ANALYSIS OF FARMERS IN SOUTH KIVU, DEMOCRATIC REPUBLIC OF CONGO

SUMMARY

This study attempted to highlight the risks threatening the peasant farmers and affecting the development of their activities in the locality of Mbinga Sud in Kalehe Territory and the localities of Bugorhe and Irhambi in Kabare Territory in South Kivu, Eastern Democratic Republic of Congo. Data were collected using a questionnaire from a sample of 150 peasant farmers randomly selected and fairly distributed in the three locations of the study area. Descriptive statistics were used in data analysis. The results showed that financial risks were not among the significant threats to farm development in the short term. Nevertheless, farm operations were more exposed to risks in Mbinga Sud Locality than in Irhambi and Bugorhe Localities with respect to financial autonomy, access to short-term liquidity and availability of investment funds. The results also revealed that natural hazards (including flooding and erosion) were not currently among the threats of farming activities. For the whole study area, the respondents confirmed that the risks of flooding and erosion are weak, at the rates of 73.3% and 78% respectively.

On the other hand, low prices of agricultural commodities, climatic disturbances, crop diseases, crop theft and raptors were the major long-term farm threats in the study area as confirmed by 60.0%, 47.3%, 40.7%, 23.3% and 45.3% of the respondents, respectively. From these findings, we recommend that the strategies for sustainable environment management should be initiated; the proximity of extension services to farmers should be enhanced; and security should be permanently kept and guaranteed.

Keywords: Risks, peasant framework, farm activities, South Kivu, Democratic Republic of Congo.

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INTRODUCTION

Nearly 80% of the world's extremely poor people live in rural areas and rely heavily on agriculture for their survival (Farouque & Takeya, 2007). Stimulating the productivity of agricultural activities can be one of the most effective ways to combat poverty as the food system provides more jobs than any other sector in many countries (Banque mondiale, 2017; Laplante, 2014; Ferraton and Touzard, 2009). According to Chausse et al. (2012), rural income growth not only reduced rural poverty, but also urban poverty when the opposite was not true. Peasants are farm households, with access to a piece of land and utilizing mainly household labor in farm production. They are located in a larger dominant economic and political system that could affect their production behavior, but fundamentally they are characterized by partial engagement in markets, which are often imperfect or incomplete (Ellis, 1992).

A big number of people farming very little land under pressing natural conditions trapped their families in a life of poverty and what has been described as “agricultural involution”; that is, increasing intensity of labor use merely to maintain an unstable subsistence (Forrest-Zangh and Donaldson, 2010). The economic importance of peasants has been highlighted by the dimensions of the food crises of the 1970s and by the recognition that they are often cheap food suppliers and a source of cheap labor for agriculture and industrial development (Deere and de Janvry, 1979).

For most developing countries, the agricultural sector is considered a key sector for their economies and one of the solutions to improving the living conditions of more of the poor people who depend on them (Rutten and Boto, 2014; Imboden, 2014; Miller and Jones, 2013; Yumkella et al, 2012). Although many African economies depend on a few raw materials or semi-processed commodities, agricultural products account for a large share of their total export earnings (AfDB, 2017) and contribute to GDP growth. According to the 2008 World Bank report on agriculture for development (World Bank, 2008), GDP growth due to agriculture is at least twice as effective in poverty reduction as GDP growth in other sectors.

Family farming has many advantages: it is an effective lever for combating poverty and undernourishment and participates extensively in the enhancement and protection of natural resources and landscapes (Ferraton and Touzard, 2009; Laplante, 2014), and produces more than 70% of the world's food, provides food security for hundreds of millions of family farmers and millions of others, and is the largest employer of the family assets that make up the bulk of this workforce (Ministry of Agriculture-French Republic, 2013). Besides, small peasant farmers are facing a lower opportunity cost of labor than big commercial farms (Barrett, 1996).

Despite a range of solutions that they can provide, family farms face a variety of challenges, such as limited access to resources, lack of succession and poor or absent training (McGlynn et al, 2013), lack of appropriate markets (FAO, 2015), the threats that climate change poses to their livelihoods (FAO, 2016), the

difficulty of accessing credit because their financial performance is low and risky (Prévitali, 2015), etc. Beyond the constraints related to production and outlets, family farmers also face with wider difficulties, including poor health conditions, lack of social protection, low level of education, lack of infrastructure and public facilities (Laplante, 2014).

The agricultural sector of the Democratic Republic of Congo (DRC) is experiencing several problems such as the degradation of production and transport infrastructure, the dependence on imports of cheap food products, the low technical level, the lack of quality inputs (seeds, fertilizers, tools, ...), the lack of agricultural credit, the failure of the agricultural extension system, the priority given to the mining sector, etc. (Lebailly et al, 2015). The province of South Kivu, while having agricultural potential that would allow it to be self-sufficient in food and to be a net exporter, is characterized by a significant proportion of a rural population, mostly poor, poverty driven by a very high unemployment rate, a very worrying nutritional situation (Mastaki, 2006), food dependence on neighboring provinces and countries (Vwima et al, 2013), a virtual absence of financial institutions in rural areas, financial services of microfinance institutions (MFIs) and savings and credit cooperatives (SACCOs) geared towards income-generating activities requiring short-cycle financing (trade, etc.), lack of diversity of financial products for agriculture (such as agricultural insurance, commercial credit, etc.), lack of adequate rural infrastructure (agricultural feeder roads, conservation infrastructure, markets, distribution networks for agricultural products, etc.), adverse business climate for investors, the problem of information asymmetry in the agricultural sector which benefits intermediaries, the rural exodus that is of great concern especially among young people, the low profitability of agricultural activities and its very risky nature which does not encourage financial institutions to lend to farmers, etc.

It is worth to note that risk plays an important role in peasant agriculture. “Different choices by farmers do not depend on differences in their attitudes towards risk, but on the differences in their subsistence needs, resource endowments and perceptions of the riskiness among competing activities. Empirical models of decision-making under risk require knowledge of the risk preferences of decision-makers” (Shahabuddin, Mestelman, & Feeny, 1986).

Many studies, such as those by Lidsky et al (2017), Cordier et al (2008) and Couty (1989), have been conducted by analyzing agricultural risks in a dispersed manner with a greater focus on their management. In this study, we focus our attention on a diversity of risks threatening agricultural holdings in the territories of Kalehe and Kabare; two areas located in the east of the Democratic Republic of Congo, where populations have experienced dramatic situations of persistent insecurity and political instability. Thus, we have grouped risks into five categories, including financial risks, natural risks, occupational risks, price risks (Cordier et al, 2008) and other risks (crop theft, crop destruction by animals, insecurity) less present in risk analysis research. The seminal work of Chayanov

in the 1920s emphasized the influence of family size and structure on peasant economic behavior, through the subjective evaluation of labor within the household, in the absence of the labor market (Chayanov, 1966).

There have been drastic developments in the agrarian farming systems that affected significantly the rural transformation (Zangh and Donaldson, 2010), but the situation in the Democratic Republic of Congo seems to be the worst in the world. In the light of the whole issues of family farming here above discussed, solutions deserve to be found to enable the agricultural sector of South Kivu Province to answer certain essential questions of sustainable development.

MATERIAL AND METHODS

The data used are from the farmers'survey carried out in May –August 2018. The questionnaire used to collect data focused on socioeconomic characteristics of the farmer respondents and mainly on the main risks associated with the faming activities. A random sample of 150 peasant farmers randomly selected and distributed equitably in the localities of Bugorhe, Irhambi in Kabre territory and the locality of Mbinga-Sud in Kalehe territory were interviewed. To this end, the sampling technique by reasoned choice was used based on the selection criteria, including the market orientation of all or part of the production and the diversification of agricultural activities.

We used the farmers' experiences for risk analysis. A three-point Likert scale (High=3, Medium=2, and Low=1) was used to test the extent at which the farmers were facing different risks. Descriptive statistics (frequencies, percentages, and means) were used in the data analysis. The z test was performed under the software of Statistical Package for Social Sciences (SPSS) to show the differences or not in the proportions between the columns (localities); the result of the z test being represented by the letters a/b in index in the results tables.

RESULTS AND DISCUSSION

The table 1 describes the characteristics of the respondents. Men represent 70% of the farmers surveyed, while women represent only 30%. The most farmers (84.7%) were aged between 20 and 59 years, with an average age of 48 years. The majority of farmers attended secondary school (44.1%), others attended primary (39.6%) and university (6.7%), while the illiterate respondents represented 8.7%.

The results from the analysis of the level at which the peasant farmers were facing different risks are summarized in Table 2. The agricultural risks were categorized as financial risks (indebtedness, low access to investment funds, high interest rate, low level of financial autonomy, problem of illiquidity in short run), natural risks (climate change, plant diseases, floods, erosion, plant devastators), price risks (price fluctuations, low prices/non-remunerating prices), professional risks (occupational diseases, exposure to the harms of pesticides, occupational accidents), and miscellaneous risks (crops theft, crops destruction, insecurity).

Table 1. Characteristics of the respondents.

Characteristics	Attribute	Frequency	Percentage
Sex	Male	105	70.0
	Female	45	30.0
Age*	20 - 59	127	84.7
	More than 59	23	15.3
Education	No education	13	8.7
	Primary	59	39.3
	Secondary	66	44.1
	University	10	6.7
	Other	2	1.3

* The mean age of the respondents was 48 years.

The results showed that the financial risks for these farmers do not pose a significant threat to the development of their farms in the short term.Debt risk and high interest rate were not part of harmful risks given that the majority of farmers (80.7% and 73.3% respectively) reported they were less exposed to them,. This could be due to the lack of financial institutions specializing in granting agricultural loans; the existing institutions are more demanding and charge high interest rate, and consequently, fewer farmers have access to credit. This supports Olagunju and Adeyemo's (2007) report that the cost for access to loan is among the big problems of farmers and the finding by Lallau et al. (2018) who stated that donors have failed to integrate sustainable financing into their development assistance programs**.**

This also contrasts Prévitali's (2015) idea who showed that farm indebtedness is one of the factors in the modernization of agriculture, because it can make it possible to enlarge the cultivated land. Consequently, the modernization of agriculture in these areas will continue to face difficulties if access to finance does not improve through the restructuring of the financial sector in the Democratic Republic of the Congo. Other problems are the lack or low level of financial autonomy, lack or low access to short-term liquidity and the issue of availability of investment funds. These are among the significant risks facing the peasant farmers as reported by more than 35%, 38%, and 21.3%, respectively. The Mbinga Sud area in Kalehe Territory is more exposed than the other two areas in Kabare Territory. This situation is crucial and cannot enable the agriculture to be a sustainable source of livelihood for the farmers in the study area, as Prévitali (2015) pointed out that such financial problems do not make the farming profession peaceful. At the 0.05 threshold, the result of the z-test (letters a and b in index) shows that there is a significant difference in the proportions between the locality of Mbinga-Sud and the localities of Bugorhe and Irhambi with regard to financial autonomy, access to short-term liquidity and the availability of capital. If better measures are not taken, these farms are likely to be vulnerable in the medium and long term.

Table 2. Levels and frequencies of appreciation of the farmers towards the risks related to farm operations (N=150)

No	Agricultural risks	High		Medium		Low		Mean	Rank
		Frequ.	Percent.	Frequ.	Percent.	Frequ.	Percent.		
	Financial risks								
1	Indebtedness	10	6.7$_a$	121	80.7$_a$	19	12.7$_a$	1.94	5
2	Availability of capital	32	21.4$_b$	53	35.3$_a$	65	43.3$_b$	1.78	7
3	High interest rate	13	8.7$_a$	27	18.0$_a$	110	73.3$_a$	1.35	13
4	Financial autonomy	21	14.0	57	38.0$_a$	72	48.0$_a$	1.66	10
5	Short-term liquidity	19	12.7$_b$	56	37.3$_a$	75	50.0$_b$	1.63	11
	Natural risks								
6	Climate change	22	14.7$_a$	71	47.3$_a$	57	38.0$_a$	1.77	8
7	Plant diseases	21	14.0$_a$	61	40.7$_a$	68	45.3$_b$	1.69	9
8	Devastating agents	31	20.7$_a$	68	45.3$_a$	51	34.0$_a$	1.87	6
9	Flooding	8	5.3$_a$	32	21.3$_a$	110	73.3$_a$	1.32	14
10	Erosion	3	2.0$_a$	30	20.0$_b$	117	78.0$_a$	1.24	16
	Price risks								
11	Price fluctuations	55	36.7$_a$	66	44.0$_a$	29	19.3$_a$	2.17	2
12	Low prices	90	60.0$_a$	35	23.3$_b$	25	16.7$_a$	2.43	1
	Professional risks								
13	Occupational diseases	12	8.0$_a$	58	38.7$_a$	80	53.3$_a$	1.55	12
14	Exposure to the harms from pesticides	10	6.7$_a$	21	14.0$_b$	119	79.3$_b$	1.27	15
15	Occupational accidents	4	2.7$_a$	28	18.7$_a$	118	78.6$_b$	1.24	16
	Miscellaneous risks								
16	Crops theft	35	23.3$_a$	75	50.0$_b$	40	26.7$_b$	1.97	4
17	Crops destruction	43	28.7$_a$	64	42.6$_a$	43	28.7$_a$	2.00	3
18	Insecurity	5	3.3$_a$	30	20.0$_a$	115	76.7$_a$	1.27	15

Note: The indices "a" and "b" indicate the results of the Z test. "a" means that there is no significant difference between the three study areas (Bugorhe, Irhambi, and Mbinga-Sud) for the scale of the concerned risk, while "b" means that at least one area is significantly different from two others.

Flooding and erosion are not currently a threat to 73.3% and 78% respectively of farms in the three communities, although the average erosion risk in Mbinga South (14.7%) is higher than in the other communities included in the study (test z, represented by letters a and b in index). This is because there are several fields in the locality of Mbinga-Sud that are located on hills compared to the localities of Bugorghe and Irhambi. On the other hand, climatic disturbances, crop diseases and grazing livestock are threats to farms, considering their average and high level of appreciation. At the 0.05 threshold, the results of the z-test show that there is no significant difference in the proportions between the three localities with regard to climatic disturbances and raptors, whereas there are differences between the locality of Bugorhe and the other two localities with regard to the risk of crop diseases. Cochereau (1989) had already mentioned the impact of insect pests on crop loss by proposing alternative solutions to address them. But the specificity for these farmers in these localities is that they are simultaneously confronted with the problem of climate change, crop diseases and grazing livestock; the first problem having an influence on the latter two. Unfortunately, we have noticed the absence of the State in solving these problems. For example, farmers do not have access to weather forecast information (Ngomba Yashele and Nsombo Mosombo, 2017) to enable them to plan their activities (Eldin, 1989). Farmers reported that in 2018 their production decreased due to rainfall disruptions and the presence of caterpillars that attacked maize, especially in Irhambi and Bugorhe. One Bugorhe farmer reported: *"I produced 1.15 tonnes of maize in season B in 2018, while for season 2017 A, I produced 4.75 tons with the same factors of production"*.

The volatility of agricultural commodity prices is a major threat to farms. For 60% of farmers, this risk is high, while it is estimated at an average level for more than 23% of farmers. Cordier et al (2008) described price volatility risk as a systemic risk because it can affect several agents at the same time, and it is a predominant risk in agriculture. It is the imposition of selling prices by buyers that is most annoying to farmers in these three communities; a factor that has a significant influence on price stability. Lidsky et al (2017) add that climate change also increases price volatility because of its unpredictable effects on crop yields. Price regulation would therefore be a possible solution to mitigate the effects of this risk in these communities. Although there is no significant difference between the proportions of high risk (36.7%) and medium risk (44%) in relation to the risk associated with fluctuations in input prices, it is important to note that this risk is a strong long-term threat to farms.

Overall, there are not too many occupational risks associated with the job of farmer in the three localities, except what occupational diseases (average risk 38.7%) resulting from bad weather (rain, cold, heat) and heavy skilled work intense risks or physical constraints by Arnaudo et al. (2013). "A disease is recognized as professional if it is a direct consequence of a worker's exposure to a physical, chemical, or biological hazard" (Lienard et al, 1998). The lack of some protective equipment (boots, gloves, etc.) exposes farmers to bites by

snakes and other insects, but also to injuries due to improper handling of agricultural tools. At 0.05 the result of the z-test shows that the exposure to plant protection products is lower in the locality of Mbinga-South, as there are few farmers who practice crops requiring the prices of these products. This is not the case for farmers in developed countries who are more exposed to chemicals. In France, for example, 42.8% of farmers are exposed to at least one chemical (Arnaudo et al, 2013) because of the intensification and modernization of their agriculture. Studies on health problems associated with exposure to phytosanitary products (Jas, 2010) should be conducted in Irhambi and Bugorhe to define strategies for controlling this risk.

Farmers regard the destruction and theft of crops as a risk and a constraint for the development of their farms. For 50% of the farmers, crop theft, although considered of average level, poses a threat to their farms. It should be noted that 23.3% of the operators stated that this risk is high. Theft is much more frequent in Mbinga-Sud and Irhambi than in the locality of Bugorhe. For some farmers, unemployment is one of the causes of crop theft; for others, theft is favored by the presence of gunmen in some villages. Nearly 29% of farmers believe crop destruction is a high risk for their farms, while for nearly 43% of farmers this is a medium risk. Theft and destruction of crops therefore pose a threat to farms in these three study areas, regardless of their level of risk.

In summary, the results show that the five most important risks of peasant farmers in the study area are the low prices, the price fluctuations, the crops destruction, the crop theft; while five least important ones are occupational accidents, erosion, insecurity, exposure to the harms of pesticides, and flooding.

CONCLUSIONS

Farmers in South Kivu, and in particular those in Kalehe territory and Kabare, are working in an uncertain and unstable environment. This is explained by the problem of access to financial and institutional services, such as access to agricultural credit due to the lack of specialized financial institutions, the absence of agricultural insurance companies, the problem of access to information about weather forecasts, the lack of basic infrastructure (market, agricultural service road, storage facilities for products), etc. Faced with this situation, farms in these two territories are exposed to innumerable risks that threaten their development.

According to survey results, financial risks are not significant threats to agricultural development in the short term. Nevertheless, farms were more at risk in the locality of South Mbinga than in the localities of Irhambi and Bugorhe with regard to financial self-sufficiency, access to short-term liquidity and the availability of investment funds. Floods and erosion are not currently a threat to 73.3% and 78% of farms in these areas. On the other hand, climatic disturbances (47.3%), crop diseases (40.7%) and devastating agents (45.3%) are major long-term risks facing peasant farmers in the study area. The low price of agricultural commodities is a major threat to 60% of farms. Price regulation would be a possible solution to mitigate the effects of this risk. There are not too many

occupational risks associated with farming in these three areas. In addition, climatic disturbances, crop and plant diseases, crop rustling and crop pests were the main long-term agricultural threats in the study area, as confirmed by 47.3%, 40.7%, 23.3% and 45.3% of respondents, respectively. On the basis of these results, we recommend that sustainable environmental management strategies be initiated, that the proximity of agricultural extension services be strengthened and that security is maintained and guaranteed on an ongoing basis.

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FIRST REPORT OF LEEK YELLOW STRIPE VIRUS ON *ALLIUM SATIVUM* L. IN UKRAINE

SUMMARY

Garlic (*Allium sativum* L.) is a valuable crop, cultivated all over the world. Viral diseases of garlic are one of the factors that reduce quality of garlic yield. *Leek yellow stripe virus* belongs to genus *Potyvirus*, family *Potyviridae*, and garlic infected with LYSV is found in many places of garlic production. In 2018, we screened garlic-growing areas in different regions of Ukraine and collected plants with symptoms characteristic of viral infection: yellow striping and streaking, mosaic, spotting and plant growth retardation. DAS-ELISA with commercial diagnostic kit for LYSV (Adgia, USA) showed that 28% of collected plants were infected with LYSV. LYSV was detected in Vinnytsia region (Bershad district), Kyiv region (Boryspil district), and Poltava region (Semenivka district) in Ukraine, suggesting wide spread of the virus. According to available literature data, LYSV is often found in coinfection with OYDV. However, we found only 7% of plants to be mixed infected (LYSV+OYDV). Subsequent transmission electron microscopy revealed viral particles of 720-800 nm in length and 16 nm in diameter as characteristic for *Potyvirus* representatives. Further, LYSV infection was also confirmed by RT-PCR with coat protein gene-specific primers generating LYSV cDNA of expected length (~409 bp). This is the first report of LYSV-infected garlic plants in Ukraine that proves LYSV is widely spread in Ukraine.

Keywords: leek yellow stripe virus, onion yellow dwarf virus, garlic, potyvirus, Ukraine.

INTRODUCTION

Garlic stocks worldwide are infected with a complex of viruses, including two potyviruses: *Onion yellow dwarf virus* (OYDV) and *Leek yellow stripe virus* (LYSV). LYSV is a member of *Potyvirus* genus belonging to the largest *Potyviridae* family of plant viruses. LYSV has flexible filamentous particles ~800 nm long containing a single-stranded positive sense genomic RNA of about 10,000 nt (King et al., 2012).

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Contrary to many other potyviruses, LYSV has narrow natural host range mostly limited to *Allium* sp. plants and induces persistent symptoms (yellow leaf striping and mosaic are most common). LYSV is mainly transmitted by aphids non-persistently, mechanically from plant to plant, as well as with planting material. The last route of transmission is especially important for commercial garlic production.

LYSV probably occurs worldwide and has been found in both temperate and subtropical regions of Asia (including China and India) (Chen et al., 2002; Gupta et al., 2013), North Africa, Europe, Oceania, and North and South America (Pappu et al., 2005; Oleas, Arahana, 2015). In Europe, LYSV was reported from Sweden, Finland, Denmark, France, Belgium, the Netherlands, Germany, Italy, Greece, Slovenia, Croatia, Serbia and Poland (Lot et al., 1994; Dovas et al., 2001; Chodorska et al., 2014; Vončina et al., 2016; Vučurović et al., 2016; EPPO, 2019).

Ukraine is one of the largest European countries enjoying strategic position between the eastern EU states and Black Sea/Middle East region, where LYSV was also detected in Turkey (Fidan, Baloglu, 2009) and Iran (Shahraeen, Lesemann, Ghotbi, 2008). Recently, authors have reported on the occurrence and possible wide spread of OYDV in Ukraine (Snihur et al., 2019). In this study, authors included the results of LYSV screening in various regions.

MATERIAL AND METHODS

Sampling was restricted to private gardens in 4 distant parts of Ukraine: Kyiv, Cherkassy, Vinnytsia, and Poltava regions. Garlic, onion and leek plants were visually examined; samples were collected from plants with LYSV-like symptoms typically including striping, mosaics, leaf discoloration, and/or stunting.

Collected samples were tested for LYSV by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), as described previously by Clark and Adams (1977), using specific polyclonal antibodies purchased from Adgia (USA) and following the manufacturer's recommendations. 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 (Greiner Bio-One, Germany) were coated with LYSV-specific broad-spectrum polyclonal antibodies (1:100) 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 LYSV in the samples was detected in 200 µL homogenate by LYSV-specific antibodies conjugated to alkaline phosphatase using *p*-nitrophenyl phosphate substrate (Sigma, USA). Absorbance values at 405 nm were measured using a Thermo Labsystems Opsis MR microtitre plate reader (USA). Absorbance values, measured 60 min after adding the substrate, greater than three times those of the negative controls were considered positive.

For transmission electron microscopy (TEM), copper grids (Sigma, USA) were coated with chloroform-dissolved 0.2% polyvinyl formaldehyde (Serva, Germany) and dried overnight on filter paper at room temperature. The samples deposited onto grids were stained with 2.5% uranyl acetate and 0.02 N lead citrate (Serva, Germany), and examined using JEM 1400 (JEOL, Japan) transmission electron microscope. The samples were photographed at a magnification of 5,000-60,000x (Mendgen, 1991).

Total RNA was extracted from naturally infected LYSV-positive plant samples using RNeasy Plant Mini kit (Qiagen, UK). The two-step reverse transcription reaction (RT-PCR) was accomplished using pairs of specific primers (1-LYSVF: 5'-ACA AGT AAG AAA CAG AAG GAC AGC-3', 2-LYSVR: 5'-GAG GTT CCA TTT TCA ATG CAC CAC-3') complementary to the part of the *CP* gene of LYSV producing the amplicon with expected size of 409 bp (Parrano et al., 2012). Total RNA extraction and PCR amplification were assessed by electrophoresis in a 1.5% agarose gel in TBE buffer (89 mM TRIS borate and 2 mM EDTA, pH 8.3) stained with ethidium bromide using HyperLadder 100 bp markers (Bioline, Germany).

RESULTS AND DISCUSSION

During the last several years, large-scale production of garlic led to wide occurrence of virus-like symptoms on *A. sativum* plants in Ukraine. In 2018, we collected garlic plants exhibiting yellowing, leaf mosaic, stunting and wilting. Transmission electron microscopy of plant sap confirmed the presence of filamentous virions (720-820 x 16 nm) typical of *Potyvirus* genus.

Using ELISA, OYDV was identified for the first time in Ukraine (Snihur et al., 2019). However, some symptomatic and TEM-positive samples were confirmed as OYDV-negative suggesting possible plant (co)infection with a different potyvirus - *Leek yellow stripe virus* (LYSV).

In 2018/2019 yy, plant samples (including garlic, onion and leek) with LYSV-like mosaic and striping, leaf discoloration, and/or stunting symptoms were collected in different regions of Ukraine (Kyiv, Cherkassy, Vinnytsia, and Poltava regions). Sampling areas mostly included private gardens with some samples collected from commercial garlic fields.

On garlic plants, LYSV typically induced yellow striping, discoloration, spotting and plant growth reduction (Fig.1A). Plants co-infected with LYSV and OYDV typically demonstrated more severe visual symptoms of the disease (Fig.1B).

Using DAS-ELISA with virus-specific antibodies, LYSV was found in garlic plants but not in onion or leek. On average, 28% of collected plants were infected with LYSV. LYSV was detected in 3/4 screened regions of the country: Vinnytsia (Bershad district), Kyiv (Boryspil district), and Poltava (Semenivka district), suggesting wide spread of the virus in Ukraine (Table 1). According to available literature data, LYSV is often found in coinfection with OYDV. However, we found only 7% of plants to be mixed infected (LYSV+OYDV).

Mixed infection of garlic with LYSV and OYDV was detected in all three regions where LYSV was found.

A B

Table 1. Double-antibody enzyme-linked immunosorbent assay for the detection of *Onion yellow dwarf virus* and *Leek yellow stripe virus* by region in Ukraine (2018/2019 yy)

Region	**Virus-positive samples (from total number of samples)**	
	OYDV	**LYSV**
Vinnytsia	13 (18)	7 (18)
Kyiv	11 (18)	4 (18)
Poltava	5 (9)	3 (9)
Cherkassy	3 (5)	0 (5)
TOTAL	**32 (50)**	**14 (50)**
Incidence of infection (%)	**64%**	**28%**

TEM of OYDV-negative and LYSV-positive plant samples showed flexuous viral particles of ~800 nm in length and ~16 nm in diameter typical for potyviruses (Fig.2) which was in line with ELISA data.

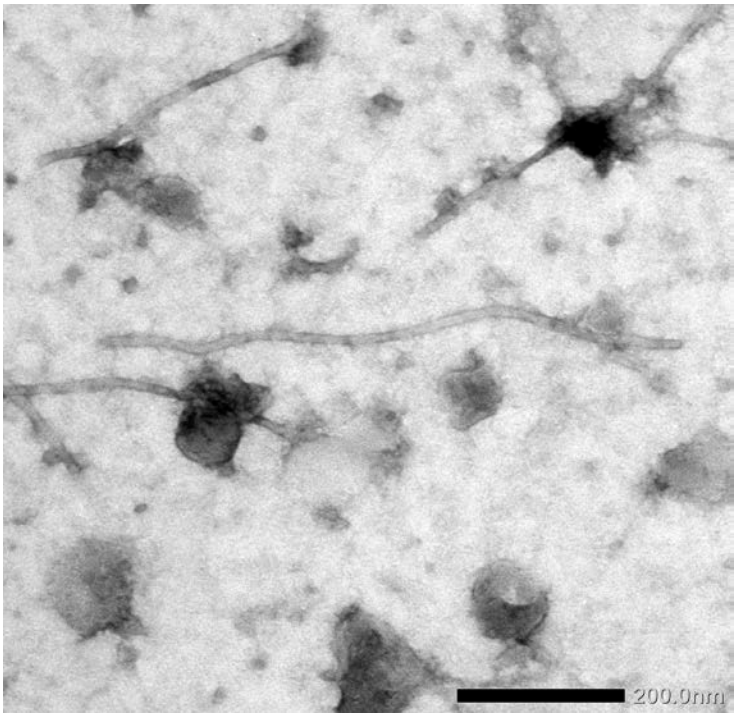

Figure 2. Transmission electron microscopy showing numerous potyvirus particles in partly purified sap of LYSV-positive garlic plant sample. Scale bar corresponds to 200 nm

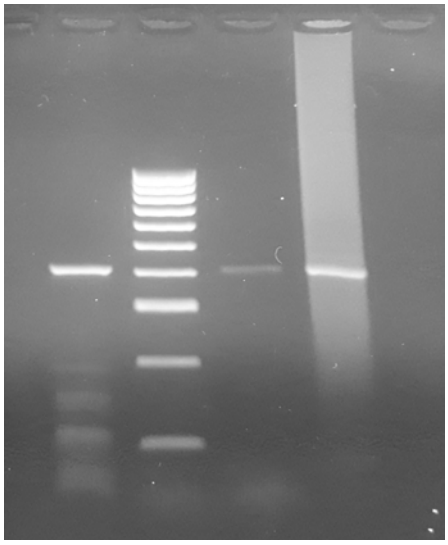

Figure 3. Amplicons of LYSV isolates corresponding to the part of *CP* gene (409 bp expected size): 1, 3, 4 – isolates from Vinnytsia, Kyiv and Poltava regions, respectively; 2 – MW markers (HyperLadder 100 bp)

Using LYSV coat protein-specific primers (Parrano et al., 2012), partial nucleotide sequence of three LYSV isolates was amplified and visualized by electrophoresis in a 1.5% agarose gel (Fig.3).

From Fig.3 it follows that all three amplified cDNAs of LYSV isolates collected from different parts of Ukraine have identical and expected size. These will be further used for phylogenetic studies of LYSV population in Ukraine.

Rather high rate of LYSV infection in private gardens (28% of symptomatic plants) may be explained by using contaminated planting material aided by flying vectors and mechanical transmission. In the view of official 'absence' of LYSV in neighboring countries (except Poland), we hypothesize that LYSV-infected seed garlic might have been the initial source of this virus in Ukraine. As we have shown before, one of the Ukrainian OYDV isolates (Accession number MK177281) was most phylogenetically related to Chinese OYDV isolates Yuhang (AJ510223) and YH1 (AJ292231) (Snihur et al., 2019), suggesting China as a potential origin of (at least) one of the OYDV isolates found in Ukraine. LYSV occurrence may follow the same pattern, as a significant part of (seed) garlic was (and is) imported to Ukraine from China.

Obtained results clearly demonstrate the importance of routine control of both imported planting/seed material and cultivated crops on a regular basis which remain highly efficient measures in preventing the spread of the mechanically and aphid-transmitted virus and reducing consequential damages.

CONCLUSIONS

Leek yellow stripe virus (LYSV) is one of the potyviruses infecting garlic in many countries including southern/eastern EU states (Vončina et al., 2016; Vučurović et al., 2017), but was never reported from Ukraine. Using double-antibody sandwich enzyme-linked immunosorbent assay with virus-specific antibodies, LYSV was detected in 28% of samples from 3/4 screened regions of Ukraine, similarly to previously described OYDV in Ukraine (Snihur et al., 2019). These findings were also supported by two other independent techniques (TEM and RT-PCR). Obtained data suggest significant spread of LYSV in Ukraine and raises questions of proper control of the quality of imported planting/seed material..

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THE SUPPRESSION OF POTATO PATHOGENS DEVELOPMENT DURING STORAGE PERIOD AS INFLUENCED BY AIR MEDIUM, CONTACTING WITH NATURAL POTASSIUM SALTS

SUMMARY

One of the urgent problems of agricultural production is preservation of the grown yield, minimizing storage losses, which can reach 30-50 percent. The ways to solve this problem are observed in given article. The analysis of existing methods of products storage based on modern technologies is fulfilled. Using of unique properties of natural potassium salts is admitted to be one of promising technologies for potato and root crops storage. The research program has been launched in Perm Federal Research Center of UB RAS to develop new technologies for storage and fertilizing seed potato based on the properties of natural potassium salts and waste products from K-Mg ores.

Natural potassium-magnesium salts have a number of specific properties affecting the air medium. One of these properties is the ability to produce light air ions due to air molecules contact with potassium and sodium cations. Such environment has the ability to inhibit the growth of many microorganisms' types, including pathogens, which is used in world practice to treat some chronic human diseases (speleotherapy). Preliminary studies conducted in used drifts of K-Mg ores deposits showed the possibility of storing potato and vegetables in underground storages. The next task is to simulate the conditions of underground drifts in typical potato storages. The most promising variations are lining the storage facilities with sylvinite tiles and aerosol treatment as a result of passing an air stream through a layer of sylvinite and the subsequent infiltration of the aerosol through the potato mass. The identification of potato pathogens in samples from the harvest of 2016 and 2017 was carried out after the main storage period. The most common diseases were fusarium, phomosis, as well as bacterial wet rot, late blight presence was insignificant. The number of infected tubers was 10.2 percent of the studied quantity.

The primary modeling of storage conditions under the influence of air ions in a special climatic chamber was carried out. The influence of saline saturated

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atmosphere on the growth of phytopathogens isolated cultures with growth inhibition assessment was determined. For this purpose phytopathogens cultures planted on potato agar bouillon were placed in sealed chambers with volume 5.28 liters. According to the results of fulfilled studies, the number of microorganisms colonies decreased by 13-63 percent compared with the control.

A system of equations has been developed that can be used to determine the optimal concentration of potassium aerosol in potato storages to achieve the best preservation of products. After fulfillment of research work in typical storages equipped with plenum ventilation system, a technology for potato storage, based on the creation of favorable air environment saturated with light negative air ions and saline aerosol, will be developed.

Keywords: potato storage, K-Mg ores, potassium salts, air ions, saline aerosol, potato pathogens

INTRODUCTION

Russia has the huge potential of agricultural products cultivation. In this regard the question of safety of the grown-up harvest, loss minimization at storage which can reach up to 30-50 percent is very relevant (Olsen, 2014,Makarova et.al., 2017).

Currently, there are known methods of storing crops in clamps, containers, bins, in storages with natural ventilation or equipped with a ventilation system, systems for maintaining and monitoring temperature and humidity (Kopylov et.al., 2012, Ponomarev, 2014) These methods do not provide high safety products laid down for long-term storage. In some Russian and foreign works, various methods have been proposed and observed for potato tubers influence in order to increase their preservation: magnetic field, electric current, and ionization (Lu,1986, Brynjolfsson,1989, Rezaee et.al., 2013 Nikitenko et.al. 2016). The researches, as a rule, applied γ-rays as ionogen and the range of absorbed radiation rates from 0.1 to 2.0 kGy. The literature sources also present a number of studies on improving potato preservation through low-temperature storage and the use of germination inhibitors (Macqueen, 1985, Cools et. al., 2014, Foukaraki et.al., 2016). According to (Douglas et. al., 2018, Kaznak, 2018) treatment with such inhibitors causes undesirable side effects, in particular the accumulation of residual chemicals. The use of biological preparations prepared from plants, for example, peppermint, avoids these undesirable effects and prevents the premature potato germination, but appeared to be ineffective method of inhibiting the potato pathogens development during storage (Sanli, 2019). Deep cooling application for potato storing at ultralow temperatures is often associated with significant economic costs and an increased risk of yield damage from hypothermia (Chourasia, 2001).

The use of bactericidal properties of the air contacting with natural potassium salts is considered to be the promising direction in potato storage. One of the largest deposits of potassium, magnesium and sodium salts, located in Russia - Verhnekamskoe, contains a third of the world's reserves, which are

mainly used for the potassium fertilizers production. However, the unique physical and chemical properties of natural potassium, sodium and magnesium salts, as well as their mechanical and filtration characteristics, the presence of huge worked out spaces (drifts) supported for a long time by hard pillars, can significantly expand the use of both natural salts and man-made underground cavities formed during mining. Natural potassium-magnesium salts have a number of specific properties affecting the air medium. One of these properties is the ability to produce light air ions due to exposure to air molecules with potassium and sodium cations. Such environment has the ability to inhibit the growth of many types of microorganisms, including pathogens, which is used in some cases in world practice to treat a number of chronic human diseases (speleotherapy) and produce rare foods (Faynburg et.al., 2008, Krasnoshtein et.al., 2008) . This feature is mainly due to the presence in the salts of the potassium isotope K-40, possessing radioactivity (beta and gamma radiation). Ionizing radiation, interacting with air molecules, "knocks out" electrons from them. A positive charge remains on the molecule, while the electron is captured by the electron-acceptor molecule and charges it negatively.

This process leads to the formation of a large number of light negative and positive air ions. Herewith, the radiation influence of natural potassium to humans does not exceed the corresponding allowable limits regulated by national Sanitary Rules (SR 2.6.1.758-99) which is due to the fact that potassium isotope content in the total mass of potassium is about 0.012 percent. Distinctive features of potassium salts thermodynamic properties are their high heat capacity and low thermal conductivity, which lead to rapid stabilization of microclimatic parameters of the atmosphere in closed space.

Thus, potassium salts have a huge potential for expanding their application. One of these options is the formation in the vegetable storages of air medium with bactericidal properties. In the 1990s, a series of experiments were conducted at the Mining Institute of the Ural Branch of the Russian Academy of Sciences, which later became part of the Perm Federal Research Center, for storing potato in the used drifts of potassium mines (Krasnoshtein et.al.,1997). Positive results were obtained, but the mechanisms of action were not studied in details. It is assumed that the main factors that positively affect the storage of potato are: an increased concentration of light aero ions and the presence in the atmosphere of a salt aerosol with bactericidal properties.

In this regard, the purpose of this research is to study the impact of these factors on the microclimate of the storage, the ability to inhibit the development of the main pathogens during storage of potato, the preservation of potato tubers during storage, and the possibility of calculated determination of the quantitative parameters of these factors.

MATERIAL AND METHODS

The equation of charged particles kinetics was used to calculate the concentration of aeroions in the air atmosphere, taking into account beta radiation

levels in potassium mines (Boyarchuk, 1999). The initial phytopathogens determination in the infected material was carried out according to morphological characteristics and microscopy observation data. Phytopathogen cultures were isolated by direct seeding on selective media from potato tubers with characteristic signs of diseases - late blight, phomosis, wet rot, etc. To isolate pathogens, wet rot (*Pseudomonas solanacearum*, *Corynebacterium sepedonicum* and others), fusarium infection (*Fusarium* spp.), phomosis (*Phoma exiqua*, var. *Foveata*) rhizoctoniosis (*Rhizoctonia solani*) and common scab (*Streptomyces* spp) were used two main media - potato agar and Chapek's medium.

Species identification of bacterial isolates was carried out by polyphase taxonomy methods in accordance with the Bergey species guide and the Bergey's Manual (Whitman et.al., 2012). The exact identification of bacteria - phytopathogens was carried out by DNA diagnostic methods: using Polymerase chain reaction (PCR) analysis of genes and subsequent sequencing of 16S rDNA genes.

For PCR analysis of 16S RNA genes of bacterial potato plant pathogens and gene sequencing, primers 27F AGAGTTTGATCCTGGCTCAG and 1391R GACGGGCGGTGWGTRCA were used. To identify phytopathogenic fungi, morphological characters were determined, including features of conidiogenesis. To detect the studied fungi and streptomycetes — phytopathogens in the mixed material, the well-known PCR systems were used (Hussain et.al.,2014, A'Hara, 2015, Xu, 2016, Khan et.al.,2017, AlHusnain, 2019).

For study the influence of salt saturated atmosphere on the growth of isolated cultures of phytopathogens and assessment of growth repression degree, phytopathogenic cultures planted on potato agar were placed in airtight chambers with linear dimensions of 200x240x110 mm (V=5.28 liters). A salt-saturated atmosphere was created in the chamber by blowing air through a column filter with dimensions of 1 = 200 mm, d = 24 mm, filled with sieved fractionated salt with a particle size in the range of 0.5-1 mm.

The distinctive feature of potassium salt, causing the interest in its use for the storage of agricultural products, is the presence in it of the natural radioactive isotope potassium-40 with β-radioactivity. Abundance ratio of potassium-40 in nature is 0.0117 percent, and the activity of one gram of isotopically pure potassium-40 is 2.65·105 Bq. Under the influence of β-radiation, the air contacting with potassium salt is ionized.

According to (Krasnoshtein et.al.,2008), β-radiation levels in potasium mines are: $4 \cdot 10^{-3}$ part/m^2·min - in rock salt used drifts; $2.8 \cdot 10^{-3}$- $3.5 \cdot 10^{-3}$ part / m^2 ·min - in sylvinite used drifts. The main reason for beta- radiation of potassium salt is the radioactivity of one of the potassium isotopes - potassium-40. Using the equation of charged particles kinetics it is possible to calculate the concentration of aero ions in the air atmosphere, taking into account the levels of β-radiation in potash mines.

Besides the generation of aeroions from the surface of salt particles, there are processes of attachment, recombination, detachment of electrons, destruction

of negative aeroions and interactions with neutral oxygen and nitrogen molecules, which are not taken into account in this analysis, since it is assumed that the lifetime of aeroions is longer than the time period during which an elementary volume of air will interact with the stored agricultural products and leave the storage. According to the data of (Kowalski, 2000), the average life span of light aeroions in a natural setting ranges from 46 to 60 s. The decrease in the concentration of aeroions in the framework of this model occurs only due to transport along with the main air flow.

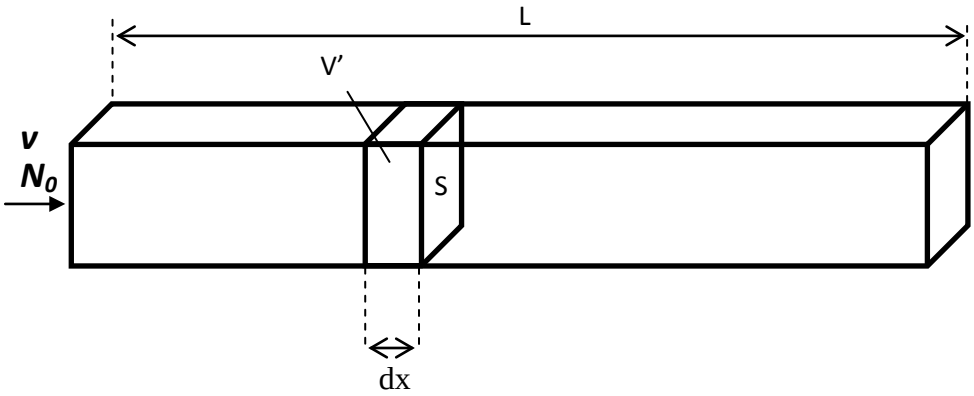

Figure 1. The model of agricultural product storage

For a storage of length L (Fig. 1), the average concentration of aeroions in the entire storage is calculated according the equation:

$$\widetilde{N} = \widetilde{N}_0 + \frac{3\rho C q_n}{v R_p}\frac{L}{2}$$

where:
$\tilde{N}$ - the average concentration of aeroions in the specific storage volume V,
$\tilde{N}_0$ - concentration of aeroions $\tilde{n}$ at the entrance to the storage;
C - concentration of salt dust in the air volume (kg / m^3);
ρ - density of the particle, kg / m^3;
q_n– component of the air ion flux vector normal to the surface of the dust particle, (β-radiation level),
part/ $м^2$ min;
R_p - the radius of the dust particle, m;
v - constant average speed of the air stream (m/min)

The maximum concentration value $\tilde{\tilde{N}}$ is reached at the end of the air path.

The influence of negative air ions on the content of microorganisms in the atmosphere is modeled by the equation:

$$\frac{\widetilde{N}_b(t)}{\widetilde{N}_b^{(0)}} = \exp\left(-Z \cdot E_{eff} \cdot t\right),$$

where: $\tilde{N}_b(t)$ – average concentration of microorganisms in storage at time t;
$\tilde{N}_b^{(0)}$- initial average concentration of microorganisms in the storage;
Z - susceptibility of bacteria to radiation, m^2/J.
E_{eff} - average beta radiation intensity, W/m^2;

The constant Z is the indicator showing the sensibility of a specific microorganism to radiation influence and, therefore, is a very important parameter in predicting the concentration of microorganisms in any storage. The value of Z should be determined experimentally for each particular type of microorganism and type of radiation (Kowalski, 2000, Peccia et.al., 2000). The constant E_{eff} depends on the concentration of aero ions in the atmosphere of the storage, on the energy of a single electron Q_e during β – decomposition of potassium-40, and on the average air velocity v.

$$E_{eff} = c_1 \cdot Q_e \cdot \widetilde{N} \cdot v.$$

The given system of equations allows calculating the dynamics of the average concentration of microorganisms in the storage over time at various concentrations of potassium aerosol (Nakaryakov, 2017, Shalimov et. al., 2018). However, before proceeding to the design of such storage facilities, it is necessary to fulfill a number of laboratory experiments that confirm in practice the possibility of exposure to pathogenic bacteria by air medium contacting with potassium salts. The results of one of these experiments are presented in this paper.

RESULTS AND DISCUSSION

To simulate such a process, isolates of phytopathogenic microorganisms were isolated by direct seeding on selective media from potato tubers with signs of characteristic diseases - late blight, phomosis, wet rot, etc. Samples of infected potato tubers of the 2016 harvest were taken from the farm “Truzhenik Ltd” (Perm Region). According to the analysis of potato phytopathogens distribution in the 2017 yield, the number of infected tubers was 10.2 percent of the studied quantity. The total number of tubers in the combined sample was 8000 sp, including: dry rot *(Fusarium* spp) 5.12 percent; wet rot (*Pseudomonas solanacearum* and others) 3.15 percent; dry rot (*Phoma exiqua*) 1.86 percent; ring rotation (*Clavibacter michiganensis* ssp. *sepedonicus*) 0.05 percent; late blight (*Phytophthora infestans*) 0.02 percent; common scab (*Streptomyces* spp) 0.0 percent 2; other 0.01 percent.

Thus, the prevailing diseases of potato tubers of the 2017 harvest were fungal infections - fusarium and fomosis, as well as bacterial wet rot. Late blight was observed in a less proportion, usually the spread of this infection reaches high values in wet seasons.

To isolate cultures of model phytopathogens, three samples of tubers with signs of late blight, wet bacterial rot, and brown rot were selected. Phytopathogenic cultures identified as representatives of the species *Phytophthora infestans* and *Pectobacterium carotovorum* were isolated.

The primary modeling of atmospheric parameters and storage conditions in a special climatic chamber was carried out. The influence of salt saturated atmosphere on the growth of isolated cultures of phytopathogens and assessment of growth repression degree were determined. For this purpose, phytopathogenic cultures planted on potato agar were placed in airtight chambers. A saturated atmosphere was created in the chamber by blowing air through a column filter. The growth of cultures was studied on Petri dishes with salt in three versions: 1) the air flow volume through the filter with salt is 1.0 litre / min; 2) the air flow volume through the filter with salt of 0.1 litre/ min; 3) sealed chamber without ventilation (with salt in open Petri dish).

The effect of air humidity in sealed chamber on the stability of pathogens has been studied. The experiments were carried out at three relative humidity values: 16, 50, 85 percent in a salt saturated medium and in an air medium passed through a sterilizing membrane filter. The growth repression of bacteria and fungi was evaluated by the number of colonies seeded on Petri dishes with potato agar with a dilution 10^{-6} (Table 1).

Table 1. The number of colony forming units (CFU) of potato pathogen cultures *Pectobacterium carotovorum* and Phytophthora infestans cultured in a salt saturated atmosphere

	Pectobacterium carotovorum			*Phytophthora infestans*		
Humidity %	Atmosphere without salt CFU/ dish	Salt saturated, atmosphere CFU/ dish	Growth repression, %	Atmosphere without salt CFU/ dish	Salt saturated, atmosphere CFU/ dish	Growth repression, %
16	255,0±6,4	194,7±4,8	23,7	187,3±4,7	113,3±4,6	39,5
50	326,0±10,6	208,0±7,1	36,2	308,7±7,3	159,7±4,8	48,3
85	383,7,0±12,1	212,7±5,4	44,6	426,0±16,5	212,7±5,4	63,1

The development of bacterial cultures of *Pectobacterium carotovorum* was maximum at a humidity of 85 percent. At the same time the saturation of medium with salt led to the suppression the growth of the phytopathogen culture by 23.7 percent at a humidity of 16 percent, by 36.2 percent with humidity of 50 percent and by 44.6 percent with humidity 85 percent. The absolute number of colonies was minimal in salt saturated medium with 16 percent humidity. The study of the influence of salt saturated atmosphere on *Phytophthora infestans* showed even greater dependence on the humidity of the environment and a greater degree of growth repression in atmosphere saturated with salt air ions. The growth inhibition of this phytopathogen was noted as 39.5 percent at 16 percent moisture

content, 48.3 percent at 50 percent humidity and 63.1 percent at 85 percent humidity.

The growth repression of phytopathogens was enhanced by 3-5 percent under the influence of convection, with little advantage of treatment with the air flow volume 1.0 litre/min but the difference between treatments was insignificant (Table 2).

Table 2. The effect of convection on the number of colony forming units of *Pectobacterium carotovorum* cultures when cultured in a saturated atmosphere

lum °	Atmosphere without salt CFU/ dish	Salt saturated, atmosphere CFU/ dish			Growth repression, %		
		No convection	Convection 0.1 litre / min	Convection 1.0 litre / min	No convect.	Convection 0.1 litre / min	Convection 1.0 litre / min
16	255,0	194,7±4,8	187,3±2,2	182,3±2,9	23,7	26,5	28,9
50	326,0	208±6,0	197,7±3,5	192,7±4,7	36,2	39,6	40,9
85	383,7	212,7±5,4	198,7±3,7	194,3±5,2	44,6	48,2	49,3

It was also discovered that with rise the air temperature from 10 to 20, and further to 30 °C, the influence of salt saturated atmosphere increases proportionally (Table 3).

Table 3. Growth repression of *Pectobacterium carotovorum* culture in a saturated atmosphere at different temperatures in version with air flow volume 0.1 litre/min, CFU / dish

Humidity, %	Atmosphere without salt CFU/ dish	The quantity of colony forming units, CFU/ dish			Growth repression, %		
		10 ºC	20 ºC	30 ºC	10 ºC	20 ºC	30 ºC
16	255,0	192,3±5,9	187,3±2,2	116,3±4,4	24,6	26,5	54,4
50	326,0	204±4,6	197,7±3,5	120,7±6,2	37,4	39,6	63,0

Such increase in the repression effect with temperature rise can be associated both with an increase in the permeability of the cell membrane for aeroions and with greater sensitivity of cells in conditions of more intensive metabolic processes. However, the practical application of temperature dependence studies should be coordinated with the physiological norms of storage temperatures for potato tubers in storage facilities, preventing their germination and moisture loss. It is well known fact, that the optimal temperatures for the preservation of the vendible and physiological value of healthy potatoes are +15 °C or less, but not lower than + 1 °C. In all treatments, the salt saturated atmosphere exerted suppression effect on phytopathogens (Fig. 2), that may be explained both by cytostatic and cytotoxic factors.

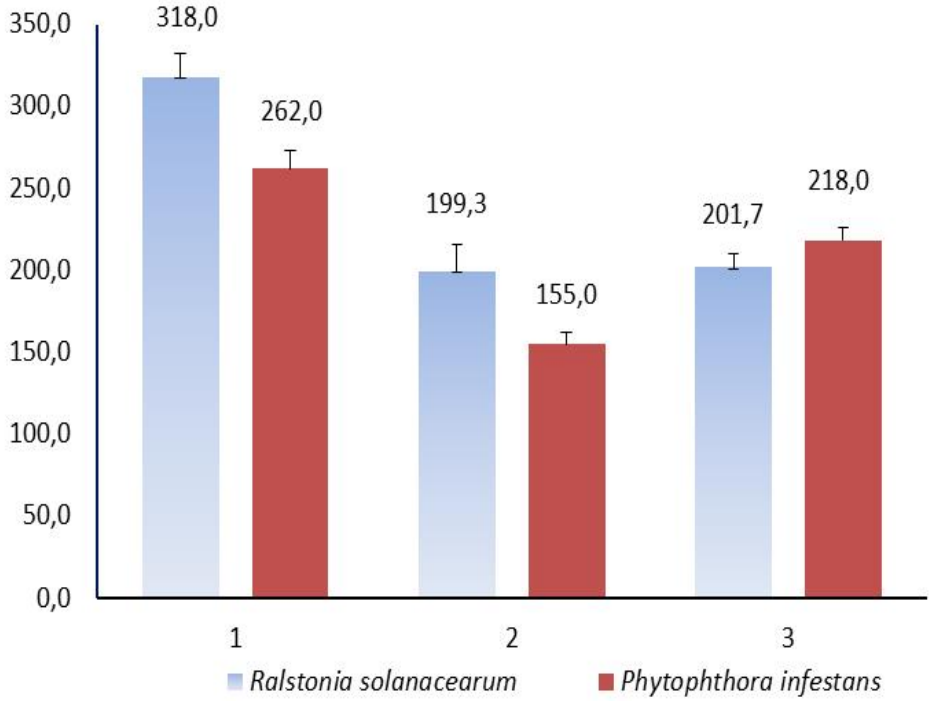

Figure 2. The number of colony forming units (CFU / cup) cultivated in a salt saturated atmosphere and growing in an atmosphere without salt.

Treatments: 1 - control, without salt; 2 - saline atmosphere; 3 - cups transferred to the atmosphere without salt after 10 hours of incubation in salt saturated atmosphere.

The usage of bacterial culture as a model phytopathogen, a cytotoxic (bactericidal) effect prevailed. Using a mushroom culture, both cytotoxic and cytostatic effects were observed. In samples removed to normal atmosphere after 10 hours of exposure in a saturated atmosphere, the number of colonies increased by 24 percent.

The effect of changes in humidity, convection, and the temperature of the air medium contacting with salts on the development and transmission rate of infections is studied. In this study 200 µl of suspension of *Pectobacterium carotovorum* or *Phytophthora infestans* with a cell density / CFU of 10^{-6} were applied and evenly distributed on the surface of potato tubers cuts treated with 70 percent ethanol.

It was shown that in salt saturated atmosphere, the effectiveness of experimental infection by transferring suspensions of *Pectobacterium carotovorum* and *Phytophthora infestans* to a potato cut reduced from 14-22 to 2-7 percent and from 16-29 to 6-11 percent, respectively.

CONCLUSIONS

The calculation results, confirmed by preliminary experiments, provide the information about the influence of the air medium contacting with potassium salts on the growth and development of major fungal and bacterial infections that accompanying potato storage process.

It was shown that storage of potato tubers in the air medium contacting with potassium salts and saturated with light negative aeroions and salt aerosol promotes growth repression of main phytopathogens and better preservation of potato during storage period, the number of microorganisms colonies decreased by 13-63 percent compared with the control.

The obtained data can be used to develop new methods for storing potatoes, root crops and vegetables, to design a new type of vegetable storages equipped with systems for maintaining optimal microclimatic parameters of the air medium, including fungi and bactericidal properties. These systems will allow to reduce significantly product loss during storage period..

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***Gordan S. KARAMAN*[1]**

THE SUBTERRANEAN SPECIES *NIPHARGUS ZAGREBENIS* S. KAR. 1950 (FAM. NIPHARGIDAE) ON BALKAN (CONTRIBUTION TO THE KNOWLEDGE OF THE AMPHIPODA 312)

SUMMARY

The subterranean species *Niphargus zagrebensis* S. Karaman, 1950 (Crustacea: Amphipoda, fam. Niphargidae) is redescribed and figured based on typical material from Zagreb, and certain variability of single taxonomical characters is given. New localities of this species are mentioned and geographic map with all known localities of *N. zagrebensis*is presented. The relation of this species regarding other similar species (*N. valachicus* Dobreanu & Manolache, 1933*; N. hrabei* S. Karaman; 1932*; N. elegans* Garbini, 1894) is discussed.

Keywords: Amphipoda, *Niphargus zagrebensis*, taxonomy, distribution, subterranean, Balkan peninsula.

INTRODUCTION

The subterranean fauna of Amphipoda on Balkan peninsula (including Slovenia) is still only partially investigated, especially family Niphargidae presented by 9 genera: *Carinurella* Sket, 1971, *Chaetoniphargus* G. Karaman & Sket, 2019, *Exniphargus* G. Karaman, 2016, *Karamaniella* Sket, 1962; *Niphargobates* Sket, 1981, *Niphargobatoides* G. Karaman, 2016; *Niphargopsis* Chevreux, 1922; *Niphargus* Schiödte, 1849; *Pontoniphargus* Dancau, 1970 (see G. Karaman & Sket, 2019).

Among them, only genus *Niphargus* is presented on Balkan by numerous species and subspecies (over 100), described during last 172 years. During so long period, the methods and descriptions with figures of species have been changed drastically. As genus *Niphargus* contains so many taxa, it is necessary even more and more detailed description and figures of each taxon. Numerous taxa, described many years ago, require more detailed redescription and additional figures, to confirm taxonomical identity of species themselves.

We redescribed *Niphargus zagrebensis* S. Karaman, 1950, species considered for a long time as a subspecies of *Niphargus valachicus* Dobreanu & Manolache, 1933, to support morphologically the species status of *Niphargus*

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Notes: The author declare that they have no conflicts of interest. Authorship Form signed online.

zagrebensis, remarkably distinct also from other two similar species, *N. hrabei* S. Kar., 1932 and *N. elegans* Garb., 1894.

MATERIAL AND METHODS

The samples of *Niphargus* were preserved in 70% ethanol. The specimens were examined and dissected in the mixture of glycerin and water, using a Wild M 20 stereomicroscope. Later, dissected specimens were transferred onto slides with Faure liquid used for final preservation of animal. The body length of examined specimens was measured by tracing individual's midtrunk lengths (tip of the rostrum to end of the telson) and drawings were made using a camera lucida attachment and manually inked.

Some morphological terminology and setae formulae follow G. Karaman's terminology (Karaman, G., 1969; 2012) for palpus article 3 of mandible [A-setae= setae on outer face of article; B-setae= setae on inner face of article; D-setae= short setae along lateral margin of article; E-setae= long setae at distal part of article], as well as for distal corner of propodus of gnathopods 1 and 2 [S-spine= corner palmar spine on outer face; L-spines= slender serrate spines sitting near S-spine on outer face; M-setae= row of long facial corner submarginal setae on outer face; R-spine= subcorner spine on inner face].

Term "setae" and "spines" are used based on its shape, not origin. This study is based on the morphological, ecological and zoogeographical data.

TAXONOMICAL PART

Family NIPHARGIDAE

NIPHARGUS ZAGREBENSIS S. KARAMAN, 1950

Figures 1-9

Niphargus (*Supraniphargus*) *valachicus zagrebensis* S. Karaman, 1950: 61, figs. 23-34; S. Karaman, 1954: 176; Sket, 1958: 67;

Niphargus (*Phaenogammarus*) *valachicus zagrebensis* S. Karaman, 1960: 83;

Niphargus elegans zagrebensis G. Karaman, 1972: 5; G. Karaman, 1974: 17; Sket, 1981: 89; Barnard & Barnard, 1983: 691; Kralj, 2001: 109;

Niphargus illidzensis dalmatinus (part.) S. Karaman, 1932: 200;

Niphargus valachicus zagrebensis Kralj, 2001: 182;

Niphargus zagrebensis Gottstein, 2010: 84; Gottstein et al., 2013: 41; Delić et al., 2017a: 4; Delić et al., 2017b: 6.

MATERIAL EXAMINED: CROATIA:

S-1645= Zagreb, April 1924, 1 exp. male (leg. S. Karaman);

Am 628= Zagreb, Rimski jarak, torrent, August 1939, 3 exp. juv. (leg. D. Rucner);

Sp.100= Zagreb, ibid., 11.4. 1948, many exp.(leg. S. Karaman);

S-6049= Zagreb, ibid., 8.5. 1949, 10 exp. (leg. S. Karaman);

S-5357= Zagreb, vicinity, 11.5.1948, leg. S. Karaman, 4 females mixed with *Gammarus fossarum* Koch, 1936;

S-4222 = Opatovina near Zagreb, 4.3. 1981, 2 males juv. mixed with *Niphargus minor* Sket, 1956 (leg. M. Kerovec);

S-8405= ibid, 20.5.1981, 2 exp. (leg. M. Krvavica);

S-2416= Between Jakovlje and Luka, Hrvatsko Zagorje, subterranean waters on the field, 27.3.1974, many exp (leg. D. Rucner);

S-3930= Forest in Turopolje, 30 km S. of Zagreb, between Poščenica and Odra rivers, 9.10. 1982, many exp. mixed with *Synurella ambulans* (F. Muller, 1846) in the forest under lives with subterranean waters (leg. G. Karaman).

DIAGNOSIS

Body strong, up to 20.0 mm long, metasomal segments along dorsoposterior margin with several short setae each; urosomal segments 1 and 2 with single lateral spines mixed with single setae. Antenna 2 with slender flagellum longer than last peduncular article. Maxilla 1 inner plate with 2-4 setae, outer plate with 7 spines (6 with one lateral tooth), palpus not reaching tip of outer plate spines. Maxilliped inner plate short, with 3-4 distal spines.

Coxae relatively short, coxa 1 with subrounded ventroanterior part, coxa 4 unlobed. Gnathopods 1 and 2 with trapezoid propodus not exceeding the size of corresponding coxa and provided with row of setae along outer margin. Article 3 of both gnathopods with one distoposterior bunch of setae.

Epimeral plates with distinctly pointed ventroposterior corner in males and females, especially on epimeral plate 3. Dactylus of pereopods 3-7 along inner margin with additional spines (3-5 spines in anterior pereopods 3-4, and 2-3 spines in posterior pereopods 6 and 7; pereopod 5 often with 1-2 spines only). Article 2 of pereopods 5-7 unlobed. Pleopods 1-3 peduncles setose, with 2 retinacula.

Uropod 1 peduncle with dorsoexternal row of spines and dorsointernal row of setae (except distal spine), in males with ventrodistal finely serrate tubercle, absent in female. Inner ramus of uropod 1 remarkably elongated in male. Uropod 3 elongated in males, with long distal article of outer ramus; in female distal article short. Telson with various number of distal, marginal and facial spines.

Sexual dimorphic characters developed (uropod 1, uropod 3; tubercle on uropod 1 peduncle in males, oostegites).

DESCRIPTION: MALE 13.2 mm (Sp. 100): Body moderately strong, mesosomal segments naked, metasomal segments 1-3 along dorsoposterior margin with up to 9 short setae (fig. 4E).

Epimeral plates 1-3 with distinctly sharply pointed ventroposterior corner progressively more produced towards epimeral plate 3 (fig. 4E). Posterior margin of epimeral plates 1 and 2 sinusoid, that of epimeral plate 3 is concave, all with 6-8 short marginal setae each. Epimeral plate 2 with 2, epimeral plate 3 with 2-3 subventral spines.

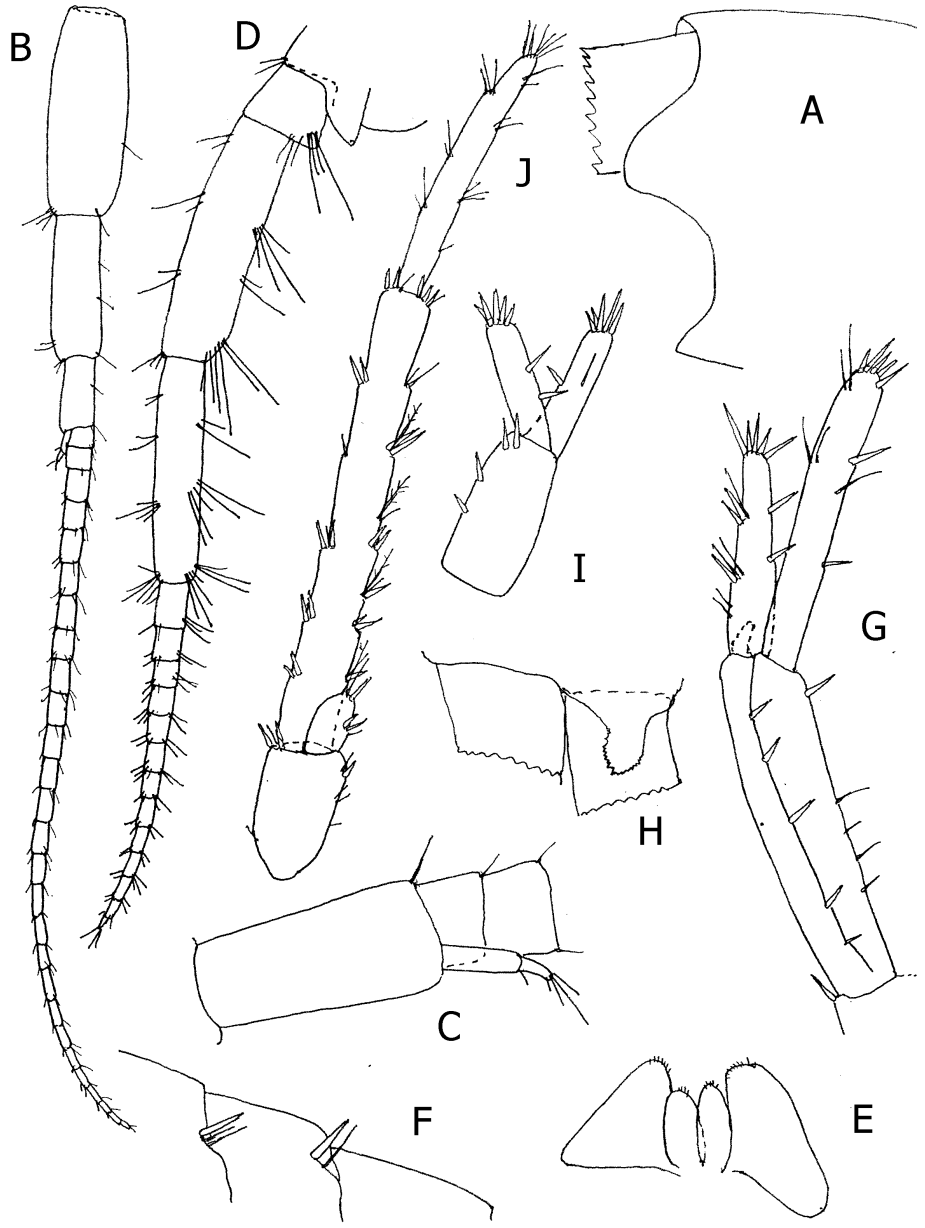

Fig. 1. *Niphargus.zagrebensis* S. Kar., 1950., Zagreb, Rimski jarak, torrent, male 13.2 mm: A= head; B= antenna 1; C= accessory flagellum; D= antenna 2; E= labium; F= urosome; G= uropod 1; H= tubercle on uropod 1 peduncle; I= uropod 2; J= uropod 3.

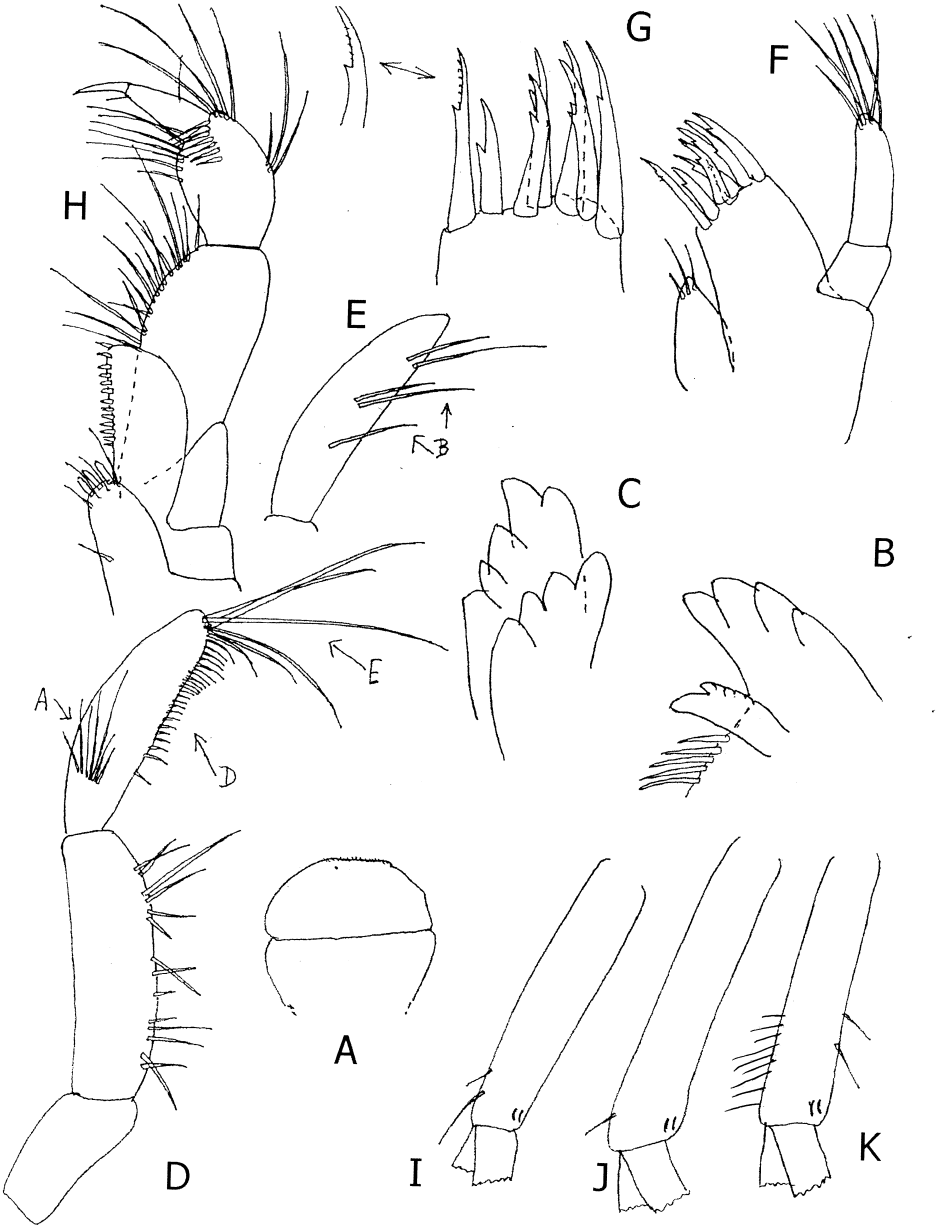

Fig. 2. *Niphargus.zagrebensis* S. Kar., 1950., Zagreb, Rimski jarak, torrent, male 13.2 mm: A= labrum; B= right mandible, incisor and lacinia mobilis; C= left mandible, molar and lacinia mobilis; D= mandibular palpus, outer face [A= A-setae; D= D-setae, E= E-setae]; E= distal article of mandibular palpus, inner face [B= B-setae]; F= maxilla 1; G= outer plate of maxilla 1; H= maxilliped; I= peduncle of pleopod 1; J= peduncle of pleopod 2; K= peduncle of pleopod 3.

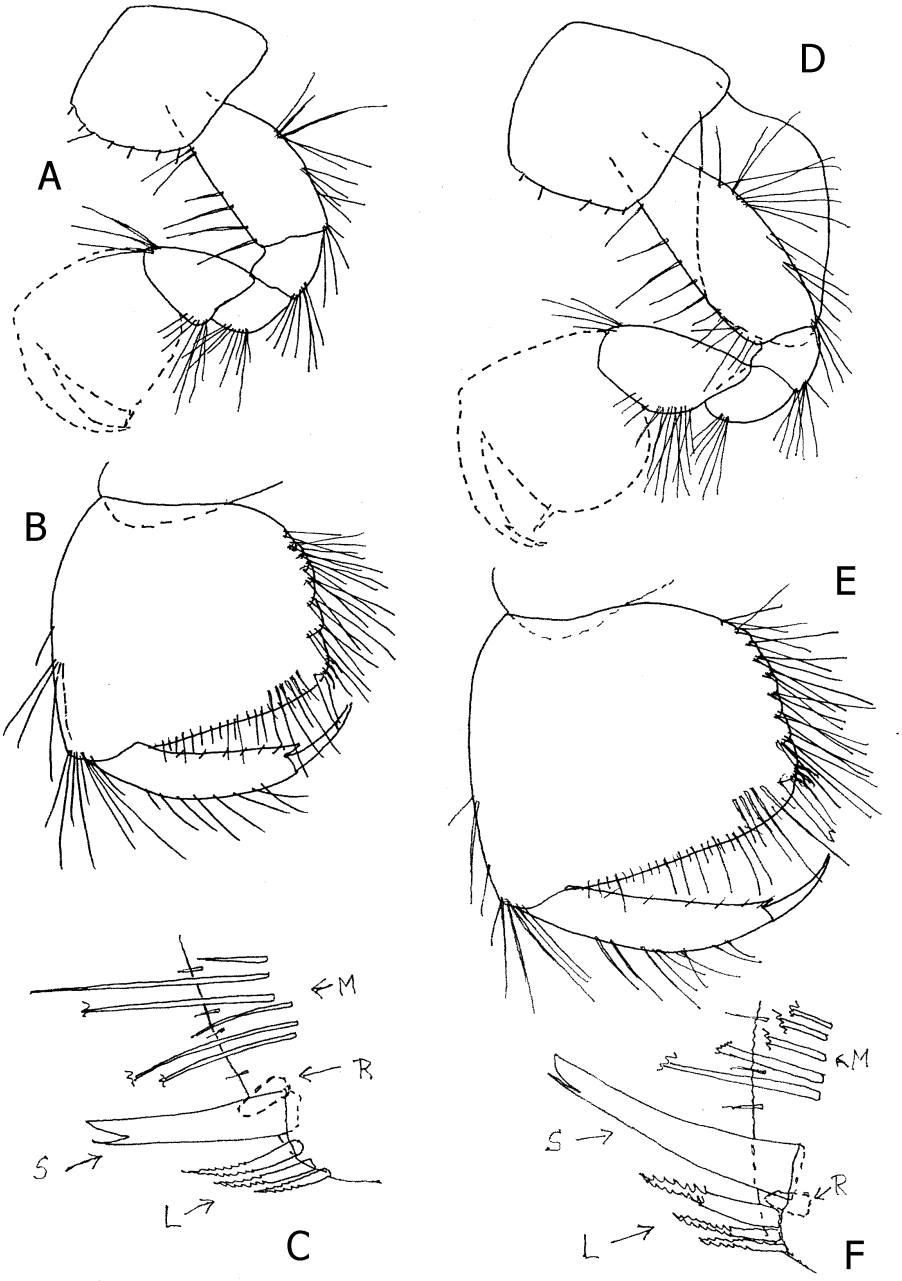

Fig. 3. *Niphargus.zagrebensis* S. Kar., 1950, Zagreb, Rimski jarak, torrent, male 13.2 mm: A-B= gnathopod 1, outer face; C= distal corner of gnathopod 1 propodus, outer face [S= corner S-spine; L= lateral L-spines; R= subcorner R-spine; M= facial M-setae]; D-E= gnathopod 2, outer face; F= distal corner of gnathopod 2 propodus, outer face [S= corner S-spine; L= lateral L-spines; R= subcorner R-spine; M= facial M-setae].

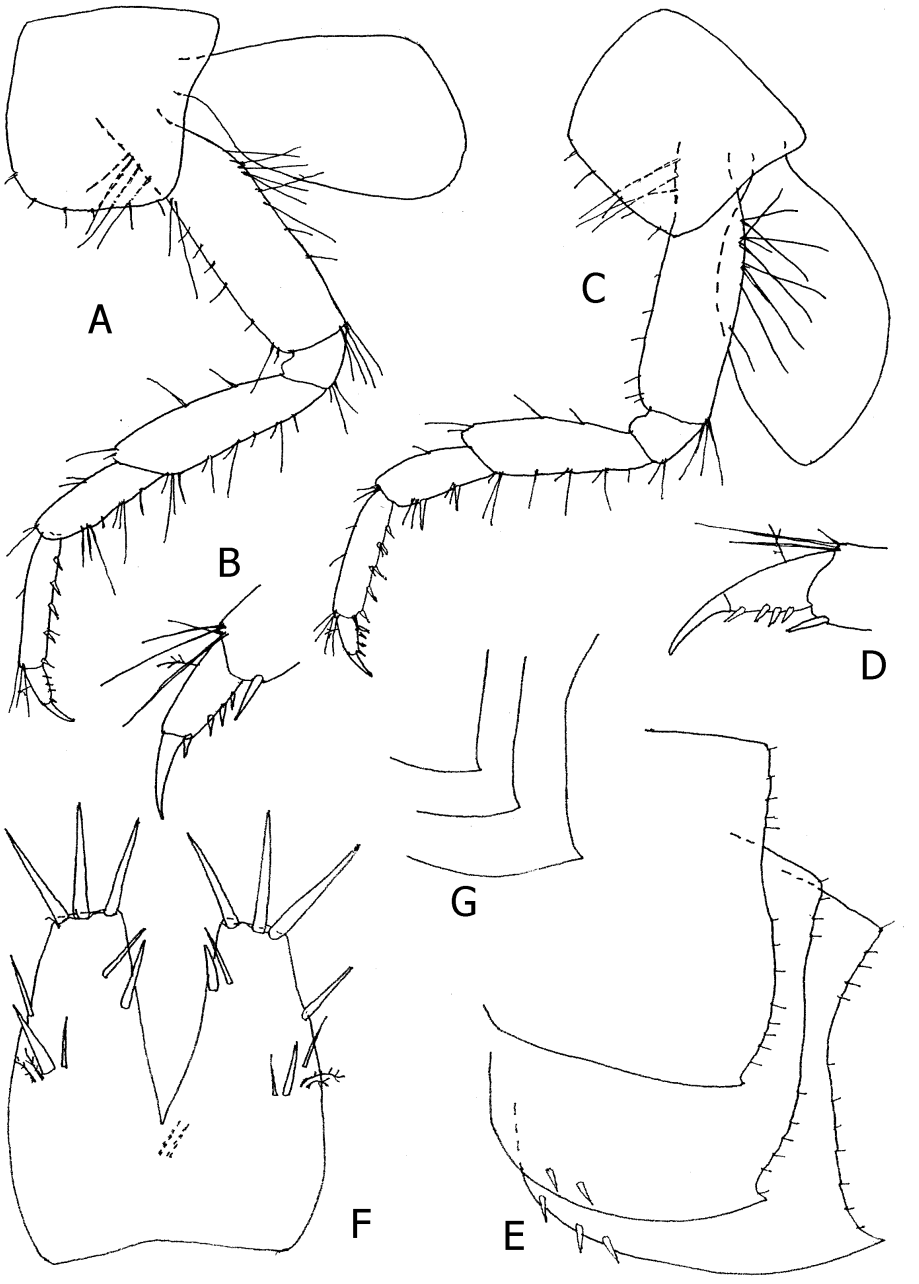

Fig. 4. *Niphargus.zagrebensis* S. Kar., 1950, Zagreb, Rimski jarak, torrent, male 13.2 mm: A= pereopod 3; B= dactylus of pereopod 3; C= pereopod 4; D= dactylus of pereopod 4; E= epimeral plates 1-3; F= telson; G= male 15.3 mm: epimeral plates 1-3.

Urosomal segment 1 on each dorsolateral side with one spine accompanied by 2 setae; urosomal segment 2 on each dorsolateral side with one spine and one seta (fig. 1F); urosomal segment 3 naked. Urosomal segment 1 on each ventroposterior corner with one spine near basis of uropod 1 peduncle (fig.1G).

Head with very short rostrum, short subrounded lateral cephalic lobes and ventral excavation, eyes absent (fig. 1A).

Antenna 1 reaching nearly half of body-length; peduncular articles 1-3 progressively shorter (ratio: 57:40:19), scarcely setose (fig. 1B); main flagellum consisting of 27 articles (most o them with one short aesthetasc), scarcely setose; accessory flagellum short, 2-articulated, almost reaching half of peduncular article 3 (fig. 1C).

Antenna 2: peduncular article 3 short, at distoventral corner with bunch of setae slightly longer than article itself (fig. 1D); article 4 longer than article 5 (ratio: 69:61), along ventral margin with 3 bunches of setae (the longest setae slightly exceeding diameter of article itself), along dorsal margin with one median spine and 4 bunches of short setae; article 5 along ventral margin with 3 bunches of setae (the longest setae are rather longer than diameter of article itself), along dorsal margin with 4 bunches of short setae. Flagellum relatively slender, slightly shorter than peduncular articles 4 and 5 combined (ratio: 95:128), scarcely setose (fig. 1D). Antennal gland cone short (fig. 1D).

Mouthparts basic. Labrum much broader than long, with poorly convex distal margin (fig. 2A). Labium broader than long, with well developed inner lobes and entire convex outer lobes (fig. 1E).

Mandibles with triturative molar. Left mandible with 5-toothed incisor and 4-toothed lacinia mobilis accompanied by 6 rakers (fig. 2C). Right mandible with 4-toothed incisor and weak bifurcate serrate lacinia mobilis accompanied by 5 rakers (fig. 2B). Palpus 3-articulated: article 1 naked, article 2 with 14 setae; article 3 nearly as long as article 2, with nearly 26 marginal D-setae and 6-7 distal E-setae (fig. 2D), on outer face by one group of 7 A-setae (fig. 2D), on inner face with 5 B-setae (1-2-2) (fig. 2E).

Maxilla 1: inner plate with 3 setae; outer plate with 7 spines (6 spines with one lateral tooth; one spine with finely serrate margin and one tooth) (fig. 2G); palpus 2-articulated, not reaching distal tip of outer plate spines (fig. 2F), provided with 6 distal setae.

Maxilla 2: outer plate slightly larger than inner on, both plates with distomarginal setae only (fig. 5A).

Maxilliped: inner plate short, with 3 distal pointed spines mixed with single setae (fig. 2H); outer plate not exceeding 2/3 of palpus article 2, along inner (mesial) margin with nearly 12 short spines; palpus 4-articulated; article 3 along outer margin with one median and one distal bunch of long setae, along inner margin with numerous setae in distal part and row of short facial spine-like setae; article 4 with short nail and bunch of 1-2 ventral setae near basis of the nail.

Coxae 1-7 relatively short. Coxa 1 as long as broad, with subrounded ventroanterior corner, provided with nearly 7 short setae (fig. 3A). Coxa 2 slightly broader than long (ratio: 50:47), along ventral margin with 5-6 short setae (fig. 2D). Coxa 3 almost as broad as long (ratio: 54:53), along ventral margin with nearly 6 setae (fig. 4A). Coxa 4 slightly broader than long (ratio: 53:50), without ventroposterior lobe, and with nearly 5-6 short marginal setae (fig. 4C).

Coxa 5 nearly as long as coxa 4, broader than long (ratio: 67:42), anterior lobe short, subrounded (fig. 5B). Coxa 6 shorter than coxa 5, broader than long (ratio: 60:37), anterior lobe relatively shallow (fig. 5D). Coxa 7 entire, broader than long (ratio: 50:26), with convex ventral margin (fig. 5F).

Gnathopods 1 and 2 relatively small, with propodus as large as corresponding coxa (fig. 3A, D). Gnathopod 1: article 2 along anterior and posterior margin with numerous long setae; article 3 along posterior margin with one distal bunch of setae; article 5 shorter than propodus (ratio: 30:40), along anterior margin with distal bunch of setae (fig. 3A). Propodus almost quadrate, broader than long (ratio: 74:70), along posterior margin with 7 transverse rows of setae; palm almost straight, inclined almost to the half of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 3 serrate L-spines and with row of 6 facial M-setae (fig. 3B, C), on inner face by one submarginal R-spine (fig. 3C). Dactylus reaching posterior margin of propodus, along outer margin with row of 8 single setae, along inner (mesial) margin with several short setae only (fig. 3B).

Gnathopod 2: article 2 along anterior margin with row of single long setae, along posterior margin with several bunches of long setae (fig. 3D); article 3 at posterior margin with one distal bunch of setae. Article 5 poorly shorter than propodus (ratio: 38:42), along anterior margin with distal bunch of setae. Propodus poorly rhomboid, broader than long (ratio: 88:78), along posterior margin with 8 transverse rows of setae (fig. 3E); palm almost straight, inclined less than half of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 3 serrate L-spines and by 5 facial M-setae, on inner face by one subcorner R-spine (fig. 3F). Dactylus reaching posterior margin of propodus, along outer margin with 9 setae (1-1-2-3-1-1), along inner margin with several short setae (fig. 3F).

Pereopods 3 and 4 moderately stout. Pereopod 3 poorly longer than pereopod 4, article 2 along anterior margin with several proximal long setae and several distal short setae (fig. 4A), along posterior margin with bunches of long setae in proximal part. Articles 4-6 of different length (ratio: 50:32:35): article 4 at posterior margin with several bunches of relatively short setae (the longest setae not exceeding diameter of article itself), at anterior margin with single facial and distal setae; article 5 along posterior margin with 4 groups of setae (the longest setae exceeding diameter of article itself); article 6 along posterior margin with 5 pairs of short spines. Dactylus strong, shorter than article 6 (ratio:

19:35), along inner margin with 3-4 strong spines, along outer margin with one median plumose seta (fig. 4B); nail shorter than pedestal (ratio: 22:30).

Pereopod 4: article 2 at anterior margin with proximal bunch of long setae and row of short distal setae, along posterior margin with numerous bunches of long setae (fig. 4C). Articles 4-6 of unequal length (ratio: 42:28:33); article 4 along both margins with setae not exceeding diameter of article itself; article 5 along posterior margin with 2 single spines accompanied by several short setae; article 6 along posterior margin with 5 groups of single or pairs of short spines. Dactylus strong, remarkably shorter than article 6 (ratio: 19:33), along ventral margin with 3-4 strong spines, along outer margin with one median plumose seta (fig. 4D); nail shorter than pedestal (ratio: 22:32).

Pereopods 5-7 relatively stout. Pereopod 5 remarkably shorter than pereopods 6 and 7 (fig. 5B, D, F), article 2 dilated, longer than broad (ratio: 72:48), along anterior margin with nearly 8 groups of spine-like setae or short setae, posterior margin slightly concave in the middle, bearing nearly 11 short marginal setae, ventroposterior lobe not developed (fig. 5B). Articles 4-6 of unequal length (ratio: 30:43:44); article 4 along anterior margin with 3 groups of short setae and distal 2 spines, along posterior margin with one median and 2 distal spines. Articles 5 and 6 along both margins with several bunches of short spines and some distal long setae. Article 6 remarkably shorter than article 2 (ratio: 44:72). Dactylus short and strong, much shorter than article 6 (ratio: 19:44), along inner margin with 1-2 strong spines, along outer margin with one median seta (fig. 5C); nail shorter than pedestal (ratio: 19:30).

Pereopod 6: article 2 dilated, longer than broad (ratio: 83:53), along anterior margin with row of 6 groups of short spine-like setae, along posterior, almost straight margin, with nearly 13 short setae, ventroposterior lobe not developed (fig. 5D). Articles 4-6 of unequal length (ratio: 55:66:72): article 4 at anterior margin with several single or bunches of short setae and single short spines, along posterior margin with 2 median and 2-3 distal spines. Articles 5 and 6 along both margins with several bunches of short spines (spines not exceeding diameter of articles). Article 6 shorter than article 2 (ratio: 72:83). Dactylus much shorter than article 6 (ratio: 22:72), at inner margin with 2 strong spines, at outer margin with one median plumose seta (fig. 5E); nail shorter than pedestal (ratio: 23:72).

Pereopod 7: article 2 longer than broad (ratio: 85:54), along anterior margin with 6 groups of spine-like setae, along posterior poorly convex margin with nearly 17 short setae, ventroposterior lobe not developed (fig. 5F). Articles 4-6 of unequal length (ratio: 49:65:87), article 2 at anterior margin with 3 groups of setae and one spine, along posterior margin with 3 groups of spines. Articles 5 and 6 along both margins with several bunches of short spines and short setae. Article 2 almost as long as article 6 (ratio: 85:87). Dactylus much shorter than article 6 (ratio: 27:87), along inner margin with 2-3 strong spines, at outer margin with one median plumose seta (fig. 5G), nail shorter than pedestal (ratio: 24:50).

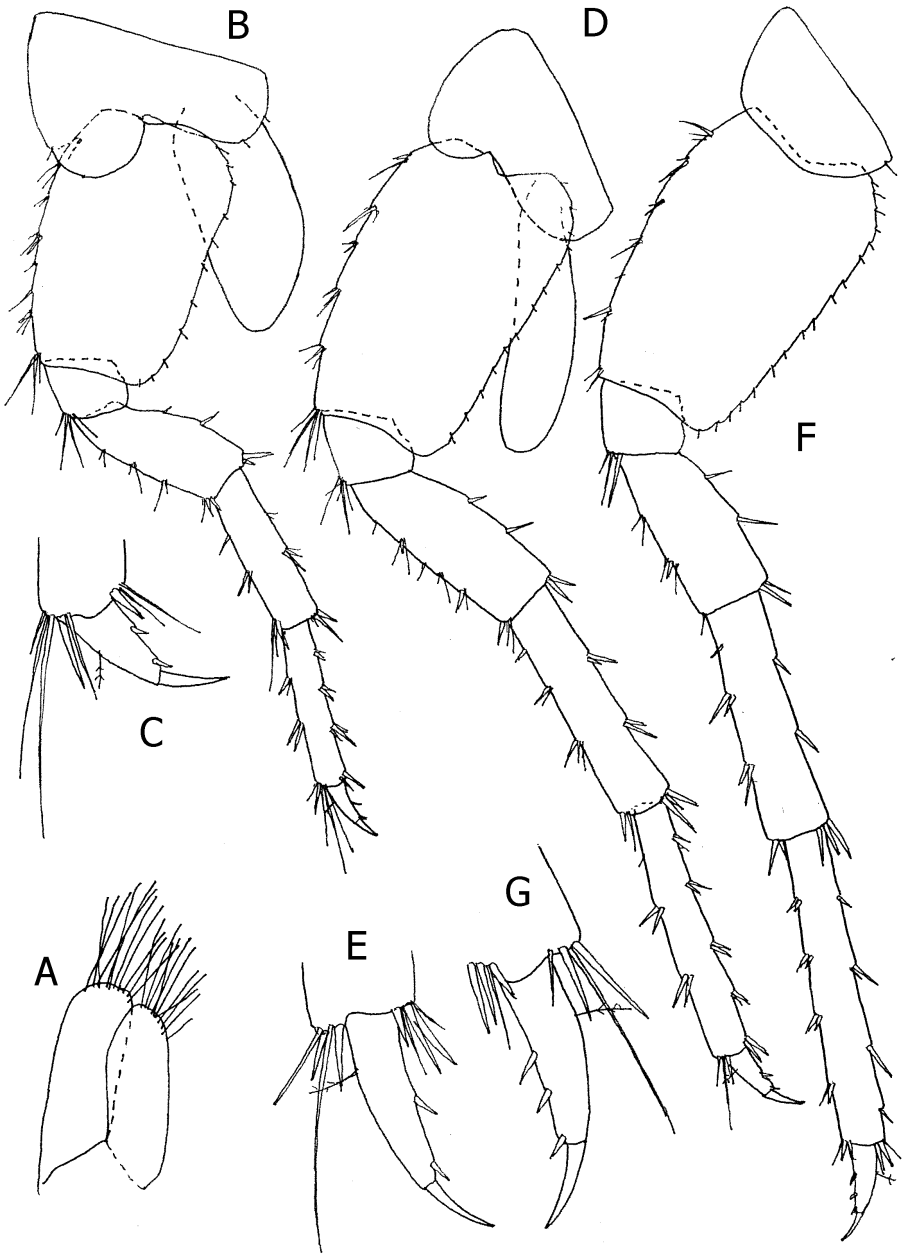

Fig. 5. *Niphargus.zagrebensis* S. Kar., 1950, Zagreb, Rimski jarak, torrent, male 13.2 mm: A= maxilla 2; B-C= pereopod 5; D-E= pereopod 6; F-G= pereopod 7.

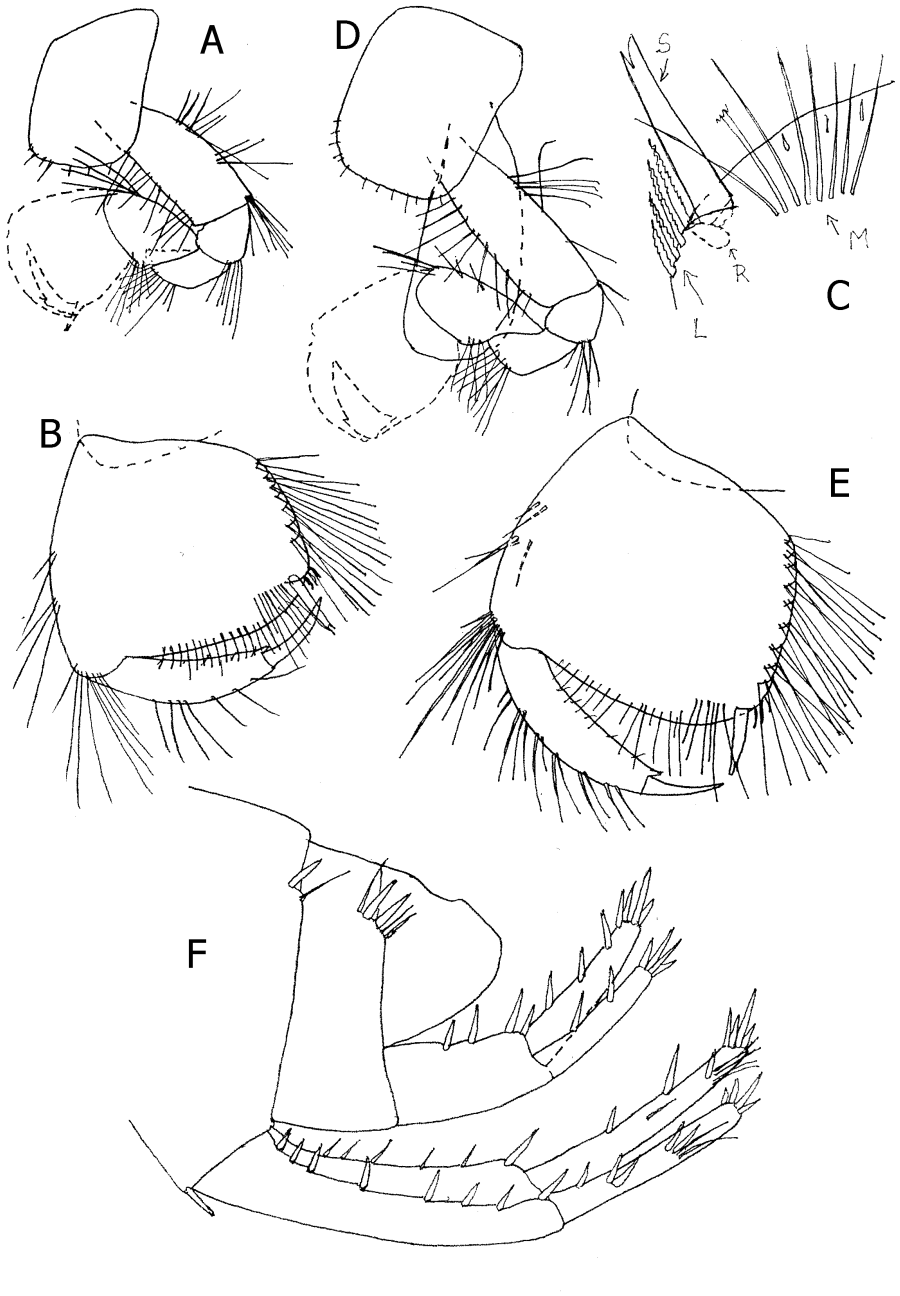

Fig. 6. *Niphargus.zagrebensis* S. Kar., 1950, Zagreb, Rimski jarak, torrent, female 13.1 mm: A-B= gnathopod 1, outer face; C= distal corner of gnathopod 1 propodus, outer face [S= corner S-spine; L= lateral L-spines; R= subcorner R-spine; M= facial M-setae]; D-E= gnathopod 2; F= urosome with uropods 1-2.

Pleopods 1-3 with 2 retinacula each. Peduncle of pleopod 1 along anterior external margin with 1-2 distal setae (fig. 2 I); peduncle of pleopod 2 at anterior external margin with one distal seta (fig. 2J); peduncle of pleopod 3 at anterior external margin with 9 median setae, along posterior margin with 2 setae (fig. 2K).

Uropod 1: peduncle with dorsoexternal row of spines and dorsointernal row of setae (except distal spine); one finely serrate tubercle is attached ventrally at top of peduncle (fig. 1H) (see detailed description in: S, Karaman 1950: 63, figs. 36, 37); Inner ramus with 5 distal and single lateral spines including 2 short setae; outer ramus much shorter than outer one, with 5 distal and several lateral spines (fig. 1G).

Uropod 2: rami of nearly subequal length, bearing single lateral and 5 distal short spines each (fig. 1 I).

Uropod 3 long, narrow: peduncle slightly longer than broad, with distal short spines; inner ramus short, scale-like, with group of lateral and distal spines. Outer ramus 2-articulated: first article along outer margin with 7 groups of short spines, along inner margin with 6 groups of short spines; single plumose setae are attached near spines at inner (mesial) side of the article (fig. 1J); second article rather shorter than first one (ratio: 73:126), with bunches of short simple setae along both margins and tip.

Telson incised less than 2/3 of telson-length, poorly longer than broad (ratio: 89:84); each lobe with 3 distal spines; single spines are attached along inner (mesial) and outer margin as well as on dorsal surface (fig. 4F); a pair of short plumose setae are attached near the median part of outer margin on each lobe.

Coxal gills on gnathopod 2 and pereopods 3-4 large, ovoid (figs. 3D; 4A, C), these of pereopods 5 and 6 rather smaller (fig. 5B, D).

FEMALE, ovigerous, with 20 eggs in marsupium, 13.1 mm:

Body strong, metasomal segments 1-3 along dorsoposterior margin with 6-8 short setae (fig. 8C). Urosomal segment 1 on each dorsolateral side with one spine and 1-3 setae (fig. 6F); urosomal segment 2 on each dorsolateral side with 2 spines and 2-3 setae; urosomal segment 3 naked. Urosomal segment 1 on each ventroposterior corner with one spine near basis of uropod 1 peduncle (fig. 6F).

Epimeral plates 1-3 similar to these in male, with ventroposterior corner distinctly pointed, progressively more developed towards epimeral plate 3 (fig. 8C). Epimeral plates 1 and 2 with poorly sinusoid posterior margin provided with several short setae each; posterior margin of epimeral plate 3 concave, provided with 7 short setae. Epimeral plate 2 with 4 and epimeral plate 3 with 4-6 subventral spines (fig. 8C).

Head like that in male. Antenna 1 almost reaching half of body-length, scarcely setose like that in male; peduncular article 3 relatively short; main flagellum consisting of 22 articles, scarcely setose; accessory flagellum reaching half of peduncular article 3 (fig. 8A).

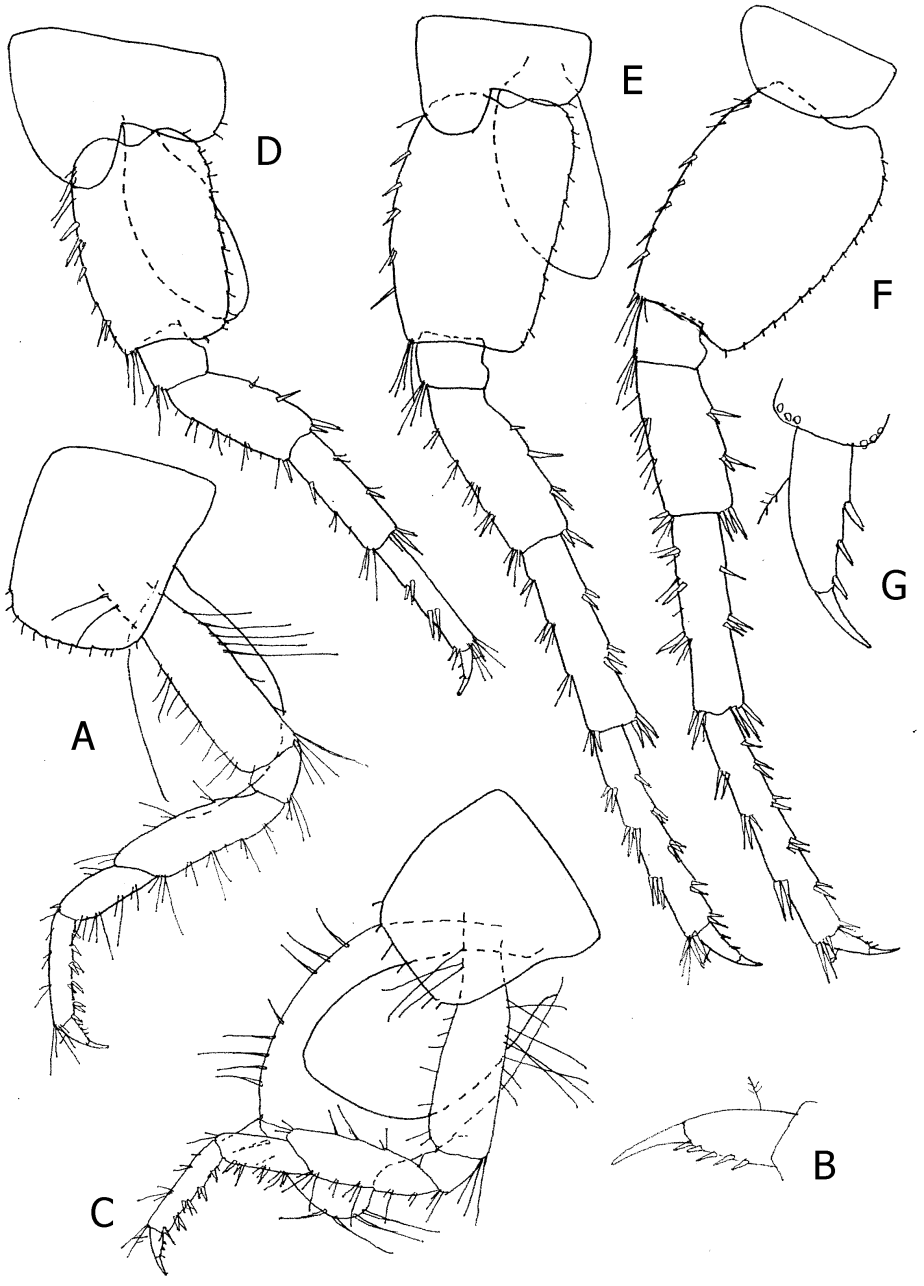

Fig. 7. *Niphargus.zagrebensis* S. Kar., 1950, Zagreb, Rimski jarak, torrent, female 13.1 mm: A-B= pereopod 3; C= pereopod 4; D= pereopod 5; E= pereopod 6; F-G= pereopod 7.

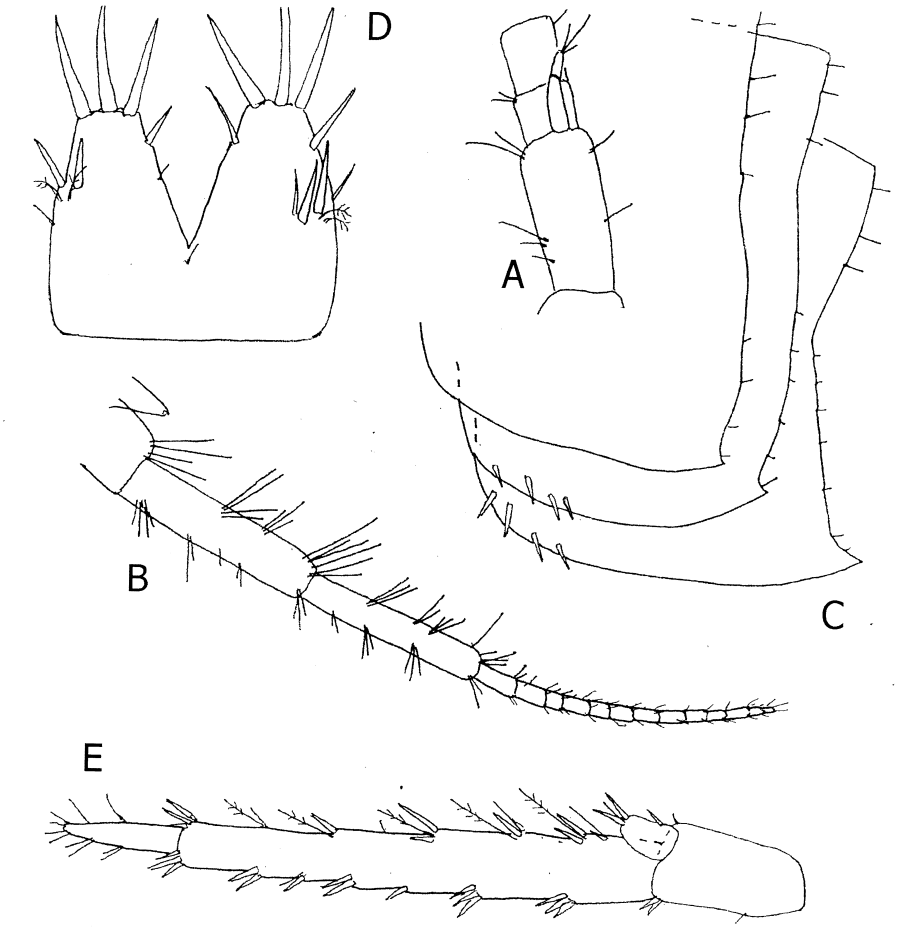

Fig. 8. *Niphargus.zagrebensis* S. Kar., 1950, Zagreb, Rimski jarak, torrent, female 13.1 mm: A= accessory flagellum; B= antenna 2; C= epimeral plates 1-3; D= telson; E= uropod 3.

Antenna 2: peduncular article 3 with distoventral bunch of long setae (fig. 8B); peduncular article 4 slightly longer than article 5 (ratio: 58:54), both articles along ventral margin with 3 bunches of ventral setae longer than diameter of articles themselves, along dorsal margin with 4 groups of short setae each. Antennal gland cone short (fig. 8B).

Mouthparts like these in male. Mandibles with 6 rakers. Mandibular palpus article 3 with nearly 30 D-setae, 5-6 E-setae, on outer face by one bunch of 7-8 A-setae, on inner face with 3 groups of B-setae (2-3-2).

Maxilla 1: inner plate with 3-4 setae, outer plate with 7 spines (6 spines with one lateral tooth, one spine with one lateral tooth and finely serrate margin, palpus 2-articulated, not reaching tip of outer plate spines and provided with 6 setae.

Maxilliped inner plate with 4 distal spines; palpus article 4 at inner margin with 2 setae near basis of nail.

Coxae 1-4 slightly longer than these in male. Coxa 1 longer than broad (ratio: 42:30), with subrounded ventroanterior corner and bearing nearly 7 marginal setae (fig. 6A). Coxa 2 slightly longer than broad (ratio: 50:40), along ventral convex margin with nearly 12 short setae (fig. 6D). Coxa 3 longer that broad (ratio: 53:45), at ventral margin with nearly 12 setae (fig. 7A). Coxa 4 as long as broad, along ventral margin with 6-8 setae (fig. 7C).

Coxa 5 broader than long (ratio: 60:41), with anterior subrounded lobe as long as coxa 4 (fig. 7D); coxa 6 distinctly smaller than coxa 5, bilobed, broader than long (ratio: 46:32) (fig. 7E); coxa 7 entire, broader than long (ratio: 43:22) (fig. 7F).

Gnathopods 1 and 2 relatively small, almost as large as corresponding coxa (fig. 6A, D). Gnathopod 1: article 2 along both margins with numerous long setae; article 3 at posterior margin with one distal bunch of setae (fig. 6A). Article 5 nearly as long as propodus, along anterior margin with distal bunch of setae. Propodus trapezoid, slightly larger than long (ratio: 69:63), along posterior margin with 7 transverse rows of setae (fig. 6B). Palm convex, inclined slightly less than half of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 3-4 serrate L-spines and 8 facial M-setae (fig. 6C), on inner face by one subcorner R-spine. Dactylus reaching posterior margin of propodus, along outer margin with row of 8 setae, along inner margin with several short setae (fig. 6B).

Gnathopod 2 rather larger than gnathopod 1: article 2 along both margins with numerous long setae; article 3 at posterior margin with one distal bunch of setae (fig. 6D). Article 5 almost as long as propodus, along anterior margin with 3 groups of setae. Propodus trapezoid, rather inclined, broader than long (ratio: 68:80), along posterior margin with 9 transverse rows of setae (fig. 6E). Palm convex, inclined nearly 1/3 of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 3 serrate L-spines and 6 facial M-setae, on inner face by one subcorner R-spine. Dactylus reaching posterior margin of

propodus, along outer margin with 13 single or paired setae, along inner margin with several short setae (fig. 6E).

Pereopods 3 and 4 moderately stout. Pereopod 3 only slightly larger and with slightly longer setae than pereopod 4, article 2 with longer proximal setae along anterior and posterior margin and with shorter setae in distal part (fig. 7A). Articles 4-6 of unequal length (ratio: 40:25:30); article 4 along both margins with several bunches of setae (the longest setae slightly exceeding diameter of article); article 5 along posterior margin with 3 bunches of setae (the longest setae exceeding diameter of article itself), along anterior margin with 2 groups of short setae; article 6 along posterior margin with 6 paired short spines, along anterior margin with 3 groups of short setae. Dactylus much shorter than article 6 (ratio: 16:30), along inner margin with 5 strong spines, nail shorter than pedestal (fig. 7B).

Pereopod 4: pilosity of articles 2-4 like that in male. Article 5 along posterior margin with 3 groups of single spines mixed with short setae, along anterior margin with distal group of short setae (fig. 7C). Dactylus like that of pereopod 3, along inner margin with 5 spines.

Pereopod 5 remarkably shorter than pereopods 6 and 7 (fig. 7D, E, F), article 2 dilated, unlobed, longer than broad (ratio: 58:38), along anterior margin with row of spine-like setae, along posterior margin with nearly 13 short setae. Articles 4-6 of equal length; article 4 at anterior margin with 5-6 groups of short setae and distal spine, along posterior margin with 2 median and 2 distal spines (fig. 7D). Article 5 along anterior margin with groups of short spines and distal anterior setae, at posterior margin with 2 groups of spines and 2 short setae; article 6 along anterior margin with 3 bunches of spines, along posterior margin with distal bunch of spines and setae. Article 2 longer than article 6 (ratio: 58:36). Dactylus much shorter than pedestal (ratio: 14:36), at inner margin with 2 spines, nail is shorter than pedestal.

Pereopod 6: article 2 longer than broad (ratio: 67:46), along anterior margin with 6 groups of spine-like setae and distal group of setae, along posterior almost straight margin with nearly 12 short setae, ventroposterior lobe not developed (fig. 7E). Articles 4-6 of unequal length (ratio: 48:54:60); article 4 along anterior margin with 5 bunches of short setae and single spines, along posterior margin with 4 groups of spines; articles 5 and 6 along both margins with several bunches of short spines often mixed with single short setae. Article 2 longer than article 6 (ratio: 67:60). Dactylus much shorter than article 6 (ratio: 19:60), at inner margin with 3 spines, nail is shorter than pedestal.

Pereopod 7: article 2 longer than broad (ratio: 70:48), along anterior margin with row of spine-like setae and distal long setae, along posterior slightly convex margin with nearly 16 short setae, ventroposterior lobe not developed (fig. 7F). Articles 4-6 of unequal length (ratio: 42:55:68); article 4 along anterior margin with several groups of short setae, along posterior margin with 3 bunches of spines; articles 5 and 6 along both margins with several bunches of short spines sometimes mixed with single short setae. Article 2 slightly longer than

article 6 (ratio: 70:68). Dactylus much shorter than pedestal (ratio: 20:68), along inner margin with 3 strong spines, along outer margin with one median plumose seta (fig. 7G); nail shorter than pedestal (ratio: 20:46).

Pleopods 1-3 with 2 retinacula. Peduncle of pleopod 1 with 5 setae along anterior external margin; peduncle of pleopod 2 with 1 seta at anterior external margin; peduncle of pleopod 3 with 9 setae along anterior external margin and 2 setae along posterior margin.

Uropod 1: peduncle with dorsoexternal row of spines and dorsointernal row of setae except distal spine (fig. 6F). Inner ramus is not produced, nearly as long as peduncle, provided with several lateral groups of strong spines; 4 spines are attached at the tip of article. Peduncle without distal ventral serrate tubercle. Outer ramus is only moderately shorter than inner one, with several strong lateral spines and with 2 simple long median setae; at the tip are attached 4 spines (fig. 6F).

Uropod 2 with inner ramus poorly longer than outer one, both rami with several lateral spines, on top of each ramus appear 4-5 short unequal spines (fig. 6F).

Uropod 3 narrow, elongated: peduncle nearly twice as long as broad (fig. 8E); inner ramus scale-like, with one lateral and 2 distal spines. Outer ramus 2-articulated: first article with 7 groups of strong spines along outer margin (fig. 8E), along inner (mesial) margin are attached several groups of single or paired spines mixed with single long plumose setae. Distal article of outer ramus much shorter than first one (ratio: 32:133), along both margins and tip with short simple setae.

Telson broader than long (ratio: 80:66), incised almost 2/3 of telson-length; each lobe with 3 long distal spines and single outer and inner marginal (mesial) slender spines, as well as with one bunch of 1-3 facial spines mixed with 0-1 short seta (fig. 8D); a pair of short plumose setae is attached near the middle of outer margin of each lobe.

Coxal gills on gnathopod 2 and pereopod 5 are ovoid, large (figs. 6D, 7A, C), these of pereopods 5 and 6 are rather smaller (fig. 7D, E).

Oostegites very large, occur on pereopods 2-5, setose along margin (fig. 7C).

VARIABILITY

Sexual dimorphic characters are visible through different uropod 3, size of inner ramus of uropod 1, slightly different length of coxae, oostegites in female, presence of sexual papillae on ventral side of metasomal segment 3 in male.

In males, inner ramus of uropod 1 is remarkably elongated, up to twice longer than outer one, in large specimens rami are paddle-shaped.

In females coxae are rather longer, telson often rather broader, inner ramus of uropod 1 only slightly longer than outer one, both rami are not paddle-shaped; oostegites present.

Size of the species is up to 20.0 mm, with antenna 1 reaching 2/5 to half of body-length.

Maxilla 1 inner plate with 2-4 setae, outer plate with 6 spines bearing one lateral tooth each, palpus not reaching tip of outer plate spines.

Maxilliped: inner plate with 3-4 distal pointed spines mixed with single setae.

Propodus of gnathopods 1-2 with 5-8 facial M-setae, 3 L-spines are attached always laterally from S-spine; dactylus of both gnathopods along outer margin with row of several single or paired setae.

Dactylus of pereopods 3-7 is with elevated number of spines along inner margin: 3-5 spines on pereopods 3 and 4; dactylus of pereopod 5 with 2, occasionally only one spine; dactylus of pereopods 6 and 7 with 2-3 spines along inner margin. We have not observed significant difference in number of spines on dactylus of pereopods in males and females.

Peduncles of pleopods 1-3 is with rather variable number of setae along anterior margin. In male 15.0 mm anterior external margin in peduncle of pleopod 1 is provided with 7 setae and posterior margin with 2 setae; anterior external margin of pleopod 2 peduncle is provided with 2 setae; anterior external margin of pleopod 3-peduncle is provided with 9 setae.

Ventroposterior corner of epimeral plates 1-3 are more or less distinctly pointed in males and females, Stanko Karaman mentioned and figured (1950) that in some large specimens (male 19.0 mm) the ventroposterior pointed corner of epimeral plates is less produced, but distinctly pointed, what agree with our observations also. We observed similar variability by various other *Niphargus* species also, that very large specimens ("senile specimens") have epimeral plates less pointed or more subrounded than adult specimens of median size within the same population. Epimeral plate 2 in both sexes is provided usually with 2-4 subventral spines, epimeral plate 3 with 2-6 subventral spines.

Urosomal segments 1 in males usually with 0-1 spine and 1-3 setae, urosomal segment 2 with 1-2 spines accompanied by single setae; urosomal segment 3 is always naked; Urosomal segment 1 in females usually with 1-2 spine often accompanied by 1-3 setae.

Uropod 1 in males with outer ramus reaching 2/3 of inner ramus (in smaller specimens) to almost as long as inner ramus (in large specimens), both rami with several lateral and 4-5 distal short spines; ventrodistal tubercle on uropod 1 in males is always present.

In females rami of uropod 1 are almost of equal length or inner ramus is poorly longer, both rami with several lateral and 4-5 distal short spines.

Uropod 2 in males and females with nearly equal length or inner ramus poorly longer than outer one, both rami with several lateral and 4-5 distal short spines.

Telson with rather variable shape, scarcely longer than broad to slightly broader than long, with 3-4 distal spines and bearing 1-2 outer marginal and mesial spines and setae; single facial spines and setae are always present.

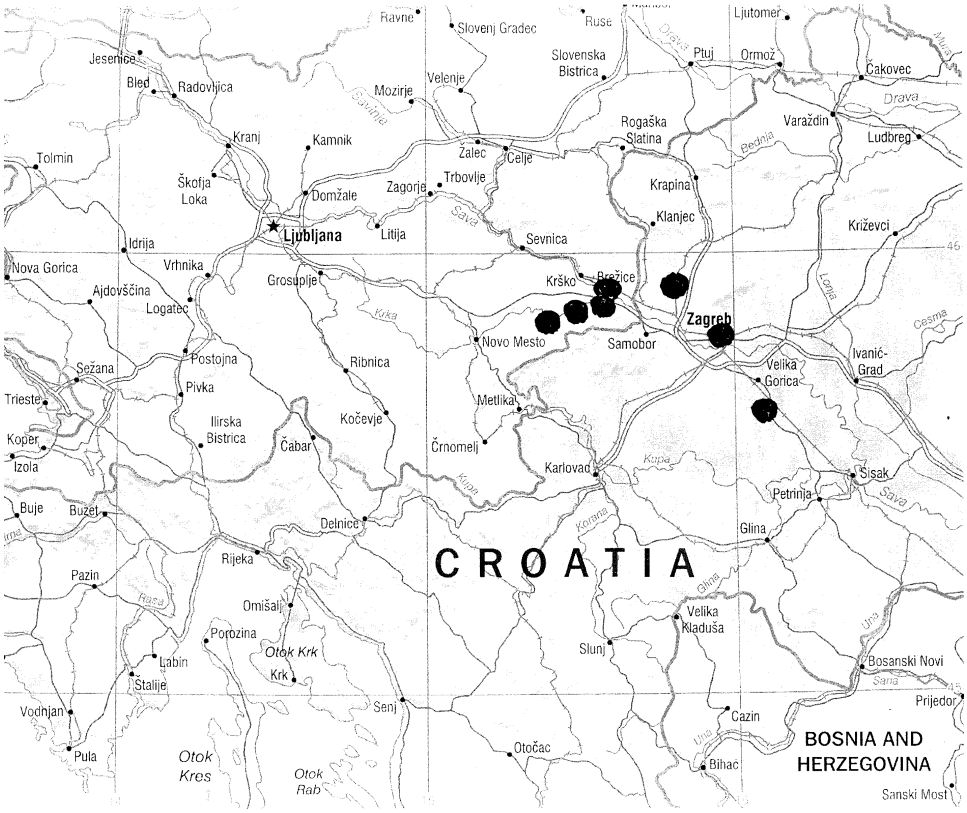

Fig. 9. Distribution of *Niphargus.zagrebensis* S. Kar., 1950 on Balkan

LOCUS TYPICUS: Zagreb, Croatia

Holotype: and paratypes are deposited in Karaman`s Collection in Podgorica, Montenegro under the numbers:Sp.100/1-7.

LOCALITIES CITED:

CROATIA:

Zagreb and vicinity (S. Karaman, 1950); Sket, 1958; G. Karaman, 1972; G. Karaman, 1974; Sket, 1981; Kralj, 2001; Delić, 2017a; present data);

Čučerje, Dubrava near Zagreb (Delić et al., 2017a);

Between Jakovlje and Luka; Turopolje (present data);

SLOVENIA:

Brežice; Cerklje; Kostanjevica in Krakovski forest; limnocrene spring near Krka River in village Malnice (Sket, 1958; G. Karaman, 1972; G. Karaman, 1974; Sket, 1981);

Kočevske poljane, Dolenjske toplice; spring in Jurišah, Juriše, Ozalj; Božakovska jama Cave, Božakovo, Metlika (Delić et al., 2017a);

Gadina, Loka, Črnomelj (Delić et al., 2017a; Delić et al., 2017b).

REMARKS AND AFFINITY

Niphargus zagrebensis belongs to the group of species with elevated number of spines on dactylus of pereopods and presence of distal tubercle on uropod 1 in male. strongly pointed ventroposterior corner of epimeral plates, by presence of ventrodistal tubercle on uropod 1 in males and by poorly unequal uropod 3 in males and females, with typus subgeneris: *Niphargus thermalis* Dudich, 1941a, including in it also *N. mediodanubialis* Dudich, 1941b, *N. hrabei* S. Karaman, 1932 and *N. valachicus* Dobreanu & Manolache, 1933.

N. thermalis Dudich, 1941 is considered as a valid species, and *N. mediodanubialis* Dudich 1941b [loc. typ.:swamps near Szeged, Southeast Hungary] and its form *aschizotelson* Dudich 1941b [loc. typ.: wells in Rėvülöp, Hungary] have uncertain taxonomical position (Balázs et al., 2015).

S. Karaman (1950) removed all *Niphargus* taxa with elevated number of spines on dactylus of pereopods into a new subgenus *Niphargus* (*Supraniphargus*) n. sbg. with typus subgeneris: *Niphargus illidzensis* Schäferna, 1922.

S. Karaman (1960) removed from subgenus *Supraniphargus* all taxa living in epigean waters with tubercle on uropod 1 in male and strongly pointed epimeral plates into subgenus *Niphargus (Phaenogammarus*) (*N. elegans* Garbini, 1894, *N. valachicus* Dobreanu & Manolache, 1933, *N. zagrebensis*, S. Karaman, 1950, *N. hrabei* S. Karaman, 1932, *N. thermalis* Dudich, 1941, *N. potamophilus* Birstein, 1954, *N. cubanicus* Birstein, 1954).

At the present time, the taxonomic categories on generic, subgeneric and species levels are in the process of redefinition and valorization by various authors, based on numerous different methods, often contradictory, and we don`t discuss this problem here.

We consider *Niphargus zagrebensis* a good distinct species, rather similar to *Niphargus hrabei* S. Karaman, 1932 described from Nana Parkan in one swamp near Danube river in Slovakia between Komorn (=Komarno) and Budapest (Hungary), later mentioned by some authors in some other localities also.

N. hrabei has also strongly acute epimeral plates, presence of distoventral tubercle on uropod 1 in males, but differs from *zagrebensis* by shorter inner ramus of uropod 1 in males, lower number of spines on some dactyls of pereopods, by shorter distal article on uropod 3 outer ramus in male, by maxilla 1, armature of telson, etc. It is necessary to redescribe *Niphargus hrabei* to understand better the taxonomical relations between these two species.

Niphargus valachicus [loc. typ.: Bucarest, Romania] differs remarkably from *N. zagrebensis* by different armature of urosomal segments 1 and 2, by very elevated number of spines on dactylus of all pereopods, etc,

Niphargus elegans Garbini, 1894 [loc. typ.: S. Pancrazio, Verona, Italy] differs from *N. zagrebensis* by higher number of spines on dactylus on pereopods 3-7, by gnathopods 1-2, by different maxilla 1, etc.

ECOLOGY:

Niphargus zagrebensis was found in the springs, wells and other subterranean waters as well as in the epigean waters in ditches with waters, but always connected with temporary or stable water sources in the vicinity.

Stanko Karaman described this species from vicinity of Zagreb (Rimski potok-torrent) and some other torrents near Zagreb), and it was collected later from the same region (Čućerje , Dubrava; Opatovina), sometimes mixed with the epigean species *Gammarus fossarum* Koch, 1836. In the Turopolje region we collected *N. zagrebensis* in small limnocrene waters under leaves in the forest, mixed with semisubterranean species *Synurella ambulans* (F. Muller, 1846). In the subterranean waters in Opatovina near Zagreb this species was collected mixed with *Niphargus minor* Sket, 1956.

Niphargus zagrebensis is semisubterranean species, blind, with strong body`s cuticula, but in the process of penetration actively again into epigean waters. Similar tendency to conquest the epigean waters it was observed by some other *Niphargus* species also: *Niphargus valachicus* Dobreanu & Manolache, 1933, species very common in Danube river basin from Slovenia till Romania, Asia Minor till Iran (G. Karaman, 1998), *N. elegans* Garbini, 1894, species also very common in superficial (epigean) waters collected sometimes with *Synurella ambulans* and various *Echinogammarus* species (G. Karaman, 1993).

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ECONOMIC ANALYSIS OF WHEAT PRODUCTION AND APPLIED MARKETING MANAGEMENT

SUMMARY

Wheat is, in quantity, the third largest crop produced in the world and therefore contributes greatly to the world economy both globally and in Serbia and Bosnia and Herzegovina - B & H. With wheat, worldwide it is sowing about 2.20 million ha annually and in Republic of Serbia wheat cultivated in 588.820 ha. The present trial was carried out to study the growth rates of yield, production and market of wheat in B & H. During the period 2010-2016, wheat was sown of Bosnia and Herzegovina on 63606.30 ha. There is trend in increasing of areas under wheat with a rate of 0.99% per year and, it varies. Average yield of wheat during the monitored period was 3.58 t ha^{-1}. Average export of wheat in the tested period was 41750 t and had a tendency to increase with a rate of 25.67% and variation (CV = 104.41%) while average import of wheat was 398750 t and had a tendency to increase with a rate of 0.82% and variation (CV = 10.37%). The highest import was achieved in 2016 (398750 t) and the lowest in 2013. External trade of agro-industrial products and trade liberalization implies the necessity of raising the technological level of production, productivity, efficiency and application of marketing management.

Keywords: area, yield, production, market, wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the oldest and most widely cultivated species to which the human race is grateful for its development and civilization. Wheat belongs to the order *Poales*, family *Poaceae*, genus *Triticum*. Wheat is an old crop grown more than 10,000 years ago in Iraq, China and Egypt, then about 5,000 years later it began to be grown in the eastern part of Europe. Wheat is part of the staple diet in every country in the world because of its high nutritional value, rich in carbohydrates and proteins, making it an extremely nutritious. Wheat is a food source for over seven billion of people and is a major food item in many countries of the world (Pavićević, 1991; 1992; Popović, 2010).

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

With the globalization, the agricultural sector is opened up with the new avenues, especially for food grains enterprises. The cereal crops are of vital importance not only in providing nutritional support but also earning additional income. Among the cereal, wheat is considered as “staple food’s grain” in the country (Asodiya Pinakin, 2014). It is grown all over the world for its highly nutritious and useful grain, as one of the top three most produced crops, along with maize and rice. It is used in the production of bread, biscuits, feeds, confectionary, etc. Wheat obviously took a very important place in international trade. Today, it is the basic food for over 70% of the country's population (7.8 billion). Wheat bread with a nutritive, vitamin and energy value of 8,500-9,400 joules is more nutritious than the bread of other types of grain, as it contains 70-78% of carbohydrates, 12-17% of total proteins, 1.2-2.5% of oil, 0.5-2.0% of mineral salts (most salts of Ca, P, Fe) is rich in vitamins B (B_1-thiamine, B_2-riboflavin and PP (nicotinamide). Besides flour, significant by-products of wheat grains milling are wheat germs rich in easily digestible proteins and high-quality edible oil and are used to prepare baby food and certain treats (Rakaščan *et al.*, 2019). The quality standards are as follows: 13% moisture, 76 kg hectolitre mass and 2% impurity. Wheat is divided into five classes: Premium: > 15% protein and hectolitre mass 80 $kghl^{-1}$; I. Class: 13.5-14.99% protein, hectolitre mass 78 $kghl^{-1}$; II. class: 12-13.49% protein and hectolitre mass 78 $kghl^{-1}$; Class III: 10.5-11.99% protein and hectolitre mass 74 kg hl^{-1}; 80 $kghl^{-1}$); Class IV: <10.49% protein and hectolitre mass <74 kg hl^{-1} (Popović, 2010).

Worldwide, wheat accounts for about one-third of the sown cereals area, or about 26%. Wheat adapts well to the climate and soil, and has many spicies and varieties; there are, also, winter and spring wheat, so it is grown almost all over the world and is included in the euro-types. The best conditions for growing winter wheat are between 30 ° and 50 ° north latitude. Conditions for growing wheat north to 60 ° and south to 16 ° north latitude are less favorable (Pedrotti, 2003). Spring wheat has much shorter vegetation than winter wheat, and is more resistant to drought and high temperatures, so it is more suitable for growing in the northern parts(45 ° - 67 ° north latitude), where such conditions prevail. The richest countries are the largest exporters of wheat, and it is very important for the economy. Due to the large income that is generated from wheat, many countries try to produce their own wheat so that they do not have to import it (Popović, 2010). Climate, soil and yield are inextricably intertwined in natural environments and should therefore be adjusted in order to produce profitable, high-quality food (Maksimović *et al.*, 2018). The success of production depends on genotype, climatic factors, soil and cultivation technology. For successful production, it is necessary to select high-yielding stable genotypes, adequate locality and to apply the correct cultivation technology. The most important climatic conditions are heat, light and water. Before the start of production, an analysis of the soil quality must be done. If the soil is poor with nutrients, extra nutrition of cultivated crops is in need. To take advantage of the high production potential of modern varieties, we must provide them with 150 kg of nitrogen and

100-120 kg of phosphorus and potassium per hectare. Wheat uses microelements and macroelements for its growth and development. Macro-elements are nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulfur (S) and magnesium (Mg) (Popović, 2010; Vukadinović and Bertić, 2013).

The yield per unit area is the result of the action of factors of variety in interaction with environmental factors. The yield is largely dependent on the genetic potential and considerably vary primarily as a result of agro-ecological conditions during the growing season (Popović *et al.*, 2011; Đekić *et al.*, 2017a; 2017b; Terzić *et al.*, 2018; Đekić *et al.*, 2019). For high yield and grain quality, it is necessary to adopt nitrogen by plants during the whole vegetation period. Understanding the fertilization, liming and rainfall effects have been a continuous endeavor toward improving farming technology and management strategy to reduce the negative impacts of these factors and to increase crop yield (Popović *et al.*, 2011; Ugrenović *et al.*, 2018; Rajičić *et al.*, 2019).

The two sides of agricultural constraints are production and marketing. From production side, smallholder farmers, particularly in developing country encounters multiple biotic and constraints abiotic such as inadequate planting material, pests and diseases, poor access to improved germplasm, low labor productivity and unreliable climatic conditions and from marketing side low market price, poor marketing infrastructure, low market integration, and this has led to low agricultural productivity and low supply of agricultural products on the market (Birachi *et al.*, 2011). HLs shows that an effective, excellent, integrated and responsive market that is marked with good performance is of crucial importance for optimal allocation of resources and stimulating households to increase output (FAO, 2003).

In Serbia, the volume of wheat production and consumption is steadily increasing, over the last two decades (Popović, 2010). The aim of this study was to analyze wheat production in Bosnia and Herzegovina and suggest improvement productivity, efficiency and application of marketing management.

MATERIAL AND METHODS

This paper analyzes the wheat production parameters in the world during the period from 2010 to 2016. The research is based on the available data already existing in related statistical publications. Data from FAO 2018 were used, http:// faostat.fao.org / and Statistical Yearbook, B&H. For the calculation of the yield and the size of the area, we used a basic statistical method comprising of the following:

-for calculation of variation degree of area size and yield coefficient of variation (CV) was applied in equation:

$$C_v = b \cdot 100 / \overline{X}$$

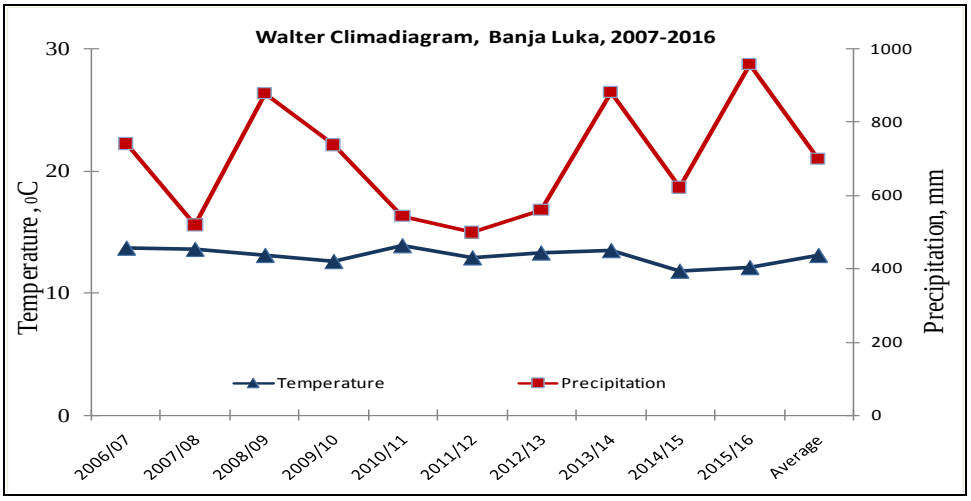
-movement of occurrences was calculated using an exponential trend in equation:

$$Yt = a \cdot b^{xi}.$$

RESULTS AND DISCUSSION

Meteorological conditions

Meteorological conditions were recorded high variability during year. In addition to the necessary reserve for the spring part of the vegetation, winter precipitation greatly influences the distribution of easily accessible nitrogen in the soil (Đekić *et al.*, 2017b, 2019; Popović *et. al.*, 2011; Terzić *et al.*, 2018). This study was conducted over a ten-year period in the Bosnia and Herzegovina. In a temperate continental climate having an average annual temperature of 13.05°C, and a rainfall amount of about 698.32 mm, Graph 1.



Graph. 1. Annual temperature, °C, and precipitation, mm, 2006/07-2015/16

The most favorable year for production was 2016 when record yields of 4.3 t ha^{-1} were achieved, while the lowest yields were achieved in 2010, 2.7 t ha^{-1}, Table1, Graph. 1.

Production of wheat in B&H

Production of wheat in Bosnia and Herzegovina, (B&H), had been growing 1.05 per cent per annum during the overall period (2007 to 2016). The coefficient of variation method was used to estimate the extent of instability in production, area and productivity of wheat crop. The coefficient of variation for wheat productivity of the B&H revealed 20.45 per cent during the study period. Popović (2010) states that the similar trend was observed in Serbia.

Wheat is planted to an average of 63606.3 hectares in B&H. There is an increasing trend of areas under wheat with a rate of 0.99% per year and variation (CV = 8.01%). The highest areas of wheat, in the B&H, was in 2016 (71394.00 ha), 2009 (68102.00 ha), 2013 (67902 ha) and 2015 (67644 ha). The smallest area under wheat was in 2010 (55454 ha) and the largest in 2016 (71,394 ha). In 2016, the area was increased by 11954 ha or 21.56% compared to 2010, Table 1.

Wheat production an average of 255780 ha in B&H. The highest production of B&H of wheat was realized in 2016 (306600 t / ha), 2013 (265150 t ha^{-1}) and 2009 (255840 t ha^{-1}). A statistically significant decrease in wheat production was achieved in 2010 (145410 t ha^{-1}) and 2014 (170000 t ha^{-1}) compared to all examined years. Wheat production was higher in 2016 by 110.85 % compared to 2010, Table 1 and Graph 2.

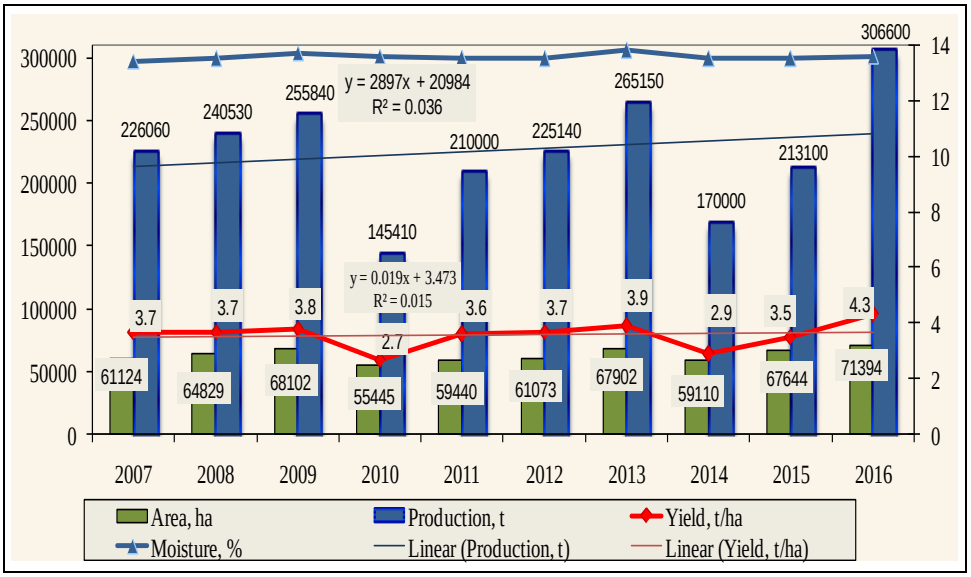
Average yield of wheat in the period 2007-2016 was 3.58 t ha^{-1} in total and have a tendency to increase with a rate of 0.45%. Yield of wheat grains recorded increasing trend and variation (CV = 13.02%). The highest yield was achieved in 2016 (4.3 t ha^{-1}) and the lowest in 2010. In 2016, a higher yield was produced for 1.60 t ha^{-1} compared to 2010, Table 1 and Graph. 2.

Table 1. Wheat area, ha, yield, t/ha, moisture, %, and production, t, B&H

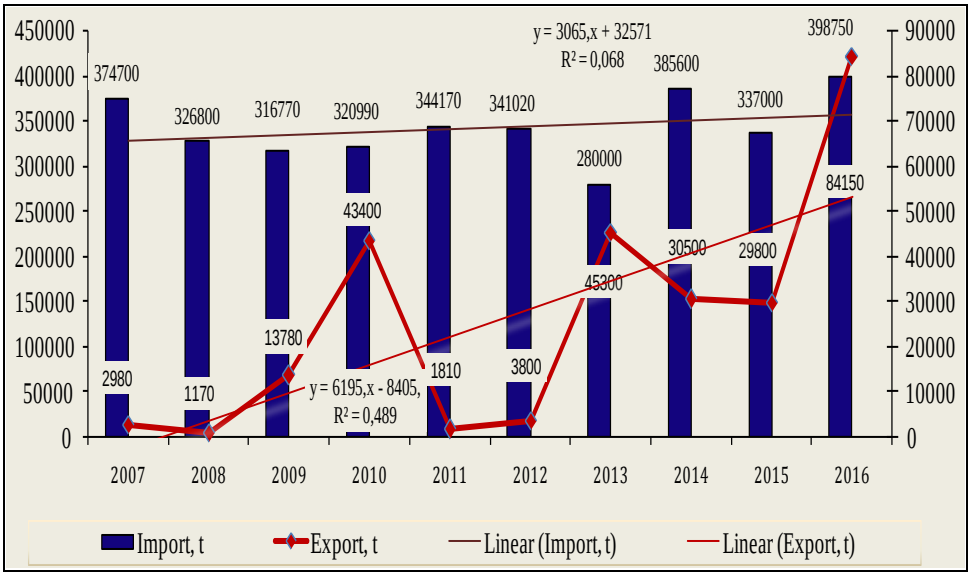
Parameter / Year	Area (ha)	Production (000 t)	Yield (t/ha)	Import (000 t)	Export (000 t)
2007	61124.00	226.06	3.70	374.70	2.98
2008	64829.00	240.53	3.70	326.80	1.17
2009	68102.00	255.84	3.80	316.77	13.78
2010	55445.00	145.41	2.70	320.99	43.40
2011	59440.00	210.00	3.60	344.17	1.81
2012	61073.00	225.14	3.71	341.02	3.80
2013	67902.00	265.15	3.90	280.00	45.30
2014	59110.00	170.00	2.90	385.60	30.50
2015	67644.00	213.10	3.50	337.00	29.80
2016	71394.00	306.60	4.31	398.75	84.15
Average	**63606.30**	**225.78**	**3.58**	**342.58**	**41.75**
Rate of change	0.99	1.05	0.45	0.82	25.67
CV	**8.01**	**20.45**	**13.02**	**10.37**	**104.41**

Average export of wheat in the tested period was 41750 t and notes a tendency to increase with a rate of 25.67% and variation (CV = 104.41%). The highest import was achieved in 2016 (84150 t) and the lowest in 2008, 2011, 2017and 2012. In 2016,significantly higher export was achieved compared to 2008. Achieved difference was of 82980 t, Table 1 and Graph. 3.

Average import of wheat in the 2007-2016 period was 398750 t and notes a tendency to increase with a rate of 0.82% and variation (CV = 10.37%). The highest import was achieved in 2016 (398750 t) and the lowest in 2013. In 2016 achieved significantly higher export compared to 2013, a difference of 118870 t was achieved, that is, 42.41%, Table 1 and Graph. 3.



Graph. 2. Wheat area, ha, yield, t/ha, moisture, %, production, B&H, 2007-16



Graph. 3. Wheat import, t, and export, t, in B&H, 2007-2016

Wheat is, in the world, the third largest crop by producing, and therefore contributes greatly to the world economy.

Wheat is the most widely grown cereal crop in the world, covering about 220 million hectares annually and giving for a total of 590 million tonnes

(Popović, 2010; Šarčević-Todosijević *et al.*, 2016; Lakić *et al.*, 2018), and so it makes at least one-fifth calories ingested by man (Glamočlija *et al.*, 2015). Average yield of wheat in Bosnia and Herzegovina in tested period 2010-2016, was about 3.58 t ha^{-1}. Serbia the world's important wheat producer.

According to FAO (2017), all types of wheat in the Republic of Serbia are cultivated in the about 588.820 ha. In addition to the main product, grain, significant quantities of by-products are remaining in the field, in warehouses and in industrial production and processing (Rakaščan *et al.*, 2019). In 2016, Serbia had a very good wheat crop of over 2.89 million tonnes, which had harvested from 595,000 ha. The initial wheat stock in 2018 was 218,000 tonnes with 3.11 million tonnes of wheat, available for consumption. Wheat needs in grain, in Serbia were about 1.55 million tonnes. For domestic consumption it required 1,200,000 tones, for supplies 200,000 tonnes and for seed production 150,000 tonnes, while the rest was intended for export (about 1.34 million tonnes). The largest exports were to Romania, 343,576 tonnes, then to Kosovo about 95,000 tonnes, and large buyers also were Bosnia and Herzegovina (108,862 tonnes), Albania (43,009 tonnes), Montenegro (28,162 tonnes), Macedonia (21,782 tonnes), Italy with (14,468 tons), Croatia (1,555 tons), Slovenia (10 tons) and other countries with 1,579 tons. In the second half of 2016, exports were slightly weaker due to the high export price of wheat produced in Serbia (Gulan, 2017).

Grain yield is the most important parameter in wheat production, however, besides grain, wheat biomass is of great importance. Harvesting residues can be used in many ways: as livestock food, livestock straw, for ploughing in, for mulching, composting or preparing artificial manure, as energy recovery material and as construction material (Manojlović and Jaćimović, 2014; Živanović *et al.*, 2017). The chemical composition and mass of the harvested residues is highly dependent on the plant species, genotype, climatic and weather conditions of the year, soil fertility and applied agro-technology. On average, plant dry matter contains about 45% carbon (C), 42% oxygen (O), 6.5% hydrogen (H), 1.5% nitrogen (N) and 0.5% minerals. Cereal straw contains about 45% of carbon while maize cob contains about 57% of it (Kastori and Tešić, 2006). The mass of crop residues (straw, trees, leaves, roots) in plant production can be quite large. For example, in production of maize up to 12 t ha^{-1}, wheat 4-6 tha^{-1},sunflower about 4-5 tha^{-1}, soybean about 4 tha^{-1}, and in sugar beets as much as 40-60 tha^{-1}. From the above data, it can be seen that, by ploughing, a significant amount of nutrients returns to the soil (Jaćimović *et al.*, 2017). External trade of agro-industrial products and trade liberalization implies the necessity of raising the technological level of production, productivity, efficiency and application of marketing management. Increased competition in the domestic market should contribute to increasing the quality and supply of goods. In order to dynamize exports, it is necessary to take advantage of the comparative advantages of the agro-industry, namely: favourable agro-ecological conditions, existence of significant capacities of the food industry, expert staff, etc. All the above factors should be in the function of creating stable export surplus products that will meet

the market's needs in quality. It is necessary to stimulate the revival of all agricultural production through appropriate economic measures, which is a necessary precondition for exports. There is also a need for constant research into the needs and requirements of the market and for agro-industrial products to be adapted to the standards that govern the market: ISO, HACCP, GLOBAL GAP; in terms of product quality, size, packaging method, type of packaging, etc. Export prices of agro-industrial products should be competitive in the market. Presnall (2003) state that price competitiveness is no longer a determining export advantage, and qualitative factors that have proven to be decisive are: design, packaging, reliability and speed of delivery, brand, the ability to meet specific consumer demands at use, services during and after purchase, warranty periods and compliance with contractual obligations in the export business, the issue of patents, introduction and use, permanent advertising in the media, representing trade interests of our country and building a positive image of the company products and promotion of national identity. The international competitiveness of the national economy is based on the measurement and comparison of macroeconomic indicators and living standards, where productivity is in focus, while in the narrow sense it is defined as a country's ability to export its products to the world market (Leko-Simic, 1999; Mihailović, 2005). Promotional activities need to be defined in order to increase exports. In order for the Republic of Serbia and Bosnia and Herzegovina to increase its exports, its products must be competitive in quality, range and prices. This implies effective, market-oriented, profitable, product programs tailored to requests and needs of consumers. Competitiveness in the market can be increased by constant changes in production and market orientation, i.e. defining products according to the needs and requirements of the foreign market. Long-term cooperation with foreign partners is necessary and it is necessary to permanently raise the quality of the product while reducing production costs.

CONCLUSIONS

Based on the results attained during the research we can conclude the following:

Wheat production an average of 255780 ha in B&H. Wheat production was higher in 2016 by 110.85 % compared to 2010. Average export of wheat in the tested period was 41750 t and notes a tendency to increase with a rate of 25.67% and average import of wheat was 398750 t and it showed tendency to increase, with a rate of 0.82%.

The basic direction for the future development of the agro-industry in B&H and in Serbia is the optimal use and available production capacities, increasing the volume of agricultural production of goods that are continuously imported. It is necessary to change the production structure in favour of intensive production intended for export, of production of high-final and high-quality products, while improving competitive position, in order to meet domestic demand and significantly reduce the import of agricultural food products.

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PREDICTORS OF WOOD QUALITY OF TREES IN PRIMARY FORESTS IN THE SOUTHERN CARPATHIANS

SUMMARY

Functional mechanisms in primary forests can help us discover structural models that can be applied to managed forests. Wood quality is important in silviculture because it influences timber price. Using the structure and tree form of primary forests in the Southern Carpathians as a model, our research objectives were to determine the relationships between wood quality and factors such as coenotic position of trees, the ratio of diameter at breast height (DBH) to tree height, and tree slenderness. Wood quality was strongly related to the coenotic position of trees (r = 0.89*–0.99***); suppressed (or dominant) trees had higher quality wood than dominant (or suppressed) trees. Wood quality and slenderness were also highly correlated (r = 0.56–0.95***) in most plots – the more slender the tree, the lower its wood quality. We found that trees with the highest quality wood were in the 30–100 cm DBH range. Therefore, DBH is a reliable predictor of wood quality for uneven-aged forests, providing quick results with little effort.

Keywords: Uneven-aged forests, wood quality, Southern Carpathians, primary forests, silvicultural models.

INTRODUCTION

Primary forests represent one of nature's assets of incalculable value (Bândiu et al. 1995), and are characterised by perfection which cannot be completely imitated by modern silviculture. The virgin forests and uneven aged forest (managed) are understood as stable ecosystems, with a specific equilibrium, from the point of view of structure and composition (Boncina et al. 2014) and provide a continuity of the stand. Concerns about primary ecosystems have increased with the negative effects caused by anthropogenic pressures, such as pollution and logging (Stoiculescu 2013, Curovic et al. 2011). In western part of Europe, because of intensive forest utilisation, most of virgin forests has disappear, but in the Southern Carpathians, large tracts of virgin forest still remain (Reininger 1997, Giurgiu 2013), which need urgent study due to the scientific value they

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have through the lack of human activities (Curovic et al 2013). The aim of such research should be to develop our understanding of primary forest structure, and to apply this knowledge in practice for managing protected areas covered by forests and for defining a model for managing uneven-aged forests (Král et al. 2018). In the past, research on the quality of wood was conducted only in even-aged forests (Pretzsch and Rais 2016). However, many old trees are found in uneven-aged forests, which could provide wood of superior quality. Therefore, it is important that such trees in uneven-aged forests are studied, because the quality of wood can be the defining property that affects timber price (Zhang 1997, Macdonald et al. 2009, Höwler et al. 2019). Such research may yield results that can act as a model for silvicultural treatments (Boncina 2014), so that trees in managed forests may be developed to imitate, as far as possible, trees shaped by nature in primary forests. In addition to engendering superior timber quality, uneven-aged forest structures also ensure high biodiversity (Paraviainen 2005, Giurgiu 2013).

The research objectives of this study were: (1) to describe the relationship between the coenotic position of trees and wood quality; (2) to determine the relationship between wood quality and the ratio of diameter at breast height (DBH) to tree height; and (3) to characterise the relationship between DBH and wood quality.

MATERIAL AND METHODS

Study area

The study areas were situated in the Southern Carpathians in the Semenic, Retezat, Făgăraș, and Penteleu Mountains (Figure 1). Identification of those types of ecosystems was determined according to identification criteria of virgin forests (Korpel 1978, Leibundgut 1982). The permanent research plots (PRPs) in this study are located in protected areas (National and Natural Parks and Natura 2000 sites) and they never been directly influenced by humans.

The PRPs had an uneven-age tree structure, with a large number of trees (between 385 and 475 trees) some of which were very old (Table 1). Altitudinally, the plots were situated between 1151 m and 1352 m in the temperate continental zone, with precipitation and temperature determined by altitude and the orientation of the mountain peaks with respect to the direction of air masses.

Ambient temperature ranged from −11 °C (Retezat) in the winter to 16 °C in the summer (Semenic and Penteleu).

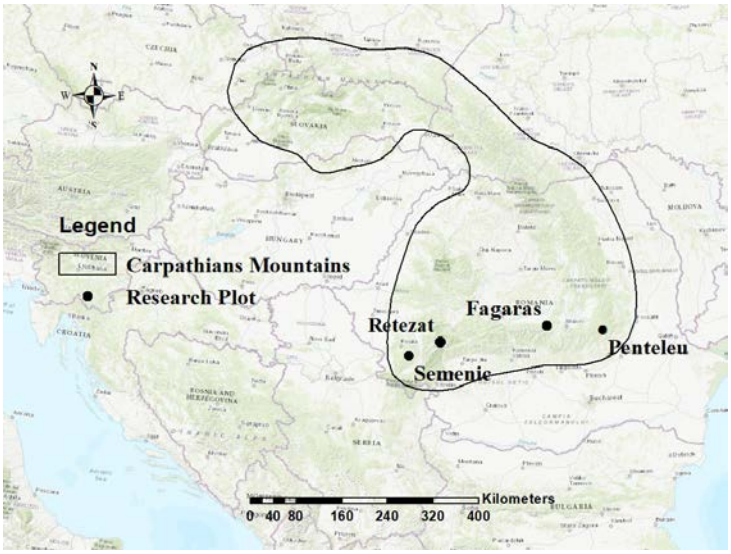

Figure 1. Location of research plots (Natural Earth 2015, Esri 2019)

Table 1. Characterisation of permanent research plots in the Southern Carpathians.

Plot	Geographic coordinates	Relief Altitude	Species composition Production class Soil type	Number of trees Area and shape Volume
Semenic	45°8'25" E 22°4'41" N	Slope: 26° 1352 m	100% beech Superior Dystric cambisol	385 1 hectare (circular) 677 m^3
Retezat	45°22'32" E 22°46'40" N	Slope: 42° 1151 m	45% beech, 29% spruce, 4% fir, 22% hardwood Middle Dystric leptosol	413 1 hectare (rectangle) 869 m^3
Făgăraș	45°39'40" E 25°10'2" N	Slope: 40° 1214 m	45% beech, 55% fir Superior Eutric cambisol	475 1 hectare (circular) 758 m^3
Penteleu	45°36'43" E 26°25'47" N	Slope: 36° 1128 m	40% fir, 30% beech, 30% spruce Superior Eutric cambisol	439 1 hectare (circular) 803 m^3

Field sampling and analyses

Each research plot had a surface area of 1 ha; three of the plots were circular (Semenic, Făgăraș, and Penteleu) and one was rectangular (Retezat) in shape. All the trees in the research plots (with DBH higher than 7 cm) were inventoried and the following tree characteristics were collected: DBH, height, species, wood quality, and coenotic position. For the diameter measurement was used a measuring tape and the height was measured with the Vertex IV hypsometer. The coenotic position was estimated by field observations.

Statistical analysis was conducted using the 'psych' package (Revelle, 2017) in R software (R Core Team, 2018), and the plot package in R software was used for graphical representation of the data. Volume was established for each individual tree using the equation:

$$\log v = a_0 + a_1 \log d + a_2 \log^2 d + a_3 \log h + a_4 \log^2 h,$$

where d is DBH (cm), h is height (m), and a_0, …, a_4 are the regression coefficients (spruce: $a_0 = -4.18161$, $a_1 = 2.08131$, $a_2 = -0.11819$, $a_3 = 0.70119$, $a_4 = 0.148181$; beech: $a_0 = -4.11122$, $a_1 = 1.30216$, $a_2 = 0.23636$, $a_3 = 1.26562$, $a_4 = -0.079661$; fir: $a_0 = -4.46414$, $a_1 = 2.19479$, $a_2 = -0.12498$, $a_3 = 1.04645$, $a_4 = -0.016848$) established for the most important species in Romanian forests (Giurgiu et al. 2004).

The methodology for determining the quality of the wood involved estimating the proportion of wood defects (stem and crown attributes) relative to the total height of the tree, according to the type of species (softwood or hardwood; Giurgiu 2004). Stem shape defects were stem eccentricity, taper, and slenderness, and crown defects were crown diameter, length, and eccentricity.

RESULTS AND DISCUSSION

Previous studies have identified a significant relationship between stand structure and wood quality, represented by stem and crown attributes (Pretzsch and Rais 2016). Most importantly, for accurate results, these attributes have to be measurable (Pretzsch and Rais 2016). Compared to trees in even-aged forests, those in uneven-aged and primary forests have a greater capacity for growth (Pretzsch and Schütze 2009, Pretzsch et al. 2010, 2015, Metz et al. 2013, Höwler et al. 2019) due to intra- and inter-specific competition, resulting in higher quality wood (Pretzsch and Rais 2016).

In this study, the PRPs contained enough trees to provide sufficient data for statistical analysis (Table 2) and ensure homogeneity of data. The number of trees varied between 385 and 475, higher than the average of 347 trees per hectare found in other studies (Curovic et al 2013). The

volume of the trees had values between 677 m^3 and 869 m^3 per hectare, which corresponds to the average values (796 m^3 per hectare) found in other virgin forests of the Eastern Carpathians and the Dinaric Alps (Keeton et al 2010, Curovic et al 2013).

Most of the trees had high quality wood, with the values falling in the upper quality classes. The wood quality values ranged between 1.80 and 2.26, and were normally distributed, with skewness close to 0 in all but one PRP. For Semenic PRP, skewness was 1.05, which only slightly exceeded 1, which is the threshold value for non-normal distribution (Hair et al. 2017).

Table 2. Statistical parameters of wood quality. Wood quality was determined as the proportion of wood defects in the stem and crown relative to the total height of the tree. Stem shape defects were stem eccentricity, taper, and slenderness, and crown defects were crown diameter, length, and eccentricity.

Research plot	Number of trees	Mean	S.D.	Min	Max	Skewness	Kurtosis
Semenic	385	1.80	0.95	1	4	1.05	0.14
Retezat	413	1.90	0.93	1	4	0.21	−1.80
Făgăraş	475	2.26	0.92	1	4	0.30	−0.74
Penteleu	439	2.01	0.97	1	4	0.53	−0.81

Wood quality in relation to stand distribution

Based on of the properties of sawn timber, trees from the PRPs were classified into four main classes: class 1included trees with the highest quality wood (those with the fewest defects) and class 4 included trees with the lowest quality wood (with many defects in stem and crown attributes), with classes 2 and 3 being intermediate in quality (Fig. 2).

Most trees in most of the plots were in classes 1 and 2 (tending towards superior wood quality) with fewer defects, except for the trees in the Retezat PRP, which were in classes 2 and 3. Other research, in those types of forests, indicate that the site class and site index not always have a good correlation (Curovic et al. 2011*) but in this case they have. Although previous research in managed forests have shown that wood quality declines with tree age (Guiman, 2007), this was not the case for trees in our plots. Our results suggest that natural selection may have resulted in the survival of trees with high-quality wood, and identification of selective pressures may provide solutions for forest management that could lead to stands with superior wood quality.

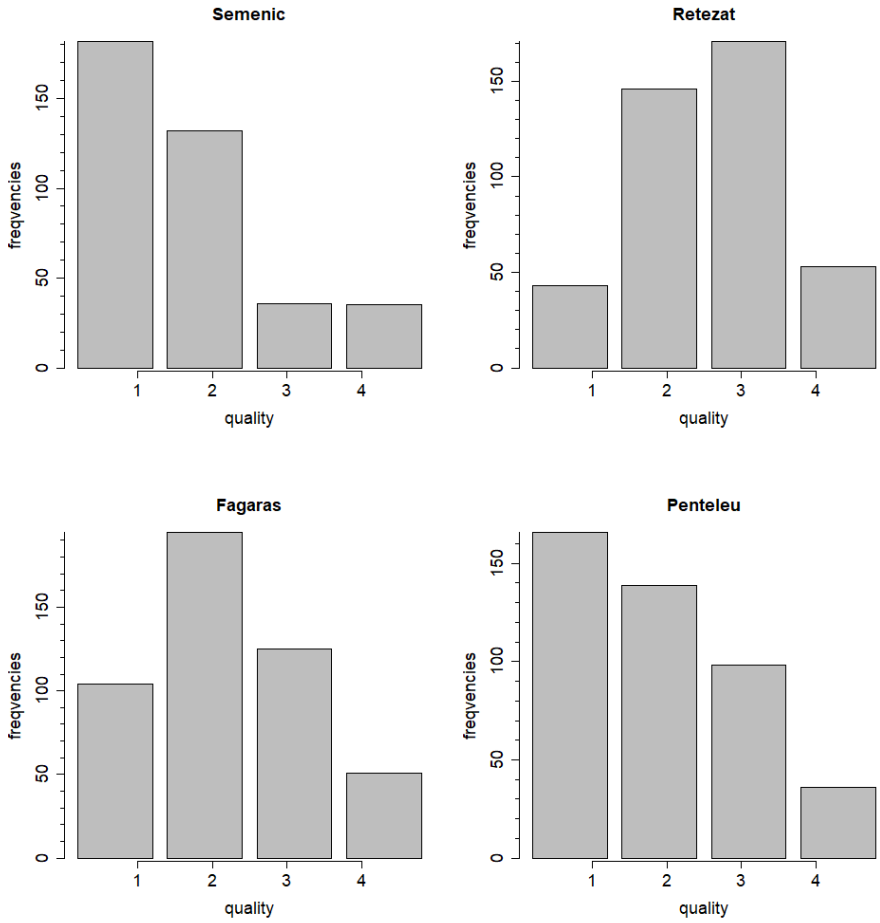

Figure 2. Frequency trees in relation to wood quality.

Relationship between wood quality and coenotic position

In uneven-aged forests and primary forests, tree stands can be characterised in relation to coenotic position as follows: inferior floor (where suppressed trees are found); middle floor (where codominant trees are found); and superior floor (where the dominant trees are found). In this study, there was a strong relationship between wood quality and coenotic position, with high correlation coefficients (r) ranging from 0.89* to 0.99*** (Fig. 3).

Our results also showed that the quality of wood was higher in the first coenotic floor and lower in the third coenotic floor in all research plots. The wood quality in all the plots was very high, with the highest identified in the Semenic PRP (1.27) and the lowest in Retezat Zănoaga (2.65).

The shape of the stem is a good indicator of the predisposition of trees to windthrow and snow-throw (Wilson and Oliver 2000, Harrington et al. 2009); stem shape is influenced by silvicultural treatment (Brazier,

1977) and coenotic position (Bruchert et al 2000). Our results show that the stands in the PRPs are stable and appear to be able to withstand uprooting by wind or snow.

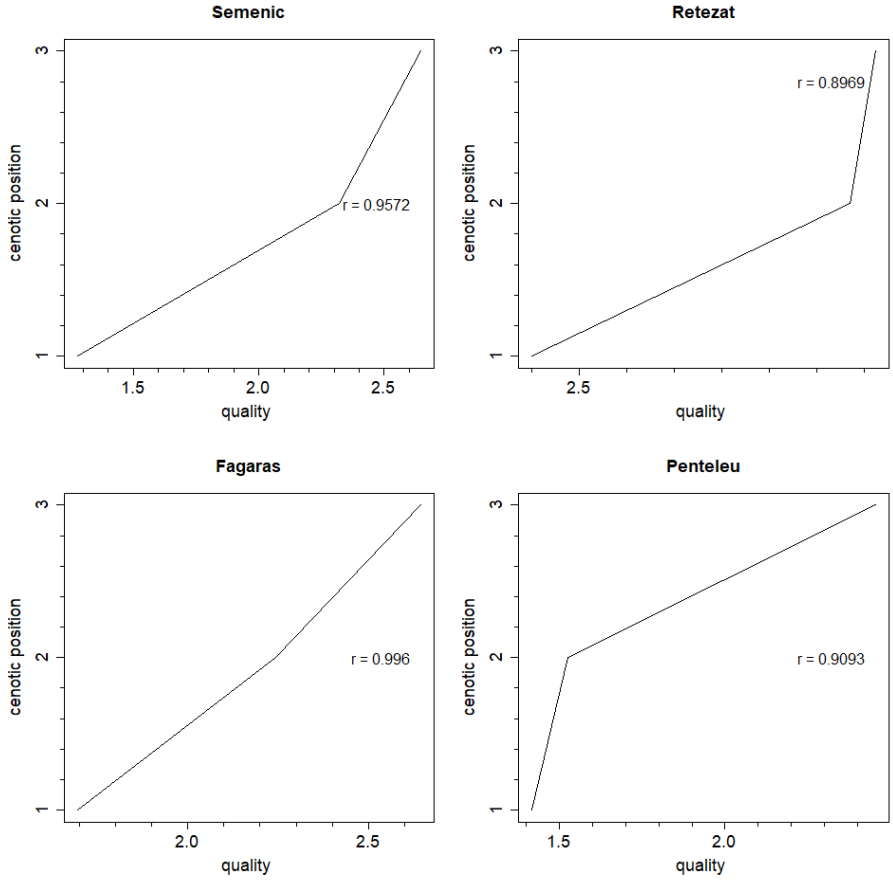

Figure 3. The relationship between wood quality and the coenotic position of trees.

Given the relationship between wood quality and coenotic position, the latter could be used as an indicator in forest management; stand quality could be determined by simply identifying the coenotic position of trees, without having to measure tree parameters relating to quality.

Relationship between slenderness (the height-to-diameter ratio) and wood quality

Many studies have shown that stem shape is a good indicator of wood quality both for individual trees and for entire stands (Roth et al. 2007, Lasserre et al. 2009, Lindstrom et al. 2009, Lenz et al. 2012, Searles 2012, Pretzsch and Rais 2016). It is important to use indicators that are easy to measure; one such is

the height-to-diameter ratio or slenderness (Pretzsch and Rais 2016), which has yielded good results for predicting wood quality in previous studies (Reukema and Smith 1987, Kijidani et al. 2009, Lindstrom et al. 2009). In our study, there was a strong correlation between wood quality and slenderness (Fig. 4), with correlation coefficients ranging between 0.56 (Retezat) and 0.95*** (Făgăraș).

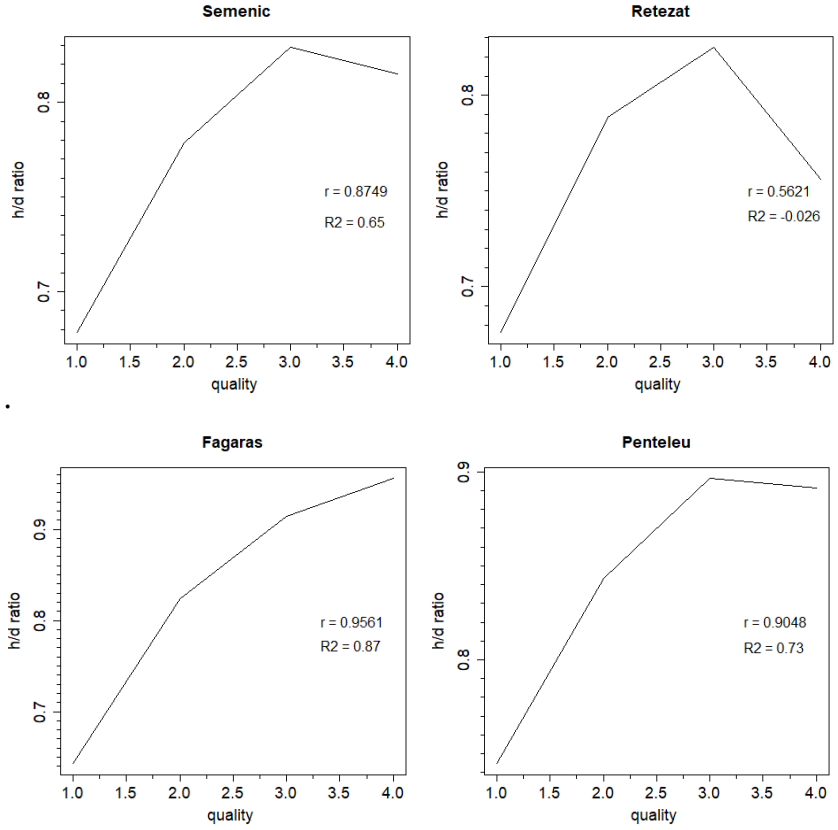

Figure 4. The relationship between wood quality and slenderness of trees.

Another aspect highlighted in this study was that the loss of wood quality was greater as slenderness increased; the slenderness index for class 1 (superior) of wood quality was about 0.7, whereas it was 0.8–0.9 for class 4 (inferior) of wood quality. All PRPs except Retezat had strong correlations between slenderness index and wood quality.

Similar results have been obtained in other studies (r^2 = 0.52; Pretzsch and Rais 2016). In the case of Retezat PRP, the number of trees with lower quality wood was high, which may have resulted in a weaker relationship between slenderness and wood quality.

Relationship between DBH and wood quality

The wood quality of the stand is determined by every individual tree within the stand, and the wood quality of individual trees is influenced by the degree of competition with surrounding trees and by silvicultural treatment (Zingg and Ramp 2003, Höwler et al. 2017). Characteristics of wood quality that can be influenced by silvicultural treatment are DBH, size and abundance of knots, crown development, stem curvature, and the proportion of juvenile wood (Hein 2008 van Leeuwen et al. 2011, Richter 2015). Among these characteristics, DBH and knottiness have the highest influence on wood quality and timber price (Ammer, 2016). Figure 5 shows a relatively stable relationship between DBH and wood quality, in which juvenile trees are found in lower wood-quality classes. This strong relationship was highlighted by statistically significant correlation coefficients ($r_{Semenic}$ =-0.66***, $r_{Penteleu}$ = -0.59***), except for Retezat (r = 0.25*) and Făgăraș (r = -0.32***) PRPs where the correlations were weak.

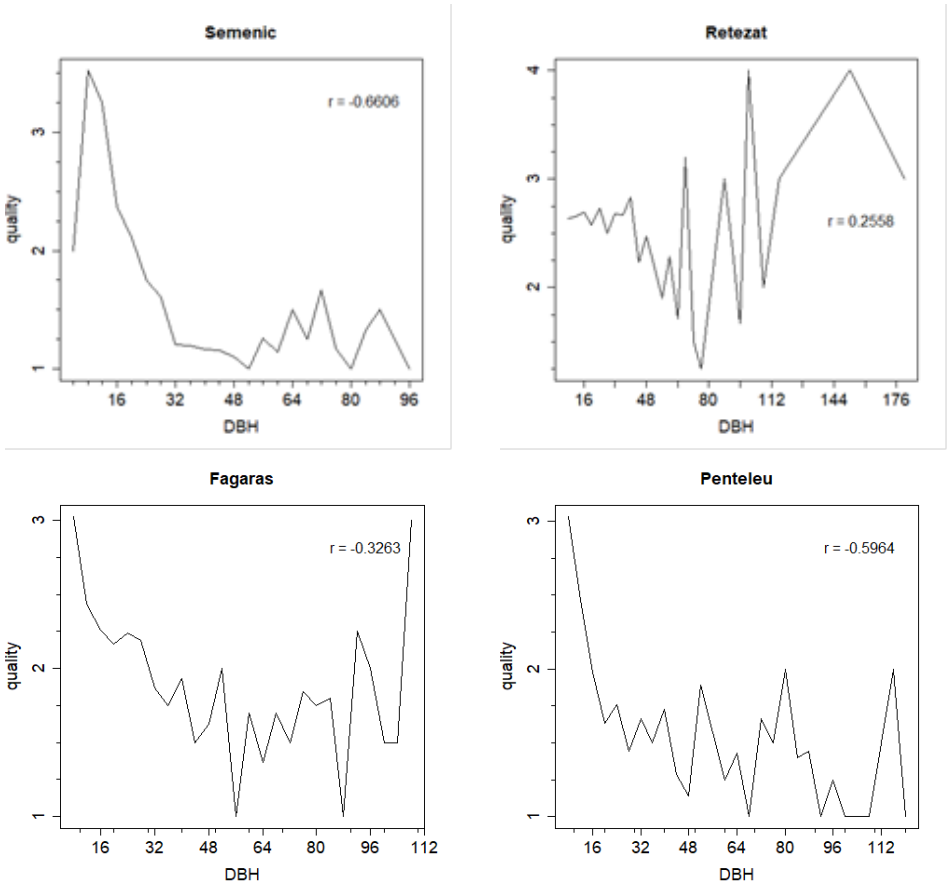

Figure 5. The relationship between wood quality and DBH of trees.

In all the plots, the DBH of trees with the highest quality wood ranged between 30 cm and 100 cm; this range is wider than the that found in other studies (e.g. 30–40 cm; Prestemon and Buongiorno 2000). The diameter limit for selective cutting, according to production class, in Romanian forests is set at 48–100 cm (MWFEP 2000, Chișca et al. 2018); our results confirm that this range is appropriate for identifying trees with higher quality wood.

CONCLUSIONS

Due to environmental changes and the impact of anthropogenic pressures, research on primary forests has intensified. Primary forests represent a worthy model for management and structuring of managed forests. In this study, we investigated aspects of wood quality in primary forests located in the Southern Carpathians to develop solutions for selected treatment of uneven-aged forests.

The statistical analysis highlighted that the wood quality of stands in most of the PRPs was normally distributed. In addition, most of the trees were found in wood quality classes 1 and 2 (superior wood quality) with fewer defects, except in the Retezat PRP where most of the trees were in quality classes 2 and 3 (intermediate quality). This suggests that natural selection may lead to better stand quality than silvicultural treatment.

The relationship between wood quality and coenotic position was strong, with statistically significant correlation coefficients. Trees from the first coenotic floor had higher quality wood than trees from the third coenotic floor. Therefore, the coenotic position of the tree could be used as an indicator for predicting the quality of its wood without measuring it for wood parameters. The relationship between slenderness and wood quality was also shown to be strong, with wood quality increasing as slenderness decreased.

Because the price of timber depends on the quality of the wood, it is important to design a simple methodology for quickly determining wood quality without having to take too many measurements. Our study found a strong correlation between DBH and wood quality; the DBH of trees with the highest quality wood ranged between 30 cm and 100 cm.

Uneven-aged forests are superior than even-aged forests because they provide greater stability, continuity, and protection for trees. In addition, they provide higher quality wood and a greater quantity of timber. Therefore, it is necessary for modern forestry to follow the model that nature offers in primary forests by imitating their actions in managed forests.

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REVIEW ON *PINNA RUDIS* (LINNAEUS, 1758) (BIVALVIA: PINNIDAE) PRESENCE IN THE MEDITERRANEAN

SUMMARY

Pinna rudis (Linnaeus, 1758) or the rough pen shell, has Atlantic-Mediterranean distribution, and prefers warmer waters and harder substrates. Species is threatened by different factors and it is listed on Annex II of the Bern and Barcelona Convention. Some changes in species distribution are evident, most probably related to climate changes. Until 2018 presence of this species in the Adriatic Sea had not been confirmed with certainty, when it was find for the first time in Boka Kotorska Bay, what also presents first certain findings of the species in the Adriatic Sea. In this paper review on species presence along Mediterranean coast is given. Collected data indicate that species is more common in west Mediterranean compare to east and north part, and that future monitoring and status of population is required.

Key words: Pinnidae, *Pinna rudis*, Mollusca, Mediterranean

INTRODUCTION

The Mediterranean Sea is a marine biodiversity hot spot with approximately 17,000 species (Coll *et al*., 2010). It is under "pressure" of alien species due to different factors such as climate changes. According to Webster (2007), Garrabou *et al*. (2009) and Mačić *et al*. (2014) the seawater temperature increase in the Mediterranean has affected the distribution and abundance of native as well as alien species. Albano (2013) indicated that some molluscs species have been proposed as descriptors of change of the biodiversity of the Mediterranean Sea under climate warming forcing. The same author described the changes in distribution of *Echinolittorina punctata* (Gmelin, 1791) and *Eastonia rugosa* (Helbling, 1779) trough Mediterranean and mentioned that some species such as *Pinna rudis* (Linnaeus, 1758) could be more difficult to monitor because of their cryptic habitat and low density. Oliverio (1997) found the *P. rudis* at unusually high latitudes in the Tyrrhenian Sea, what according to Albano (2013), gives some signal of change which are probably related to climate changes. Bearing in mind that it is known that the indigenous thermophilic species which inhabit warmer waters are able to appear in more northern and colder parts of the

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Mediterranean due to the global warming (Coll *et al*., 2010), finds of *P. rudis* in the Adriatic Sea are not surprising.

Changes in marine ecosystem usually have several causes, and their joint effect with degrading pressures on the living world of the sea has a critical role in maintaining diversity of species, ecosystem health and achieving good ecological status. Seas and oceans hide huge quantities of waste under the surface, representing a global landfill for decades. Waste accumulation and poor pollution prevention is a threat that will increasingly cost the generations to come (Mandić *et al*., 2018).

Studies on *P. rudis* population are scare and one of the main reasons is most probably linked to the low population densities (García-March & Kersting, 2006). Because of that each individual is important for the population sustainability. Although species is distributed through the Atlantic Ocean and the Mediterranean, it is not common as *Pinna nobilis* (Linnaeus, 1758).

The aim of this paper was to collect all available literature data about *P. rudis* presence in the Mediterranean and to provide map about its distribution.

MATERIAL AND METHODS

Papers for this review were collected mainly through keyword searches of Google scholar databases. We focused on the peer-reviewed journal literature, and also included material from books, reports as well as conference papers. Based on available literature the map about *P. rudis* presence/findings in the Mediterranean is given. The references indicated near some country names means that the species is mentioned as occurring near this country but without a precise location. For clarity, those publications indicating the existence of *P. rudis* in the Mediterranean Sea without offering a precise locality have been excluded. For example, Cosentino & Giacobbe (2006) mentioned that individuals of *P. nobilis* and *P. rudis* were collected from North Africa and the West and East Mediterranean, including the Adriatic Sea, but did not mention from which region which species was collected, therefore this publication is excluded.

RESULTS AND DISCUSSION

The rough pen shell *Pinna rudis* (Linnaeus, 1758)

Classification (WoRMS, 2019)
Kingdom: Animalia
Phylum: Mollusca
Class: Bivalvia
Subclass: Pteriomorphia
Order: Ostreida
Superfamily: Pinnoidea
Family: Pinnidae
Genus: Pinna
Species: *Pinna rudis* (Linnaeus, 1758)

Synonyms (WoRMS, 2019)
Pinna chautardi var. annobonensis (Alvarado & Alvarez, 1964)
Pinna elongata (Röding, 1798)
Pinna ferrugines (Röding, 1798)
Pinna ferruginosa (Röding, 1798)
Pinna mucronata (Poli, 1795)
Pinna paulucciae (Rochebrune, 1883)
Pinna pernula (Chemnitz, 1785)
Pinna rudis var. belma (de Gregorio, 1885)
Pinna rudis var. blama (de Gregorio, 1885)

Description

The Pinnidae family is a small family of large, fan-shaped "pen shells", belonging to order of Ostreida (Lemer *et al*., 2016). The family includes two genera, Atrina and Pinna (Lemer *et al*., 2014) and 61 species (Vásquez-Luis *et al*., 2017), which live either completely or half-buried in sand, mud or gravel bottoms, anchored in the substrate by byssus threads.

The species are large in size, usually between 15 – 35 cm, but they can be up to 120 cm (*Pinna nobilis* Linnaeus, 1758) (Huber, 2010). Most species have an Indo-Pacific distribution, but some species have been reported from the Caribbean Sea, West Africa, the Mediterranean Sea, the North East Atlantic, and west of America (Vásquez-Luis *et al*., 2017).

P. rudis (Linnaeus, 1758) is distributed throughout the warm waters of the Mediterranean Sea as well as the Atlantic Ocean (Poppe & Goto, 1993; Huber, 2010). It prefers gravel bottoms from below the low tide line down to 40 m (Poppe & Goto, 1993), but it is also found in Posidonia meadows (Garzía-March & Kersting, 2006).

The species is threatened by different factors, including anchoring, fishing, collection and habitat disruption (Tunesi *et al*., 2006) and it is listed by Annex II of the Bern Convention as a strictly protected species and by Annex II of the Barcelona Convention as a threatened or endangered marine species.

Species can reach dimensions up to 50 cm (Garzía-March & Kersting, 2006). The external color of the valves is reddish brown. The valves are symmetrical, triangular, transparent at the ends and also very fragile with 5 to 10 radiating ribs, covered with large scales arranged in quite regular rows. Morphologically, *P. rudis* can be confused with *P. nobilis* juveniles but in *P. rudis* the shell is more triangular and robust with fewer and larger protruding scales.

Adult *P. nobilis* exceed the size of *P. rudis* and lose the protuberances on the surface of the shell. The mantle border is usually white and iridescent in *P. rudis* and pink in *P. nobilis*. The nacre lobes on the inner side of the valves are of a similar size in *P. rudis* (Garzía-March & Nardo, 2006).

Mediterranean records

Most authors, e.g., Poppe & Goto (1993), UNEP/MAP/MED POL (2005) and Huber (2010), mentioned that *P. rudis* is present only in the warmest area of the Western Mediterranean. Based on all collected data, it is evident that species is more abundant in western Mediterranean waters, especially along the Spanish coast. Data about *P. rudis* presence in the East Mediterranean are rare (Öztürk & Çevik, 2000; IOPR, 2003; El-Komi *et al*., 2007; UNEP, 2011; Salomidi *et al*., 2016; Aguilar *et al*., 2018), while presence of species in the Adriatic Sea was certainly confirmed recently (Petović, 2018). Records of *P. rudis* trough Mediterranean waters, based on all collected literature data, are given in Figure 1.

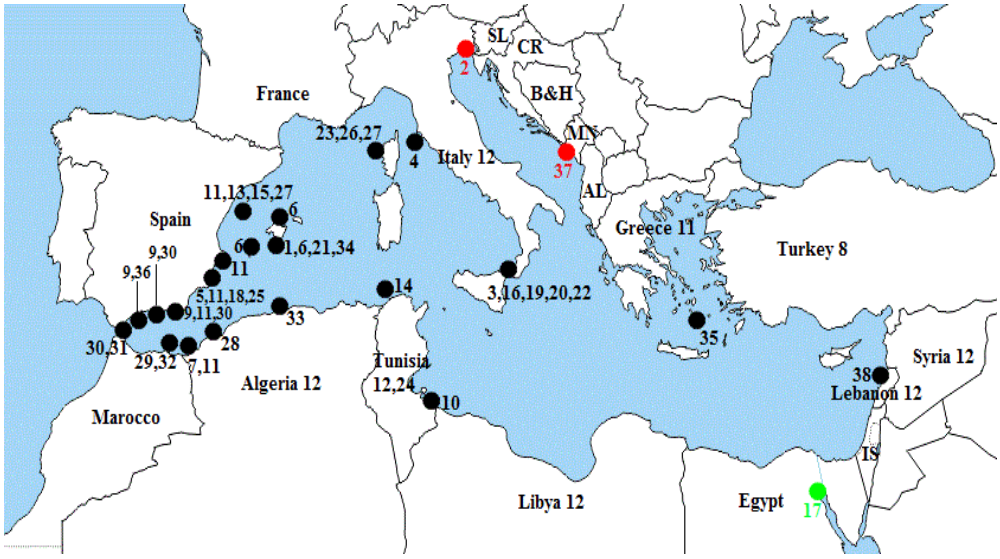

Figure 1. Mediterranean records of *Pinna rudis.* SL – Slovenia, CR – Croatia, B&H – Bosnia and Herzegovina, MN – Montenegro, IS – Israel.

1. Hidalgo (1917); 2. Stackowitsch (1984); 3. Giacobbe & Rinelli (1991); 4. Oliverio (1997); 5. Richardson *et al*. (1997); 6. Ballesteros (1998); 7. Guallart (2000); 8. Öztürk & Çevik (2000); 9. Templado (2001); 10. Mustapha *et al*. (2002); 11. Garzía-March (2003); 12. IOPR (2003); 13. Garzía-March & Kersting (2006); 14. Garzía-March & Nardo (2006); 15. Sempere *et al*. (2006); 16. Cosentino & Giacobbe (2007); 17. El-Komi *et al*. (2007); 18. Giménez-Casalduero *et al*. (2009); 19. Crocetta *et al*. (2009); 20. Giacobbe *et al*. (2009); 21. Jimenez *et al*. (2010); 22. Vazzana (2010); 23. Vicente (2010); 24. UNEP (2011); 25. Giménez-Casalduero *et al*. (2011); 26. Vicente & Trigos (2012); 27. Trigos *et al*. (2013); 28. Boumediene & Djillali (2014); 29. Espinosa *et al*. (2014); 30. García-Gómez *et al*. (2014); 31. Department of the Environment and Climate Change (2015); 32. Espinosa *et al*. (2015); 33. Bachertazi et al. (2016); 34. Nebot-Colomer *et al*. (2016); 35. Salomidi *et al*. (2016); 36. Urra *et al*. (2016); 37. Petović (2018); 38. Aguilar *et al*., 2018

Spain (including Gibraltar)

Based on all collected data we found the highest number of *P. rudis* records along the Spanish coast, mainly southern part of the coast as well as Balearic, Columbretes and Chafarinas islands. Hidalgo (1917) reported *P. pernula* (synonym for *P. rudis*), for Cabrera islands. Richardson *et al*. (1997) examined *P. rudis* and *P. nobilis* shell on presence of *Pontonia pinnophylax* (Otto, 1821) in Carboneras, Aguamarga and Villaricos. Ballesteros (1998) reported *P. rudis* for the whole area of Balearic archipelago and mentioned that it is not abundant as *P. nobilis*. In soft sediments of Chafarinas islands, Guallart (2000) have been found mean density of *P. rudis* 0.7 ind./m^2, and rates *P. nobilis*/*P.rudis* of 4.6:1. Templado (2001) observed *P. rudis* in area between Gibraltar Strait and Almería Bay, with greater density of individuals respect to *P. nobilis*. Garzía-March (2003) report that the relative abundance of *P. rudis* grows southwards, and that species is abundant in the Almería seagrass meadows. Author has also been found species in the Moraira bay, the Chafarinas islands, Murcia and Almería, inside the meadow of *P. oceanica* coexisting with *P. nobilis*. Garzía-March & Kersting (2006) gave the data about distribution and density of *P. rudis* and *P. nobilis* in the Columbretes Islands Marine Reserve.

By island groups, authors observed the densest population in El Carallot (1 ind./100 m^2), followed by L'Illa Grossa (0.3 ind./100 m^2), La Ferrera (0.3 ind./100 m^2) and La Forada (0.2 ind./100 m^2). For the same area Sempere *et al*. (2006) indicate *P. rudis* as not so abundant species and gave morphometric parameters of some individuals. Jiminez *et al*. (2010) indicate *P. rudis* presence in area of Cabrera islands (Balearic islands). Giménez-Casalduero *et al*. (2011) listed *P. rudis* in coastal part of Murcia as protected species listed on Annex II of Barselona and Bern Convention. In Columbrete islands low density of *P. rudis*, 0.3 ind./100 m^2, is reported by Trigos *et al*. (2013). Authors also reported that individuals are apparently separated from each others. Presence of *P. rudis* in area of Gibraltar is reported by García-Gómez *et al*. (2014) and Department of the Environment and Climate Change (2015). García-Gómez *et al*. (2014) indicate *P. rudis* on different types of substrates used in the construction of port structures and breakwaters (dolomitic quarried rock, cubic concrete block and concrete tetrapod) in the Strait of Gibraltar and the Mediterranean coast of Andalucía and Gibraltar, specifically in Ceuta, Tarifa, Algeciras, La Línea, Gibraltar, La Mamola and Motril, while in Department of the Environment and Climate Change (2015) *P. rudis* was listed for British Gibraltar territorial waters on sub-tidal rocky reef, both natural and artificial. Nebot-Colomer *et al*. (2016) in their study provides quantitative information on the population structure, age and growth of the pen shell in a marine protected area (Archipelago of Cabrera – Balearic islands) and due to the low densities observed, authors noted that each individual is important for the sustainability of the population. On the coastline between Punta de Calaburras and Calahonda presence of *P. rudis* is noted by Urra *et al*. (2016). Although, during 2017 along Spanish coast mass mortality of

P. nobilis due to parasite infection was mentioned (Vázquez-Luis *et al*., 2017; Martinović *et al*., 2019), *P. rudis* was not affected (Vázquez-Luis *et al*., 2017).

Marocco

In Marocco waters *P. rudis* is mentioned by (Espinosa *et al*., 2014; 2015) in Cap des Trois Fourches (Northern Marocco). Espinosa *et al.* (2014) did characterizing of the marine biodiversity in Cap des Trois Fourches, while Espinosa *et al*. (2015) gave spatial distribution of dominant assemblages in order to allow the implementation of MPA in the Cap des Trois Fourches. In both studies *P. rudis* was listed as endangered species present in this area.

Italy (without Adriatic coast)

In IOPR (2003) species is just mentioned for the Italy and it is concluded that inventory on this species is one of future topic that should be developed. Oliverio (1997) observed *P. rudis* at unusually high latitudes in the Tyrrhenian Sea (Tuscan Archipelago). Five other studies listed species as present in area of Messina. Giacobbe & Rinelli (1991) examined shell of *Pinna nobilis* and *Pinna rudis* as habitat of the rare echinoid *Arbaciella elegans* (Ole Theodor Jensen Mortensen, 1910). Consentino & Giacobbe (2007) gave composition and structure of epizoobiontic mollusc assemblages on both *P. nobilis* and *P. rudis* shell. Crocetta *et al.* (2009) listed the most common macro-molluscs, including *P. rudis*, in association with alien Mollusca along the Calabrian shores of the Messina Strait. Giacobbe *et al.* (2009) suggest that the occurrence of both *P. nobilis* and *P. rudis* in the Faro lake had in the ancient age a remarkable symbolic value, probably linked to the economic importance of the byssus production, and that the occurrence of these two protected species adds a high historical and cultural value to the ecological heritage of the protected area of Capo Peloro. Vazzana (2010) studied the Mollusks fauna occurring in the bottom sediment off Scilla (Messina Strait) and listed *P. rudis* as present in this area.

France

Only one location (Nature Reserve Scandola – Corsica) with *P. rudis* presence is listed for France (Vicente, 2010; Vicente & Trigos 2012; Trigos *et al*., 2013). Results about *P. rudis* and *P. nobilis* monitoring in Nature Reserve Scandola are given in two reports (Vicente, 2010; Vicente & Trigos, 2012). Species is described as not so common and a few individuals are monitored since 2000 year. Vicente & Trigos (2012) indicate that most probably larvae of *P. rudis* originated from Spain and by sea currents arrived to the coast of Corsica. Trigos *et al.* (2013) reported low density of *P. rudis*, 0.25 ind./m^2 in this Nature Reserve.

Tunisia

In Tunisia IOPR (2003) and UNEP (2011) mentioned species without precise location. As for the Italy coast, also for Tunisia in IOPR (2003) this

species is just mentioned without precise locality, while UNEP (2011) just gave the list of Tunisia Mollusks including *P. rudis*. Mustapha *et al*. (2002) described Tunisian mega benthos from infra and circalittoral sites and found largely spread coverage of *P. rudis* and *P. nobilis* in El Bibane lagoon where authors also found very special kind of sponge reefs "buildups", recorded for the first time in the Mediterranean. Garzia-March & Nardo (2006) reported a dense population of both *P. rudis* and *P. nobilis* estimated to consist of more than 30 ind./100 m^2 in Galite Islands Marine Park.

Algeria

In Algeria waters IOPR (2003) also listed *P. rudis* without precise location, and indicate that inventory on this species should be developed in future. Boumediene & Djillali (2014) in their observation of the macrozoobenthos biodiversity of Oran coastal area indicate *P. rudis* as part of middle horizon biocenosis (3-15 m depth), while Barchertazi *et al*. (2016) observed a significant number of ecological, patrimonial, protected statuses, threatened, endemic and invasive species in their study about biodiversity of Agueli island, and mentioned *P. rudis* as protected one in this area.

Israel

Israel is the country for which any literature data about *P. rudis* presence was not found, even any data which just mention species as present on the Israel coast.

Egypt

In Egypt species is indicated in Suez Canal, but in El-Ein El-Sukhna station (El-Komi *et al*., 2007) which is actually part of the Red Sea, so it can not be consider as Mediterranean record. When we have in mind only Mediterranean waters Egypt can also be considered as the country without any record of *P. rudis*.

Turkey, Syria, Libya

For Turkey, Syria and Libya only one record of each of the country was found, and in those sources, species was mentioned without a precise location (Öztürk & Çevik, 2000; IOPR, 2003). Öztürk & Çevik (2000) just indicate that three members of Pinnidae family, *P. rudis*, *P. nobilis* and *Atrina pectinata* inhabit the coast of Turkey. IOPR (2003) just mentioned *P. rudis* for Syria and Libya.

Lebanon

For Lebanon also IOPR (2003) just mentioned *P. rudis*, while Aguilar *et al*. (2018) in „Deep Sea Lebanon" publication, report *P. rudis* in area of Batroun in abundance of 1-10 ind./area in detritic sediments. In other investigated areas (Beirut, Jounieh, Sayniq and St. George) species has not been found.

Greece

Two literature sources were found for Greece. IOPR (2003) indicate species for Greece waters without a precise location. Salomidi *et al*. (2016) indicate *P. rudis* for Santorini island complex in the Aegean Sea (Nea Kameni island, Aspronisi island and south-west part of Thera island) in the study about getting qualitative and quantitative information on the current ecological state and future potential of the Santorini island as MPA.

Adriatic Sea (Adriatic coast of Italy, Slovenia, Croatia, Bosnia and Herzegovina, Montenegro, Albania)

Based on the available literature, there was only one record of the species for the Adriatic Sea, provided by Stackowitsch (1984) who reported *P. pernula* (synonym for *P. rudis*) from the Gulf of Trieste (Italy). This author mentioned a high abundance (approx. 5 ind. /m^2) and that all the individuals were dead, but did not give any pictures of individuals and did not provide any further discussion. Abundance, 5 ind./m^2 is quite questionable, as so high abundance was not mentioned for western areas where species is more common. By 2016 the presence of *P. rudis* in the Adriatic Sea had not at any point been confirmed with certainty (Prvan & Jakl, 2016).

First certain record of *P. rudis* for the Adriatic Sea is reported by Petović (2018). Author mentioned species (only empty shells) from Kostanjica to Risan (Boka Kotorska Bay) on muddy-sandy substrate at about 20 m depth. Petović (2018) indicate that presence of the species in the Bay is not a new, but in the literature was missing for an unknown reason. The first data on the macromolluscs of the Boka Kotorska Bay dates from the 1970s and 1980s (Stjepčević, 1967; Karaman & Gamulin-Brida, 1970; Stjepčević & Parenzan, 1980; Stjepčević & Parenzan, 1982). In these studies, only *P. nobilis* and *P. pectinata* (Linnaeus, 1758) were mentioned as representatives of the Pinnidae family. Petović & Marković (2017) and Petović *et al*. (2017) also listed these two Pinnidae species for the Boka Kotorska Bay. One of reasons why species was not earlier listed for the Boka Kotorska Bay and most part of the Adriatic Sea is that maybe authors confused it with other Pinnidae species, while on the other hand it is possible that authors did not record it simply because its less numerous populations and fact that prefers deeper water. Third reason can be related to the fact that species did not inhabit Adriatic Sea in the past, and that climate changes affect it spreading on the north.

CONCLUSIONS

Data about *P. rudis* populations are scarce, including it biology, ecology and distribution. Low population density is present at almost all sites where the presence is confirmed. Monitoring and status of the species populations, especially in the central, east and north Mediterranean is required, although low density and cryptic habitat hinders the monitoring. Trigos *et al*. (2013) suggested that MPAs are supposed to be the best environmental tool to guarantee the

protection of endangered species. Future step on the national level should be including of *P. rudis* on the list of protected marine species in Montenegro. Finding of *P. rudis* in Montenegrin waters and fact that species is listed by Annex II of the Bern Convention as a strictly protected species and by Annex II of the Barcelona Convention as a threatened or endangered marine species and that inhabits Posidonia beds and Reefs (habitats listed as priority in EU habitat directive), should have positive effects towards the soonest possible promulgation of MPA in Montenegro.

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EFFECT OF CULTIVAR AND INCREASED NITROGEN QUANTITIES ON SOME PRODUCTIVE TRAITS OF TRITICALE

SUMMARY

In the research carried out in the period from 2009–2012, in the north of Montenegro, the influence of cultivar and different amounts of nitrogen on the productive traits of winter triticale was examined. The research covered 5 cultivars of winter triticale (Odyssey, Kg-20, Triumph, Rtanj and Tango). The experiment was set up by random block system in four repetitions. Unfertilized plot (the control) and three steps of N fertilization (60, 90 and 120 kg ha^{-1} N) on the same level of phosphorus and potassium (80 kg $ha^{-1}P_2O_5$ + 80 kg ha^{-1} K_2O) were applied.

The results showed that genotypes respond to the application of mineral nutrients, as well as to the increased nitrogen levels by changing productive properties.

Variety Tango had the highest average grain yield, while Kg-20 had the lowest. Also, Tango had the highest value of the 1000 grain weight, while variety Triumph had the highest value of hectolitre weight. The application of fertilizers has led to a very large and significant increase of yield compared to the control. Based on the analysis of variance, it can be concluded that there were highly significant differences in grain yield among years of the research and significant differences at the 1000 grain weight and hectolitre weight.

Key words: *fertilization, yield components, variety, winter triticale.*

INTRODUCTION

Triticale, a hybrid species created by crossbreeding wheat and rye, is a promising species that takes a significant place in both crop and livestock production. As a species, it exhibited high adaptability in our agroecological conditions, which resulted in stable yields. Triticale is suitable for cultivation at higher altitudes, on soils with poor physicochemical properties, saline and acidic soil with pronounced resistance to biotic and abiotic stresses (Epure et al. 2015; Fras et al. 2016). According to the results of Stošović (2009), triticale achieved high yields in the hilly-mountainous region and in the application of lower cultivation technology, while under optimal conditions, in terms of grain yield, it

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has mostly reached the leading varieties of wheat, and outreached the varieties of rye, barley and oats (Biberdžić et al. 2012). Also, Estrada-Campuzano et al. (2012) point out that new cultivars of triticale achieve higher yields and show better adaptation to soil and climatic conditions compared to wheat.

The great possibility of using triticale for different purposes, as well as the emphasized varietal differences, require the need and importance of more detailed studying of new varieties, with the aim of their more efficient utilization in wide production. Triticale plants, which are characterized by very rapid growth and development, are adaptable to different cultivation technologies and achieve high and stable grain yields (Kendal and Sayar 2016).

Different cultivars have different requirements for applying agrotechnical measures. Nitrogen is an element that has the greatest influence on the vegetative growth of the plant, its photosynthetic capacity and yield. Also, nitrogen positively affects the activity of the root system and its penetration into deeper soil layers. However, it makes a big difference for the plants in what ration they will adopt the necessary elements. It is known that increasing the amount of nitrogen in the soil increases the needs of plants for phosphorus and potassium, and the needs of plants for mineral elements vary greatly depending on climatic factors, primarily from precipitation (Biberdžić et al. 2011).

Popović et al. (2011), Jelić et al. (2013) and Rajičić et al (2019) underlined that mineral fertilizers play a vital role in increasing crop yields, but one of the main constraints in achieving proven crop potential is an unbalanced use of nutrients, in particular a low use of phosphorus in comparison to nitrogen. The optimum rate of phosphorus is important for grain yields improving. Djekić et al. (2014) pointed out that the lack of nutrients in the soil causes shortening of the period of grain and grain filling, which has a negative effect on the yield and quality of grain.

Cultivars of triticale are distinguished by high genetic potential for yield, and that is why triticale can be considered a perspective plant species, especially from the point of view of climate change, which through increasing temperatures and drought show an even more intense influence on cultivated plants. For this reason, it is necessary to spread species, such as triticale, that are more tolerant to stress conditions, in the production.

The aim of this research was to study the influence of the increased nitrogen levels on the productive properties on the tested cultivars, for this area a relatively new plant species. Also, the goal was to distinguish those cultivars of triticale which, under certain conditions of growth, give the highest yield of grain and the best quality.

MATERIAL AND METHODS

The three-year experiment (2009-2012) was carried out in the agroecological conditions of the north of Montenegro in the vicinity of Bijelo Polje (Sutivan) (43°09′ N, 19°77′ E). The soil on which the experiment was carried out belongs to the type of Eutric Cambisol (CM-eu) on alluvial coating.

The experiment was set up by random block system in four repetitions, with the size of an elementary plot of $6m^2$ (3x2 m), including 5 cultivars (Odyssey, Kg-20, Triumph, Rtanj and Tango) of winter triticale (×*Triticosecale Wittmack*). The study included the following varieties of fertilizers: unfertilized plot (0 - the control) and three variants of fertilization (N_1 – 60 kg ha^{-1} N, 80 kg ha^{-1} P_2O_5, 80 kg ha^{-1} K_2O; N_2 – 90 kg ha^{-1} N, 80 kg ha^{-1} P_2O_5, 80 kg ha^{-1} K_2O and N_3-120 kg ha^{-1} N, 80 kg ha^{-1} P_2O_5, 80 kg ha^{-1} K_2O). Phosphorus and potassium were used in equal amounts (80 kg ha^{-1}) before the sowing period, while nitrogen was used in small amounts before the sowing period, and the rest of the planned amount was used as fertilization at the end of March. Common agronomical practices were used in the experiment. Sowing was carried out by manual method in optimal term (second decade of October). At full maturity, a random sample of 30 plants was collected from each plot for hectoliter weight and 1000 grain weight measurements. Grain yield was determined after the harvest. Upon harvest, the grain yield of each plot was measured and calculated as t ha^{-1}.

Soil and weather conditions

Before setting up the experiment, soil sampling was carried out from two depths from 0 to 10 cm and from 10 to 30 cm. Samples were analysed in the Agrochemical Laboratory of the Centre for Cereal Grains in Kragujevac. The applied soil testing methods were adopted by the Yugoslav Society for Soil Studies (JDPZ). In the tested soil samples, which were pre-dried to air-dry state and crushed in a porcelain mortar to a particle size of 2 mm, the following chemical and physical properties were determined: land reaction (pHKCl) by the combined glass electrode, the content of humus by the Kotzman method, content of $CaCO_3$ volumetric, using Scheibler calcimeter (Džamić et al., 1996) and the content of the easy-access P and K by the Al-method, by Egner-Riehm (1960). The obtained results showed that the tested soil had an acid reaction, well supplied by humus 3.35–3.96% and poor in available phosphorus (5.12–4.24 mg 100 g^{-1} soil) and potassium (7.5–3.8 mg 100 g^{-1} soil). The soil was weakly calcareous, too (the total content of carbonate being 2.4–2.44%).

Meteorological conditions, temperature and precipitation are the main non-genetic factors that determine the success of cultivation of winter triticale and other small grains. In the growing season 2009–2010 there were 881 mm of rainfall which is 116 mm more than in the second year and 329 mm more compared to the third year of the research.

Figure 1 showed that in the period from October to July 2009–2010, the highest rainfall was recorded in October, and the lowest in June. In this same interval during 2010–2011, the highest rainfall was in December, and the lowest in March, while in the third year of the study the highest precipitation was recorded in February, and the lowest in November. From the above it can be concluded that conditions for germination and autumn plant development were considerably favourable during the first two years compared to the third year of research. Also, the amount of rainfall in the period from April to June in the first year of research was higher compared to the other two years of research.

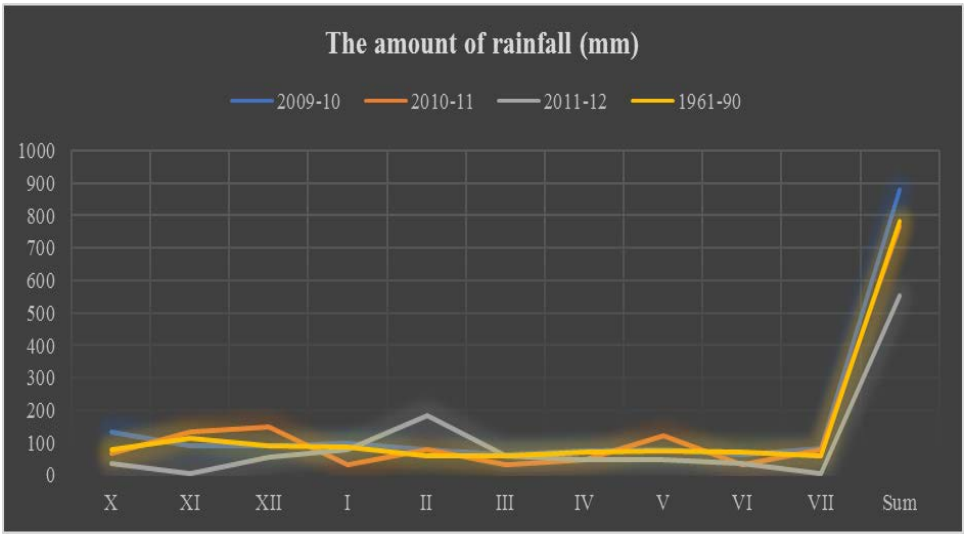

Figure 1. Rainfall distribution during the growing seasons (2009–2012) and multi-year average

Unlike the first two years of the study, in the second decade of November in 2011, air temperatures were significantly lower, which slowed down the development of plants in the initial stages (Fig. 2).

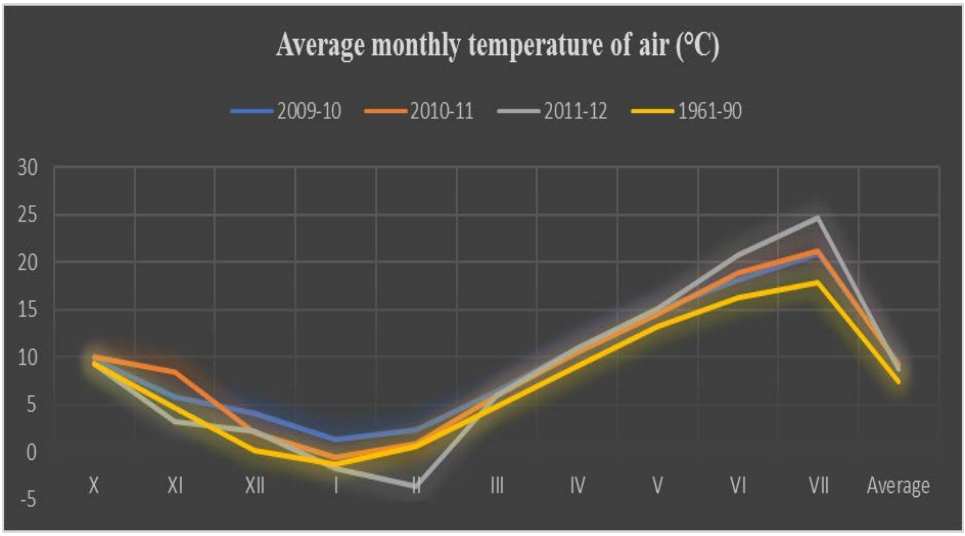

Figure 2. Middle monthly air temperature during the growing seasons (2009–2012) and multilevel average (1961-1990)

The biggest differences were observed in the coldest months, December, January and February. In February 2012, a lot of low temperatures were observed, especially in the first decade, when the temperature was −9.6°C. The

snow cover in this year has significantly contributed to the protection of plants from freezing, but its long retention slowed down the vegetation in the spring.

The favourable air temperatures in May and June, in the first two years of the study, followed by a satisfactory amount of precipitation allowed the proper formation and filling of grains in relation to the third year. The high air temperatures in July 2012 with the modest moisture reserves led to distortion of the filling phase and forced ripening, which had a negative impact on the yield.

Computation and data analysis

Analysis of variance (ANOVA) was performed using the WASP 1.0. statistical package with two-way factors, fertilization and variety, to establish the treatment effects. The importance of differences among the observed elements was tested by Fisher′s protected least significant difference (LSD) test at $P \leq 0.05$ and $P \leq 0.01$.

RESULTS AND DISCUSSION

The results of our research have shown that there are significant differences in the values of 1000 grain weight among the tested triticale cultivars (Table 1.). Also, it was noted that the conditions of the external environment as well as the certain nutritional elements significantly influenced the observed trait. The application of the highest nitrogen dose in combination with phosphorus and potassium, in all three years of the research, resulted in the highest average 1000 grain weight in all cultivars included in the study, which is in agreement with Jaćimović et al. (2008) who found that 1000 grain weight is significantly higher in intensive fertilization treatments, especially by nitrogen.

Our results are in agreement with the results of Djekić et al. (2010; 2012), Djurić et al. (2013) and Jelić et al. (2013) which pointed out that 1000 grain weight is a cultivar specific trait, with considerably higher variations being observed among genotypes than among treatments or factors of environment. Bielski et al. (2015), according to their research, pointed out that the application of nitrogen in the amount of 120kg ha-1 led to a significant decrease of 1000 grain weight compared to the application of nitrogen in the amount of 30kg ha-1. However, the same author emphasized that the application of nitrogen in the amount of 150kg ha-1 led slightly to the increase of 1000 grain weight.

The highest average 1000 grain weight in the three-year study period was achieved by the cultivar Tango (48.2 g), and the lowest by the cultivar Kg-20 (32.8 g). Also, the lowest 1000 grain weight was noted in the first year of the research (40.9 g), which was the climate most favourable, while the highest one was achieved in the third year (44.2 g). Climatic conditions are especially important during the filling of the grain, because the lack of moisture and high temperature during this period influence the reduction of the 1000 grains weight (Bielski 2015), which is confirmed by the results of this research.

Table 1. 1000 grain weight (g) per cultivars, fertilizing variants and years

Varieties (A)	1000 grain weight (g)														
	2009-2010					2010-2011					2011-2012				
	Fertilization variant (B)														
	0	N_1	N_2	N_3	Aver.	0	N_1	N_2	N_3	Aver.	0	N_1	N_2	N_3	Aver.
Odyssey	39.0	40.3	42.8	45.7	41.9	39.4	40.2	40.5	42.3	40.6	41.4	44.0	45.5	47.9	44.7
Kg-20	27.0	31.7	32.9	33.4	31.6	30.7	35.7	31.3	34.6	33.1	28.8	31.8	35.8	38.1	33.6
Triumph	38.2	41.8	40.6	45.1	41.4	39.0	40.7	41.2	42.8	40.9	43.4	47.8	48.1	48.9	47.0
Rtanj	37.2	41.5	43.2	43.8	41.4	44.4	46.1	46.4	46.3	45.8	44.7	45.8	46.7	49.0	46.5
Tango	45.0	48.3	50.7	49.8	48.4	44.5	48.0	47.1	48.9	47.1	43.8	46.8	52.6	53.8	49.2
Average	37.3	40.7	42.0	43.6	40.9	39.6	42.1	41.3	43.0	41.5	40.4	43.2	45.7	47.5	44.2

Table 2. The analysis of variance for 1000 grain weight

Source of variation	Degrees of freedom	2009-2010			2010-2011			2011-2012		
		Sum of squares	Mean sum of squares	F	Sum of squares	Mean sum of squares	F	Sum of squares	Mean sum of squares	F
Replicat.	2	3.826	1.913	1.510	2.073	1.037	0.761	1.460	0.730	0.631
Treatm.	19	2193.690	115.457	91.150	1598.655	84.140	61.795	2323.199	122.274	105.697
Factor A	4	1827.354	456.838	360.659**	1469.241	367.310	269.766**	1814.758	453.689	392.182**
Factor B	3	322.452	107.484	84.855**	92.909	30.970	22.745**	430.427	143.476	124.025**
A x B	12	43.883	3.657	2.887**	36.506	3.048	2.234**	78.014	6.501	5.620**
Error	38	48.134	1.267	-	51.740	1.362	-	43.960	1.157	-
Total	59	-	-	-	-	-	-	-	-	-
LSD		0.05		0.01	0.05		0.01	0.05		0.01
A		0.930		1.246	0.964		1.292	0.889		1.191
B		0.832		1.115	0.862		1.156	0.795		1.065
A x B		1.860		2.492	1.928		2.584	1.777		2.382

*significant at 0.05; ** significant at 0.01

From the data in Table 3. it can be noticed that all cultivars had the lowest value of hectolitre weight in control. The application of fertilizers led to a very significant increase in the value of this trait in all three years of the research, while the application of different fertilization variants did not significantly affect this trait. Also, the influence of the year on the observed trait was noticed, since the lowest average value of the hectolitre weight was recorded in the third year, according to climatic conditions, the most unfavourable year. The influence of meteorological factors on this trait was confirmed earlier in the research by Djekić et al. (2009) and Stošović et al. (2010).

Grain yield is the safest indicator of the existence of differences in productivity among varieties and their specificity towards mineral nutrition, because it is precisely the end result of both external factors on the plant and the biorhythmic activity of certain physiological and biochemical processes. The data in the Table 5. show that the use of mineral fertilizers has led to a significantly very high yield increase in all tested cultivars compared to the control.

The research has shown that winter triticale achieves high yields in the use of nitrogen in the amount of 120 kg ha^{-1} and phosphorus and potassium in the amount of 80 kg ha^{-1}, as confirmed by Knapowski et al. (2009) and Pecio (2010), who, in their researches, obtained the highest grain yields when using nitrogen in the amount of 120 kg ha^{-1}. In the trial run by Bielski (2015) the highest yield was harvested from treatments fertilised with the highest dose of nitrogen (150 kg ha^{-1}).

Table 3. Hectolitre weight (kg) per cultivars, fertilizing variants and years

Varieties (A)	Hectolitre weight (kg)														
	2009-2010					2010-2011					2011-2012				
	Fertilization variant (B)														
	0	N_1	N_2	N_3	Aver.	0	N_1	N_2	N_3	Aver.	0	N_1	N_2	N_3	Aver.
Odyssey	65.9	76.2	76.3	73.2	72.9	66.2	70.8	67.0	68.3	68.1	65.8	67.6	69.2	69.2	68.0
Kg-20	61.5	67.4	66.3	68.9	66.0	69.1	69.5	70.3	71.7	70.1	64.2	65.5	63.2	62.3	63.8
Triumph	72.5	75.7	76.6	77.5	75.6	69.2	69.5	69.9	71.6	70.0	68.6	70.5	71.4	70.3	70.2
Rtanj	68.3	69.1	71.8	71.3	70.1	65.6	64.9	66.7	68.4	66.4	67.1	67.9	64.8	65.2	66.2
Tango	73.0	72.7	72.9	73.3	73.0	63.8	65.3	64.6	66.8	65.1	67.7	69.6	68.4	71.2	69.2
Average	68.2	72.2	72.8	72.8	71.5	66.8	68.0	67.7	69.4	67.9	66.7	68.2	67.4	67.6	67.5

Table 4. The analysis of variance for hectolitre weight

Source of variation	Degrees of freedom	2009-2010			2010-2011			2011-2012		
		Sum of squares	Mean sum of squares	F	Sum of squares	Mean sum of squares	F	Sum of squares	Mean sum of squares	F
Replicat.	2	7.314	3.657	2.182	5.055	2.528	0.930	1.801	0.901	0.208
Treatm.	19	1000.872	52.677	31.426	337.501	17.763	6.532	400.156	21.061	4.861
Factor A	4	629.628	157.407	93.904**	244.580	61.145	22.486**	304.721	76.068	17.556**
Factor B	3	216.619	72.206	43.076**	48.536	16.179	5.950	23.002	7.667	1.770**
A x B	12	154.625	12.885	7.687**	44.385	3.699	1.360**	72.883	6.074	1.402**
Error	38	63.698	1.676	-	103.332	2.719	-	164.649	4.333	-
Total	59	-	-	-	-	-	-	-	-	-
LSD		0.05		0.01	0.05		0.01	0.05		0.01
A		1.070		1.433	1.363		1.826	1.720		2.305
B		0.957		1.282	1.219		1.633	1.538		2.061
A x B		2.140		2.867	2.725		3.651	3.440		4.609

*significant at 0.05; ** significant at 0.01

Table 5. Grain yield (t ha^{-1}) per cultivars, fertilizing variants and years

Varieties (A)	Grain yield (t ha^{-1})														
	2009-2010					2010-2011					2011-2012				
	Fertilization variant (B)														
	0	N_1	N_2	N_3	Aver.	0	N_1	N_2	N_3	Aver.	0	N_1	N_2	N_3	Aver.
Odyssey	4.31	5.43	5.53	6.28	5.39	3.75	5.03	5.24	5.49	4.88	3.12	4.94	5.02	5.23	4.58
Kg-20	3.74	4.52	5.45	5.28	4.75	3.12	4.61	4.73	4.86	4.33	2.99	4.54	4.42	4.45	4.10
Triumph	4.11	5.28	6.13	6.25	5.44	3.66	5.21	5.81	5.88	5.14	3.23	5.01	4.84	4.90	4.49
Rtanj	3.87	5.02	5.93	6.00	5.20	3.59	5.57	5.68	6.24	5.27	3.34	4.97	5.11	5.22	4.66
Tango	4.17	5.64	6.73	7.14	5.92	3.96	6.03	6.39	6.73	5.78	3.40	5.14	5.21	5.29	4.76
Average	4.04	5.18	5.95	6.19	5.34	3.62	5.29	5.57	5.84	5.08	3.22	4.92	4.92	5.02	4.52

Table 6. The analysis of variance for grain yield

Source of variation	Degrees of freedom	2009-2010			2010-2011			2011-2012		
		Sum of squares	Mean sum of squares	F	Sum of squares	Mean sum of squares	F	Sum of squares	Mean sum of squares	F
Replicat.	2	0.429	0.215	3.093	0.159	0.080	0.429	1.621	0.811	2.587
Treatm.	19	53.122	2.796	40.284	61.245	3.223	17.380	37.714	1.985	6.334
Factor A	4	7.341	1.835	26.443**	11.588	2.897	15.620**	3.105	0.776	2.477*
Factor B	3	42.527	14.176	204.246**	47.288	15.763	84.988**	34.106	11.369	36.277**
A x B	12	3.254	0.271	3.908**	2.368	0.197	1.064**	0.502	0.042	0.133 **
Error	38	2.637	0.069	-	7.048	0.185	-	11.909	0.313	-
Total	59	-	-	-	-	-	-	-	-	-
LSD		0.05		0.01	0.05		0.01	0.05		0.01
A		0.218		0.292	0.356		0.477	0.463		0.620
B		0.195		0.261	0.318		0.426	0.414		0.554
A x B		0.435		0.583	0.712		0.954	0.925		1.240

*significant at 0.05; ** significant at 0.01

The greatest impact on the yield and yield structure had the years during which the experiment was carried out, followed by cultivar and distribution of nitrogen fertilization dosing (Alaru et al. 2004). The positive effects of mineral nutrition, especially nitrogen, on the grain yield of triticale were pointed out earlier and by other authors (Bojović 2010; Lestingi et al. 2010). As a result of the influence of various climatic factors, variation in yields by the years of testing was also noted. The highest grain yield was recorded in the vegetation year 2009/10, which was the most favourable in terms of quantity and precipitation. The unfavourable weather conditions for the cultivation of winter triticale in the third year of the study had a negative impact on the yield level in relation to the previous two years. Significantly smaller amounts of precipitation in the last three months of the year, which are important for planting and initial development of plants, resulted in later incomplete emergence. Also, the heavy snowfall in February 2012, as well as the long retention of snow cover led to a slowdown of vegetation in spring, later sowing and flowering. The high air temperatures in June and July, in the same year, caused a shortening of the period of filling of the grain, accelerated ripening and reduction of the yield.

The results of this study showed that the influence of nitrogen fertilization on the grain yield, components of the yield and the quality of grain, do not depend only on the dose of fertilizers, but also on the climatic conditions, as Janušauskaitė (2013), Ivanova and Kirchev (2014), Djurić et al. (2015), Madić et al. (2018), Terzić et al. (2018) and Đekić et al. (2019) had confirmed previously in their researches. (Djekić et al. 2012; 2014), as well as many other authors, point out that the high quality of grain and the best triticale yields are obtained in the years with warm bright spring weather conditions and cold summers without excessive precipitation during the grain filling stage, which is in agreement with this study.

CONCLUSIONS

The results of this study showed that the values of the examined productive traits of the genotypes of winter triticale varied depending on the applied dose of nitrogen, weather conditions and cultivar. On average over the three-year period of the study, the N_3 variant of fertilization had the greatest positive effect on most parameters affecting productivity. The cultivar Tango had the highest average yield (5.49 t ha^{-1}) while Kg-20 had the lowest (4.39 t ha^{-1}). Also, Tango had the highest value of 1000 grain weight, while Triumph had the highest value of hectolitre weight. The differences in yields that have been shown in the cultivars included in the research are the result of varietal specificity, which is largely genetically conditioned. The results of the research can be of great importance for the popularization of triticale as a species in this production area for the improvement of production practice in Montenegro and beyond. Also, the results are important for cattle-oriented farms, where the main priority is to ensure a sufficient amount of quality food.

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Abdenour KHELOUFI, Lahouaria Mounia MANSOURI [1]

ANATOMICAL CHANGES INDUCED BY SALINITY STRESS IN ROOT AND STEM OF TWO ACACIA SPECIES (*A. KARROO* AND *A. SALIGNA*)

SUMMARY

Soil salinity is one of the main abiotic constraints limiting plant growth. This paper focuses on the concept of internal adaptation in relation to salt tolerance during the vegetative phase. Under saline conditions, we evaluated some anatomical changes in stems (area, perimeter, cortex thickness, stele area, stele perimeter, pith area) and roots (thickness, cortex thickness and stele thickness) of two acacia species (*A. karroo* and *A. saligna*). Plants of 90 days old were cultured at various concentrations of NaCl (0, 200, 400 and 600 mM) for 21 days. The experiment was laid out in completely randomized design with four replications. For microscopic analysis, the stem tissues were cross-sectioned and the root were profile viewing. Results showed that salt caused remarkable changes in some anatomical-related parameters. Microscopic studies showed that every acacia species had made its own anatomical changes in stem and root by increasing/decreasing organ area, such as cortex thickness, stele thickness and pith area compared to control. In conclusion, under saline regimes, both species adapted specific characteristics of the roots and stems for better survival under saline environments.

Keywords: Acacia, forestry, salt tolerance, NaCl, osmoprotection.

INTRODUCTION

The response of plants to salinity can be described in two main phases: Shoot ion-independent reaction occurs first (within minutes to days) and is thought to be related to Na^+ detection and signaling (Roy *et al*., 2014). In this first phase, the effects of salinity can be significant on water relations, causing stomatal closure and inhibition of leaf expansion (Miller *et al*., 2010). The second phase, the salt-dependent response to salinity, develops over a longer period (days to weeks) and leads to the accumulation of toxic ions in the stem, particularly in old leaves, leading to premature leaves senescence (Munns and Tester, 2008. Salinity can affect the growth and yield of most plants, inducing a reduction in cell division in roots and leaves, auxesis, cell differentiation, as well as genetic, anatomical, biochemical, physiological processes, morphological, ecological, with their complex interactions followed by significant tissue

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damage, resulting in plant senescence under prolonged exposure to salinity (Reynolds *et al*., 2005 ; Negrão *et al*., 2017, Kheloufi *et al*., 2018a).

In Algeria, it has been reported that *A. karroo* could germinate under 400 mM NaCl with 66% of final germination (Kheloufi *et al*., 2017a). However, the seeds of *A. saligna* could only germinate at 150 mM with only 18% of final germination (Kheloufi *et al*., 2016). Indeed, in previous studies (Kheloufi *et al*., 2016 ; Kheloufi *et al*. 2017a ; Kheloufi *et al*., 2018b), we showed that *A. karroo* was the most salt-tolerant species compared to *A. saligna* whose seeds were sensitive to the germination stage. *A. saligna* seems very sensitive compared to *A. karroo* but can be considered as a salt-tolerant glycophyte. However, the status of 'Sensitive' is only given as a comparison between *A. saligna* and *A. karroo*. Effectively, *A. saligna* is a salt-tolerant glycophyte that has been proven to contribute to the prevention of soil erosion and revegetation with moderate salinity (Sekkour, 2008 ; Mansouri, 2011). Thus, the introduction of *A. karroo* and/or *A. saligna*, as salt-tolerant species, could be an important strategy for the conservation of ecology and wood production in the Algerian salt-affected regions. In addition, no studies have been undertaken to characterize and understand the internal anatomical mechanisms associated with the adaptation of *A. karroo* and *A. saligna* under salt stress. Therefore, in this study, we sought to examine the effects of various levels of salinity on some anatomical changes in both *A. karroo* and *A. saligna* plants under greenhouse.

MATERIAL AND METHODS

Plant material, growth condition and salt treatment

The seeds of *A. karroo* Hayne were collected from Aïn El Baïda salt farm area (Oran, Algeria) (latitude: 35°39'34.96" N; longitude: 0°40'4.68" W; elevation: 136 m) and those of *A. saligna* from the region of Terga (Aïn Temouchent, Algeria) (latitude : 35°26'32.26"N ; longitude : 1°13'42.80"W ; elevation : 2 m). Pods were collected from 10 trees and the seeds were then mixed. The thousand-seed-weight of *A. karroo* and *A. saligna* were 39 g and 15 g, respectively. Sieving and flotation were used to sort out seeds. The clean seeds were then spread on filter paper to dry. Once dried, the seeds undergo a chemical treatment which consisted of immersion in 96% sulphuric acid for 30 minutes for *A. karroo* (Kheloufi, 2017) and 90 minutes for *A. saligna* (Kheloufi *et al*., 2017b), followed by washing in distilled water. *A. karroo* and *A. saligna* seeds need this pre-treatment to break down the seed coat and induce a high germination rate in a short time (Kheloufi, 2017).

Seeds were germinated in plastic pot (Top diameter: 10 cm; Bottom diameter: 7 cm; Height: 14 cm) (Figure 1) containing 1 kg of mixed substrate (two volumes of sand mixed with one volume of compost) (EC = 49 $mS.m^{-1}$; pH = 6.2; N = 89 $g.m^{-3}$; P_2O_5 = 42 $g.m^{-3}$; K_2O = 27 $g.m^{-3}$) and arranged according to the method of complete randomized blocks with four replicates under greenhouse conditions. Sand was sieved at 2 mm to eliminate wastes and coarser material then washed repeatedly with tap water to eliminate all carbonates and chlorides.

The experiment was conducted in the green house of Ecology and Environment Department, University of Batna 2, Algeria (latitude: 35°38'10.32"N; longitude: 6°16'31.52"E; elevation: 926 m).

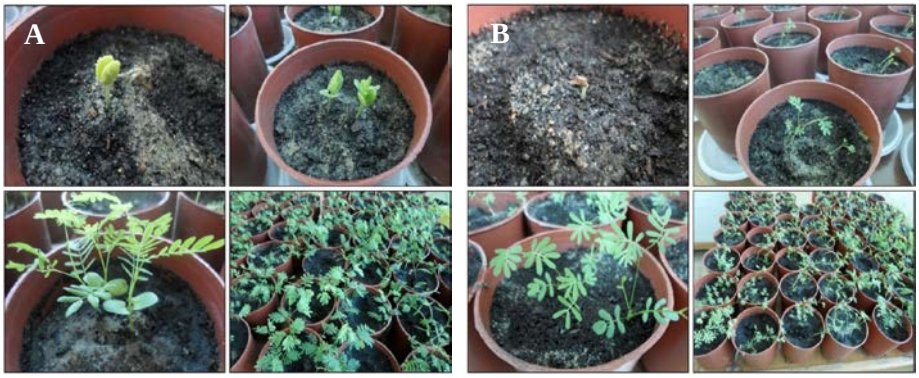

Figure 1. Experimental design and different stages of plant development of two acacia species: (A) *A. karroo* and (B) *A. saligna.*

Three months (90 days) old healthy seedlings of uniform size were selected as initial material and further grown in KNOP's nutrient medium. Plants were subjected to salt treatment by supplementing the nutrient medium with varied sodium chloride (NaCl) concentrations (200, 400 and 600 mM). The control plants were grown in the nutrient medium devoid of NaCl. The nutrient solutions were replaced with freshly prepared solutions at every 7 days intervals. After 21 days of salt treatment, stem and root samples were harvested from control and NaCl-treated plants for estimation of various parameters. It should be noted that for each measurement or assay, a number of 4 replications were used. In addition, and to ensure the study of the effect of salinity on all parameters with the same conditions of growth and development, a considerable number of plants were used (4 plants × 4 treatments × 2 species) (Kheloufi *et al*., 2019).

Measurement of anatomical parameters

Osmotic adjustments induced by salinity were deduced by cutting the stems and roots with a razor blade (Gillette). Sections of both control and NaCl-treated plants were observed immediately after cutting the tissues (about 1.5 cm below the root apex and 1.5 cm from the shoot apex).

All sections were viewed under light microscope at 40X magnification and without staining. This microscope (Zeiss Microscope camera) has an integrated camera connected to a computer. The best observations were then captured as images (JPG). These images were subjects of several dimensional analyzes with Motic Image Plus 2.0 software (Motic Instruments Inc., Canada).

For cross sections of a stem:

SA : Stem area (mm^2)

SP : Stem perimeter (mm)

SCT : Stem cortex thickness (mm)
SSA : Stem stele area (mm^2)
SSP : Stem stele perimeter (mm)
SPA : Stem pith area (mm^2)

For a root profile observation (without crushing):

RT : Root thickness (mm)
RCT : Root cortex thickness (mm)
RST : Root stele thickness (mm)

Statistical analyzes

All experiments were conducted with four replicates (n = 4) and the results were expressed in average (± standard deviation). All data were subjected to one-way analysis of variance (Treatment), two factors (Treatment and Species) (ANOVA) and Duncan's multiple comparison test ($p < 0,05$) using SAS Version 9.0 (Statistical Analysis System) (2002). Charts were made with Excel 2016.

RESULTS AND DISCUSSION

This anatomical study was performed on the 21st day of the application of salt stress by sodium chloride. Our observations suggest that the anatomical characteristics of stems and roots are significantly altered when plants are exposed to salinization of the environment. Indeed, stress due to salt had a very significant effect ($p < 0,0001$) (Table 1) on the internal cell morphology, and the plants subjected to salt stress showed an adjustment of the anatomical characteristics in order to minimize the damage caused by the presence of an excessive amount of NaCl.

Effect of salinity on stem anatomy

As salt concentration increases, a decrease or increase in the cross-sectional area of the stems was observed in *A. auriculiformis* (Rahman *et al*., 2017) and *A. ampliceps* (Theerawitaya *et al*., 2015). Akcin *et al*. (2017) have associated the stem succulence with the adaptation mechanisms to saline conditions in a halophytic species *Salicornia freitagii*. According to Figure 3 and Table 1, the anatomy of the stems has been modified when applying various doses of salt water. For *A. karroo*, the stem area has been gradually reduced compared to the increase in salt concentration up to 400 mM NaCl and then this area tries to recover its initial size at 600 mM NaCl. For *A. saligna*, the SA has also undergone a reduction but stops at 200 mM. Indeed, at 400 and 600 mM NaCl, the SA reached its maximum with an increase of 70% compared to control (Figure 3A).

The stem perimeter of *A. karroo* plants has endured a 35% decrease under the extreme concentrations of NaCl (400 and 600 mM) compared to control and 200 mM NaCl. However, the SP of *A. saligna* plants has undergone a gradual increase that stabilizes at 400 and 600 mM NaCl with a 35% increase over the control (Figure 3B).

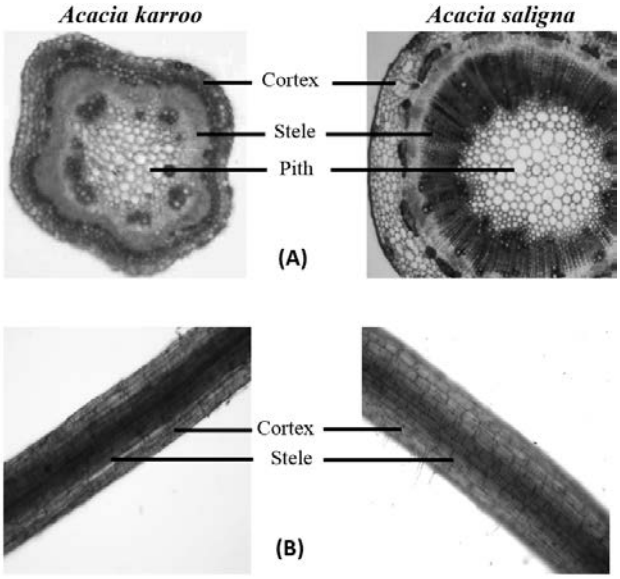

Figure 2. Anatomical parameters studied of (A) the stem (cross section) and (B) the root (Profile view) at 1.5 cm from the shoot apex and root apex at 21st day of the application of different levels of salinity (0, 200, 400 et 600 mM NaCl) on *A. karroo* and *A. saligna* plants.

In addition, it has been found that increasing the thickness of the stems could increase the storage capacity in order to preserve more water and overcome the adverse moisture conditions under the effect of salt stress (Rodriguez *et al*., 2012). An increase in stem cell thickness under saline conditions has also been reported in *Citrus* (Rewald *et al*., 2012) and *Tamarix* (Zhang *et al*., 2016). In general, salinity can cause a reduction in the stem area (Bader *et al*., 2015). However, in the population of *A. saligna*, the SA showed a considerable increase under salt regime (Figure 3A). This change in the stem dimensions can help to store extra water and can contribute to some resistance to water loss by stem cells to ensure plant survival in an unfavourable environment (Feikema *et al*., 2010).

The decrease in the SCT is mainly due to the collapse of cortical cells in response to salinity (Al-Tardeh and Iraki, 2013). This can be beneficial for limiting growth under saline conditions by conserving the energy for survival (Naz *et al*., 2013 ; Quartararo, 2018). According to Figure 3C, the SCT of *A. karroo* was gradually reduced compared to the increase in salt concentration up to 400 mM where this thickness was reduced by 35%. At 600 mM NaCl, the stem cortex regains a thickness equivalent to that recorded in plants subjected to 200 mM NaCl where the difference compared to the control was just 20%. The SCT of the control plants of *A. saligna* was reduced by 30% under 200 mM NaCl. However, at 400 mM NaCl, the cortex regained a thickness equivalent to that of the control and reached its maximum at 600 mM with a value that exceeds the control by 50% (Figure 3C). Decreasing in cortical area of the stems could be

a defensive strategy for plants to limit growth under the salt regime by conserving the essential energy for plant survival in a difficult environment (Chen and Polle, 2010). In contrast, increasing stem cortex thickness in the presence of high salinity may be considered as a defensive strategy to reduce Na^+ toxicity (Mudgal *et al*., 2010).

Table 1. Analysis of the variance of the effects of salinity on anatomical parameters of the stems (SA : Stem area, SP : Stem perimeter, SCT : Stem cortex thickness, SSA : Stem stele area, SSP : Stem stele perimeter, SPA : Stem pith area) and the roots (RT : Root thickness, RCT : Root cortex thickness, RST : Root stele thickness) in two acacia species (*A. karroo* and *A. saligna*) (SP) under saline treatment (TRT).

Organs	Parameter	Sources of variables	Degree of freedom	F of Fisher	*P*
STEM	SA	TRT	3	55,54	<0,0001
		SP	1	424,06	<0,0001
		TRT×SP	3	137,83	<0,0001
	SP	TRT	3	0,60	0,6202
		SP	1	10,29	0,0038
		TRT×SP	3	22,12	<0,0001
	SCT	TRT	3	45,33	<0,0001
		SP	1	56,84	<0,0001
		TRT×SP	3	46,92	<0,0001
	SSA	TRT	3	17,47	<0,0001
		SP	1	521,00	<0,0001
		TRT×SP	3	106,72	<0,0001
	SSP	TRT	3	5,09	0,0072
		SP	1	455,54	<0,0001
		TRT×SP	3	108,60	<0,0001
	SPA	TRT	3	241,02	<0,0001
		SP	1	4455,50	<0,0001
		TRT×SP	3	487,53	<0,0001
ROOT	RT	TRT	3	17,91	<0,0001
		SP	1	1,25	0,2752
		TRT×SP	3	1,58	0,2204
	RCT	TRT	3	21,99	<0,0001
		SP	1	0,00	0,9471
		TRT×SP	3	11,48	<0,0001
	RST	TRT	3	4,77	0,0095
		SP	1	14,89	0,0008
		TRT×SP	3	21,85	<0,0001

For *A. karroo*, the SSA decreased with increasing stress and stabilizes from 400 mM with a 45% loss in size compared to the control. However, the SST of *A. saligna* increased gradually with increasing salt levels reaching a considerable value compared to the control of a difference of 80% at 400 mM NaCl and 50% at 600 mM NaCl (Figure 3D). Figure 3E showed that the SSP of

A. karroo decreased with increasing salt concentration by stabilizing from 400 mM NaCl with a slight loss of 23% compared to the control. On the other hand, the SSP of *A. saligna* increased progressively with the increase in stress level reaching a gain of 32% at 400 mM NaCl and 22% under the extreme concentration of 600 mM NaCl (Figure 3E).

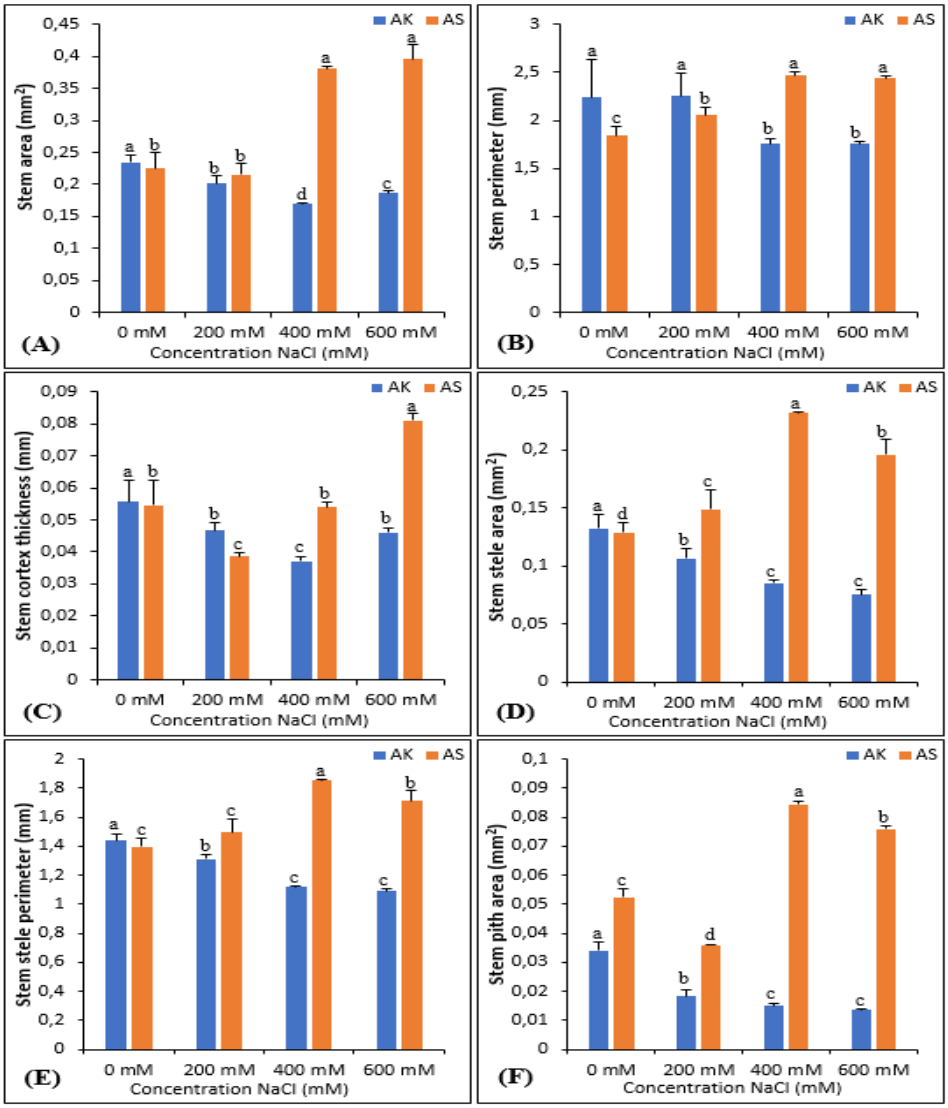

Figure 3. Effects of salt stress on the anatomical parameters of the stem: (A) Stem area, (B) Stem perimeter, (C) Stem cortex thickness, (D) Stem stele area, (E) Stem stele perimeter and (F) Stem pith area, in two acacia species (*A. karroo* and *A. saligna*) after 21 days of treatment at different levels. Means, in each box, with similar letters are not significantly different at the 5% probability level using Duncan's test.

Decreased in the stele area under excessive saline conditions was considered an adaptation strategy to reduce water loss in several forest species in arid zones (Rewald *et al*., 2011a). Reduced stele size may also imply a reduction in saline water absorption by xylem and a reduction in water loss through transpiration (Rewald *et al,* 2011b) and this is the case of the plants of *A. karroo* in our study. On the other hand, the increase in the stele thickness of *A. saligna* with the increase of the salinity level can also constitute a defensive characteristic in saline conditions as it has already been reported by Srikanth *et al*. (2016).

According to Figure 3F, the SPA of *A. karroo* decreases with the increase in the degree of stress and stabilizes from 400 mM with a loss of 55% compared to the control. Cependant, the SPA of *A. saligna* increased progressively with the progression of salt levels reaching a considerable value compared to the control of a difference of 61% at 400 mM NaCl and 46% at 600 mM NaCl (Figure 3F). It should be noted that in this species, the SPA recorded a 30% loss at 200 mM NaCl compared to the control. These changes in anatomical dimensions can be considered as an adaptive mechanism of *A. saligna* in order to maintain a regular water movement in aerial parts of plants, especially under saline conditions (Polle and Chen, 2015). Unlike *A. saligna*, the plants of *A. karroo* of this study and the case of many plants have undergone a decrease in the stem pith under salt regime: *Leptochloa fusca* (Ola *et al*., 2012); *Leucaena leucocephala* (El-LAmey, 2015); *Phaseolus vulgaris* (Bargaz *et al*., 2016); *Salicornia freitagii* (Akcin *et al*., 2017). These same authors have pointed out that this reduction due to salinity is indeed another strategy of salinity and water-stress tolerance.

Effect of salinity on root anatomy

The root system is the main organ affected by salinity and is known to rearrange its anatomical characteristics in order to confer adaptation to the species under adverse environmental changes induced by salinity (Aroca *et al*., 2011; Patakas, 2012). Figure 4A showed that the root thickness is constant at 200 and 400 mM NaCl and increased with the increase in salt stress and this was observed in both acacia species. Indeed, the RT recorded highest values under 600 mM NaCl with a gain of 41% in *A. karroo* and 37% in *A. saligna*. This increase in root thickness under salt stress could be an adaptation of these two species to reduce the transpiration rate and thus to maintain the water content in the internal tissues of the root.

The increase in root and stem thickness not only improves the plant water-use efficiency, but also provides additional compartment for Na^+ sequestration in cells (Parida and Jha, 2010). In contrast to our study, the root thickness was reduced in soybean with different levels of salinity (Dolatabadian *et al*., 2011), suggesting that there is a differential anatomical-adaptation between halophytes and glycophytes in response to salinity. Based on our results, the main increase in root thickness occurred to prevent the influx of Na^+.

The same observation was indicated for the cortex thickness of the root where the root preserved its size compared to the control at 200 and 400 mM

NaCl in *A. saligna*, recording a 55% increase over the control under 600 mM NaCl. For *A. karroo* plants, The RCT increased at 200 and 600 mM NaCl recording a gain of 49% and 33%, respectively (Figure 4B). Salinity is known to stimulate suberization and increased the thickness of root cortical cells (Franco *et al*., 2011; Byrt *et al*., 2018). These results are in agreement with those of the present study where the root cortex had undergone an increase proportional to the salt levels for the two populations of acacia. This is the characteristic of salt-tolerant species which will certainly avoid the water loss through the roots (Muchate *et al*., 2016). In fact, halophytes or salt-tolerant species generally have a thick root cortex, which is an effective mechanism against water loss in case of combined stress between salinity and drought (Rewald *et al*., 2013).

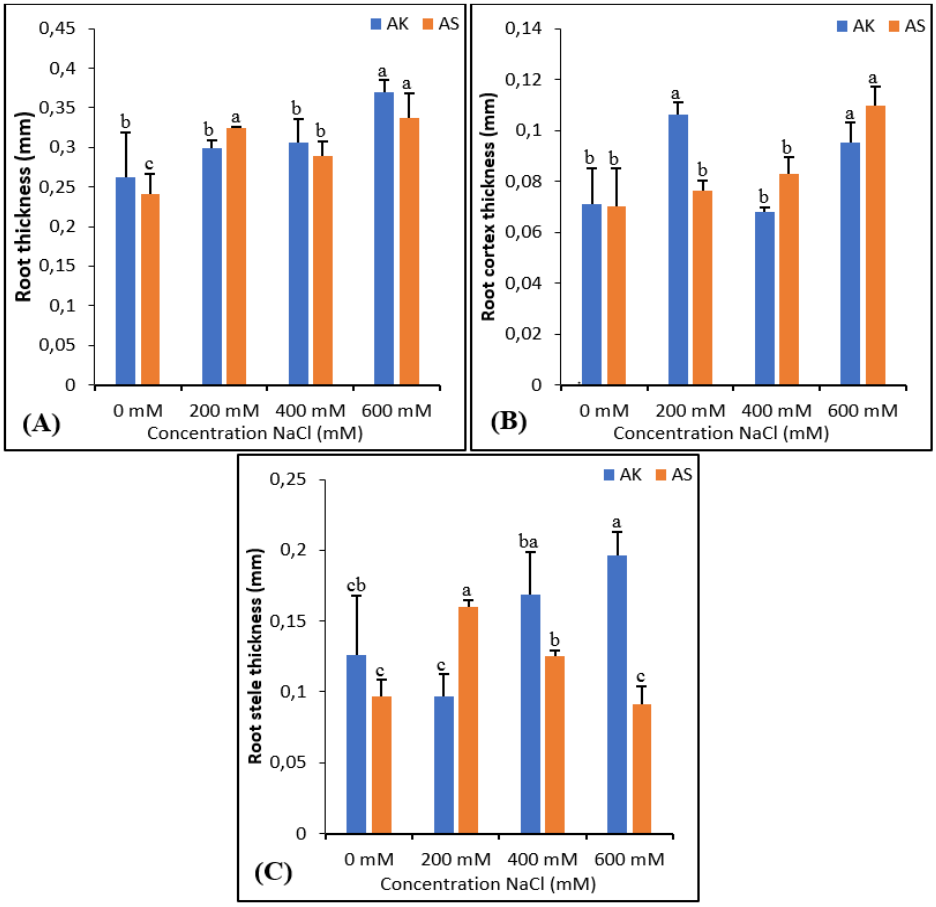

Figure 4. Effects of salt stress on the anatomical parameters of the root: (A) Root thickness, (B) Root cortex thickness and (C) Root stele thickness, in two acacia species (*A. karroo* and *A. saligna*) after 21 days of treatment at different levels. Means, in each box, with similar letters are not significantly different at the 5% probability level using Duncan's test.

Figure 4C showed in turn that the increase in salt stress had an effect on the stele thickness of the root by increasing its thickness in *A. karroo* and reaching its maximum under 600 mM NaCl with more than 55% compared to the control. The RST of *A. saligna* behaved differently where it reaches its maximum just at 200 mM NaCl with a 64% increase over the control. However, the RST tend to decrease after this concentration and stabilized at 600 mM recording the same value as the control (Figure 4C). When size and thickness of the root stele increased, the water storage capacity increased to overcome the conditions of water stress conditioned by salt stress (Karimi *et al*., 2012). It has also been reported that the roots water-potential is much more dependent on the size of the internal structure such as stele and vessels (Munns and Gilliham, 2015). The structure and the stele area are an adaptive mechanism for storing or transmitting water under salt regime (Deinlein *et al*., 2014). In previous studies, it has been reported that the expansion of the stele plays an important role in the plant adaptation to salinity (Abbas *et al*., 2013). When transporting water and mineral elements such as Na^+ ions from the rhizoderm to the stele; the lignification of the exoderm, endoderm, metaxylem and protoxylem, prevents the excessive ion movements (Han *et al*., 2015). Indeed, the endoderm layer is considered to be the most important barrier against the passive flow of ions in root tissues (Singh and Stasolla, 2016).

Overall, Na^+ exclusion, tissue tolerance, osmoprotection and mineral homeostasis play a key role in salt acclimation and tolerance in these two acacia species. *A. karroo* is considered tolerant also on the basis of total dry biomass of stressed plants (Kheloufi et al., 2018b). Under saline conditions: the synthesis of the results of several authors such as Läuchli and Epstein (1990) ; Blumwald (2000) ; Yamaguchi and Blumwald (2005) ; Munns and Tester (2008) ; Hauser and Horie (2010) ; Wu *et al*. (2015) ; Mansouri and Kheloufi (2017) and Hamed *et al*. (2018) summarize the plant acclimation by three main mechanisms: Ion-exclusion - Net exclusion of toxic ions - Compartmentalization of toxic ions in specific subcellular tissues, cells and organelles - Maintaining growth and water absorption - Anatomical changes in root, stem and leaves.

CONCLUSION

In conclusion, under saline regime, both acacia species adapted specific characteristics of the roots and stems for better survival under saline environments. The parameters estimated during this study are valid only for the case of young plants in pots and under defined conditions of salinity. It would be necessary to validate our results *in situ* where these species grow naturally on saline soils.

However, this study does not exclude that these acacia species are considered as potential halophytic species to be cultivated in saline lands, thus making them favourable to agroforestry practices, especially since these forests leguminous have the capacity to revegetate nutrient-poor soils.

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CORRELATION BETWEEN THE MASS AND CHARACTERISTICS OF EGG STRUCTURE IN DIFFERENT AGE GROUPS OF LAYING HENS

SUMMARY

In order to determine the characteristic structure of consumer eggs of light line hybrids Isa Brown, and the correlation links between the eggs mass and the examined characteristics, the research was conducted on the eggs taken from the private farm "Poljoprom" in Vojkovici, Bosnia and Herzegovina. The eggs were taken from three periods of production cycle, 20th, 28th and 48th week of age of laying hens. In these periods, using random sample method, the appropriate number of eggs was taken for a detailed examination, analysis and statistical processing of eggs structure characteristics. During the production cycle the hens were raised in modern facilities and adequate technology was used for each particular aspect of production with all technological phases automatically regulated.

Along with the age of laying hens, the absolute value of the eggs structure (the mass of the shell and the membrane, egg whites and egg yolks) are usually statistically increased at the level of $P < 0.01$, except at the mass of the shell of laying hens in 48th week of age, where the significance was absent. The largest relative share of shell (14.41%) was in full production, 28th week of age (WA28), and the smallest (11.80%) in the middle of the production (WA48). Between WA20 and WA28, the differences in relative part of the shell were not statistically confirmed, while other differences were statistically significant at the level of $P < 0.01$. The largest share of egg whites in the mass of the egg was in WA48 (62.56%), and the lowest in WA28 (60.89%). The largest share of egg yolk (26.80%) was at the peak of production (WA48), and the smallest (22.50%) at the beginning of the production (WA20). All differences in terms of the share of egg yolks and egg whites were statistically confirmed.

Keywords: eggs weight; egg structure; yolk; egg whites; correlation.

INTRODUCTION

Many authors have found through their research that different factors affect the structure of the eggs for consumption, including an important role of the genotype and the age of the hens. The eggs are composed of three basic parts: the egg yolk, the egg whites and the shell. The yolk is situated in the center of the

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egg, wrapped in membrane, fixed with halazama (thick part of egg whites). Egg white is wrapped in with two membranes (inner - thinner and outer - thicker). The shell is the hard part, composed of three layers, makes 11% of the egg mass, and 98% of calcium makes the shell (Jacqueline et al., 2000). The mass of egg is a significant feature, because all the components of an egg are dependent on mass (Hartmann et al., 2000). According to Li-Chang et al. (1995) the share of components in the mass of the entire egg mass of an average 57 g is the following: yolk 28-29%; egg whites 60-63%; shell 9-11%; while Juric at al. (2005) give slightly different values, that is, the share of components in the mass of the entire egg mass of an average 58 g is: yolk 31-33%; egg whites 57-58%; shell 10-11%. Rossi et Pompei (1995), Suk et Park (2001), Van den Brand et al. (2004), Rizzi et Chiericato (2005), Tůmová et Ledvinka (2009) concluded in their research that along with age, the weight and proportion of egg yolk and a mass of egg whites significantly increases, and the share of egg whites in the total mass of the egg decreases with the age. Basmaciúolu et Ergul (2005) found that genotype ISA Brown had average values for the egg yolk mass at 15.25 g and the egg yolk share in the total mass of the egg 25.07%, and mass of egg whites at 39.25 g and share of 65.04%. Also, the average mass of the shell is 5.99 g, while the share of shell in the total mass of the egg amounts 9.92%.

The influence of genotype and age on the egg structure characteristics were examined by Lukas et al. (2009). Based on the results of experiment they concluded that the age has statistically significant ($P < 0.001$) impact on mass of the shell, egg yolk and egg whites, as well as their share in the total mass of the egg. Along with age, the mass and share of egg yolk increased, as well as mass of the shell and mass of egg whites. Also, along with age the share of shell and egg whites decreased. The correlation between the mass of eggs and the share of egg yolk was positive ($r_p = 0.182$) and statistically significant ($P < 0.001$). Negative, statistically confirmed phenotypic correlation occurred between the mass of eggs and the share of egg whites ($r_p = - 0.039$), ($P < 0.001$) and between the mass of eggs and the share of the shell ($r_p = - 0.400$), ($P < 0.001$).

In order to determine the impact of the age of the laying hens hybrid Lohmann Brown on the mass and structure of the eggs, as well as their interconnection and Pandurevic et al. (2013) carried out an experiment on two samples of eggs from laying hens who were 20 and 28 weeks old. Based on the obtained results they have noticed that older hens produced heavier eggs than younger hens; the share of egg whites in the mass of eggs was smaller, the share of the shell was similar, and the share of egg yolk was larger. At 20-weeks-old laying hens a statistically significant ($P < 0,001$) negative correlation coefficient is determined between the mass of the egg and the share of the shell in egg mass, while at 28-weeks-old laying hens was also determined a negative correlation coefficient that was not statistically significant ($P > 0.05$). Between the mass of eggs and egg yolk's share, at both groups of laying hens, a negative correlation coefficient was determined, statistically significant ($P < 0,001$) for elderly hens, while for younger hens the significance has not been confirmed ($P > 0.05$).

Along with age, the percentage share of egg whites in the egg mass increased, with the difference that for elderly hens was statistically significant ($P < 0.001$), while for younger hens the significance has not been confirmed ($P > 0.05$).

Rajicic et al. (2008) presented in their work results for the mass shell of hybrids Hisex Brown and Shaver 579. The mass of raw shell mass gradually increased along with age. With a hybrid Shaver 579 determined correlation coefficient ($r_p = 0.887$) has showed a very strong dependence between the age and the mass of the shell, and it was statistically significant ($P < 0.001$). The same dependence for hybrid Hisex Brown was high and it was not significant ($r_p = 0.579$), ($P > 0.05$).

The aim of this paper was somewhat similar to the previous examinations, with the difference to the individual characteristics of examination of the egg structure for the light line laying hens hybrid Isa Brown of different ages, i.e. the laying hens old 20, 28 and 48 weeks, as well as determining the strength and statistical significance of examined characteristics by calculating the phenotypic correlations.

MATERIAL AND METHODS

Laying hens od light line hybrids Isa Brown were raised in accordance with the current technological recommendations of selector (https://www.isa-poultry.com) and they were moved to the farm "Poljoprom" in the village of Vojkovici, localized in the Republika Srpska, Bosnia and Herzegovina at the age of 18 weeks. During the production period, they were held in a classic battery (cage) system to accommodate hens. The hens were fed with standard diet mixture for laying hens, at will (*ad libitum*).

According to the recommendations of the selector of a line of light hybrids Lohmann Brown, the forage during the production cycle should contain from 2,750 to 2,800 kcal ME and 17.5% of crude protein (load capacity below 5%), or 2,800 kcal ME and about 18% of crude protein (capacity over 5%). This food is used all the way until achieving the maximum production (28 week of age), and depending on the production of eggs and body mass, after which the concentrate diet is added with specific supplements of adequate content.

At the hens` age of 20 weeks (egg production initial stage), 28 weeks (peak load - the maximum laying eggs intensity) and 48 weeks, the random sample was taken by 120 eggs per each age stage. So, in the course of the entire production cycle there were three control measurements of the eggs (total eggs 360), that is, statistically speaking, there were three treatments of 120 repetitions. The eggs were packed and transported with air conditioning car to the experimental laboratory of the Faculty of Agriculture in East Sarajevo, University of East Sarajevo.

In all three examined groups of lying hens (WA20, WA28, WA48), we have determined the egg weight (g), the shares of raw shell (g; %), yolk (g; %) and egg whites (g; %). The egg weight was determined by individual egg measurements using special technical weighing scale, brand „KERN$_{PFB}$“, which

is accurate to 0.01 g. To determine the structure of the egg, each egg was broken after the measurement. Then the raw shell, egg yolk and egg white were also measured. The weight of raw shell was measured by breaking the egg, separating its content from the shell and measuring its mass, together with egg membranes. The values are expressed in grams (absolute value) and as a percentage of the total egg mass. The yolk mass was measured on technical weighing scale, after the yolk was previously separated from the egg white by separator. The mass of the egg white is obtained by calculating the difference between the egg mass on one side, and the mass of the shell and yolk on the other side. Absolute measures were used to determine the shares of shell, yolk and egg white. The percentages of raw shell, yolk and egg white in the egg mass were calculated.

Based on sample data and defined distribution frequency of examined occurrences, the following parameters were determined for tracked characteristics: the average value ($\bar{X}$), the standard error of the mean ($S_{\bar{x}}$), standard deviation (*S)* and the coefficient of variation (CV, %). Determined differences between the parameters of the structure of the egg of laying hens aged 20, 28 and 48 weeks, were tested with student's t-test. The phenotype correlation coefficients, (r_p) between the weight of the eggs and tracked the characteristics of the structure of the eggs were calculated by standard form (SAS).

RESULTS AND DISCUSSION

In this paper we examined the characteristics of the egg structure of different age laying hens, during the production cycle. For these tracked characteristics of egg structure, by random sample method, a 120 eggs was taken for the analysis during various stages of the production cycle.

The average values and variabilities of the basic indicators of the structure of eggs for consumption of light line hybrids Isa Brown, are shown in table 1. From table 1 we can see that the average value of the mass of the shell increased through the first two periods of the studies (WA20 – 6.10 g and WA28 – 7.03 g), and in the third period of examination the mass of the shell was slightly lower (WA48 – 6.07 g). The share of the shell in total egg mass were reduced with age, while at the time of maximum production (WA28) the average was slightly higher (14.41%). A slightly lower values in terms of share of the shell were found by Dikmen et al. (2017), Gjorgovska et al. (2016), and Zaheer (2015)

Along with the aging, the average absolute values of the egg whites mass increased (28.15 g; 36.30 g; 41.79 g), the mass of egg yolk (10.68 g; 14.37 g; 16.60 g) as well as the average relative values of the egg whites mass, except when WA28 (60.89%), when it was slightly lower than the previous one, while the share of egg yolks in the mass increased to WA28, and then dropped in the WA48 (25.39%). As for the egg whites, a slightly lower share was found by Kralik and Ljuboja (2017). A slightly lower values of the mass and yolk share were noted by Dikmen et al. (2017), while higher values were found by Kralik and Ljuboja (2017), Gjorgovska et al. (2016).

-Table 1. The average values and variabilities of eggs structure in certain stages of the production cycle.

Indicators	The age of laying hen	$\bar{x}$	S	$S_{\bar{x}}$	C.V.
The shell mass, g	WA_{20}	6.10	0.77	0.06	12.90
	WA_{28}	7.03	0.70	0.04	7.97
	WA_{48}	6.07	0.43	0.03	5.20
The share of the shell, %	WA_{20}	13.20	1.35	0.10	10.15
	WA_{28}	14.41	0.80	0.06	6.22
	WA_{48}	11.80	0.73	0.05	6.22
The egg whites mass, g	WA_{20}	28.15	3.02	0.22	10.36
	WA_{28}	36.30	3.14	0.24	8.69
	WA_{48}	41.79	4.03	0.29	9.38
The share of egg whites, %	WA_{20}	61.13	2.14	0.15	3.32
	WA_{28}	60.89	2.11	0.15	3.39
	WA_{48}	62.56	1.85	0.12	2.86
The egg yolk mass, g	WA_{20}	10.68	1.43	0.11	12.31
	WA_{28}	14.37	0.68	0.04	4.53
	WA_{48}	16.60	0.74	0.06	4.78
The share of egg yolk, %	WA_{20}	22.50	1.65	0.14	7.02
	WA_{28}	26.80	1.30	0.10	5.15
	WA_{48}	25.39	1.11	0.08	4.47

In the following table are the values that indicate the significance of the calculated difference structure of consumer eggs hybrids Isa Brown, in three age periods of production (table 2).

From the results in table 2 we can see that with the age of laying hens the absolute value of the eggs structure generally increased. Heavier eggs originating from older laying hens had significantly higher absolute mass of egg whites ($P < 0.01$) and egg yolks than smaller (lighter eggs) originally from young laying hens.Also, determined differences in the absolute mass at the shell of the egg were statistically significant ($P < 0.01$), except the differences between the second and third sampling, when significance has not been confirmed ($P > 0.05$).

The percentage share of egg whites and egg yolks, a statistical significance was found in all three periods of the studies ($P < 0.01$, $P < 0.05$), while at the percentage share of the shell, statistical significance occurs between the first and third, that is, the second and third period, and between the first and second periods there is no statistical significance ($P > 0.05$).Similar results, with three different genotype (Isa Brown, Hisex Brown and Moravia BSL), were found by Dikmen et al. (2017).

Phenotype correlation connection between the mass and structure of the eggs.

The mass of the egg represents a significant characteristic, because each component of the egg depends on it. In support of this, in table 3 are calculated

coefficients of phenotypic correlation between the mass of eggs originating from three age groups of laying hens (WA20, WA28 and WA48) and the examined characteristic of egg structure.

Table 2. Significance of difference structure of eggs in certain stages of the production cycle

Indicators	The age of laying hen	Average value	Difference	Significance
The shell mass, g	WA_{20}	6.10-7.03	-0.93	**
	WA_{28}	6.10-6.07	0.03	**
	WA_{48}	7.03-6.07	0.96	ns
The share of the shell, %	WA_{20}	13.20-14.41	-1.21	ns
	WA_{28}	13.20-11.80	1.40	**
	WA_{48}	14.41-11.80	2.61	**
The egg whites mass, g	WA_{20}	28.15-36.30	-8.15	**
	WA_{28}	28.15-41.79	-13.64	**
	WA_{48}	36.30-41.79	-5.49	**
The share of egg whites, %	WA_{20}	61.13-60.89	0.24	**
	WA_{28}	61.13-62.56	-1.43	*
	WA_{48}	60.89-62.56	-1.67	**
The egg yolk mass, g	WA_{20}	10.68-14.37	-3.69	**
	WA_{28}	10.68-16.60	-5.92	**
	WA_{48}	14.37-16.60	-2.23	**
The share of egg yolk, %	WA_{20}	22.50-26.80	-4.30	**
	WA_{28}	22.50-25.39	-2.89	**
	WA_{48}	26.80-25.39	1.41	**

Based on the results from table 3 we can see that in young laying hens (WA20) the correlation coefficient between the egg mass and the mass of the shells amounted to $r_p = 0.086$ and was not statistically significant, while the correlation coefficients for older laying hens showed a stronger connection between these two characterestics, as well as the statistical significance.

Unlike the previous one, the coefficients of phenotypic correlation between eggs mass and shell shares were negative for all periods and statistically significant.

A positive correlation coefficients were found between the mass of eggs and mass of egg whites, as well as the mass of the egg yolk, and all of them were statistically significant.

From table 3 we can see that the young hens did not have a significant correlation coefficients, calculated between the mass of eggs and shares of egg whites and egg yolks. With the age of laying hens, the correlation coefficients were significant at the level of ($P < 0.01$).

In the work of the Dikmen et al. (2017), the authors have found similar results of phenotypic correlations for all three components of the structure of eggs, with the difference that the level of significance in this paper was $P < 0.01$,

while at authors mentioned above was at the level of *P < 0.01*, where the comparisons with tabular values showed a statistically significant difference.

Table 3. Phenotypic coefficients of correlation between the mass of eggs and shell, egg yolk and egg whites.

Indicators	The age of laying hen	r_p	t_{exp}
The shell mass, g	WA_{20}	0.086^{ns}	1.211
	WA_{28}	0.655**	11.012
	WA_{48}	0.410**	5.748
The share of the shell, %	WA_{20}	-0.415**	5.899 1.703
	WA_{28}	-0.132^{ns}	15.978
	WA_{48}	-0.782**	
The egg whites mass, g	WA_{20}	0.823**	18.080
	WA_{28}	0.904**	26.109
	WA_{48}	0.945**	37.050
The share of egg whites, %	WA_{20}	0.084^{ns}	1.080 7.938
	WA_{28}	0.533**	14.427
	WA_{48}	0.753**	
The egg yolk mass, g	WA_{20}	0.811**	17.359
	WA_{28}	0.604**	9.600
	WA_{48}	0.738**	13.745
The share of egg yolk, %	WA_{20}	-0.090^{ns}	1.170
	WA_{28}	-0.772**	15.459
	WA_{48}	-0.703*	10.452

***P<0.01; *P<0.05; ns P>0.05*

CONCLUSIONS

In order to determine the characteristic structure of consumer eggs of light line hybrids Isa Brown, and the correlation links between the eggs mass and the examined characteristics, the research was conducted on the eggs taken from the private farm "Poljoprom" in Vojkovici, Bosnia and Herzegovina, from three periods of production cycle, 20^{th}, 28^{th} and 48^{th} week of age of laying hens. In these periods, using random sample method, the appropriate number of eggs was taken for a detailed examination, analysis and statistical processing of eggs structure characteristics.

Based on the examinations of the eggs structure that originated from laying hens of different ages during the production cycle (WA20, WA28 and WA48), the following conclusions can be drawn:

Along with the age of laying hens the absolute value of the eggs structure (the masses of the shell and membranes, egg whites and egg yolks) are usually a statistically increased at the level of *P < 0.01*, except at shells mass of laying hens aged 48 weeks, where the significance is absent.

The largest relative share of shell (14.41%) was in full production, 28^{th} week of age (WA28), and the smallest (11.80%) in the middle of the production

(WA48). Between WA20 and WA28, the differences in relative part of the shell were not statistically confirmed, while other differences were statistically significant at the level of $P < 0.01$. The largest share of egg whites in the mass of the egg was in WA48 (62.56%), and the lowest in WA28 (60.89%). All differences were statistically confirmed.

The largest share of egg yolk (26.80%) was at the peak of production (WA48), and the smallest (22.50%) at the beginning of the production (WA20). All differences were statistically significant.

In young lying hens (WA20), the correlation coefficient between the egg mass and shell mass was not statistically significant, while in older hens the correlation coefficients showed a stronger correlation between these two traits, as well as statistical significance.

The phenotypic correlation coefficients between the egg mass and shell share were negative for all periods and statistically significant.

Positive correlation coefficients were determined between the egg mass and the mass of yolk, as well as the mass of egg whites, and they were all statistically significant.

The young lying hens did not have significant correlation coefficients calculated between the egg mass and the shares of yolk and egg whites.

Taken as a whole, it can be concluded that in the analyzed commercial flock of light Isa Brown hybrid on the poultry farm "Poljoprom" in Vojkovici, Bosnia and Herzegovina, the age of the lying hens influenced certain characteristics of the structure of consumer eggs, for all three examined periods, as shown by correlation between egg mass and examined characteristics of the egg structure.

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OPPORTUNITIES TO PROFIT UNDER COMPETITIVE MARKET CONDITIONS

SUMMARY

Building a recognizable brand for the high-quality bottled wines is opportunity for the wineries to strengthen the international market position. This paper attempts to identify the opportunities to profit under competitive market condition of the Macedonian wineries. For this reason, we first interpret evidence on structural determinants of Macedonian wineries' profitability, then we interpret evidence on the existing marketing strategies of the Macedonian wineries, and finally we present successful case study of a winery that have succeeded to create a recognized brand internationally. The results from this analysis suggest definition of a successful winery that exhibits increasing opportunities to profit under competitive market conditions. The defined winery may be used as a guideline to reinforce possibilities for Macedonian wineries to be able to follow future market signals, considering that they still struggle to adjust to the imposed market-oriented production.

Key words: profitability strategy, marketing strategy, brand creation, competitive position.

INTRODUCTION

With the market globalization process, the agri-food sector in the Western Balkan economies is faced with many challenges. These challenges rise even more with the aspiration of these countries to join the European Union (Palevic et al., 2019). The tough market competition within the EU may negatively affect the small economies from the Western Balkans, if the countries join EU.

The transition process in the Republic of North Macedonia (RNM) has given rise to major structural and economic changes, limiting the competitive market position of the Macedonian agri-food companies. Today, decentralization has led to the agri-food sector being composed of small-scale units that adjust more difficultly to the new market conditions (Lerman et al., 2002).

The wine sub-sector is one of the most perspective agri-food industries for the RNM that could be competitive on the international markets. The favorable

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microclimatic conditions make a great potential for the country to produce high-quality wine (Vlahovic et al., 2017). So far, Macedonian wines have a good reputation on the domestic and regional markets. However, the domestic agricultural policy aims to increase this reputation and recognition for the Macedonian wine more globally, by allocation of financial support to investments in sophisticated equipment for increased production of quality bottled wine and for marketing technologies. Besides, the investments in marketing to increase the export prices is one of the main objectives of the policies, resulting in additional increase of the export of bottled wine in the total export (Ministry of Agriculture, Forestry and Water Economy 2014-2020). However, the country is still recognized as a producer of a bulk wine.

Building a recognizable brand for the high-quality bottled wines is the core to a strengthened international market position. Moreover, it allows pricing flexibility which is important for overcoming the competitive pressures. Therefore, this paper attempts to identify the competitive (dis)advantages of the Macedonian wine sub-sector.

We first interpret evidence on structural determinants of Macedonian wineries' profitability, grouped into three categories: earnings, capital and financial business structures. Then we interpret evidence on the existing marketing strategies of the Macedonian wineries, and finally we present successful case studies of wineries that have succeeded to create a recognized brand internationally.

The results from this analysis suggest definition of a successful winery that exhibits increasing opportunities to profit under competitive market conditions. The defined winery may be used as a guideline to reinforce possibilities for Macedonian wineries to be able to follow future market signals, considering that they still struggle to adjust to the imposed market-oriented production. In this regard, the results and the analysis may have a wider applicability if the study is applied to other related national or international cases (Vlahovic et al., 2018). The following section briefly describes the determinants of wineries profitability, followed by a description of the existing marketing strategies among the Macedonian wineries and the successful cases for creation of brand equity in the wine sub-sector. At the end conclusions are drawn followed by a short discussion.

MATERIAL AND METHODS

To present opportunities profit under competitive market conditions of the Macedonian wineries, we use three different approaches presented in figure 1. First, we use the financial analysis tool so to provide evidence on the financial condition of the Macedonian wineries, as a starting point in determination of their future possibilities to profit. Second, we use field research methods so to capture the level of Macedonian wineries' commitment towards IPR, as an important marketing strategy in creation of competitive market advantages. And finally, we use the case study approach to present a successful case of a

Macedonian winery in creation of a recognized brand internationally through an effective use of Intellectual Property Rights (IPR).

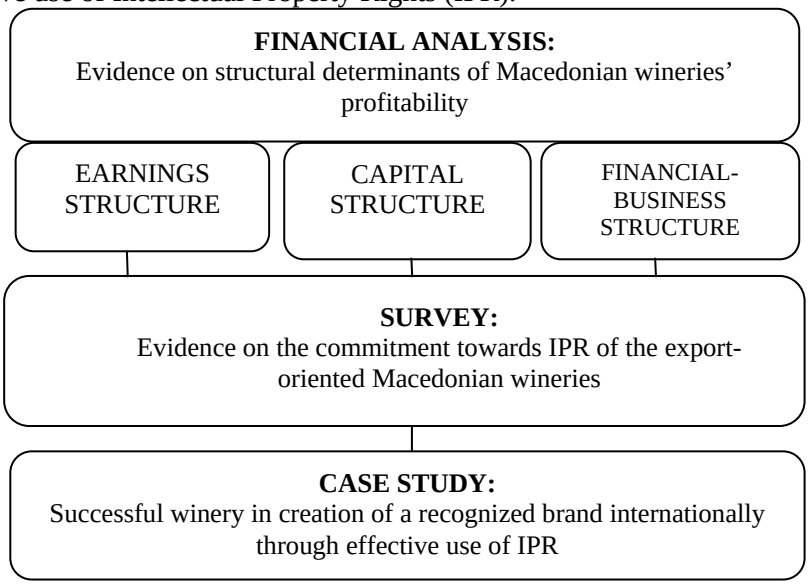

Figure 1. Methodological framework

Financial analysis approach

Financial analysis is a common tool that is used as a standard procedure in the assessment of economic entities' creditworthiness (Altman, 1968) and allows a determination of economic entities' financial position, which is a base for proposal of new investment activities (Smith and Reilly, 1975). The financial ratio analysis provides assessment of the three criteria that determine the economic utility of economic entities, which are, increasing profitability, reducing risk levels and providing liquidity (Barry et al., 2000). By this way, it allows recognition of critical moments that may jeopardize the financial performance, and accordingly indicates if any corrective measures should be undertaken while managing the business so to create basis for better financial performance (profitability).

This tool includes analysis of financial indicators of profitability, liquidity, activity and leverage; however, we give emphasis on the structural determinants of profitability, such as the earnings, capital and financial-business structure, defined in table 1.

The profitability determinants are obtained for 16 Macedonian wineries observed during the period from 2011 to 2016. These wineries cover 98.4% of the installed capacity for wine production in the RM. The official records on wineries, in the form of financial reporting statements (balance sheets and income statements) are provided by the Central Register of the Republic of Macedonia (CRM, 2017).

Table 1. Structural determinants on profitability

Determinants	Code	Definition	Benchmark
		Earnings Structure	
Asset turnover ratio	AT	$AT = \frac{SALES_{T1}}{TOTALASSETS_{\overline{T1+T0}}}$	(C)*
Net profit margin	NPM	$NPM = \frac{NETINCOME_T}{SALES_T}$	(A)*
		Capital structure	
Debt-to-equity ratio	DTER	$DTER = \frac{TOTALDEBT_T}{TOTALEQUITY_T}$	<1*
Debt ratio	DR	$DR = \frac{TOTALDEBT_T}{TOTALASSETS_T}$	<1*
		Financial-business structure	
Return on assets	ROA	$ROA = \frac{NETINCOME_{T1}}{TOTALASSETS_{\overline{T1+T0}}}$	(A)*
Current liquidity ratio	CLR	$CLR = \frac{CURRENTASSETS_T}{CURRENTLIABILITIES_T}$	>2*
Quick-liquidity ratio	QLR	$QLR = \frac{(CURRENTASSETS_T - INVENTORY_T)}{CURRENTLIABILITIES_T}$	>1*

Legend:
* Interpretation depends on the industry.
(A) The higher the ratio indicates higher profitability of the company.
(C) The higher the indicator is, the higher the efficiency is in terms of the use of funds.

The wineries earnings structure is explained by the asset turnover and the net profit margin. It shows the winery's strategy in increasing profit opportunities. The asset turnover is the ratio of net sales over total average assets measured between two time periods. It is a measure of sales income per employed assets. In order to determine the financial strategy of wineries to increase their opportunities for profit, we consider two indicators on capital structure. The debt-to-equity ratio, which is a ratio of total debt over total equity, measures the farms' leverage as proposed in the literature (Abu-Rub, 2012). On the other hand, the debt ratio shows the proportion of total assets financed by external sources of capital. Financing through debt is considered to offer the lowest cost of capital. And finally, we consider three indicators of the financial-business structure of the wineries so to determine their profitability and liquidity position. Return on assets, also recognized as to return on investments, measures the rate of return on assets employed by the company and shows how profitably the winery is using its assets. Liquidity ratios show the ability of the winery to timely payback its liabilities. Liquidity is provided by owning liquid assets or possessing the capacity to borrow additional funds. If the winery is not liquid, it

means that its survival is threatened. The difference between the current ratio and the quick liquidity ratio is that the later ratio excludes inventory from current assets, as being low liquid asset.

The procedure for calculation of these financial indicators is simple, but their interpretation is specific and depends on several factors, especially important is the type of the industry (Helfert, 1997), in this case the wine sub-sector. There are three main types of comparison for assessing the financial indicators: trend analysis, benchmark analysis and cross-industry comparison (Horngren et al., 2010); however, considering the data type we only use the first two approaches so to provide evidence on the structural determinants of profitability of the selected Macedonian wineries.

Wineries' commitment towards IPR

In order to observe the IPR commitment of the Macedonian wineries, structured questionnaire that follows five stages of the model of intellectual property portfolio (IP portfolio) was conducted to 30 export-oriented wineries, of which 14 responded to the questionnaire. The table 2 presents the sample distribution by total capacity.

Table 2. Distribution of the wineries according to production capacity

Size	Number of wineries	Total capacities (l)
Micro	3	<50.000
Small	1	50.000 – 100.000
Medium	2	100.000– 1.000.000
Large	8	>1.000.000

RESULTS AND DISCUSSION

Profitability determinants of the wineries

The profitability determinants are obtained for 16 Macedonian wineries observed during the period from 2011 to 2016. These wineries cover 98.4% of the capacity for wine production in the RNM. The official records on wineries, in the form of financial reporting statements (balance sheets and income statements) are provided by the Central Register of the Republic of Macedonia (CRM, 2017). The results are presented in table 1.

The return on assets reflects (ROA) the profitability of wineries on how well they utilize their fixed assets in making earnings, or simply, the earnings per asset unit. Arsov (2008) considers 5% as the floor limit of the return on assets for selected Macedonian companies, including food processors. However, our findings on return on assets for the Macedonian wineries is 3%, and peaking at 5%, likely due to an improvement in the investment environment supported by lowered credit interest rates.

The wineries earnings structure is explained by the asset turnover and the net profit margin. It shows the winery's strategy in increasing profit opportunities. The asset turnover is the ratio of net sales over total average assets

measured between two time periods. It is a measure of sales income per employed assets, varying between 0.34 and 0.5 for wineries. The net profit margin indicates the amount of net income available to equity holders. The higher the net profit margin the more pricing flexibility a company has in its operations. For Macedonian wineries, there is a negative relationship between asset turnover and net profit margin, which is decreasing over time probably due to financial rigidities.

Table 3 Summary statistics for 26 agribusiness companies

Variable names	*Abbreviations*	*Mean*	*Overall Std. Dev*	*Yearly Min.*	*Yearly Max.*
Winery Performance	***WP***				
Return on assets	ROA	0.03	0.07	-0.17	0.39
Earnings Structure	***ES***				
Asset turnover ratio	AT				
Net profit margin	NPM	0.02	0.32	-2.42	1.10
Capital Structure	***CS***				
Debt-to-equity ratio	DTER	1.23	1.19	0.05	6.91
Debt ratio	DR	0.52	0.26	0.05	1.00
Liquidity Structure	***LS***				
Current liquidity ratio	CL	2.05	1.50	0.01	7.29
Quick-liquidity ratio	QL	0.82	0.73	0.01	5.57

In order to determine the financial strategy of wineries to increase or decrease their opportunities for profit, we consider two indicators on capital structure. The debt-to-equity ratio, which is a ratio of total debt over total equity, measures the farms' leverage as proposed in the literature (Abu-Rub, 2012) as an indicator of a farm's capital structure. Financing through debt is considered to offer the lowest cost of capital. The debt-to-equity ratio for Macedonian wineries is 1.23 which is uncommon situation in agriculture, since farms usually have a simple capital structure having a debt-to-equity ratio of between 0.5 and 1 (Barry *et al*., 2000) due to simplicities in the hierarchy of decision processes. However, wineries are more complex production units, and depending on size, they may have more complex hierarchical composition than farms. Considering the debt ratio, which is a ratio of total debt over total assets, it measures the financial risk wineries face. Wineries that hold more assets than debt have a ratio of 0.5. In this regard, their financial behaviour is similar to individual farms. In fact, Barry *et al*. (2000) set a floor limit of this ratio for farms to be lower than 0.5. On average, the debt ratio remains constant over the observed period.

According to Devic and Krstic (2001), size is one of the most important determinants in financial decisions *i.e.* larger wineries have higher leverage than smaller ones. They measure size by taking the logarithms of net sales. However, we calculate size as the share of the farm company's individual net sales in

relation to the total net sales for all companies in the sample. Large and well-established wineries operating at a higher size percentage may show a better performance than smaller companies, under the average percentage size. This is probably due to established marketing arrangements.

Inventory assets serve as a buffer to meet market uncertainties which can easily be turned into liquid assets. The days in inventory for wineries are on average 411 days. The longest period for holding inventory assets is observed during 2010 probably due to the limited purchasing power of the wine consumers as a result of the global financial crises started in 2008.

The quick-liquidity ratio shows the wineries' ability to cover current liabilities capturing determinants in the business cycle, as an industrial development indicator. The wide variation of the quick-liquidity ratio among wineries indicates that there are high liquidity constraints among some of the wineries, while others are highly liquid. Actually, holding more debt than equity decreases the liquidity of wineries, and increases their exposure to the financial risk (Barry *et al.*, 2000). The highest quick-liquidity ratio is 1.04, and the lowest 0.67.

There is a weak return on invested capital among the wineries, only 3% in comparison to 5% for other industries. Low profitability of farm companies is induced by low net profit margins, which is not specific for the wine sub-sector since it allows added value production aligned with high net-profit margins. This shows that wineries have not yet adopted a market-oriented strategy, using the pricing flexibility to cope the competition. Instead, volume production is preferred which can be seen from the large asset turnover as opposed to the low net-profit margins. Assets are preferred over debt, indicating fewer investment activities of Macedonian wineries.

Macedonian wineries' commitment towards IPR

The marketing strategies of the wineries in the RNM are observed through a survey among 14 export-oriented wineries by using a structured questionnaire. These wineries hold the greatest proportion of the domestic market share. Despite the domestic market, the selected wineries are well recognized on regional markets.

The questionnaire gives an insight in the winery IP related behavior in order to understand the perception of wineries about the role of the marketing strategies play as part of the wineries' business strategies, where the special attention is paid on the intellectual property rights' (IPR) commitment. This issue is becoming more and more attractive with the aspiration of the RNM to join the EU creating a demand for strong system of IPR enforcement in practice.

There is low awareness of the benefits of IPR among wineries. Implementation remains a challenge, and the system of collective management of these rights is still underdeveloped (European Commission, 2017).

Small firms often lack or cannot afford to build up specific competencies. They also lack the financial capability to defend the infringed IPR. As most

important variables when explaining the use of IPR, Keupp M., *et al*., 2009, point to the firm size. Lopez 2009, argue that very little is known about the appropriability strategies displayed by different groups of firms, or the ways in which different kinds of innovations are protected in developing countries. He points out the reason that micro-level studies are, relatively scarce, making it difficult to learn about the determinants of the use of IPR in different types of firms and sectors in developing countries. Furthermore, there is a lack of evidence regarding the perception of domestic firms in developing countries about the role that IPR play, or might play, in the context of their innovation strategies. The wine sub-sector in the RNM is characterized with low of awareness of the economic benefits of protection and enforcement of IPR (Anastasovska-Dabovic and Zdraveva, 2009).

Implementation of IP and level of protection fairly much differs between sizes of the wineries and product finalization. Innovative strategy is present in 71% of the wineries, which invest particularly in new product, new technology, replacement of old equipment and patents and licensing. From the wineries, 64% have already protected different IPR, mostly trademarks (70%) and industrial design (30%).

As main reasons for not protecting IP rights, wineries state that finances are the largest barrier, then lack of knowledge about legal procedures for protection and lack of information about different types and possibilities for protection. This is mostly evident in the case of small wineries that should consider the possibility of strategic protection (especially in innovating strategy, vision for new markets and constant marketing investments) of those rights that would gain economic benefit to the winery. However, 70% of the wineries believe that protection justified the costs, taking in to account the potential benefits that could arise from it (Figure 2).

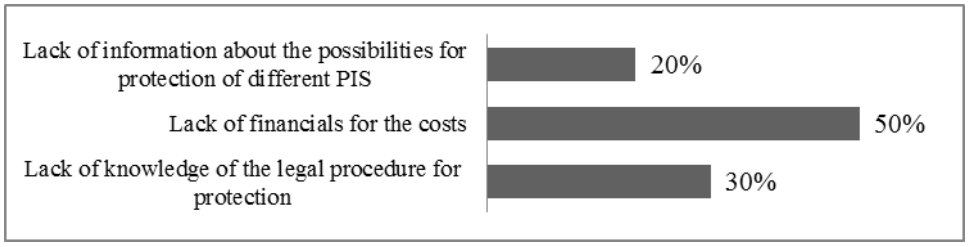

Figure 2 Main reasons for sufficient IPR protection

IPR-committed wineries with protected trademarks and industrial designs that constantly invest in marketing successfully create product differentiation and brands. The non-IPR committed wineries have faced with lack of information about the possibilities for protection and financial barriers for conducting the procedures, so IPR is perceived as investment that require additional costs.

Regarding export orientation, 71% believe that increased export of bottled wines increased the need for IPR protection, which is in line with Keupp M., *et*

al., 2009, who stressed that exporting companies are, to some extent, more likely to use IPR protections. It depends mostly of the winery strategy and the exporting market. In case of export orientation, crucial to be considered is the fact that larger, open and competitive markets bring higher risk of counterfeit of the package and label (the case with large Macedonian winery), creative label (micro winery) and creative idea (small winery). In case of counterfeit, IPR user that first protects the right of ownership could withdraw the competitive products.

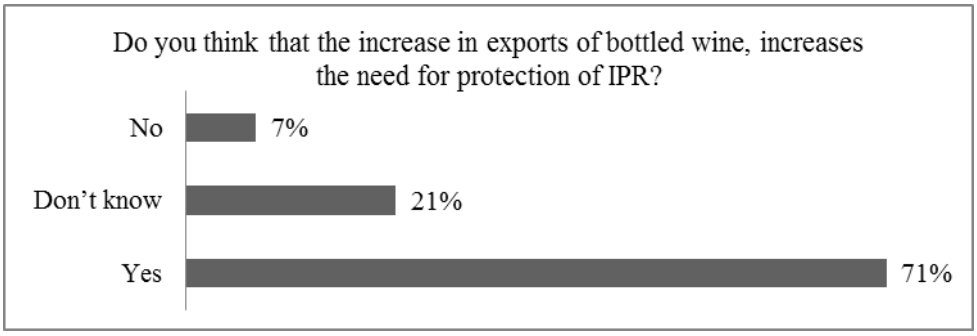

Figure 3 Importance of IPR protection in export orientation

Main motives for protection of IPR are wineries intention to build reputation and to prevent counterfeits and imitation. Additionally, wineries protect IPR in order to build strategy for differentiation and competitiveness of products in the market. These motives are related to the overall strategy of building corporative brands, because consumers' perspectives are ranked with lower priorities. In fact, it presents that wineries are directed mostly to international markets, and perceived the domestic market as monopoly in terms of competition. However, according to the forthcoming period and tendency in the agri-food trade and policy, as well as globalization of the markets, this trend could be considerably changed in the future.

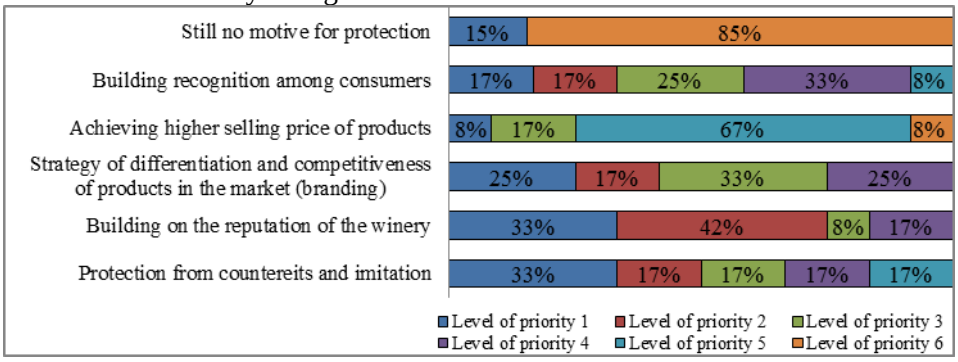

Figure 4 Motives for protection IPR

Exceptional potentials for long term benefits and income from protection of IPR are considered by large and market-oriented wineries especially when

they use marketing strategies for branding. Accordingly, micro and small wineries with bulk production has no potentials at all. However, almost all wineries have equally or higher expectations for gaining incomes of strategic use of protection of IP assets.

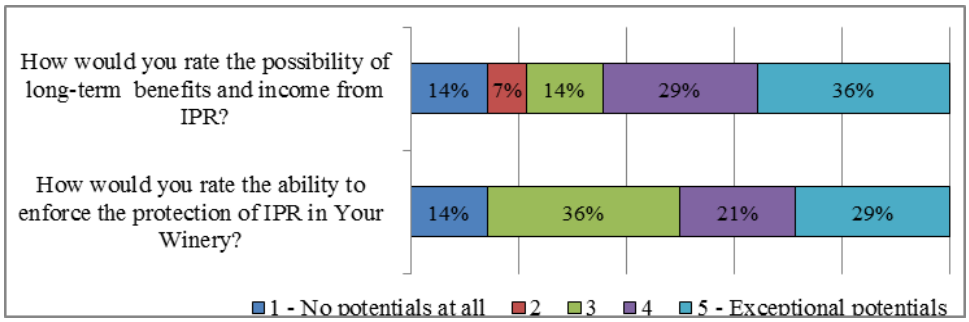

Figure 5 Possibilities for benefits and ability to enforce of IPR protection

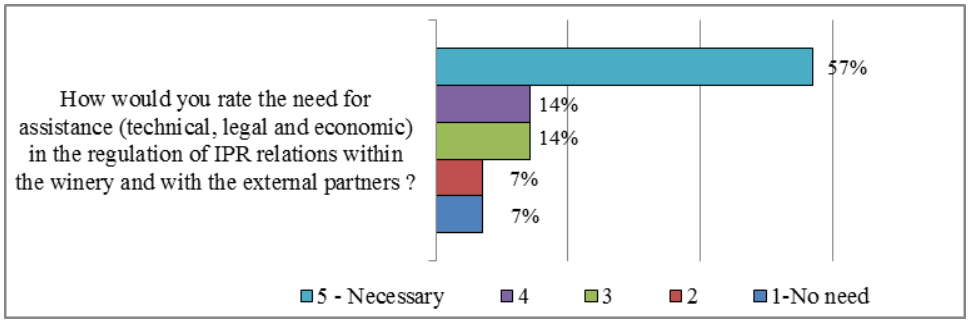

Figure 6 Need for technical, legal and economic assistance in IPR procedures

In 36% of the wineries, education in the field of IPR is provided as an integral part of training courses to employees or managers in the winery. This present represents the large wineries that mostly exist on the domestic and regional markets and use a strategy of product differentiation and building brand identity. Other wineries that do not provide education are small, micro or those that do not use sufficient marketing investment for further development of individual or corporative brand.

A case of a successful winery's brand equity creation

A single case study of a successful winery is presented to guide future competitive strategies in the wine sub-sector in the RNM. The selection of the case was based on the level of importance of the wine sub-sector for the RNM and on a distinctive IP experience that this winery has. The wine production is a crucial strategic sub-sector for the country, confirmed by the following facts: 1) 70% export-oriented, 2) growing trend of development, 3) high degree of specialization, and 4) positive investment trends in sophisticated equipment and marketing skills (CBI, USAID, 2012). In the next period, the development of this

sub-sector will be intensified by positioning wines as the most important exported and competitive brands from the agri-food industry in RNM (MAFWE, 2014-2020).

The high-level IPR committed winery successfully transforms IP assets into high brand equity by continuously investing in marketing strategies. As a result, it holds the dominant domestic market position with a 65% market share of the total production of bottled wines (or 75% market share in terms of sales value). It is also successfully established on the regional markets, holding award of the most recognized regional brand. The persistent innovative strategies followed by a strategic use and protection of IP assets, the permanent marketing investments and the investments in education in the field of IPR as an integral part of training courses, distinguishes this winery as a representative case.

The case study is of a high-level IPR committed winery that successfully transforms IP assets into brand equity and simultaneously protects IPR. By this, it was ranked on the list of 100 most perspective global brands (M&M Global, 2012). In this regard, the following marketing strategies and activities were undertaken: *1) Constant innovation, 2) National and international trademarks protection, 3) Product differentiation through branding and designs, 4) New international markets' penetration and 5) Constant marketing campaign.*

In the past years, the winery invested more than 20 million Euros in modernization of technology, equipment and processes. Their main objective is to rapidly raise the product quality so to offer competitive brand on international markets. Therefore, they rely on both, product and process innovative strategies. The long-term strategy of the winery is to continuously invest in technology, new product development, human creativity and knowledge. The permanent IPR protection enables the winery to prevent counterfeit, thus strengthening its market position and increasing its commercial value.

The winery protects mostly trademarks; around 80 domestically and 50 in each regional country. This contributed to increased brand visibility, exclusivity and prevention from counterfeit. For instance, the winery was faced with many counterfeits of the packages and the labels; however, due to its commitment towards IPR, it effectively coped against the infringement of the protected rights.

The main motives for protection of IPR are winery intention to build reputation and to prevent counterfeits and imitation. Additionally, it protects IPR in order to build strategy for differentiation and competitiveness of products in the market. These motives are related to the overall strategy of building corporative brand, because consumer perspectives are ranked with lower priorities. The corporative brand is perceived as fastest growing brands in the RNM. In the process of brand creation, the winery constantly introduces new and improved products, adopts new production techniques and marketing of product and services and protects them with IPR. Its long-term marketing strategy resulted in quality products that created an emotional relation between its brands and consumers.

With regards to the new international markets' penetration, the winery considers that export orientation and penetration of new markets, such as countries from the European Union, USA, Canada, China and Australia, increase the need for IPR protection. It is widely known that the level of protection depends of the company strategy and the exporting market. Having in mind that, larger, competitive and globalized markets bring higher possibilities for counterfeit, they usually make the decisions strategically, based on the economic interest. The procedure is costly, so the protection in new international markets is mostly directed towards protection of trademarks and industrial designs that evolved into strong brands.

Even though by the Macedonian Law on Assessment only the trademark's financial value is determined, the winery regularly conducts research for valuation of the emotional brand perspective. These findings are helping the winery to define the current brands' positioning, as well as the key indicators of the brand strength, domestic and regional consumers' perception and the potential for launching new products. Based on these findings, the winery performs in-depth analysis, necessary for marketing strategies development, as well as defines plans for development and improvement of products and for evolution of their brands.

Due to these strategies, including IPR protection supported with marketing investments, the winery has increased its market share and has gained higher business opportunities. Thus, within a period of ten years, its products' average sales price increased by 75%, mainly due to the brand image.

CONCLUSIONS

This research covers three aspects in order to depict the opportunities to profit under competitive market conditions of the Macedonian wineries. These aspects are: a) the existing profitability strategies; b) the general marketing strategies, and c) the distinguished marketing strategies for a brand creation.

The earnings structure suggests that Macedonian wineries in the short run are limited by pricing flexibility undertaking different strategies in order to increase profitability. More efficient investments are undertaken by growing wineries. By holding small inventories, wineries may easily follow market signals adjusting production capacity.

Wineries in RNM rely more on debt than on equity to operate activities. However, statistical evidence does not support the hypothesis that high-leverage increases opportunities for wineries to profit. Probably due to asymmetries between the national capital and credit markets and farm companies, increasing risk exposure. However, in line with Arsov (2008) results suggest that wineries prefer more assets than debt, considering financial risk in the long run decisions. This strategy seems to be a good financial strategy to increase profitability of wineries with positive net sales growth while the opposite is valid for those companies operating at negative net sales growth. Wineries facing a negative equity signal financial distress, without the ability to generate sufficient liquidity.

Considering the preceding, we may define a typical winery that exhibits increasing opportunities to profit, as follows: a low-levered winery showing a positive net sales growth, relaying on assets rather than debt, larger than the average size in terms of total capacity, able to cover current liabilities taking advantage of the fluctuations of the business cycle.

However, the wine sub-sector needs to establish and maintain competitive international position by imposing intensive marketing strategies in creating strong brand equity.

The wineries' use and protection of IPR depends on the size of the wineries, availability of finance for protection and investments in marketing as well as accessibility to information and knowledge about IPR protection and use. There is a high protection of trademarks and designs, but economic value of IP assets is not sufficiently used. It means that most of the cases do not transform trademarks into strong brands because lack of marketing investments. The field experience implies that only the biggest and successful wineries show high commitment to IPR so far, thus investing in marketing and building a corporative brand of the winery or individual brand of the products. Furthermore, general awareness for IPR exists, but there is low expertise within the wineries and cooperation with IPR experts that result in insufficient IPR enforcement. The effective use of IPR requires that they should be well incorporated into a firm's overall strategy. This is more often the case with large Macedonian wineries than the smaller ones, which do not use IPR in the same way as larger companies. Small wineries predominate with 90% of the total number, usually faced with financial constraints and low marketing budget, so, commitment to IPR is perceived as investment that require additional costs.

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Mersida JANDRIĆ , Željko SAVIĆ [1]

INFLUENCE OF MILK ON THE CONTENT AND CHANGES OF MILK FAT DURING PRODUCTION OF SJENIČKI WHITE CHEESE

SUMMARY

The quality and overall values of the cheese depend on the type and quality of milk. Milk fat is the most important parameters of quality which determines randman, consistency, rheological characteristics and sensory properties of cheese dough. The studies aimed to determine the significance of milk type for the percentage of milk fat content after cheese making, changes during ripening, as well as the content of mature cheeses for a 45-day ripening period when it comes to Sjenica cheese of industrial production. Studies have shown that the milk fat content in cottage cheeses were for cow milk cheese 21.33% and 23:43% for sheep milk cheese. During ripening, the milk fat content in both kinds of cheese for the whole period of ripening steadily increased. After 45-day period of ripening cow milk cheese had an average content of 25.66%, and sheep milk cheese had 29.36% milk fat.

At the end of the ripening period of 45 days average content of milk fat was in cow milk cheese 25.66% and 29.36% in sheep milk cheese. Dynamics of milk fat in dry matter had a different trend. In cow milk cheese was recorded a decrease in the first 30 days and a slight increase in the last 15 days. Sheep milk cheese had a trend of balanced increase for the entire period of ripening. The content of milk fat in dry matter (MF in DM) in mature kinds of cheese in average was 51.07% in cow milk cheese and 52.72% in sheep milk cheese. These results showed that the cheese had a high content of fat in dry matter and belong to the group of full-fat kind of cheese.

Key words: Sjenički cheese, milk fat, ripening.

INTRODUCTION

Sjenički cheese is one of the famous white brined cheese in the Republic of Serbia. Center of the production are mountain plateau, with a rich, healthy and high-quality natural meadows and pastures on the Pester plateau that surround the town Sjenica. It is produced by the indigenous technology on individual farms, and now more and more in industrial conditions. The raw material for the production is fresh whole fat cow's and sheep's milk without the application of heat treatment in process of making cheese.

The quality and overall value of the cheese depends on chemical composition and quality of milk, whereby a fat content is of particular

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

importance. The fat content of milk depends on the species, breed, diet, lactation, etc. Sheep's milk has high values of chemical components, and it is the best raw material for the production of cheese, because it gives twice higher randman than cow's milk. Milk fat is one of the most valuable parameters of the chemical composition of milk on which depend the quality and the overall value of the cheese.

The content of milk fat in cheese affects on its energetic and nutritional value, chemical composition and rheological characteristics, respectively, its structure and consistency. Milk fat in cheese is mainly in solid, aggregate state and it is distributed in a protein matrix.

Together with water, even though to a less extent, it is the carrier of viscous properties of the cheese. The milk fat content is determined by the size of fat droplets in milk, standardization, coagulation and curd processing (Bringe and Kinsella 1986; Fox and Cogan 2000; Lopez et al., 1998).

Djordjević (1987) states that the size of fat droplets affects the retention of fat in the cheese lump and its transition into the whey. If fat droplets are larger and if process of making cheese lasts longer, the transition of milk fat into the whey is greater during the curd processing.

The main function of fat is reflected in its contribution to the sensory properties of cheese. The milk fat, due to the high share of low and medium fat acids, as well as the characteristic melting temperature, which is close to the temperature of a human body, has a very pronounced effect on the taste and smell of cheese. Therefore, the cheese with high fat content is characterized by a fuller taste and smell (Puđa, 2009).

Milk fat, with proteins, represents the predominant part of the dry matter of cheese. Changes in milk fat during ripening are at much lower level in response to changes of proteins. A smaller volume of fat change is the result of very limited accessibility of substrates. The milk fat during cheese production retains the shape of fat droplets, the form in which the milk fat is originally present in the milk. During cheese ripening membrane of fat droplets constitutes a sort of protection to lipids content, so that the lipases presented in the cheese mass significantly come more difficult into contact with the substrate. The decomposition of fat by enzymes lipase is done during the ripening process, which means, hydrolysis of the triglycerides to free fat acids and small amounts of diglycerides and monoglycerides is occurred. It is generally accepted that the lipase is largely responsible for the release of free fat acids, which contain more than four carbon (C-4) atoms. The free fat acids affect the taste of the cheese, and serve as a substrate for the formation of other compounds such as: alcohols, estri, aldehydes, ketones, et al. (Fox et al., 1993; and Fenelon Guinee, 2000).

Milk fat and its degradation products influence the formation of the sensory properties of the cheese. However, in terms of the uncontrolled maturation, changes in free fat acids can cause the appearance of rancid taste (Wilkinson, 1990).

MATERIAL AND METHODS

The experiments with the industrial production of cheese are made in the dairy "Pester" in Sjenica, Republic of Serbia. The both types of cheese are made from raw whole cow's and sheep's milk. Preparation of milk included primary treatment (squeezing- filtering) and reheating at a temperature, equal to the temperature needed for the process of making cheese, of 32 ^{0}C. Milk coagulation lasted 50 min. Curd was cut into cubes of 5x5 cm. Whey separation and the formation of curd was carried out by filtration in the course of 1.5 h. The cheese was sliced into slices of 10x10 cm. The research was aimed to determine the content of fat and fat in dry matter (MF in DM) in cheese after the preparation (1stday), and then to monitor their changes after 15,30 and 45 days of ripening which was optimal ripening period.

Cheese analysis was conducted in the chemical laboratory of the Veterinary specialist institute, by the following methods:

-Determination of fat percentage by Van-Gulik Acidobutyrometric method (Carić et al., 2000).

- The both types of cheese were made in 5 repetitions, and the total sample was 10.

Rate of statistical significance was carried out over a difference of arithmetic means and measures of variations, standard deviation (SD) and coefficient of variation (CV). Student's t-test was used to test the difference of means (Stanković et al., 1989).

Determination of % fat by Van Gulik method

Apparatus and accessories: Analytical balance; Butyrometer for cheese; Automatic pipette 10 ml; Gerber's centrifuge; Water bath, t = 65-70 ^{o}C.

Reagents: sulfuric acid; Amyl alcohol.

Procedure: In a butyrometer glass 3 g cheese sample is weighed. We put the glass in butyrometer, and through upper opening of butyrometer, we pour sulfuric acid so that the acid level covers the contents of the cup. We close the butyrometer, shake it strongly and place it in a water bath temperature of 65-70 ^{0}C, with intent of cheese dissolution. It is needed to jumble the content of butyrometer every 15 min. Agitation of butyrometer is steadily growing until the complete dissolution of cheese, whereby the liquid gets darkly violet color. When the sample is dissolved, butyrometer is removed from the bath and using automatic pipette we add 1 ml amilo alcohol, then we well shake the content and add sulfuric acid approximately to the upper line of the scale. Shaken butyrometer is kept in a water bath for 5 min at a temperature of 65 °C. Then we clean off the butyrometer and put in a Gerber's centrifuge. Centrifuge it for 5min, remove the butyrometer and place it with facing down lower stoppers in a water bath of 65 ^{o}C, for 3-5 min.

Isolated fat in dried part of butyrometer is the amount of fat in weight percentages directly seen on a scale of butyrometer.

RESULTS AND DISCUSSION

Milk fat is an important nutritive component of milk and cheese and greatly contributes to the formation of specific sensory and functional properties of the cheese. Milk fat which is incorporated into a protein matrix, which presents the base of cheese structure, provides a so-called "Smooth taste of whole fat milk cheese" (Miočinović, 2013).

Research results related to the dynamics of fat during cheese ripening are given in Table 1.

Table 1. Dynamics of fat during cheese ripening in %

Cheese type	Parameters	Ripening period (days)			
		1	15	30	45
Cow's milk cheese	Min	20.43	19.90	18.70	21.39
	Max	22.10	24.71	27.34	27.63
	$\bar{x}$ =(n=5)	21.33	22.26	24.29	25.66
	Sd	0.60	1.55	2.94	2.28
	Cv %	2.83	6.96	12.10	8.91
Sheep's milk cheese	Min	22.03	24.76	26.62	27.63
	Max	24.71	26.25	30.78	29.85
	$\bar{x}$ =(n=5)	23.43	25.94	28.31	29.36
	Sd	0.87	0.76	1.77	1.33
	Cv %	3.71	2.95	6.26	4.50

Testing the significance of arithmetic means

Ripening period-days	t-calculated	X_1-X_2
1	4.46	2.10**
15	4.77	3.68**
30	2.62	4.02*
45	3.13	3.70**

The theoretical values of the „t" arrangement refer to the number of degrees of freedom (df) 8 and are: $p < 0.05 = 2.179$; $p < 0.01 = 3.055$

At the beginning of ripening cow's milk cheese had an average milk fat of 21:33%, and sheep's milk cheese 23:43%. The analysis of the results showed that these differences were statistically significant. Differences in fat content are the result of different water content, as well as the raw material that is used for making cheese.

As a ripening period develops, an increasing content of milk fat for the entire period of maturity in both types of cheese is noticed, whereby this increase was even.

After 15-days of ripening average increase of milk fat in cow's milk cheese amounted 0.93% and in sheep's milk cheese 2:51%, so that the cow's milk cheese had an average of 22:26% of milk fat and sheep's milk cheese

25.94%. Processing of the results showed that the differences between the both types of cheese were highly significant.

Table 2. Dynamic of milk fat in dry mater (MF in DM) during ripening period in %

Cheese type	Parameters	Ripening period (days)			
		1	15	30	45
Cow's milk cheese	min	49.37	47.29	42.58	45.94
	max	52.88	50.94	50.98	53.52
	$\bar{x}$ =(n=5)	51.76	49.16	48.33	51.07
	Sd	1.26	1.42	2.94	1.64
	Cv %	2.44	2.90	6.09	3.22
Sheep's milk cheese	min	47.81	49.01	52.22	49.11
	max	51.50	52.20	54.64	55.33
	$\bar{x}$ =(n=5)	49.44	50.46	51.21	52.72
	Sd	1.26	1.35	2.73	2.66
	Cv %	2.55	2.68	5.34	5.04

Testing the significance of arithmetic means

Ripening period-days	t-calculated	X_1-X_2
1	2.91	2.32*
15	1.48	1.30
30	1.60	2.88
45	1.18	1.65

The theoretical values of the „t" arrangement refer to the number of degrees of freedom (df) 8 and are: $p < 0.05 = 2.179$; $p < 0.01 = 3.055$

The fat content trend increasing has continued in the period of 15-30 days of ripening. This increase in cow's milk cheese was 2:03%, and 2:37% in sheep's milk cheese. After 30 days of ripening cow's milk cheese is the average had 24.29% of milk fat and sheep's milk cheese had 28.31% of milk fat. Analysis of the data showed that the differences between the cheeses were statistically significant.

During the last 15 days of ripening, a further increase of fat content was observed. After a set period of ripening, the average content of milk fat was 25.66% in cow's milk cheese and 29.36% in sheep's milk cheese.

Analysis of the data resulted in statistically highly significant differences among both kinds of cheese.

If we compare both types of the cheese at the beginning and end of the ripening period, we can conclude that the milk fat content increased in cow's milk cheese for 4:33% and 5.93% for sheep's milk cheese.

If we analyze the correlation between fat content and moisture content of the cheese, it leads to the conclusion that the reduction in moisture is in

proportion to the increase of milk fat content. Proof of this is sheep cheese, which contains the most milk fat, and contains a minimum of moisture.

The obtained results of milk fat content are in accordance with the results of similar types of cheese in this group, and which state the following authors: fat cheese 26.32%, Pljevaljski cheese 25.44% Polimsko-Vasojevićki 29.38% Polimsko-Sjenicki 24.92% (Dozet et al. 1996); Domiati 22.75% (Abd El Salam 1993); White cheese-slice 19.87% (Živić, 1989); Homoljski 28.42% (Jovanovic et al., 2004); Sjenički 27.22%, 27.70% Zlatarski (Maćej et al., 2006); Sjenički cheese 26.38%, Sjenički type cheese 24.63% (Savić, 2011); Polimsko-Vasojevićki 29.85% (Konotar, 2006).

Savić et al., (2016) state that the basis of curd structure is composed of proteins, while milk fat plays a filler role and is incorporated into the curd network and contributes to the soft consistency of Sjenica cheese and good sensory properties.

During the cheese ripening, different processes occur, some of which lead to the concentration of milk fat in dry matter. The fat content in dry matter depends on the amount of salt (NaCl), because the salt drawing water from the cheese increases dry matter. Among other processes that affect the concentration of milk fat in dry matter, it includes also the transition of soluble nitrogen matter of cheese into brine for ripening, as well as the decline of the salt concentration in the cheese. Results in the content and dynamics of milk fat in dry matter (MF in DM) are given in Table 2.

Based on these results, it can be seen that dynamics of fat in dry matter had a trend of balanced increase for the entire fixed period of maturity of 49.44% to 52.72% in sheep's milk cheese. In cow's milk cheese it is recorded a declining trend for the period 1-30 day maturity from 51.76% to 48.33%, and the increase in the last 15 days of ripening for 51.07%.

At the end of the set period of ripening, the milk fat content in the dry matter, in average was 51.07% in cow's milk cheese and 52.72% in sheep's milk cheese. Processing of the results showed that the differences between two types of cheese were not statistically significant. Bearing in mind the fact that fresh whole milk is used for making cheese, therefore cheese is characterized by a high content of fat in dry matter (51.07%, 52.72) due to which belong to the group of full-fat cheese.

CONCLUSION

Milk fat is one of the most valuable parameters of chemical composition and quality of the milk, and the physical properties, chemical composition of curd, structure and randman of cheese production depend on it.

Milk fat content in cheese effects on its energetic and nutritional value, chemical composition and rheological properties and on its viscosities consistency structure. The type of fat cheese has softer coexistence, while the cheese with lower milk fat has tougher dough and firmer consistency.

Basic function of fat is reflected in her contribution, formation of sensory and functional properties of cheese, and lower and middle fat acids. Milk fat as a very pronounced effect on the taste and smell of cheese, which is a characteristic of sheep's milk cheese.

Cheese is characterized by a high content of fat and fat in dry matter (MF in DM), since the raw material for making cheese was whole cow's and sheep's milk. On the basis of fat content in dry matter (51.07% and 52.72%), all types belong to the group of full-fat types of cheese.

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MULTIVARIATE ANALYSIS FOR WHEAT GENOTYPES CULTIVATED IN BRAZILIAN SAVANNA (CERRADO)

SUMMARY

The Cerrado is an important agricultural region for the production of food, feed and (bio)fuel, with great potential for the cultivation of tropical wheat (*Triticum aestivum* L.). However, it is necessary to understand how some genotypes of that crop would fit into this biome, as well as to identify possible physiological and chemical markers that may contribute to the selection of the best plant materials. The objective of this research was to evaluate the metabolic, physiological, biometric and productive characteristics of six wheat cultivars, commonly cultivated in the Cerrado of Minas Gerais, through multivariate analysis (Principal Components Analysis: PCA), in order to characterize the performance of these cultivars in the Cerrado conditions, in two years (2016 and 2017). The PCA showed that monosaccharides content (glucose and fructose) and net CO_2 assimilation rate were highly correlated in both years. Significant differences in rainfall between the two years resulted in different responses of the cultivars and their respective metabolic, physiological, biometric and productive behaviour. Furthermore, it was demonstrated that the CD 151 and Bio Sintonia cultivars grew better when exposed to favourable rainfall conditions, whereas BRS 264 and BRS 394 were the cultivars most suited to lower rainfall.

Keywords: *Triticum aestivum* L., physiology, sugars, dryland cultivation

INTRODUCTION

The Cerrrado, a Brazilian neotropical Savanna, covers 204.7 million hectares in the centre of the country (Sano *et al*., 2010), and it can be considered a region with great potential for the production of tropical wheat (*Triticum aestivum* L.) (Pasinato *et al*., 2018). Specifically, in the state of Minas Gerais, the land used by agriculture covers 2,122,452 hectares and the wheat cultivation, in 2018, occupied approximately 84,000 hectares (CONAB, 2018).

The Cerrado biome is characterized by a dry period from May to September (Wolf, 1977), which hampers agricultural production (Assad *et al*., 1993). For the last 40 years there has been a search for better wheat cultivars for the Cerrrado and, currently, agronomists specialising in the tropical wheat crop

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seek genetic materials adapted to dryland or irrigated conditions (Caierão *et al*., 2014). The successful cultivation of tropical wheat in the dry climate of the Cerrado is explained by the early sowing (between February and March) of cultivars with a short growth-cycle on to water retentive soils (Pasinato *et al*., 2018). Furthermore, this crop stands out because it is harvested at a different time compared to the wheat grown in the main cereal producing regions in Brazil (Ribeiro *et al*., 2012). Approximately 20% of the total proteins and calories consumed by the world's population are supplied by the wheat crop and, because of this global significance, it is vital that cultivars are developed that are adapted to environmental changes, such as: increasing temperature, carbon dioxide, drought and salinity (Shiferaw *et al*., 2013; Curtis and Halford, 2014). In order to combat such environmental stresses, plants are likely to have developed the use of some sugars as 'signalling molecules', which control the plants growth and development (Sheen *et al*., 1999; Miller *et al*., 2010; Keunen *et al*., 2013) and protect the plants tissues under inhospitable conditions (Couée *et al*., 2006). These latter four papers suggest that many carbohydrates function as 'adjustment markers' in plants under adverse conditions, with important signalling in their growth and reproductive stages.

The identification of those biological processes that act as markers in tropical wheat is essential for its cultivation across new agricultural frontiers, the promotion of new farming practices, and the attainment of higher yields. The aim of this research is to determine the physiological, metabolic and production characteristics of wheat cultivars grown under dryland conditions in the Cerrado.

MATERIAL AND METHODS

Two experiments were conducted in 2016 and 2017 at the Cooperativa Agropecuária do Alto Paranaíba (COOPADAP), Rio Paranaíba, MG, Brazil, whose geographic coordinates are 19º 12' 26" S and 46º 10' 05" W, with 1,140 m of altitude. The Köppen classification of the climate is Aw, with tropical and dry winter, as seen in the course of the experiments (Table 1).

Table 1. Means of air relative humidity (%), precipitation (mm) and air temperature (°C) in experimental area (COOPADAP), Rio Paranaíba (MG), during two years of analysis.

	2016			**2017**		
	RH (%)	Precipitation (mm)	Temperature (°C)	RH (%)	Precipitation (mm)	Temperature (°C)
April	68.60	19.50	21.90	81.75	34.20	23.11
May	74.60	9.39	19.50	75.86	95.20	20.23
June	73.40	28.46	17.40	70.28	12.40	19.10
July	61.50	0.00	18.60	65.26	0.20	16.84
August	58.30	13.22	19.90	54.00	0.00	20.80
Mean	67.28	14.11	19.46	69.43	28.4	20.01

The seeds for the first experiment were planted on 04.24.2016, and for the second on 04.17.2017. Five wheat cultivars were studied in both experiments: Coodetec CD 151 and Coodetec 1104 (Coodetec), together with Biotrigo Sintonia (Biotrigo), BRS 264 and BRS 394 (Embrapa).

Both experiments consisted of four replicates of each cultivar, with each replicate consisting of 5 rows, 5 m in length and spaced at 17 cm, using 400 seeds m^{-2}. Measurements were taken solely from the three central rows of each replicate. Following chemical analysis of the soil in the experimental area, 300 Kg ha^{-1} of Mono-ammonium-phosphate fertilizer (MAP) was applied at planting, and 250 kg ha^{-1} of formulated 21-00-21 (N-P_2O_5-K_2O) during the vegetative stages. Weeds, pests and diseases were controlled, according to the needs of the crop, by products registered for use on wheat crops in Brazil.

The gas exchange analyses were done by a portable infrared gas analyser (IRGA), model LI-6400XT (LI-COR, Lincoln, Nebraska, USA), during the 'boot' stage (Feekes 10.0 phenological stage) (Large, 1954), at 53 days after plant emergence (APE) in the first experiment, and 51 days APE in the second experiment. In both experiments were measured: photosynthetic rate (*A* - μmol m^{-2} s^{-1}); stomatal conductance (*gs* - mol m^{-2} s^{-1}); transpiration rate (*E* - mol H_2O m^{-2} s^{-1}); and intracellular CO_2 concentration (*Ci* - Pa). These measurements were taken between 8:30 am and 11:30 am, under photosynthetic active radiation of 1,500 μmol photons m^{-2} s^{-1}.

A ruler was used to measure plant height (PH) and source-sink distance (SD): i.e. the sum of flag leaf length and distance between the upper culm node and spike insertion (Fioreze and Rodrigues, 2014). Also measured were the numbers of total tillers per plant (TP) and of fertile tillers per plant (FT). The productive components were measured, at 105 days APE in 2016 and at 103 days APE in 2017, by counting: the number of spikelets per plant (NSP); the number of grains per spikelet (NGS); and the total number of grains per plant (NG).

Flag leaves were collected for glucose and fructose analyses between the phenological stages: Feekes 9.0 (ligule of flag leaf visible) and Feekes 10.0 (boot stage), according to the Feekes-Large scale (Large, 1954). After collection, the flag leaves were put into 50 mL Falcon® tubes, which were then placed in Styrofoam boxes containing ice and taken to the laboratory, where approximately 150 mg of leaves were grounded in liquid nitrogen to obtain a fine powder and extracted with 1.5 ml of an ethanol/water mixture (80/20; v/v) during 1 h, then the samples were centrifuged at 3000×*g*, at 4 °C, during 10 min. The supernatant was recovered and evaporated at reduced pressure. The extract was finally dissolved in 1ml water, filtered at 0.45 m and analyzed (Guignard *et al*., 2005). The concentrations of sugars were measured by High Performance Liquid Chromatography (HPLC), using the model LC-10 of Shimadzu Co., with a refractive index detector (Shimadzu Co. model RID 6A) and column (Shimadzu Co. model CLC-NH2 (M)), 15 cm x 6.0 mm, with amine groups chemically bonded to silica. The mobile phase consisted of a mixture of acetonitrile and ultrapure water at a ratio of 80:20 v/v, with a flow of 2.0 mL min-1, at oven

temperature used was 40 °C. The sample was filtered with Millipore disposable filter (Hidrophilic PVDF) with membranes 0.45 µm of pore, and stored in vial of 1.5 ml, being the injected volume equal to 20 µL.

Multivariate principal component analysis (PCA) was applied to identify possible clusters between all dependent variables evaluated in both experimental years. The data were transformed for standardization (a procedure necessary to homogenize the scales), and the calculation of the Eigen vectors values was adopted to determine the importance of each variable (McGarigal *et al*., 2000). The data were analysed using the software PAST - Paleontological Statistics (Hammer *et al*., 2001).

RESULTS AND DISCUSSION

In our research we observed that water scarcity inhibited the genetic potential of crop growth and production, this behaviour is expected in plants under drought stress, according (Fotouhi et al., 2017) this is one of the several environmental factors that greatly limiting crop production and plant establishment, and we adopted the multivariate analysis for understand different mechanisms in wheat plant, under different water conditions by two consecutive years.

By recognizing patterns, rather than classifying data, PCA reveals the existence (or not) of relations or groupings between observed samples (Lyra *et al*., 2010). In this research PCA was used to assess the relation between six wheat cultivars and their physiological and biochemical properties, during two years of cultivation under dryland conditions. According (Bro and Smilde, 2014), the components of PCA explain the variation in the whole dataset in a certain sense, and the technique is able to identify some phenomena. The results of this research showed how the components of PCA could explain 59.98% of total variances obtained in 2016 and 49.85% in 2017 (Table 2). Where it was observed that, in the first year of evaluation, principal component 1 (PC1) explained 37.38 % of variance and principal component 2 (PC2) explained 22.60% of variance, while in the second year PC1 explained 30.48 % of variance and PC2 explained 19.37% of variance (Table 2).

For the 2016 crop, the biplot graph (Figure 1) showed the existence of an independent gradient, characterized by a positive grouping among glucose, fructose, number of grains per spikelet (NGS) and photosynthetic rate (*A*) in the right lower quadrant. The parameters more directly involved with the regulation of water status, i.e. transpiration (*E*), stomatal conductance (*gs*) and source-sink distance (SD) of the wheat plants, are also located in the right upper quadrant (Figure 1). With regard to cultivars in 2016, it was observed that BRS 264 and BRS 394 showed a close relationship to *gs*, *E* and SD. For PC2 another independent gradient was seen, characterized by the grouping of productive parameters: number of spikelets per plant (NSP), fertile tillers (FT) and number of grains per plant (NG), all positively correlated (Figure 1).

Table 2: Eigen values and variances for each principal component in the 2016 and 2017 crop seasons.

PC	2016		2017	
	Eigenvalue	**% Variance**	**Eigenvalue**	**% Variance**
1	4.850	37.388	3.963	30.483
2	2.930	22.600	2.518	19.371
3	1.482	11.407	2.226	17.120
4	0.962	7.403	1.501	11.547
5	0.891	6.857	1.114	8.570
6	0.693	5.338	0.740	5.694
7	0.466	3.591	0.457	3.519
8	0.353	2.719	0.249	1.916
9	0.226	1.743	0.113	0.869
10	0.076	0.585	0.102	0.784
11	0.033	0.256	0.009	0.073
12	0.011	0.091	0.004	0.034
13	0.002	0.017	0.003	0.022

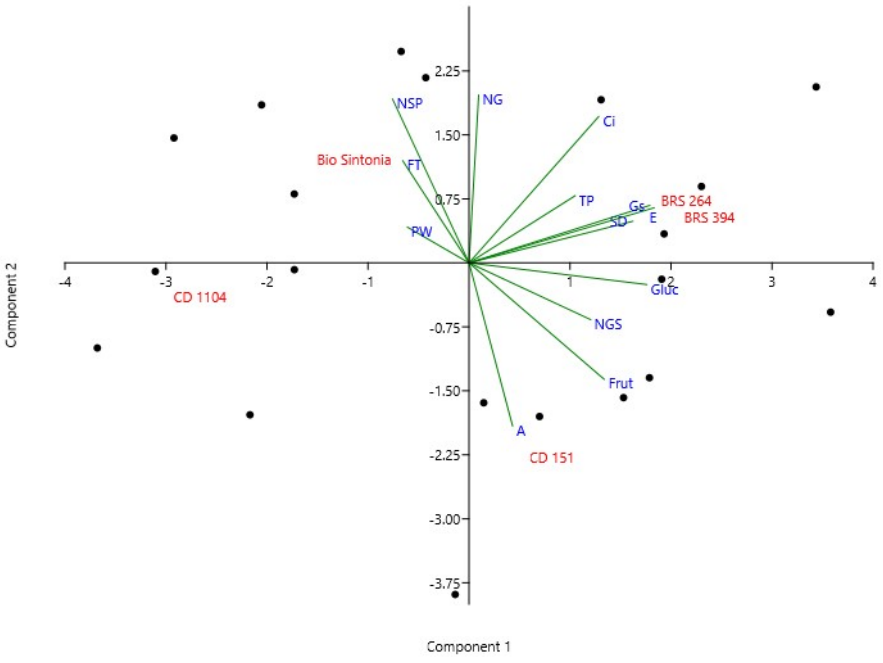

Figure 1: Biplot graph for cultivars in relation to physiologic, biometric and production parameters in 2016 crop session.

It is believed that this plant response was due to major divergent environmental conditions between the two years of analysis: specifically, the low precipitation of 2016. This result is similar to the model proposed by (Zhou *et al*., 2013), which asserts that water levels are strong limiters of stomatal conductance, but this is not the only restricting effect of water loss, and the plant finds different strategies to combat water deficit in order to maintain its stable metabolism.

In 2017 there was an inversion in the phenomena observed, in relation to physiology, biochemistry and productive parameters, where the non-structural carbohydrates (glucose and fructose) and photosynthetic rate (*A*) remained grouped, but located in the left on the PC1, and the productive parameters (NSP, FT and NG) are grouped on the right of PC1 (Figure 2). This observation represents a positive grouping of these components with water status in the environment, because in 2017 the rainfall was double that of 2016 (Table 1). For PC2 there was a positive grouping of cultivars BRS 264 and BRS 394 for the productive parameter NG.

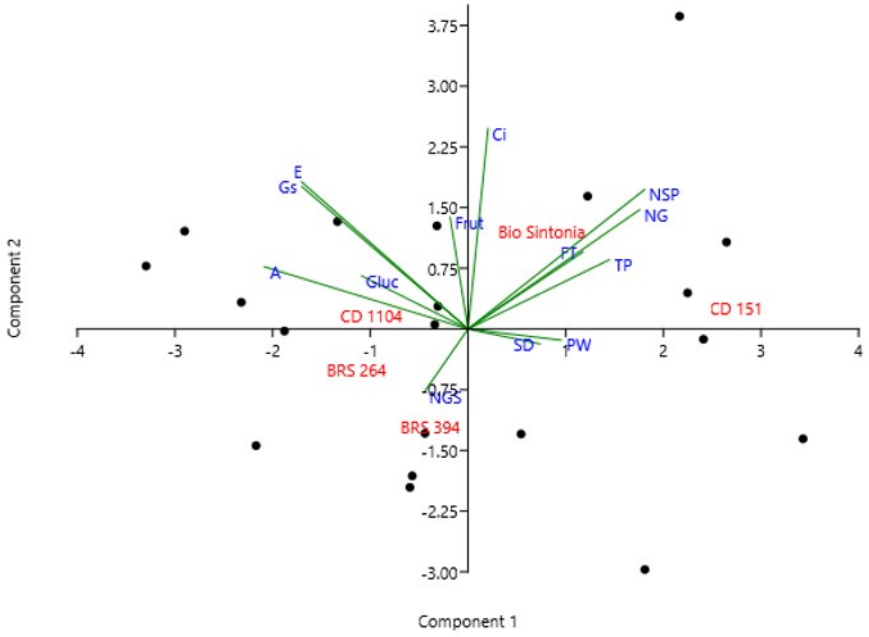

Figure 2: Biplot graph for cultivars in relation to physiologic, biometric and production parameters in 2017 crop session.

There were distinct differences in the growth of the crop in 2017 compared to 2016, possibly due to the amount of water available to the crop. In

2017 the cultivars BRS 264 and BRS 394 are in the negative quadrant of the biplot graph, and the Bio Sintonia and CD 151 genotypes are grouped in the same quadrant, and highly correlated to the productive parameters, because CD 1104 was highly correlated with the non-structural carbohydrates (Figure 2).

It was verified that, in the years 2016 and 2017, the concentration of both carbohydrates evaluated (glucose and fructose) were always found in the same quadrant as photosynthetic rate, and there was a positive grouping of these carbohydrates and photosynthetic rate (*A*) with the cultivars CD 151 and CD 1104, respectively, in 2016 and 2017 (Figures 1 and 2). The cultivar BioTrigo stood out by being influenced mainly by the NSP and FT parameters in both years, with very similar responses, inferring that these productive characteristics are very important for this cultivar (Figures 1 and 2).

Different genotypes of wheat present distinct photosynthetic and source-sink responses, so that some cultivars are better in reallocating their carbohydrates in more expressive sinks (Zhang *et al*., 2014), Moreover, the leaves are not the only carbohydrate source of wheat plants: the ears and stalks can contribute to the synthesis of photoassimilates (Sanchez-Bragado *et al*., 2014; Zhang *et al*., 2014), thus justifying the variation observed in the number of grains per plant and per spikelet in 2016, despite low photosynthesis. Also, these results show that glucose and fructose have a key role in the regulation in wheat: this phenomenon was confirmed by the research of (Kameli and Lösel, 1993) into the physiological adjustment in wheat plants under water stress.

In 2016 PC1showed that the biometric and productive indices: plant height (PH), fertile tiller (FT) and number of spikelets per plant (NSP) occupied a negative position in the biplot graph, whereas for 2017, the majority of the biometric and productive parameters were found on the positive side of the biplot graph, demonstrating the sensitivity of the wheat crop to changes in the Cerrado environment. This research did not find a positive link between the concentrations of glucose and fructose and grain index per plant (Figures 1 and 2). According to (Tack *et al*., 2015), a favourable environment, with stable rainfall rates during the crop's growth cycle, will not only stimulate the growth of the crop, but will also aid in the reduction of stress caused by an elevated temperature, which is otherwise likely to result in major damage to wheat production.

In this study we verify distinct responses between wheat cultivars, under two cycle life and contrasting environments, according (Rakašćan *et al*, 2019) this fact occurs mainly by impact of different agro-ecological conditions they were submitted. So the recognition of morphological and physiological characters and traits that affecting the drought tolerance (the ability of a plant to survive under on water restriction) are important for improvement of wheat productivity under inhospitable conditions, where the severity, frequency, and duration of drought stress are essential factors for choosing the place of cultivation and selection of genotypes (Mohammadi and Abdulahi, 2016).

CONCLUSIONS

The performance of the wheat cultivars was distinct in both years. Physiological indicators enabled the differences between the genotypes to be understood, within the imposed environmental conditions. There was a strong, positive correlation between photosynthetic rate and sugar content. The cultivars of EMBRAPA adjusted their concentrations of glucose and fructose to cope with low rainfall. The increased rainfall in 2017 seemingly caused the productive parameters to overlap with the physiological and metabolic parameters, reversing the analyses compared to 2016.

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THE INFLUENCE OF SOW DENSITY ON PRODUCTIVITY AND MOISTURE IN BUCKWHEAT GRAIN *(Fagopyrum esculentum* Moench.) IN CONDITION OF STUBBLE SOWING AND IRRIGATION

SUMMARY

The important role of buckwheat in moder human diet and contemporary, sustainable agriculture derives from its chemical composition of grain and modest growing requirements. Consequently, there is an increasing trend of areas under buckwheat with a rate of 22.46% per year. Buckwheat arouses great interest as essential ingredient for functional foods because of its health benefits. Its grain is one of the best sources of high quality protein in the plant kingdom. Buckwheat is very sensitive to the lack of moisture, especially during the first development phases, in period of rooting, during flowering and yielding period. So, the aim of investigation was to estimate the optimal seed rate of buckwheat to achieve the productive, economic and other objectives, especially important for ecological agriculture, in conditions of stubble sowing and irrigation. The investigation included 12 genotypes and three sowing density (80, 120 and 160 grains m^{-2}).

The highest average grain yield is obtained in density of 160 grains per m^{-2}, then in density of 120 grains per m^{-2}, but the lowest in 80 grains per m^{-2} density. Difference in grain yield between 160 grains per m^{-2} and 120 grains per m^{-2} density variants is a slight, only 80 kg or 3.49% that means both sowing density variant could be applied successfully in buckwheat planting technology, but sowing density of 120 grains per m^{-2} has priority because of production economics.

Key words: buckwheat, density, grain yield, irrigation.

INTRODUCTION

Buckwheat is plant species known for centuries and has cultivated, according to recent evidence, more than 7000 years (Ohnishi et al., 1998). It originates from the mountainous parts of central and northeast Asia, Junan and Sečuan provinces. Due to nomadic tribes, buckwheat has been spread to the

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Balkan Peninsula in the, most likely, 13th century. It is interesting that buckwheat is one of the few plant species transferred from Europe to America, during 16th century. For a long time, it has been neglected plant species, excluded from production, human and animal nutrition. Buckwheat exhibits, very often, characteristics of wild, uncultivated plant species, sensitive to mineral fertilizers (Kreft et al., 2008). Such characteristics like modest demands, economy and good competitive ability recommends it for ecological farming system (Gadzo et al., 2009; Dedic, 2012).

Nowadays, buckwheat is planted to an average of 2.113 million hectares worldwide. There is an increasing trend of areas under buckwheat with a rate of 22.46% per year. The highest production of buckwheat of 93.67 % in the world was in Europe and on Asian continent (1.133 ha, 846.799 ha), that is 53.61 % and 40.06 %, respectively. The most significant producers of buckwheat in the world are: China (34.25 %), Russia (32.43 %) and Ukraine (11.46 %) (Popovic et al., 2014).

Buckwheat arouses great interest as essential ingredient for functional foods because of its health benefits (Li et al., 2001; Zhang et al., 2012). Its grain is one of the best sources of high quality protein in the plant kingdom. It contains about 52.11% starch, 11-12.55% of the total protein, 8.7% of the pulp, 2.23% oil, 11% water and 1.75% of N protein (Popovic et al., 2013). The most important ingredients of this plant are flavonoids (Arsic et al., 2008). Grain contains a large amount of indispensable amino acids (EAA), primarily lysine and methionine and dietary protein, vitamins B_1 and B_2, minerals like Fe, Zn and Se (Bavec and Bavec, 2006). The presence of antioxidants like rutin, tannin and fagopyrin has special importance (Kreft et al., 2006). Complete grain is used as a nutritive supplement for different stews, while flour is used for preparation of porridge (polenta) or mixed with wheat or rye for preparation of bread with higher digestion value. Because of the beneficial chemical composition, and the absence of adhesive proteins, buckwheat is suitable for diabetics and children diets. Like a honey plant, buckwheat is very useful for bees throughout the entire summer and fall period in absence of other flowered plants. Buckwheat honey contains increased amounts of bioflavonoids that have antibacterial effects (Janovska et al., 2008).

At the same time, buckwheat crops protect the soil from erosion, attract beneficial insects and raise the level of organic matter in the soil (Wallace, 2001). Barac et al. (2012) emphasized the importance of the threshing device on a harvester and quality of harvesting for the quality of the threshed grain. The properly done this phase is very important to keep and care quality of seed products.

The aim of investigation was to estimate the optimal seed rate of buckwheat to achieve the productive, economic and other objectives, especially important for ecological agriculture, in conditions of stubble sowing and irrigation.

MATERIAL AND METHODS

Climate and soil conditions during experiment conducting

Buckwheat is very sensitive to the lack of moisture, especially during the first development phases, in period of rooting, during flowering and yielding period. During the later phases, excess of moisture causes a lot of negative effects. It has very high transpiration coefficient, up to 700 (Janovska et al., 2008). Influences of temperatures are rather strong, too.

During 2010, 360.4mm precipitation were in May and Jyly, while 125.6mm precipitation only or almost three times less were in the same period in 2011. It means that 2010. was more favorable in term of soil moisture, necessary for germination. During July and August, almost the same precipitation sums (120mm, averagely) were in the both of investigation years in generative phase (impregnation and grain filling phases) (Table 1).

Table 1. Distribution and total precipitation (mm) in growing season at Sombor locality

Year	Month				Total precipitation in growing season (mm)
	V	VI	VII	VIII	
2010	166	194.4	77	44.2	481.6
2011	67.8	57.8	66.6	50.4	242.6

This precipitation sum could be limiting factor to obtain more yield per unit area because it is critical period for buckwheat. Several irrigation doses, however, made goog conditions for yielding.

Air temperatures in vegetative phase of development (intensive growth of trees and branching) were very favorable during the both of investigation years (Table 2).

Table 2. Air temperatures (^{0}C) during vegetative seasons at Sombor locality

Year	Month											
	V			VI			VII			VIII		
	min	X	max	min	X	Max	min	X	Max	min	X	max
2010	12.1	16.9	21.8	15.6	20.3	24.9	17.6	23.1	28.6	15.7	21.8	27.9
2011	10.2	16.6	23.0	15.1	21.0	26.9	15.8	21.9	28.0	15.6	22.7	29.7

Buckwheat does not tolerate high temperatures. Growth is already slowing down at 28^{o}C, as the percentage of impregnation and grain formation (Glamoclija, 2011).The optimal temperature for grow and development is 20°C while temperatures above 30°C, during flowering phase, followed by low air moisture, cause drying and falling flowers and bad pollination (Pauseva, 1976).

There were not extreme temperatures which could limit normaly fertilization and yielding during this experiment.

The trial was set up on carbonate meadow black soil, with wheat as preceding crop, on field of APS "Sombor" in Sombor, Serbia as double cropping system, during 2010. and 2011., under irrigation. The investigation included 12 genotypes: P2, P4, P5, P6, P9, P10 and P11, as domestic and foreign populations, Darja, Bamby, Oberon and Bylly, as foreign cultivars and Novosadska, as domestic cultivar. The experiment was designed as block system, in three replications, with area of elementary plot $20m^2$. Distance between rows was 25 cm. Sowing was in acordiance with planned sowing density (80, 120 and 160 grains m^{-2}). The sowing was done on 15th July in 2010, but on 9th July in 2011. The plants were irrigated three times: in phase of intensive growth of trees and branching, 15 days after and in phase of intensive flowering and yielding, with 25l water per m^{-2} , each time. The harvest was done in moment when 2/3 of yield were riped respectively when grains of the latest genotype were dark brown. The harvest was one – phased, done on 20th August, in 2010. and on 12th August, in 2011. The grain yield was measured per each plot and appropriate sowing density. On the base of that, average yield was calculated and expressed as grain yield with 13% of moisture.

Statisticall analysis

Data were analyzed statistically using two factorial model of analysis of variance according to Hadzivukovic (1973). The differences of means were tested using an LSD test at p=0.05 and p=0.01 probability levels.

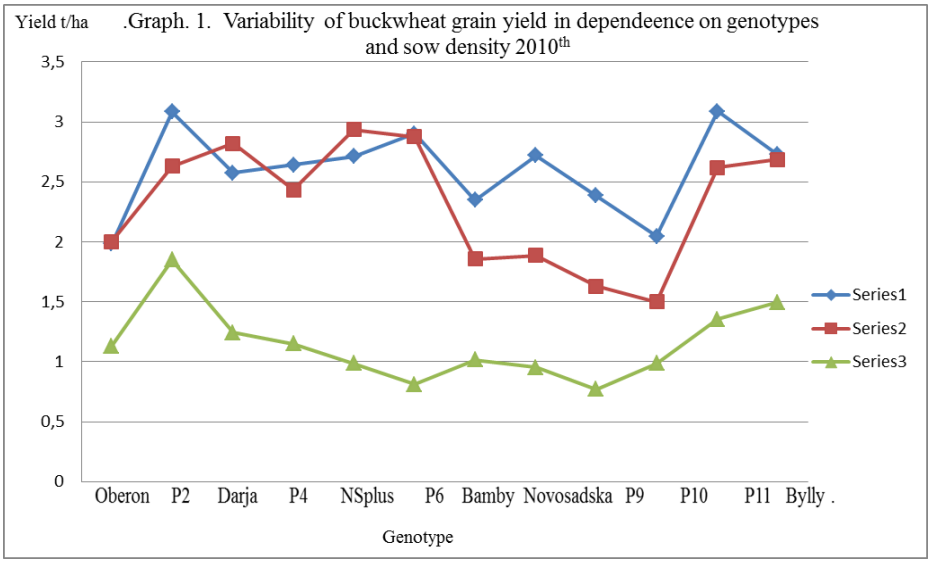
RESULTS AND DISCUSSION

Grain yield

Buckwheat is grown primarily due to grain. Grain yield is quantitative trait influenced by genotype, external factors and applied growing technology. After harvest, it is necessary to free the grain from the harvest residues and inert matter, as soon as it is possible.

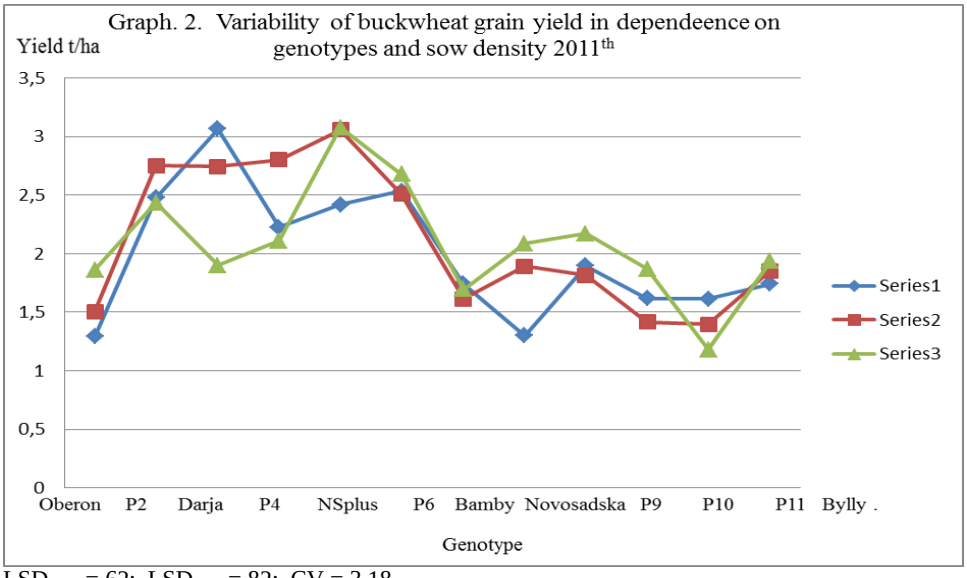
Investigated genotypes obtained high and stabile grain yield in sowing density of 160 grains m^{-2} in 2010. P_2 and P_{11} gained yield above 3 t ha^{-1}. The most of genotypes (P_2, Darja, P_4, NS plus, P_6, P_{11}, Bylly) gained yield between 2.5 and 3 t ha^{-1} in sowing density of 120 grains m^{-2} , while significant grain yield decline was noticed in all genotypes, in sowing density of 80 grains m^{-2} (Graph.1).

According to grain yield and its dependence on sowing density, during 2011, buckwheat genotypes were divided in two groups: with more variable and higher grain yield above 2 t ha^{-1} (P_2, Darja, P_4, NS plus, P_6) and with less variable and medium grain yield - between 1 and 2 t ha $^{-1}$ (Oberon, Bamby, Novosadska, P_9, P_{10}, P_{11}, Bylly) (Graph.2).



$LSD_{0.05}$ = 111; $LSD_{0.01}$ = 148; CV = 5.86
Legend: series 1 - 160 grains m^{-2}; series 2 - 120 grains m^{-2}; series 3 - 80 grains m^{-2}

Graph 1. Variability of buckwheat grain yield depending on genotype and sowing density in 2010.



$LSD_{0.05}$ = 62; $LSD_{0.01}$ = 82; CV = 3.18
Legend: series 1-160 grains m^{-2}; series 2-120 grains m^{-2}; series 3-80 grains m^{-2}

Graph 2. Variability of buckwheat grain yield depending on genotype and sowing density in 2011.

These results are in accordinace with previous results reported by Popovic et al. (2014) and Gadzo et al. (2007). However, cited authors planted buckwheat as main crop, without irrigation. In such conditions, influences of external factors are more expressed. Further analysis leads to observation that adequate yields can be achieved by reduction of seed quantity, with applying new growing technologies and more productive genotypes and populations. These two years results show that sowing density of 120 grains m^{-2} is economical and efficient in the given conditions.

Considering average grain yield, during each of investigated years, at all of three sow rates, it can be observed that almost a half of genotypes (Oberon, P2, Bamby, Novosadska, P10) obtained stable average yields and showed less sensitivity to environmental influence during investigation years. Population P2 stands out, with yields over 2.5 t ha-1. The strongest environmental influence was registered on genotype NS plus and population P11 (Tab.3).

Table 3. Stability of grain yield of investigated buckwheat genotypes in 2010. and 2011.

Year	Genotype											
	Oberon	P2	Darja	P4	NS plus	P6	Bamby	Novo sadska	P9	P10	P11	Bylly
2010	1.56	2.56	2.57	2.38	2.85	2.57	1.68	1.76	1.96	1.64	1.40	1.84
2011	1.71	2.52	2.22	2.07	2.21	2.20	1.74	1.85	1.60	1.51	2.35	2.30

Popovic et al. (2014) noticed that favorable climatic conditions and adequate planting technology have important influence on yield while this investigation pointed out importance of genetic structure of genotype and population and interaction genotype x environment.

This investigation points to the possibility of buckwheat croping in lowland areas, irrigation and double cropping system obtaining more yields than average ones in world.

Table 4. Average buckwheat grain yield in dependence on sowing density

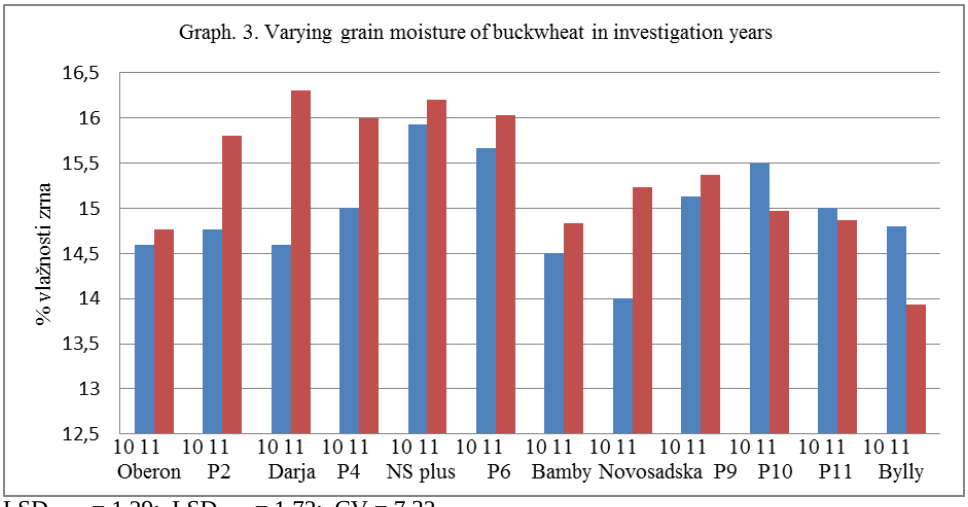
Year		Sowing density (grain number m^{-2})			Yield (kg)	LSD		CV
		160	120	80		0.05	0.01	
2010	Grain	2.601	2.323	1.145	2.023	111	148	5.86
2011	yield	1.994	2.114	2.083	2.064	62	82	3.18
2010/2011	(kg)	2.298	2.218	1.614	2.043	63	83	4.69

Different sowing density influnces grain yield of some genotypes and average grain yield in different sowing density variants while average grain yield in investigation years do not vary significantly (Tab. 4). The highest average grain yield is obtained in density of 160 grains per m^{-2}, then in density of 120 grains per m^{-2}, but the lowest in 80 grains per m^{-2} density. The increases of grain yield in 160 grains per m^{-2} and 120 grains per m^{-2} density variants in relation to

variant of 80 grain per m^{-2} are 684kg (29.77%) and 604kg (27.24%), respectively. Difference in grain yield between 160 grains per m^{-2} and 120 grains per m^{-2} density variants is a slight, only 80 kg or 3.49%. It means that both sowing density variant could be applied successfully in buckwheat planting technology, but sowing density of 120 grains per m^{-2} has priority because of production economics. Less sowing density, 80 kg per ha^{-1}, is recommended for earlier sowing times, but later sowing, like double cropping system, requires a higher amount of seeds, above 100 kg per ha^{-1} (Rotim et al., 2012). Lower sowing density decreases production cost, but sowing density, however, have to provide such crop structure that will improve the effectiveness of weed control (Bavec et. al., 2006). Omid Beygi et al. (2002) studied effects of different nitrogen amounts and sowing density on plants growing, grain yield and rutin content and concluded that density of 100 plants per m^{-2} and total amount of 100 kgN per ha^{-1} give the best results. Gavric et al. (2018) registered significant influence of sowing density and meteorological conditions on grain yield of buckwheat, too, but not to chemical compose of grain. Increasing sowing rate had positive effect on grain yield, but it had no effect on chemical compose of kernel. The same authors noticed that unfavorable weather conditions (drought and high temperature) resulted in low average yield, but in high grain quality i. e. high content of crude protein, starch and total phenol in grain.

Grain moisture

Harvesting buckwheat is the most important agrotechnical procedure in system of plamting technology required flawless planning and proper doing. It can be as one phase, like in this trial and two phases process.



$LSD_{0.05}$ = 1.29; $LSD_{0.01}$ = 1.72; CV = 7.32
Legend: series 10-2010; series 11-2011.

Graph 3. Varying grain moisture of buckwheat in investigation years

One phase harvest is performed at the moment of high percent of moisture and inert matters in grains, catching up parts of biomass (leaves, branches and stems). Therefore, it is necessary to carry out the grain drying and separation, immediately after harvest. Two phases haarvest means mowing and hay drying and, after that, treshing on different treshing machines. The importance of determinaation of grain moisture is reflected in decrease losses, drying and entire production costs as well as preserving the grain quality of buckwheat for a longer period.

Grain moisture varried from 14.0% to 15.93%, and from 13.93 to 16.3%. in 2010. and 2011., respectively. Differences in grain moisture between investigation years are not significant for most of genotypes, except for Darja (1.7%) and NS buckwheat (1.23%) (Graph. 3.)

Results presented in Table 5. show that .there are not differences in grain moisture in various sowing density variants in both of years.

Table 5. Average values of grain moisture of buckwheat depending on sowing density

Year	Grain moisture (%)	Sowing density (grain number m^{-2})			Average (%)	LSD		C_V
		160	120	80		0.05	0.01	
2010		14.09	15.79	14.99	14.96	1.79	2.42	7.09
2011		16.28	15.50	14.29	15.36	1.95	2.64	7.52
2010/2011		15.19	15.65	14.64	15.16	1.29	1.72	7.32

CONCLUSIONS

Different sowing density influnces grain yield of some genotypes and average grain yield in different sowing density variants while average grain yield in investigation years do not vary significantly. The highest average grain yield is obtained in density of 160 grains per m^{-2}, then in density of 120 grains per m^{-2}, but the lowest in 80 grains per m^{-2} density. Difference in grain yield between 160 grains per m^{-2} and 120 grains per m^{-2} density variants is a slight, only 80 kg or 3.49% that means both sowing density variant could be applied successfully in buckwheat planting technology, but sowing density of 120 grains per m^{-2} has priority because of production economics. Various sowing density did not influence grain moisture in both of years.

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ASSESSMENT OF SOIL EROSION, SEDIMENT YIELD AND MAXIMUM OUTFLOW, USING IntErO MODEL (CASE STUDY: S8-intA SHIRINDARREH WATERSHED, IRAN)

ABSTRACT

The complexity of hydrological processes, spatial and temporal variation of all effective factors and lack of essential measured filed data convinced researchers to use empirical models in various scales. The IntErO model was used to predict maximum outflow (Qmax), soil erosion intensity (W year) and sediment yield (G year) in one of the internal sub-watersheds of Shirindarreh watershed, northeast of Iran. The results showed that the peak flow was 13.51 m^3s^{-1} for a return period of 100 years. As an internal area, the peak flow of all upstream sub-watersheds should be also considered to be added to the predicted peak flow. The value of Z coefficient of 0.696 indicated that the river basin belongs to III destruction category and the strength of the erosion process was medium, and according to the erosion type, it was intrusive erosion. The predicted gross soil erosion in the study sub-watershed was 8.06 ton ha^{-1} $year^{-1}$. The coefficient of the deposit retention (Ru) or sediment delivery ratio (SDR) was 0.124 and therefore, the sediment yield was 1.00 ton ha^{-1} $year^{-1}$. According to Gavrilovic, the study sub-watershed is a region of very weak erosion.

Keywords: IntErO Model, Land Use, Runoff, Sediment Delivery Ratio.

INTRODUCTION

Soil erosion is one of the most important environmental issues worldwide and is a cause of various problems (Toy et al., 2002). Soil loss is a serious problem in developing countries (Wolancho, 2012) that cause in great concern (Gholami et al., 2013). For the appropriate watershed management, land use and landscape planning, which will more effectively meet national or local needs and assists in assessing the consequences of the alternatives the important issue is to

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

quantify the sediments and to estimate sediment yield at the river basin scale (Chalise et al., 2019; Spalevic, 2019; Curovic et al, 2019; Parsipour et al, 2019, Fikfak et al, 2017; Popovic et al, 2018). On one hand, it is difficult to accurately measure the soil erosion generated in the field (Conoscenti, 2008; Rawat et al., 2011) and on the other hand, in many of the hydrometric stations, the sediment concentration are determined only in some severe storms without any evaluation of time distribution pattern of sediment (Khaledi Darvishan et al., 2010). Therefore, the modelling of the erosion process has progressed rapidly and a variety of models have been developed to predict both runoff and soil loss (Spalevic, 2017a; 2017b; Barovic et al., 2015). Soil erosion process models can be used for long as well as short periods (Sadeghi et al., 2013). Calculation of sediment yield is one of the basic necessities to achieve integrated land management and soil and water conservation (Khaledi Darvishan et al., 2014). Therefore, among the available soil erosion and sediment yield models, those which can predict both soil erosion and sediment yield can be more useful and more widely used. Because of the complexity of factors influencing sediment delivery ratio (SDR) as the ratio between soil erosion and sediment yield, it is very important to predict and use of this ratio correctly (Behzadfar et al., 2014; Khaledi Darvishan et al., 2012; Vujacic et al., 2017; Vujacic et al., 2015).

Among several empirical models, Erosion Potential Method – EPM, originally developed for Yugoslavia by Gavrilovic (1972), was in recent times repeatedly applied in several watersheds in the Balkan region (Blinkov and Kostadinov, 2010; Kostadinov et al., 2006, 2014; Milevski et al., 2008; Ristic et al., 2012; Spalevic et al. 2013, Spalevic et al. 2014; Spalevic et al. 2015; Spalevic et al. 2016; Stefanovic, 2004; Tazioli, 2009; Zorn and Komac, 2008; Tavares et al., 2019), and also in arid and semi-arid areas of the south-western USA (Gavrilovic Z., 1988), Saudi Arabia (Aburas Al-Ghamdi, 2010). The method was based on the factors affecting erosion in a watershed; its parameters were dependent on the temperature, the mean annual rainfall, the soil use, the geological properties and some other factors in the watershed scale (Khaledi Darvishan et al., 2016; 2017). The synergic influences of climate and human abandonment could have triggered erosion processes (Vitali et al., 2019).

The Intensity of Erosion and Outflow - IntErO program package (Spalevic, 2011) with the Erosion Potential Method – EPM (Gavrilovic, 1972) embedded in the algorithm of this computer-graphic method, was developed to predict the runoff peak discharge and the intensity of soil erosion in a watershed scale. This model is a computer-graphic method based on the Erosion Potential Method - EPM, which is embedded in its algorithm. The use of this model has been reported in various countries all around the world including Bosnia & Herzegovina, Bulgaria, Croatia, Czech Republic, Italy, Iran, Montenegro, Macedonia, Serbia, Slovenia, Morocco, Brazil, Nepal and Japan. The efficiency of IntErO model to predict peak outflow, soil erosion and sediment yield was also assessed in some cases and the results showed that this model can be use in variety of watershed sizes with various land uses (Spalevic, 2011).

The present study was therefore conducted to use the IntErO model to predict peak outflow, soil erosion and sediment yield for S8-intA sub-watershed in Shirindarreh watershed located in the northeast of Iran.

MATERIALS AND METHODS

Study area. The study area is an internal sub-watershed located in the north part of Shirindarreh watershed, northeast of Iran (Figure 1).

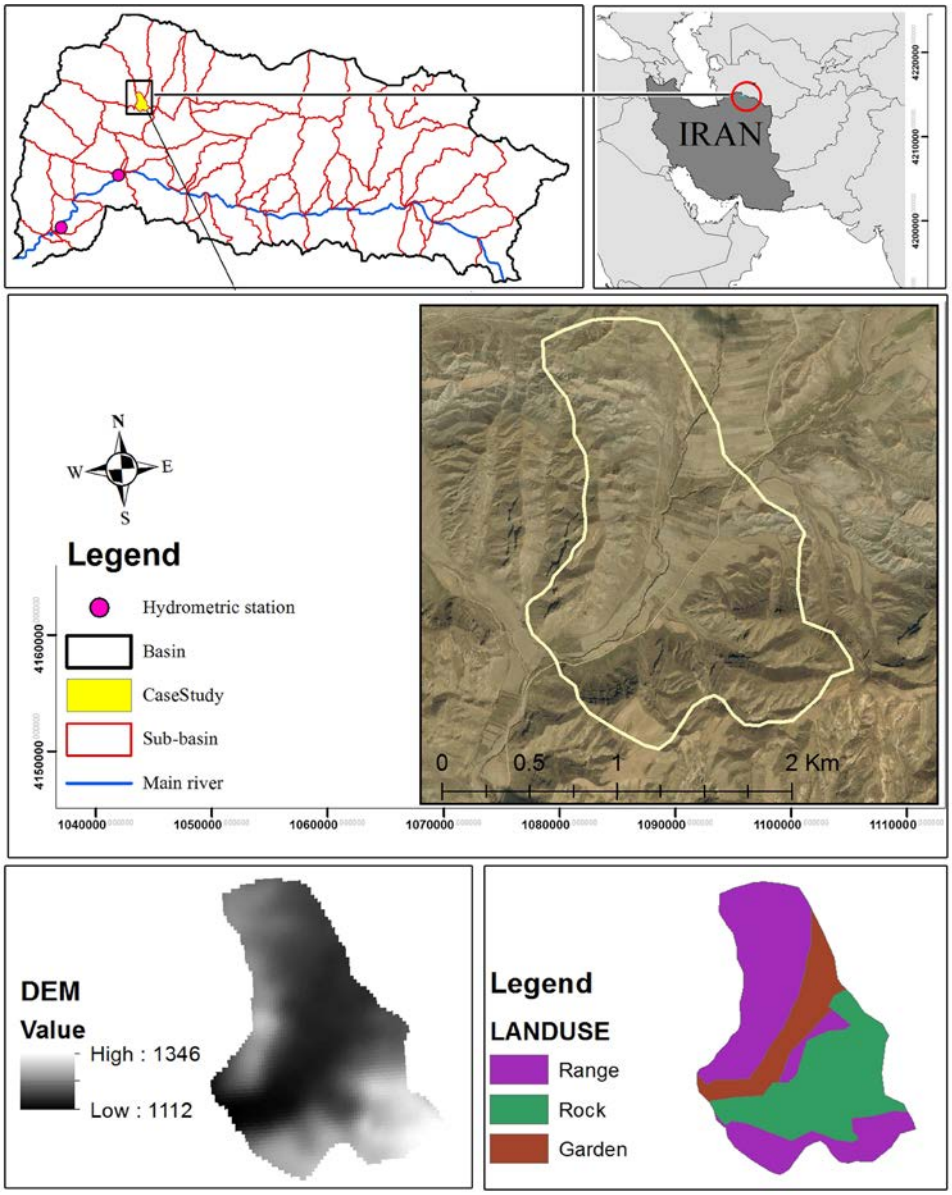

Figure 1. Location of the S8-intA basin of the Shirindarreh watershed, Iran

The name of the study area is S8-intA which means that a series of sub-watersheds drain into it. In other words, three upstream sub-watersheds (S8-1, S8-2 and S8-3) drain in a small inter-basin area. The location of the study area in Shirindarreh watershed, northeast of Iran is shown in.

Application of IntERO model. The Intensity of Erosion and Outflow - IntErO program package was used to estimate maximum peak outflow, the intensity of soil erosion and sediment yield for the study sub-watershed. All the input data required for the model can be extracted from some simple available maps including topography or Digital Elevation Model (DEM), geology map, land use/land cover map and also some easy obtained climatic variables such as temperature and precipitation.

The average annual air temperature (t0) and the average annual precipitation (Hyear) were 12.4 °C and 293.3 mm, respectively. The geological required data was extracted from the geological map of Iran (Bolourchi et al., 1987). According to the analysis, the total area of the study sub-watershed was totally under grass, meadows, pastures and orchards (100%). The coefficient of the river basin planning (Xa) was calculated about 0.7. All the model inputs calculated using the maps and data provided by Natural Resources and Watershed Management Office, North Khorasan province are shown in Table 1.

Table 1. IntErO model inputs for the study area of the S8-intA basin

Input		Value	Unit
River basin area	F	2.62	km²
The length of the watershed	O	7.44	km
Natural length of the main watercourse	Lv	1.93	km
The shortest distance between the fountainhead and mouth	Lm	1.5	km
The total length of the watercourse with tributaries of I&II class	ΣL	4.07	km
River basin length measured by a series of parallel lines	Lb	10.9	km
The area of the bigger river basin part	Fv	1.55	km²
The area of the smaller river basin part	Fm	1.07	km²
Contour line length	Liz	4.8	km
		2.79	km
The area between the two neighboring contour lines	fiz	1.65	km²
		0.96	km²
		0.01	km²
Altitude of the first contour line	h0	1200	m
Equidistance	Δh	100	m
The lowest river basin elevation	Hmin	1120	m
The highest river basin elevation	Hmax	1363	m
A part of the river basin with very permeable rocks	fp	0.15	
A part of the basin area consisted of medium permeable rocks	fpp	0.35	
A part of the basin consisted of poor water permeability rocks	fo	0.5	
A part of the river basin under forests	ff	0	
A part of the basin under grass, meadows, pastures and orchards	ft	1	
A part of the basin under plough-land and without grass	fg	0	
The volume of the torrent rain	hb	32.71	mm

Incidence	Up	100	years
Average annual air temperature	t0	12.4	°C
Average annual precipitation	Hgod	293.3	mm
Types of soil products and related types	Y	0.9	
River basin planning, coefficient of the river basin planning	Xa	0.7	
Numeral equivalents of clearly exposed erosion process	φ	0.54	

RESULTS AND DISCUSSIONS

After preparing the inputs required for IntErO model, the model was ran and all the model outputs were obtained and shown in Table 2.

Table 2. The IntErO model outputs for the study area of S8-intA basin

Output		Value	Unit
Coefficient of the river basin form	A	0.75	
Coefficient of the watershed development	m	0.34	
Average river basin width	B	0.24	km
(A)symmetry of the river basin	a	0.37	
Density of the river network of the basin	G	1.55	
Coefficient of the river basin tortuousness	K	1.29	
Average river basin altitude	Hsr	1193.63	m
Average elevation difference of the river basin	D	73.63	m
Average river basin decline	Isr	28.97	%
The height of the local erosion base of the river basin	Hleb	243	m
Coefficient of the erosion energy of the river basin's relief	Er	60.8	
Coefficient of the region's permeability	S1	0.81	
Coefficient of the vegetation cover	S2	0.8	
Analytical presentation of the water retention in inflow	W	0.4535	m
Energetic potential of water flow during torrent rains	2gDF^½	61.52	m km s
Maximal outflow from the river basin	Qmax	13.51	m^3/s
Temperature coefficient of the region	T	1.16	
Coefficient of the river basin erosion	Z	0.696	
Production of erosion material in the river basin	Wgod	1624.361	m^3/god
Coefficient of the deposit retention	Ru	0.124	
Real soil losses	Ggod	201.55	m^3/god
Real soil losses per km^2	Ggod/km²	76.93	m^3/km^2 god

The coefficient of the river basin form, A, calculated as 0.75 using IntErO software. Coefficient of the watershed development, m, was 0.34 and the average river basin width, B, was 0.24 km. (A)symmetry of the river basin, a, which indicates that there is a possibility for large flood waves to appear in the outlet of the study area, was calculated as 0.37. In addition as mentioned before, the study area in a kind of inter basin which three more upstream sub-watersheds (S8-1, S8-2 and S8-3) drain into it. It means that it is possible to receive large amounts of peak flows.

Drainage density, G, was calculated as 1.55 km km^{-2} which corresponds to low density of the hydrographic network. Because of the location of the study area as a low slope inter basin in the middle of high slopes surrounded, the drainage density was not high.

The value of Z coefficient as 0.696 indicated that the strength of the erosion process in the study sub-watershed is low and according to the erosion type, it is surface erosion.

For the current state of land use, calculated peak flow was 13.51 m^3s^{-1} for a return period of 100 years. It is very important to emphasize again the location of the study area in downstream of three other sub-watersheds. So that the total peak flow of the river in the outlet of the study area can be calculated by adding 13.51 m^3s^{-1} with other three peaks (58.34, 75.53 and 89.47 m^3s^{-1}). Therefore, the total peak flow of 236.85 m^3s^{-1} is expected to be seen at the outlet of the study area for a return period of 100 years.

The total production of sediments, or total soil erosion occurred in the study area, Wyear, was predicted as 1624.361 m^3 $year^{-1}$ (8.06 ton ha^{-1} $year^{-1}$); and the coefficient of the deposit retention, Ru, was 0.124 which indicated that about 12.4% of the eroded materials will reach to the river network of the studied sub-watershed. Therefore, the sediment yield, or real/net soil loss at sub-watershed outlet (Gyear) was predicted as 76.93 m^3 km^{-2} $year^{-1}$ (1.00 ton ha^{-1} $year^{-1}$).

According to the results shown in Table 2, it seems that the study sub-watershed is a region of very weak erosion and the surface erosion has taken place in all the soils on the slopes as the dominant erosion form in the studied sub-watershed which is the most pronounced on the steep slopes with scarce vegetation cover especially in meadow land use.

CONCLUSION

The Isr value of 28.97% indicates that in the river basin prevails steep slopes. The value of Z coefficient of 0.696 indicates that the river basin belongs to III destruction category. The strength of the erosion process is medium, and according. The value of 76.93 m^3 km^{-2} $year^{-1}$ (about 1.00 ton ha^{-1} $year^{-1}$) indicates, according to Gavrilovic, that the river basin belongs to V category; region of very weak erosion.

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'KHODERI' OLIVE CULTIVAR AS AN EFFICIENT POLLINISER FOR SOME FRENCH AND ITALIAN OLIVE CULTIVARS

SUMMARY

The research was conducted in 2017 and 2019 seasons in Bouka center for research and plant production, Latakia, Syria, in order to investigate the ability of using Syrian olive cultivar 'Khoderi' as polliniser for 'Tanche',' Picholine' (French) and 'Frantoio' (Italian) olive cultivars. Pistil abortion (%), pollen germination (%), flowering period, self, open and cross-pollination with 'Khoderi' were studied. Results showed that all cultivars were characterized by good pollen germination ratio. Both season and cultivar affected pistil abortion significantly. The flowering periods were overlapping for all studied cultivars but with some delay of 'Frantoio'. 'Picholine' was highly self-incompatible (ISI= 0.11), while 'Tanche' was partially self-incompatible (0.33- 0.35). The highest self-compatibility was for 'Frantoio' (ISI= 0.52- 0.73). Pollination results showed that using 'Khoderi' as polliniser increased final fruit set over open- and self-pollination for all cultivars and seasons. The results indicated that 'Khoderi' was efficient polliniser for 'Tanche', 'Picholine' and 'Frantoio' olive cultivars under Syrian Coast conditions.

Keywords: Pollination, abortion, fruit set, self- incompatibility

INTRODUCTION

Olive (*Olea europaea* L.) is one of the most important fruit trees worldwide. Olive cultivation is close related to the culture and traditions of the Mediterranean countries. Syria is considered as one of the native origins of olive, also it's an active olive producer with ~850 thousand tons (MOAAR, 2017).

Traditionally, olive was considered self-fertile, but the recent studies mentioned that most olive cultivars are self- incompatible (Saumitou-Laprade *et al*., 2017). Self-incompatibility (SI), a common physiological phenomenon in flowering plants, leads to rejection of self- pollen. Although self- incompatibility encourages biodiversity by inhibiting self-fertilization, but this could lead to lower fruit set of olive cultivars planting without pollinisers. In Mediterranean as well as non-Mediterranean countries, many studies were conducted to find the efficient pollinisers for local and imported olive cultivars in each area and in different planting systems (Zhu *et al*., 2013; Lodolini *et al*., 2018; Sanchez-

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Estrada and Cuevas, 2019). In Croatia, 'Leccino', 'Drobinca' and 'Oblica' were partially self- incompatible cultivars, also 'Levantinka' and ' Oblica ' were cross-compatible in both directions (Selak *et al*., 2011). In Turkey, Mete *et al*. (2016) reported several Turkish cultivars to be efficient pollinisers for the new olive cultivar 'Hayat'. Farinelli *et al*. (2012) mentioned that the most efficient polliniser for 'Ascolana Tenera' was 'Carolea' followed by 'Picholine'. In Mexico, which is non- traditional olive producer, self- and open-pollination were reported to give low fruit set in mono-cultivar orchards of 'Manzanillo', fruit set was increased when using 'Barouni' as polliniser (Sanchez-Estrada and Cuevas, 2019).

Breton and Berville (2012) used fruit set estimation and fertility index to predict self-incompatibility alleles in olive; they reported six SI- alleles in olive with dominance relationships among them. Using pollen growth observation in stigma tissue, paternity analysis and fruit set estimation, Saumitou-Laprade *et al*., (2017) reported two SI groups in olive G1 (with S1S2 genotype) and G2 (with S1S1 genotype). Till now S-locus in olive still undiscovered in the molecular level and many phenomena must be studied to full understand SI in olive (Alagna *et al*., 2019). In general, cross-compatibility response was reported to differ depending on location and seasons (Gonzalez and Cuevas, 2012), which leads to conduct local experiments in each area in order to have an accurate evaluation of the pollinisers.

Pollination experiments are fairly recent in Syria. Mhnna *et al*. (2015) reported that imported cultivars 'Picholine' and 'Frantoio' were self- incompatible and had good performance under Syrian coastal area. So, this study aimed to evaluate the ability of local olive cultivar 'Khoderi' to pollinate those two cultivars, 'Picholine' and 'Frantoio', with 'Tanche' another French cultivar also proved good performance in the area.

MATERIAL AND METHODS

Study location and Plant material:

The experiments were conducted for two seasons (2017 and 2019) in Bouka center for research and plant production, Latakia province, Syria (latitude 35º32' North, 35º48' East), 40 m above sea level, characterized by clay calcareous soil. More than 40 olive cultivars are grown in the germoplasm collection. Average monthly maximum and minimum temperatures (c°) and Precipitations (mm) were taken from Sit- Kheris station located less than 8 km from the experimental orchard. Three olive cultivars were involved as pollen receptors: 'Tanche', 'Picholine' (French) and Italian olive cultivar 'Frantoio'. Autochthonous olive cultivar 'Khoderi' was used as pollen donor. Trees are about 30 years old, planted under rain fed conditions.

Methods:

Three uniform trees of each of 'Tanche', 'Picholine' and 'Frantoio' olive cultivars were chosen, a sample of 50 inflorescences was taken randomly from one year branches in white bud stage randomly distributed around the canopy of each tree. Average number of flowers per inflorescence and pistil abortion (%)

were estimated. Pollen was extracted and stored for several days in 2017 season, but cultured immediately in 2019 season in a media according to Ferri *et al*. (2008), with some modifications containing 15% commercial sugar, 50 ppm boric acid and 0.6% agar. Germination was observed after 24 hours of incubation on 26± 2°C. Germination (%) was estimated by taking three fields, each field containing more than 50 pollen grains (Koubouris *et al*., 2009) in each petri dish (four petri dishes for each cultivar). Pollen was considered germinated when pollen tube length was longer than twice the pollen diameter.

Pollination treatments:

Three one year old branches on each side of the trees were tagged in white bud stage (twelve on each tree), number of inflorescence were counted and unified. Two of the three branches were enclosed by white paper bags.

Cross-pollination: at full bloom, paper bag enclosing one of the two branches in each side of the trees were opened and flowering branch of 'Khoderi' with open flowers taken from the same orchard were inserted into the bag. Bags were enclosed and shaken daily for three days.

Self-pollination: the second branch on each side of the trees was kept enclosed to force self-pollination.

Open pollination: The third branch in each side of the trees was kept without enclosing for open (free) pollination.

Initial and final fruit (%) set were estimated 30 and 60 days (Selak *et al*., 2011) after pollination, respectively. 'Picholine' was studied for only one season (2017).

Index of self-incompatibility was calculated as fruit set obtained by self-pollination / fruit set obtained by open or cross-pollination. Evaluation was as follows: ISI= 0: comletley self-incompatible, 0.1 to 0.2 highly self-incompatibile, 0.2- 0.9 partial self-incompatibile, ≥1: completely self-compatibile (Zapata and Arroyo, 1978).

Fertility index (R) was estimated and evaluated according to Moutier (2002) as follows:

R = number of fruits in the case of cross-pollination / number of fruits in the case of free- pollination: R=0 to 0.33: bad polliniser, R= 0.33 to 0.66: passable polliniser, R= 0.66 to 1 or higher: good polliniser.

Experimental design and statistical analysis:

Completely randomized blocks design was used with three replications (trees). Data were arcsine transferred and subjected to ANOVA. Means were separated using Duncan multiple range test ($P \leq 0.05$) using (CoStat version 6.400 Copyright(c) 1998- 2008 CoHort software, CA, USA).

RESULTS AND DISCUSSION

Meteorological data:

Climatic factors play major rule in physiology of fruit trees, affecting the transition from one phenological phase to another (Marra *et al*., 2018). Figure (1) shows that the climatic conditions were different between 2017 and 2019

seasons. January and February of 2017 season were colder than 2019, while the period from March to June was warmer.

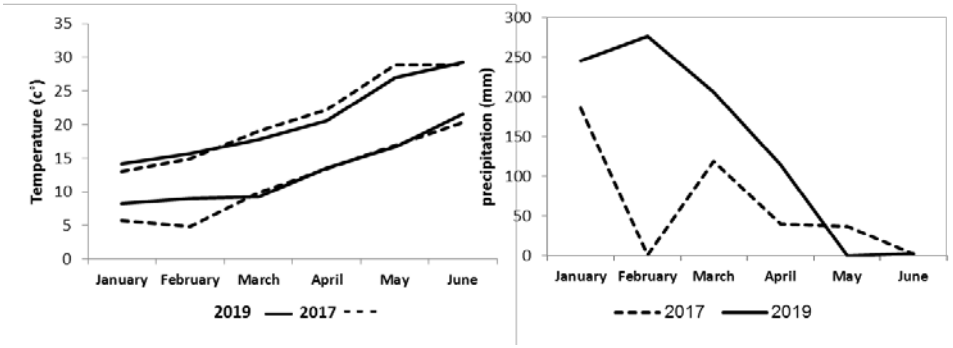

Figure 1. Monthly minimum and maximum temperatures (°C) (on the left) and precipitation (mm) (on the right) for the period from January to June of 2017 and 2019 seasons.

Precipitation behavior was aslo different among seasons, precipitation almost stopped in February 2017, then lasted until June. In 2019 season, precipitation was higher but stopped in June resulting in droght in that month.

Flowering period:

The flowering period is a key factor when choosing pollinisers. Figure (2) shows that flowering started earlier in 2017 comparing with 2019 season. Differneces in flowering could be due to higher precipitation in April and higher flowering load of 2019 season comparing with 2017 (Data from visiual observation). Mehri and Mehri-Kamoun (2007) reported differences in flowering periods depending on year and study site.

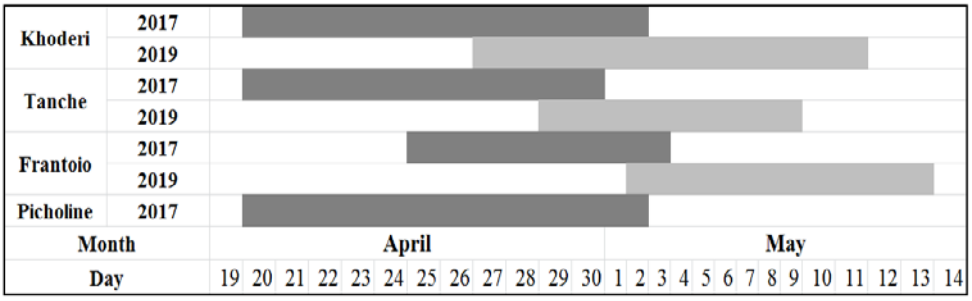

Figure 2. Flowering periods of 'Khoderi', 'Tanche', 'Frantoio' and 'Picholine' olive cultivars in 2017 and 2019 seasons.

All the studied cultivars started flowering by the same time in 2017 except for 'Frantoio' whose flowering was delayed by five days. In 2019 season, 'Khoderi' was the earliest flowering followed by 'Tanche' and 'Frantoio'. In general 'Khoderi' had the longest flowering period and overlaped or covered the entire flowering periods of the other cultivars. This meets the fact that when choosing pollinisers, the pollen donor (polliniser) and the receptor plant must

overlap in flowering periods that ensures the presenence of pollen grains by the time of flower opening of the receptor cultivars (Selak *et al*., 2018).

Pistil abortion and Number of flowers per inflorescence:

Data analysis showed that both cultivar and season affected pistil abortion; the main effect was of the cultivar. Figure (3-A) shows that 'Picholine' had the highest pistil abortion (%) and 'Frantoio' had the lowest. Significant differences between seasons were observed in 'Tanche' and 'Picholine' but not in 'Frantoio'.

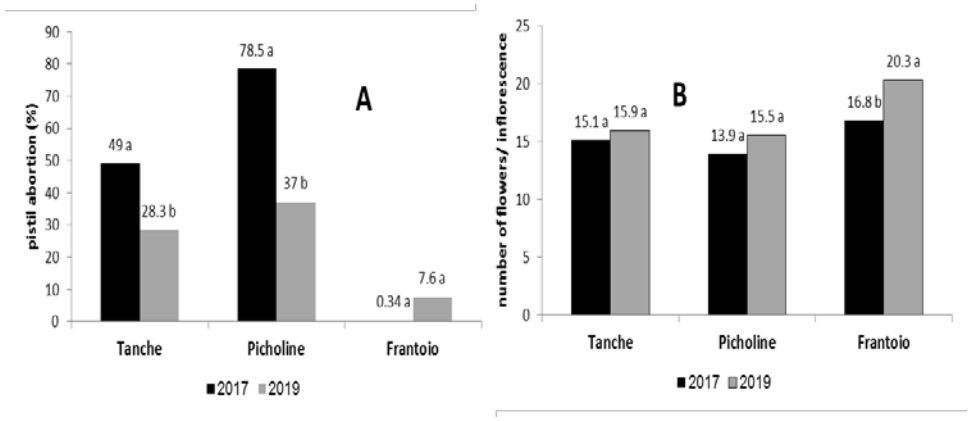

Figure 3. Pistil abortion (%) (A) and Average number of flowers per inflorescence (B) of 'Tanche', 'Picholine' and 'Frantoio' olive cultivars in 2017 and 2019 seasons. Different letters on the columns indicate significant difference ($P \leq 0.05$) among seasons of the same cultivar using LSD test.

Generally, several factors reported to influence pistil abortion like genetic factor, season, competition for resources and other factors (Rosati *et al*., 2011; Rosati *et al*., 2012; Alagna *et al*., 2016). Rapoport *et al*. (2012) found that water deficits in the stage of inflorescence formation reduced the hermaphrodite flowers ratio. In the study site buds began to burst in February, so lacking moisture in this period (Figure 1) could negatively affect the inflorescence formation. The number of flowers per inflorescence (Figure 3-B) was lower in 2017 than in 2019 season, this could be due to similar reason even though this reduction was insignificant in all studied cultivars except 'Frantoio'.

Pollen germination:

Pollen germination differed depending on cultivar and season (Figure 4). 'Frantoio' and 'Tanche' had the highest germination ratio in 2017 and 2019 seasons, respectively, while 'Picholine' had the lowest in both seasons. Significant differences were observed between 2017 and 2019 seasons, 2019 season was characterized by higher germination ratio. This could be due to fact that pollen was cultured immediately in 2019 season while stored for several days before culturing in 2017. Pollen germination could also be influenced by the number of the pollen produced by the tree, nutritional status and climatic conditions (Ferri *et al*., 2008; Mazzeo *et al*., 2010).

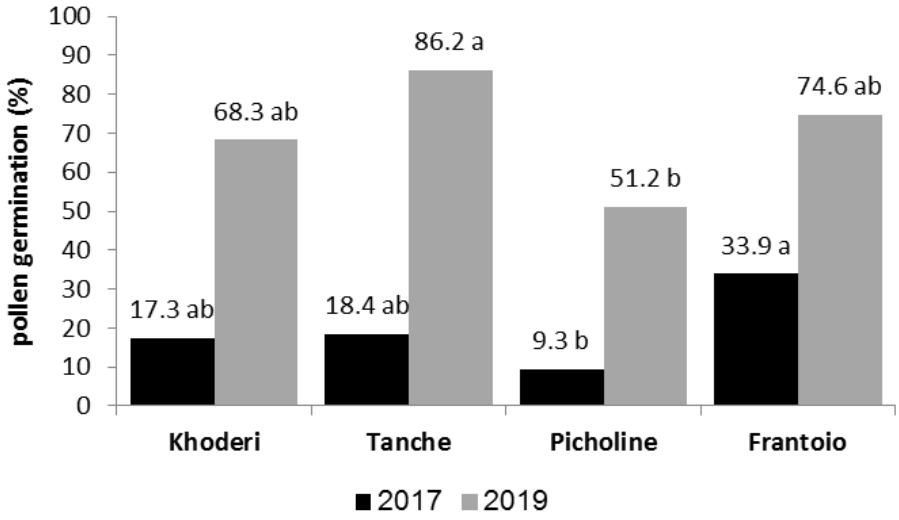

Figure (4): pollen germination (%) of 'Khoderi', 'Tanche', 'Picholine' and 'Frantoio' olive cultivars in 2017 and 2019 seasons. Different letters on the columns indicate significant difference ($P \leq 0.05$) among cultivars in the same season using Duncan's test.

Fruit set:

Data presented in table (1) shows that initial fruit set (%) was influenced by pollination type. In 2017 season, open pollination and cross- pollination by 'Khoderi' resulted in the highest initial fruit set, while lowest initial fruit set was of self-pollination. In 'Frantoio' cultivar, the differences in initial fruit set for all pollination type were insignificant. In 2019, initial fruit set of 'Tanche' was higher than 2017 and behaved the same. In 'Frantoio', cross pollination with 'Khoderi' resulted in the highest initial fruit set. Significant effect of pollinisers on initial fruit set for number of olive cultivars was reported in Croatia (Selak *et al*., 2011).

Final fruit set was used to evaluate the fruit set from different pollination types in most pollination studies (Selak *et al*., 2011; Sánchez-Estrada and Cuevas, 2018). Data presented in table (1) shows similar response of 'Tanche' and 'Picholine' to pollination type, i.e. cross-pollination with 'Khoderi' resulted in the highest final fruit set followed by open pollination, while self-pollination resulted in the lowest final fruit set. After initial fruit set massive fruit abscission normally occurs mainly due to fruit competition for substrate (Rapoport and Rallo, 1991), this could be the reason for higher fruit drop of 'Tanche' fruits resulting from cross-pollination with 'Khoderi' in 2019 comparing to 2017 season and to other treatments were fruit sets were lower.

'Frantoio' behaved little different. No significant differences between all pollination types were observed in 2017, but in 2019 final fruit set resulted from cross pollination with 'Khoderi' was significantly higher than fruit set resulted

from open and self-pollination. Generally, cross-pollination with 'Khoderi' resulted in the highest final fruit set for all studied cultivars and seasons.

Several studies reported the significance of cross-pollination in olive cultivars, Farinelli *et al*. (2006) found that cross pollination of 'Sorani' with 'Gordal', 'Moresca' and 'Picholine marocaine' resulted in fruit set similar to open pollination; by the same way, 'Gemlik' olive cultivar was reported to increase fruit set of 'Hayat' when used as pollen donor (Mete *et al*., 2016).

Table 1. Initial and final fruit set (%) of 'Tanche' 'Picholine' and 'Frantoio' olive cultivars as affected by cross-pollination with 'Khoderi', self-pollination and open pollination in 2017 and 2019 seasons*

Initial fruit set	**Tanche**		**Picholine**		**Frantoio**	
	2017	**2019**	**2017**	**2019**	**2017**	**2019**
Cross pollination with 'Khoderi'	2.62 a	7.47 a	1.75 a	---	5.97 a	3.36 ab
Self- pollination	0.81 b	0.62 c	0.19 b	---	2.42 a	1.54 b
Open pollination	2.78 a	2.39 b	2.22 a	---	5.19 a	5.45 a
p values	.0281	.0009	.0043	---	.1781	.0323
Final fruit set	**Tanche**		**Picholine**		**Frantoio**	
	2017	2019	2017	**2019**	2017	2019
Cross pollination with 'Khoderi'	2.49 a	2.73 a	1.65 a	---	4.60 a	3.02 a
Self- pollination	0.74 b	0.50 b	0.18 b	---	2.13 a	1.15 c
Open pollination	2.21 a	1.42 ab	1.59 a	---	4.12 a	1.57 b
p values	.0399	.0203	.0090	---	.2898	.0018

* Different letters in the columns indicate significant difference at $p \leq 0.05$ using Duncan's test.

Many studies reported increasing fruit set under open pollination over cross-pollination (Pinillos and Cuevas, 2009; Selak *et al*., 2011; Sanchez- Estrada and Cuevas, 2018), this could be a result of more favorable environmental conditions in open branches comparing with enclosed bags (Selak *et al*., 2011), and/or the presence of more compatible pollinisers than those used in cross-pollination. However, in the present study the opposite situation was found, cross-pollination results were higher than open pollination, which clearly indicate that 'Khoderi' is highly compatible with the studied cultivars. Enhancing fruit set under cross-pollination over open-pollination is a case more important in areas with little olive diversity or mono-cultivar olive orchards of 'Tanche', 'Picholine' and 'Frantoio'.

Self-incompatibility and Fertility index:

Data presented in table (2) shows that 'Picholine' was highly self-incompatible cultivar with ISI= 0.11. Previous studies in the same region

reported that 'Picholine' was highly self-incompatible with ISI= 0-0.09 (Mhnna *et al*., 2015), which is in accordance with Moutier (2002) who found that 'Picholine' is highly self-incompatible in France. 'Tanche' and 'Frantoio' were partially self-incompatible, but 'Frantoio' was more self-fertile than 'Tanche'. Spinardi and Bassi (2012) found that ISI of 'Frantoio' was 0.25; this difference could be related to different environmental conditions. Gonzalez and Cuevas (2012) reported different ISI values of 'Arbequina' depending on season and study location. This cultivar was self-compatible in one region and incompatible in another. The same cultivar was reported to be infertile in Montenegro conditions with concern of the productivity in mono-cultivar orchards (Lazović *et al*., 2017).

Table (2) Index of self- incompatibility (ISI) and evaluation of 'Khoderi' as polliniser for 'Tanche' 'Picholine' and 'Frantoio' olive cultivars using fertility index (R index) in 2017 and 2019 seasons*

	Tanche			Picholine			Frantoio		
	2017	2019	Eva.	2017	2019	Eva.	2017	2019	Eva.
ISI	0.33	0.35	PSI	0.11	---	HSI	0.52	0.73	PSI
RI	1.13	1.92	Good	1.04	---	Good	1.12	1.92	Good

*PSI: partially self- incompatible, HSI: Highly self- incompatible, RI: fertility index, Eva: evaluation.

Fertility index (R-index) was used to evaluate olive pollinisers by different authors (Moutier, 2002; Mete *et al*., 2016, etc.). In our study, based on R-index 'Khoderi' was good (efficient) polliniser for 'Tanche', 'Picholine' and 'Frantoio' olive cultivars in both study seasons. Recent studies also reported that most olive cultivars are self-incompatible (Saumitou-Laprade *et al*., 2017), and there is a need of cross-pollination with compatible cultivars to increase fruit set even for self-compatible cultivars (Mete *et al*., 2016).

CONCLUSIONS

'Picholine' was highly self-incompatible olive cultivar, while 'Tanche' and 'Frantoio' were partially self-incompatible. 'Khoderi' was efficient polliniser for all the studied cultivars, characterized by good pollen germinability and extended flowering period that overlaps the flowering period of the receptor cultivars. Overall results suggest 'Khoderi' as efficient polliniser for 'Picholine', 'Tanche' and 'Frantoio' olives in order to enhance fruit set and consequently productivity**.**

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WEST NILE VIRUS DETECTION PROGRAMME, SURVEILLANCE OF *Culex sp*. IN MONTENEGRO, 2019

SUMMARY

Vectors and vector-borne diseases are fascinating systems to study in addition to their importance to human and animal health, a single environmental factor may have opposite impact on the system at different points in time. Interdisciplinary collaboration is essential for proper survey and it's requires awareness of the differences between disciplines and the ability to effectively communicate with each other. It is only by forming multi-disciplinary groups to focus on specific vector-host-pathogen systems we will be able to answer the most pressing problems.

During season 2019 interdisciplinary collaborative "Programme for WNV detection in Montenegro" has been launched based on One Health Concept. This approach is essential for studying such systems: 'a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of healthcare for humans, animals and the environment'. At paper we present first year preliminary results of collaboration.

Keywords: *Culex sp.*, surveillance, West Nile Virus, Montenegro

INTRODUCTION

In a sense, vectors and vector-borne disease epidemics are like hurricanes, or tornadoes: they occur sporadically and their precise time and location are not possible to predict. Above that they are quickly forgotten and neglected. One of the major reasons vector-borne diseases are so difficult to predict is the complex interaction of multiple organisms—vector, vertebrate host, pathogen—in space and time (Moore, 2008). Also, it is not possible to present the precise cost-benefit analysis on investing in vector and vector-borne disease surveillance and control. It is much easier to do it for the agricultural pests, (measuring the pest control effects on the crops yield), than to estimate the value of the human life lost. Montenegro as a candidate to become the Member State of EU has imperative to harmonize and to apply regulations in the domain of public health and environmental protection with EU legislative, including legislative and actions related to the mosquitoes and vectors at all.

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Beside some vector-borne diseases like Bluetongue Disease and some explosive epidemics like Lumpy Skin Diseases and African Swine Fever on animals; particular and permanent interest in public health in Montenegro are on Dengue (DENV), Chikungunya (CHIKV), West Nile Virus (WNV) and Leishmania; not just in Montenegro but also in other Southeast Europe countries.

West Nile Virus (WNV) (*Encephalitis Nili occidentalis*) infection is a zoonosis generally transmitted through the bites of infected mosquitoes, mainly of the *Culex* genus (rarely transmission occurs through blood transfusion or organ transplantations). WNV is an enveloped RNA virus belonging to the Japanese encephalitis virus sero-complex (*Flavivirus* genus, *Flaviviridae* family) (Petersen, 2013); as well as a "member" of diverse group: Arboviruses - viruses that transmit hematophagic arthropods i.e. Yellow Fever Virus, Dengue Virus, Saint Louis Encephalitis Virus, Japanese Encephalitis Virus,... Today WNV is most widespread arbovirus in the World (Figure 1).

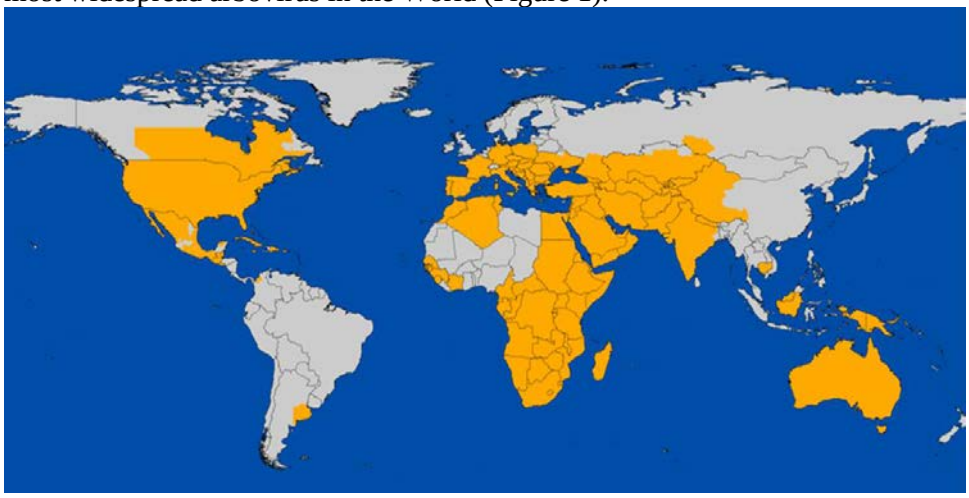

Figure 1: Global distribution of West Nile virus, 2006 (Reisen, 2013).

Virus was discovered before II World War in Uganda. From that time till late 90's sporadic cases were detected over the World (similar happened with Zika virus). After several decades without any reported cases in Europe, the virus re-emerged in Romania in 1996 resulting in a major outbreak with 393 confirmed cases including 17 deaths (Ceianu et al., 2001). WNV is maintained in an enzootic cycle, where mosquitoes serve as vectors and birds are the main amplifying hosts (Petersen, 2013). The transmission cycle is linked to the vector activity period from April to November (Barrett, 2018). Humans and other mammals, such as equids, are considered dead-end hosts (Bowen & Nemeth, 2007). It is certain that WNV is not transmitted to humans through contact, nor from infected birds without mosquito bites.

Most humans infected with WNV remain asymptomatic, however about 20% develop flu-like symptoms and less than 1% develop severe symptoms such as encephalitis, meningo-encephalitis or meningitis (Kramer et al., 2007). Flu-

like symptoms are usually misdiagnosed like “summer flu”; no matter that there is no flu during the summer. Anyway, important fact for virus detection is that infection develops immunity in human’s body. Elderly, over 50 years, mainly male and immuno-compromised persons are at particular risk of severe symptoms (Lim et al., 2011): strong headache, high temperature, neck stiffness, high sensitivity on light, confusion, loss of consciousness, paralysis, coma and death. These symptoms occur in 1 out of 150 infected patients, last for several weeks and lead to irreversible neurological damage. The mortality rate in patients with severe clinical symptoms is 3-15%.

Surveillance of human WNV infections can be done effectively just through surveillance of mosquitoes. Overall surveillance needs to be complemented by surveillance of equids, birds and dead birds, as well with prevention of human-to-human transmission via donation of contaminated substances of human origin. Based on surveillance results synchronized alert to physicians, public health professionals and the public about ongoing virus circulation is important as additional activity.

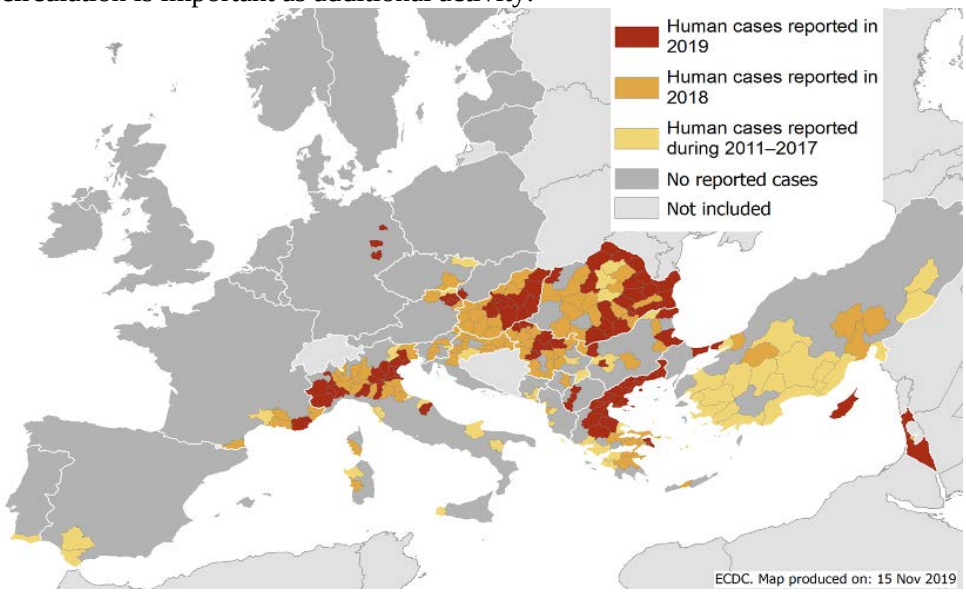

Figure 2: West Nile Virus infections by affected areas, in the EU/EEA Member States and EU neighboring countries 2011-2016.; 2017. and 2018 transmission season. Source: https://www.ecdc.europa.eu

During season 2019 special “Programme for WNV detection in Montenegro” has been launched. Beside fact that Montenegro need to apply EU regulations in the domain, five detected human cases of WNV were reported in Montenegro. Data considering countries affected with WNV in Europe (Figure 2) were important for launching the program; particularly during 2018 when epidemics reached significant number of human cases (Table 1). According ECDC data 30 countries reported human cases of WNV (period 2011-2019) in

EU and EU neighboring countries. In four of them for the whole period human cases were reported; Italy (980; 16.58% reported cases from the total), Romania (606; 10.25%), Israel (577; 9.76%) and Hungary (396; 6.70%). Additional problem is that virus is circulating in all countries in East Mediterranean from Italy on north to the Egypt on south, except Lebanon (no data due to the lack of surveillance) and in Balkan without any exception. From all 30 countries and from total 5.912 cases most of them were reported in Serbia (1.007 or 17.03%). Montenegro reported 1 case in 2012 and 4 cases in 2013; or 0.08% of all reported cases in Europe and Mediterranean basin (Table 1).

		2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Cases/country	% of total
1	Albania		2									**2**	0.03
2	Austria	+				1	7	5	5	20	4	**42**	0.71
3	Bosnia&Herzegovina				3	13						**16**	0.27
4	Bulgaria			2			2	2	1	15	5	**27**	0.46
5	Croatia			5	16			1	5	53		**80**	1.35
6	Cyprus							1		1	16	**18**	0.30
7	Czech Republic				1					5		**6**	0.10
8	France						1		1	27	2	**31**	0.52
9	Germany										4	**4**	0.07
10	Greece	+	100	161	86	15			48	311	223	**944**	15.97
11	Hungary	+	3	17	31	11	18	44	21	215	36	**396**	6.70
12	Italy	+	14	50	69	24	61	76	57	576	53	**980**	16.58
13	Kosovo			4						14		**18**	0.30
14	**Montenegro**			**1**	**4**							**5**	**0.08**
15	North Macedonia		4	6	1						6	**17**	0.29
16	Portugal						1					**1**	0.02
17	Romania	+	11	14	24	23	32	93	66	277	66	**606**	10.25
18	Russia	+	153	447	177	29	39	135				**980**	16.58
19	Serbia			69	302	76	28	41	49	415	27	**1007**	17.03
20	Slovakia										1	**1**	0.02
21	Slovenia				1					3		**4**	0.07
22	Spain							3				**3**	0.05
23	Turkey	+	3					2	7	23	9	**44**	0.74
24	Ukraine		8	12	1			1				**22**	0.37
25	Israel	+	39	83	63	17	125	84	28	128	10	**577**	9.76
26	Palestine			2		1	1					**4**	0.07
27	Tunisia		3	63	6			1				**73**	1.23
28	Algeria			1								**1**	0.02
29	Siria							2				**2**	0.03
30	Egypt							1				**1**	0.02
	Total		**340**	**937**	**785**	**210**	**315**	**492**	**288**	**2083**	**462**	**5912**	

Table 1: Reported human cases of WNV in the EU/EEA Member States and EU neighboring countries 2010-2019. Source: https://www.ecdc.europa.eu

The "Programme for WNV detection in Montenegro" partly relies on results of HERIC/LOVCEN project where survey of citizen opinion on different aspects of vectors (VECs) and vector-borne diseases (VBDs) in Montenegro were conducted. During survey 1.643 citizens from all municipalities were interviewed providing a valuable indication on how to proceed and make the result of LOVCEN sustainable. The analysis of answers provided the three reasons for the establishment of Programme: a) 97 % of citizens are supporting VEC and VBD surveillance; b) 95% are supporting independent evaluation of mosquito control; and c) 59% are willing to personally support programme of surveillance of VEC and VBD and independent quality control of mosquito treatments (Salasan et al., 2015).

MATERIAL AND METHODS

Methods for mosquitoes trapping are specialized as much as possible. Common mosquito trapping methods are (1) "oviposition traps"; using ovitraps we can determine the presence of a species and approximately calculate their abundance. For purpose of *Culex* sp. and WNV surveillance they are not useful (Figure 3a). (2) BG Sentinel (Figure 3b) are traps with attractants for particular mosquito species. They are more used for *Aedes* species trapping and are not suitable for *Culex* sp. mosquitoes' mass-trapping. (3) CDC traps (Figure 4a) have CO_2/dry ice as an attraction for mosquitoes. This type of trap is excellent for catching large number of females present in the area. However for us CDC traps were not suitable due to the fact that in Montenegro procurement of dry ice is not reliable, cost per kilo is high and procurement procedure is quite complicate. (4) "Resting" traps, the least developed mosquito trapping system. This type of trap has the greatest potential for trapping mosquitoes, but unfortunately, it is not fully technically developed, so it is the least used (Figure 4b). For "Programme for WNV detection in Montenegro" we used Gravid traps in surveillance of *Culex* sp. mosquitoes (Figure 5).

Figure 3: a: Ovitrap; b: BG Sentinel trap

Figure 4: a: CDC trap; b: Resting trap

One of the main goals of the Program is to detect the presence of WNV in mosquitoes. WNV is present in mosquito females after taking a “blood meal” on infected host, not before. For this reason, the name of the trap comes from "pregnant" females: gravid in terms of pregnancy, not gravity. This fact is advantage of Gravid traps compared with all previously mentioned trapping systems (Miller et al., 2015). Usually used CDC traps capture large number of present mosquitoes due to CO_2 emissions, especially females that have not taken blood (not infected jet) looking for hosts based on increased emissions and concentrations of CO_2 (Petrovic et al., 2018). Gravid traps, on opposite, capture far fewer mosquitoes, mainly "pregnant" females after taking a “blood meal” (or after been infected) looking for a suitable place to lay eggs. Gravid traps capture few males and other non-Culex species. Attraction of Gravid traps is actually convenient place for laying eggs. Culex females lay eggs standing on the surface of the water. The females are attracted by water and after landing on the surface they are sucked into the trap. In summary this capturing system for the purposes of determining WNV is the most convenient, considering all negative and positives sides.

Figure 5: Gravid traps

RESULTS AND DISCUSSION

Natural reservoirs for WNV are birds; mosquitoes are natural vectors from infected birds to “dead-end hosts” - mammals (particularly humans and equids). General meaning of the term “natural vector” is transmitter of infectious human or/and animal diseases; on the way that the causative agent does not affect the vector itself. Vector serves only to transmit causative agent from the infected to a healthy host organism.

In rare occasions it has been noted that infection can be transmitted through transfusion of infected blood, transplantation of organs and from mother to child transplacental or during lactation. It is not clear can virus be transmitted to carnivorous animals by eating the meat of infected animals. Although WNV is not isolated from any other vector even that in theory they can become vectors of

the virus, especially ticks. Beside these WNV is transmitted to humans by the bite of an infected mosquito, practically exclusively.

Up to now virus has been isolated from 60 mosquito species, mostly from the genus *Culex*. During the first season of surveillance at the territory of Montenegro we find several WNV vector species.

Invasive species *Aedes albopictus*, even highly anthropophilic, can transmit WNV. Species is present all over South and Central part of the State (Pajovic et al., 2013). It is interesting that this invasive species appear every year in North Region (Plužine, Šavnik, Bijelo Polje, Andrijevica) but can not overwinter there due to the climatic characteristics. Essential for *Aedes albopicitus* control is to have permanent survey above this species not only concerning WNV, than other mosquito borne diseases (Petric et al., 2018). The study of the geographical distribution of invasive species can also provide valuable information about their invasiveness (Sherpa et al., 2019).

Less important for WNV, but still possible vectors of virus, are *Anopheles* mosquitoes. They, so called "malaria mosquitoes", are present in: Herceg Novi, Kotor, Tivat, Budva, Bar, Ulcinj, Cetinje, Podgorica, Tuzi, Danilovgrad, Žabljak, Šavnik, Bijelo Polje, Berane and Gusinje. Never detected in Montenegro, but worth to mention as possible vectors for WNV, are *Mimomyia* mosquitoes.

In 80% of cases WNV has been transmitted by: *Culex pipiens* "house mosquito", species present all over Montenegro. During last several years species has been found in Kotor, Tivat, Budva, Bar, Ulcinj, Cetinje, Podgorica, Tuzi, Danilovgrad, Žabljak, Šavnik, Plužine, Pljevlja, Mojkovac, Kolašin, Bijelo Polje, Berane Andrijevica, Plav, Gusinje, and Rožaje. *Culex modestus* is present in Southern and Central part of the State. And *Coquillettidia richiardi* is present at Budva, Ulcinj, Cetinje, Podgorica and Danilovgrad municipalities.

All mentioned species are living in anthropogenic environment suitable for their reproduction cycle (stagnant water). For transmission of virus to next season it is important to note that: once infected mosquitoes transmit the pathogen for the rest of their lives; transovarial transmission of WNV is noted in some *Culex* species (John et al., 2012) and WNV was detected in overwintering mosquitoes in Europe (Rudolf et al., 2017). For survey crucial information is that *Cx. pipiens*, *Cx. modestus*, *Coq. richiardi* + *Ae. albopictus* are detected in our country. Most dangerous *Cx. pipiens* is spread throughout whole Country in urban and semi-urban places, as native species.

In accordance with 1) data we have from the last 5 years about mosquito abundance and geographical distribution; 2) recorded cases of WNV in humans and 3) the total population of Montenegro (the possibility of transmission of the virus on a larger scale), we selected the sites where monitoring was carried out. Of great importance is the anthropophilicity of these species but also their ability to take "blood meals" not only from humans but also from animals, fact of high importance in the virus-vector-host (human) chain.

Considering *Aedes albopictus*, it's important to emphasize possibility for transmission over long distances and in regions where it is not an indigenous

species, by human's activity primarily by all forms of traffic. This is important for decision to catch mosquitoes in Plužine, town where we have recorded species entries but without overwintering. As such, species is introduced (by passing vehicles / passengers / tourists) every year, but there is no possibility for overwintering (for now) in the given climatic conditions. Then again, during following season, species will be brought into the same living space. It is similar to Berane municipality in which additionally we have a confirmed "malarial mosquito" records.

Based on all data we consider Montenegro as lower risk territory for possible WNV transmission on a larger scale. During the 2019 season, adult capture was performed seven times; collection in May was absent due to heavy rains. Trapping of mosquitoes has been performed in: Podgorica (mid June); Berane (mid-July); Podgorica (late July and early August); Plužine (early September); Ulcinj (late September) and two times at end of the season in Podgorica (early and mid-October).

Table 02.: Captured adult mosquitoes during season 2019.

City	*Date*	*Culex sp.* ♀	*Ae. albopictus*
Podgorica	17-18th June	255	0
Berane	15-16th July	228	0
Podgorica	29-30th July	102	44
Plužine	09-10th September	17	0
Ulcinj	23-24th September	49	5
Podgorica	07-08th October	51	34
Podgorica	14-15th October	38	11
	Total:	**740**	**94**

Trapping was performed in period of the season with highest abundance and activity of mosquitoes / highest possibility for WNV transmission; June-October. Reiter-Cummings gravid traps were placed in semi-urban and urban previously selected localities in the indicated cities during the morning and were left to operate in average next 22-24 hours. After that time mosquitoes were collected, put on dry ice in the field and maintained under cold conditions throughout the testing process. They were brought to the Diagnostic Veterinary Laboratory or Laboratory for Applied Zoology of the Biotechnical Faculty where the mosquitoes were identified to the species level, counted and pooled according to date, location, and species. After determination mosquitoes were left in deep freezer until further analysis. The idea was, in case of WNV detection at a specific locality, to repeat trapping with all functional traps.

In total 740 *Culex pipiens* females were delivered to a Diagnostic Veterinary Laboratory, including a pair of *Cx. modestus*. There were no individuals of *Coquillettidia richiardi*. Table 02 also shows the number of captured *Aedes albopictus* specimens. Presented number is high considering that

these traps are not specialized in capturing particular species. Most likely females of the species were attracted by water as an attractant because the summer was dry and other common places where they lay eggs were not functional.

CONCLUSIONS

Mosquito vectors and mosquito-borne diseases are raising threat to Europe, which impact strength is difficult to predict. The main infection sources are dependent on vector and environmental factors; hence the best choice for prevention and control of diseases is surveillance and control of mosquito vectors. WNV main vectors in South-East Europe are *Culex pipiens*, *Culex modestus* and *Coquillettidia richiardi*, all native in Montenegro. Including invasive species *Aedes albopictus*.

During season 2019 interdisciplinary collaborative "Programme for WNV detection in Montenegro" has been launched based on One Health Concept. Important for launching the program was complex epidemiological situation in South-East Europe. One of the reasons for such situation is mosquito abundance. Season 2018 has been very favorable for mosquitoes development; the dry period started with high temperatures unusually early in May (as opposed to 2019), extended into deep autumn and exceeded the total number of cases in all previous years (between 2010 and 2017) (Haussig et al., 2018). Important reason is that at least eight phylogenetic lineages of virus have been described (Fall et al., 2017), but only lineage 1 and 2 are associated with disease in humans (Petersen, 2013). In Europe, prior to the emergence of a lineage 2 in Hungary in 2004, sporadic cases and occasional outbreaks in animals and humans were due to lineage 1. Since 2008, the WNV lineage 2 has spread over central Europe and the eastern Mediterranean region (Chaskopoulou et al., 2016; Rizzo et al., 2016), including possibly Montenegro.

As obvious, importance to human and animal health of mosquito (vector) – mosquito borne diseases (MBD) interaction can be answered only through interdisciplinary collaboration. This approach is essential for studying such systems, but this requires awareness of the differences between disciplines and the ability to effectively communicate with each other (Moore, 2008). Interdisciplinary approach is consistent with the One Health concept, defined by the One Health Initiative (http://www.onehealthinitiative.com) as 'a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of healthcare for humans, animals and the environment' (Jourdain et al., 2019).

There is no WNV vaccine, so the most effective way to control the disease is survey, monitoring and control of vectors. Personal protection measures are more than useful in mosquito bites prevention: usage of repellents; installation and usage of safety nets on windows and doors; drainage of all unnecessary stagnant water; removal of all unnecessary items that can hold water from the garden; regular cleaning of gutters and other water collection and drainage

channels; twice a week replacement of water from the vessels in which it is kept; covering vessels for technical water storage...

We described the epidemiology of WNV infections in the EU and EU Enlargement countries, presence of vector species in Montenegro and results of surveillance in first year which will help for better understand the geographical extension of vectors and support Country to prepare for possible future WNV outbreaks.

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THE PRODUCTION OF BIOFUEL FEEDSTOCK ON RECLAIMED LAND BASED ON SWEET SORGHUM BIOMASS

SUMMARY

Almost all of the studied sorghum hybrids showed a good growth both on the black soil mass and on loess-like loam. The yield of fresh biomass was reached at the level of 50-100 t ha^{-1}. The juice amount in the stems of Ukrainian hybrids was more than in American ones by 5-10%. At the same time, the concentration of sugar in it was less, 16-19% as against 18-21%. The theoretical ethanol yield for highly productive hybrids (Zubr, Medove, Mohawk, SS506) was 2500-3600 L ha^{-1}, and for low-output hybrids (Sioux and Silosne-42) 705-1600 L ha^{-1}. Hybrids Zubr, Medove, Mohawk, SS506 and G1990 were selected as the most promising for cultivation on reclaimed lands. Addition of the plant biomass to sludge activates the thermal behavior of the composite mix. As a result, the combustion level rises to 41.4%.

Keywords: sweet sorghum, reclaimed lands, biomass productivity, theoretical ethanol yield, thermal effects.

INTRODUCTION

Sweet sorghum is a promising, drought-resistant plant which has a number of useful features and a great potential for use in various areas of the national economy of Ukraine (Mostenska et al., 2013; Rakhmetov et al., 2018 The juice extracted from the fresh stem can be used to produce sugar, syrup and first generation bioethanol (Sipos et al., 2009; Kim and Day, 2011). Bagasse can be used as fodder, fertiliser, second generation bioethanol or as a raw material for the paper industry (Betancur and Pereira, 2010). Sorghum stems contain readily available soluble carbohydrates, so enzymatic conversion of starch into sugar is not necessary. This gives sorghum an economic advantage over other starch-based crops. High sugar content and ease of extractability make sweet sorghum one of the leading feedstock crops for biofuels (Taylor et al., 2006, Mathur et al., 2017). Agronomic traits like short life cycle of about 4 months, C4 photosynthesis which contributes to higher water and nutrient use efficiency,

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

unpretentiousness and low cost of cultivation are especially helpful for its adoption as a raw material (Reddy et al., 2005; Shoemaker et al., 2010; Rutto et al., 2013; Regassa and Wortmann, 2014). The unpretentiousness of sorghum to environmental conditions determines the prospect of growing this plant on unproductive and reclaimed lands, thus avoiding a conflict between food production and biofuels (Ameen et al., 2017; Mehmood et al., 2017). There are some data of sorghum cultivation in marginal lands. For example, in Northern and Eastern China, the biomass yield, nutrient (N, P, and K) accumulation and energy productivity of sorghum on marginal lands conditions under arid and semi arid conditions were studied (Ren et al., 2012; Fu at al., 2016). In USA, Holou and Stevens (2012) investigated the optimum nitrogen fertilizer rate for producing sweet sorghum juice, sugar, and bagasse on silt loam, sandy loam, and clay soils. Asif Ameen and colleagues studied the potential energy sorghum biomass production and uptake of nitrogen (N), phosphorus (P), and potassium (K) on a sandy loam marginal land (Ameen et al., 2017). Given the specifics of reclaimed land, the selection of energy sorghum genotypes in such conditions should be based on the following criteria: fast and homogeneous germination; the ability to produce a stably high biomass yield; high sugar content in stem juice; disease resistance (Adeyanju et al., 2015). Nevertheless, the matter of selection of the most productive hybrids and the technologies of sorghum cultivation under unproductive lands conditions have not been sufficiently studied, so the need for research in this direction is urgent.

The utilization of different kinds of biomass residues become an important part of future bio-energy concept (Curovic et al., 2016; Steite et al., 2011). Last years the sewage sludge as residual waste of sewage plants is applied to mix with different kinds of crop residues for pelletization (Li et al., 2015).

The main objective was to give the bio-energetic assessment of the production of bio-fuel feedstock on reclaimed land based on sweet sorghum biomass.

MATERIAL AND METHODS

This research was carried out under Ukraine steppe zone conditions at Pokrov land reclamation station of Dnipro State Agrarian and Economic University in 2017 and 2018. Four sweet sorghum hybrids of Ukrainian selection (Medove, Zubr, Pokrovske, Silosne-42) and four American hybrids (SS506, Sioux, Mohawk, G1990) were investigated in the field. Experience was carried out in two versions. In the first case the plants were grown on long-term plant meliorated loess-like loam (LLL), in the second on the black soil (BS) mass taken in stockpiling. The humus content in the loess-like loam is about 1.1%, in the black soil is 3.3. The ratio of humic and fulvic acids is 0.65 (LLL) and 1.36 (BS). Sorghum seeds were sown in early May. Biometric indices, productivity, conservative sugar yield, and theoretical ethanol yield were researched. The plant height was measured using a measuring line. To determine the yield of above-ground biomass, each cultivar was harvested after the grain reached hard dough

stage by cutting at the height of 10 cm from the ground level and weighed. After that, the biomass was dried to constant weight, and then weighed again. The sugar concentration in sweet sorghum stalks is measured in Brix units, which represents the percent soluble sugars. One degree Brix is equal to 1 g of sugar per 100 g of juice. Brix was determined using a hand-held refractometer "RHBO–50ATC". Conservative sugar yield (t ha^{-1}) was calculated based on an approach assuming that the sugar concentration is 75% of Brix expressed in g kg^{-1} sugar juice (Wortmann et al., 2010; Ekefre et al., 2017). It was used the equation: CSY= (FSY–DSY)*Brix*0.75. Where, CSY is conservative sugar yield (t ha^{-1}), FSY is fresh stem yield (t ha^{-1}), DSY is dry stem yield (t ha^{-1}). Theoretical ethanol yield was calculated as sugar yield multiplied by a conversion factor (0.58 L ethanol per kg of sugar): TEY=CSY*0.58 (Rutto et al., 2013; Ekefre et al., 2017). Where, TEY is theoretical ethanol yield (L ha^{-1}), CSY is conservative sugar yield (kg ha^{-1}).

The sorghum biomass and dewatered sewage sludge were taken as raw material. The sludge was obtained from an urban sewage station in Kiev city (Ukraine). Sludge and sorghum biomass sample were mixed manually with ratio of 50wt.%. The thermal analysis of plant biomass was carried out using the derivatograph Q-1500D of the "F. Paulik-J. Paulik-L. Erdey" system. Differential mass loss and heating effects were recorded. The results of the measurements were processed with the software package supplied with the device. Samples of biomass were analyzed dynamically at a heating rate of 10°C/min in an air atmosphere. The mass of samples was 100 mg.

RESULTS AND DISCUSSION

The cultivation of sweet sorghum on different mining substrates detected some variations in growth indicators. Cultivars Zubr and Medove showed the best growth characteristics on loess-like loam whereas Pokrovske, Silosne-42 and all American hybrids grew better on black soil (Figure 1).

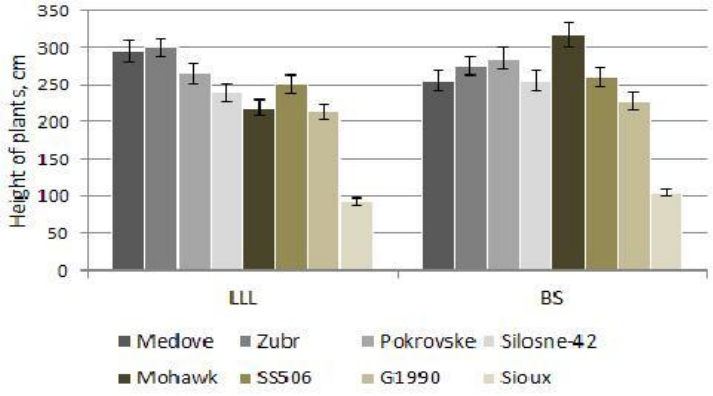

Figure 1. Height of sweet sorghum hybrids grown on reclaimed lands.

Mainly, the height of the studied cultivars was lower than the potentially average, by 9-25% on black soil, and by 22-37% on loess-like loam. The

smallest height was noted for Sioux on both substrates. It was not more than 95-105 cm with a potential height of 200-250 cm. By different assessments (Almorades and Hadi, 2009; Erickson et al., 2011; Regassa and Wortmann, 2014; Cavalaris et al., 2017), depending upon the cultivar, climate and soil conditions, and the planting date etc., productivity of sorghum fresh biomass can vary within a wide ranges, from 35 to 145 t ha^{-1}. In our experiment, as shown in the Figure 2, fresh biomass yield was recorded on loess-like loam from 18.9 t ha^{-1} (Sioux) to 101.0 t ha^{-1} (Zubr), and on black soil from 37.5 t ha^{-1} (Sioux) to 80.7 t ha^{-1} (SS506).

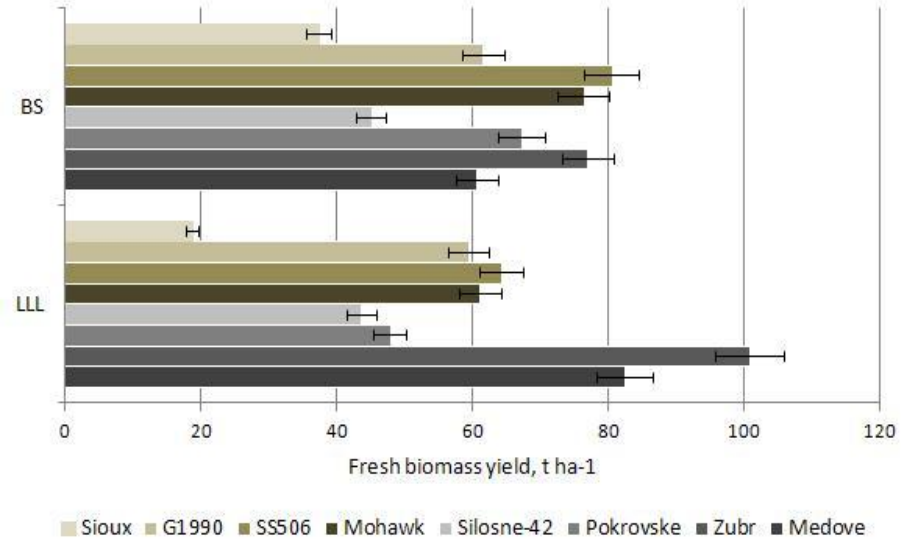

Figure 2. Fresh biomass yield of sweet sorghum hybrids grown on reclaimed lands.

During the experiment, it was defined that juice amount in the stems of Ukrainian hybrids was more than in American ones by 5-10%. At the same time, the concentration of sugar in it was less, 16-19% as against 18-21%. The plants grown on the black soil mass had Brix values slightly higher than on loess-like loam. As a result, on loess-like loam conservative sugar yield varied from 1.2 to 6.2 t ha^{-1}, and on black soil from 2.2 to 4.9 t ha^{-1}. The highest yield was noted for Zubr, and lowest for Sioux. The theoretical ethanol yield for highly productive hybrids (Zubr, Medove, Mohawk, SS506) was 2500-3600 L ha^{-1}, and for low-output hybrids (Sioux and Silosne-42) 705-1600 L ha^{-1} (Figure 3). Medove and Zubr were more productivity on the loess-like loam, and other hybrids on the black soil.

Thermal analysis of sorghum biomass, sludge, and composite mix of sludge and biomass showed the almost complete absence of sludge thermal degradation. The process occurs in the temperature range from 60^{o}C to 550^{o}C, and the level of decomposition is only 15.5% (Figure 4).

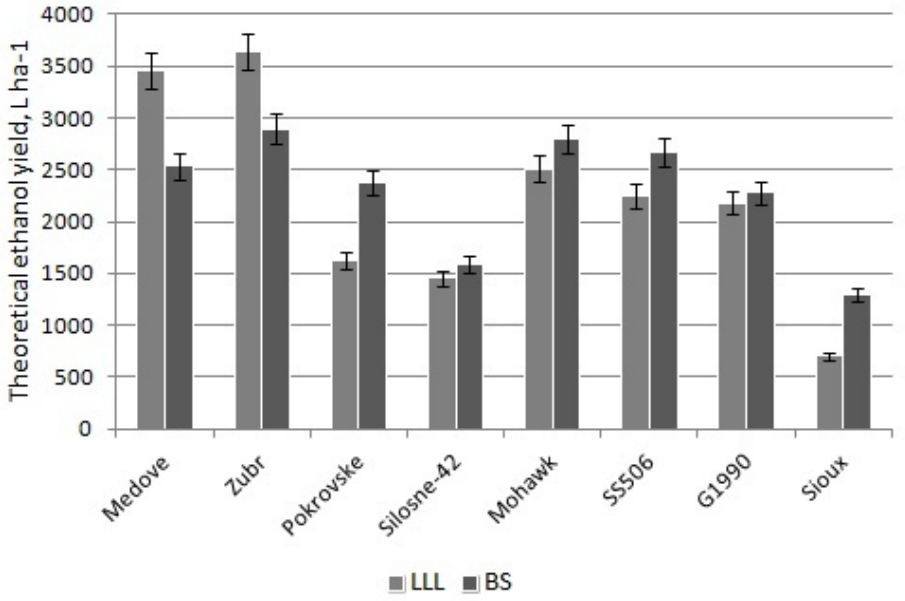

Figure 3. Potential ethanol yield per area in sweet sorghum hybrids grown on reclaimed lands.

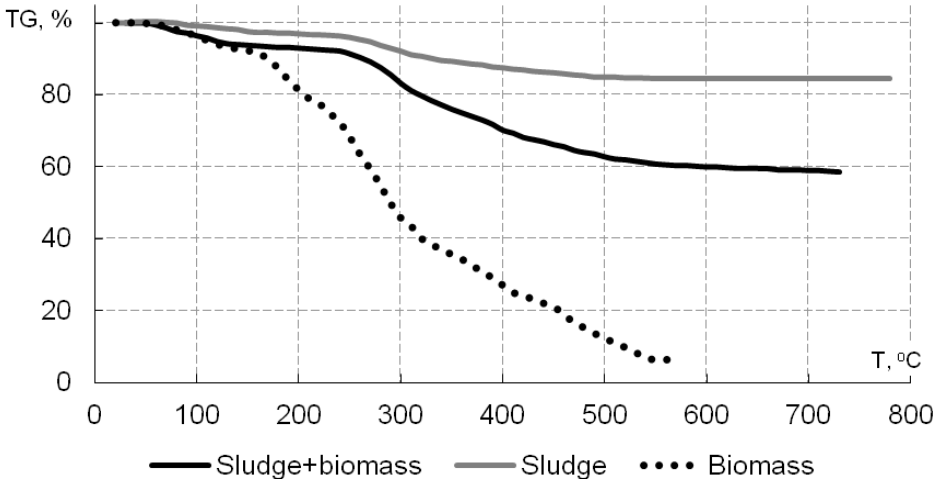

Figure 4. TG curves of biomass, sludge, and mix of sludge and biomass thermal destruction

Addition of the plant biomass to sludge activates the thermal behavior of the composite mix, as a result, the combustion level rises to 41.4%. The process takes place in the range of 40-730 ^{o}C and includes three stages with characteristic peaks of decomposition rate at temperatures of 90 ^{o}C, 290 ^{o}C and 370 ^{o}C. Pure biomass is consumed to

93.6% in the temperature range from 30 °C to 560 °C and embraced four stages. In all three samples, the first stage of the removal of volatile components is accompanied by the presence of endothermic reactions (Figure 5).

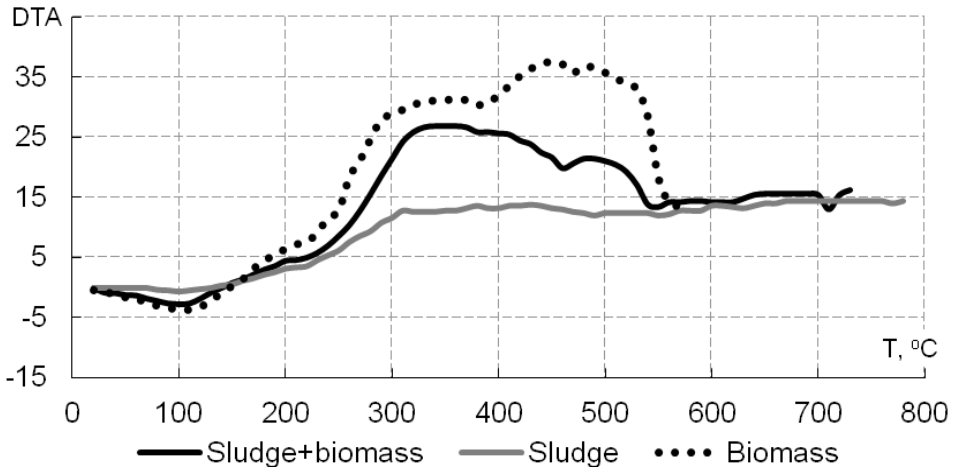

Figure 5. DTA curves of biomass, sludge, and mix of sludge and biomass thermal destruction

The starting of exothermic reactions is observed at a temperature of 140-160 °C. The greatest exothermic effect was noted in the sorghum biomass sample in the temperature ranges 350-370 °C and 440-460 °C. Exothermic effects in the sludge sample were very weak.

CONCLUSIONS

Almost all studied sorghum hybrids showed a good growth both on the black soil mass and on loess-like loam. Although these indicators were lower than potentially average ones by 9-37%, the yield of fresh biomass was reached at the level of 50-100 t ha^{-1}. All American hybrids and Ukrainian variety Pokrovske were more productive on the black soil. The other Ukrainian cultivars were high-yielding on the loess-like loam. The juice amount in the stems of Ukrainian hybrids was more than in American ones by 5-10%. At the same time, the concentration of sugar in it was less, 16-19% as against 18-21%. The plants grown on the black soil mass had Brix values slightly higher than on loess-like loam. Thus, the theoretical ethanol yield for highly productive hybrids (Zubr, Medove, Mohawk, SS506) was 2500-3600 L ha^{-1}, and for low-output hybrids (Sioux and Silosne-42) 705-1600 L ha^{-1}.

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MODELLING OF SOIL EROSION PROCESSES AND RUNOFF FOR SUSTAINABLE WATERSHED MANAGEMENT: CASE STUDY OUED EL ABID WATERSHED, MOROCCO

ABSTRACT

The study was carried out in the watershed of Oued El Abid that is located upstream of the Bin El Ouidane Dam in Morocco. Looking for a sustainable watershed management practices, we estimated soil losses from the river basin and the sediment yield deposited in the dam of Bin El Ouidane, using the Intensity of Erosion and Outflow - IntErO model, based on the Erosion Potential Method - EPM. The watershed of the lake receiving the waters and sediments from the two mean water courses Oued El Abid and Assif Ahansal, therefore before to proceed the calculation, the watershed was divided in two sub basins; the soil erosion and sediment yield were calculated for each sub basin. The result of calculation for the studied Oued El Abid river basin showed that the production of erosion material in the river basin is 3.960.115 m^3yr^{-1}. Coefficient of the deposit retention, calculated using the IntErO model, was 0.3 and as a consequence, real soil losses were calculated on 1.188.657 m^3yr^{-1}; specific real soil losses per km^2 402 $m^3km^2yr^{-1}$. Our findings, based on Gavrilovic classification, pointed out that the studied area is with a medium potential of soil erosion risk, due to the steep land slope and low vegetation cover in the watershed. The model outcome is validated using the Bathymetry measurements in the Dam of Bin El Ouidane.

Keywords: Soil erosion, IntErO Model, Land Use, Runoff, Soil erosion, Soil conservation

INTRODUCTION

Soil erosion is one of the most important causes of land degradation and one of the key global environmental hazards; especially for developing countries

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(Eswaran *et al*, 2001), affecting all natural and human-managed ecosystems (Kalibová *et al*., 2016; Gholami *et al*., 2013). Soil erosion by water affects 1,094 million hectares of arable land worldwide (Noor *et al*, 2013).

According to the various available reports 33% of the continental land is degraded, mainly by the use of agrochemicals, deforestation and water erosion. Among them, water erosion is considered the principal and most widespread agent of soil degradation, causing the reduction of productive capacity and the environmental devastation (Kinnell, 2016). In the Mediterranean neighbourhood of the studied basin, in Europe, data on trends in soil erosion are lacking and erosion estimates are based on modelling studies. In the 1990s, water erosion affected 105 million hectares of soil or 16% of Europe's total land area. In 2006, it was estimated that the surface area affected by water erosion 37 in the EU-27 was 130 million hectares (Spalevic, 2011). In 2014-15, approximately 11.4 % of the EU territory was affected by moderate to high level water erosion rate (more than 5 tonnes per hectare per year). The reduction of this rate against 1990s by 4.6 % is mainly due to the application of water erosion control practices which have been applied during the last decade in the EU.

For the appropriate sustainable watershed management, land use and landscape planning, which will more effectively meet national or local needs and assists in assessing the consequences of the alternatives the important issue is to quantify the sediments and to estimate sediment yield at the river basin scale (Chalise *et al*., 2019; Spalevic, 2019; Curovic *et al*, 2019; Parsipour *et al*, 2019, Fikfak *et al*, 2017; Popovic *et al*, 2018). Sediment sampling in the rivers need a lot of time and costly laboratorial works. Therefore, developing models with ability of estimating total amount of sediment is an inevitable need (Das and Agrawal, 1990; Khaledi Darvishan *et al*, 2010). Modelling of the erosion process is of a vital importance and various research teams developed models to predict soil loss - sediment yield, peak discharge – runoff. That can be used for the analysis of long as well as short periods of time (Spalevic, 2017a; 2017b; Barovic *et al*., 2015, Khaledi Darvishan *et al*., 2014; Sadeghi *et al*., 2013).

In the recent time we recorded the shortfall of rain and the problems of the storage capacity of the river dams in Morocco. In Bin El Ouidane Dam, in the Middle Atlas, the reservoir reached an unpreceded level of storage, almost 16% of its total capacity, just as the empty concrete pipes that snake in the plains to irrigate more than 100,000 hectares of beet, cereals and fruit trees; the point where the agriculture irrigation was no longer possible. The consequences can be disastrous while the country depends on agriculture, this sector, which draws 88% of the country's water consumption, supports 40% of the population and accounts for 15% of GDP.

This alert called for more interest to dam siltation problem caused by soil erosion and sediment yield, including the reservoir sedimentation in the Oued el Abid Watershed (Sabri *et al*. 2017).

We used the computer-graphic "River Basin" model of Spalevic (Spalevic, 1999) and the IntErO model (Spalevic, 2011) for prediction of soil erosion

intensity and sediment yield in the Bin El Ouidane Dam. This models calculate inputs using analytics of the Erosion Potential Method (EPM), originally developed by Gavrilovic (1972). This approach has been tasted earlier in many catchments area in Bosnia & Herzegovina, Bulgaria, Croatia, Czech Republic, Italy, Iran, Montenegro, Macedonia, Serbia and Slovenia and Morocco (Behzadfar, *et al*., 2014; Kostadinov *et al*., 2006; Gholami *et al*, 2016; Khaledi Darvishan *et al*, 2016, 2017; Vujacic *et al*, 2017). In Morocco have been successfully used in the Region of Western Rif of Morocco (Ouallali *et al*. 2016).

This study aims to identify the erosion processes in relation to the recent state of the sediment yield in the dam of Bin El Ouidane downstream the watershed of Oued el Abid in Morroco. This included the previous research results on the same watershed and precedent bathymetry measurements in the same dam, using different way of modelling sediment yield by the IntErO model that could be used for the efficient management and protection in the basins with similar climate and physical-geographical conditions.

MATERIALS AND METHODS

Study Area. Morocco, where the study area of Oued El Abid watershed is located, is in Northern Africa, bordering the North Atlantic Ocean and the Mediterranean Sea, between Algeria and Western Sahara. Position of this area is strategic with the location along Strait of Gibraltar; and it is the only African country to have both Atlantic and Mediterranean coastlines.

Terrain is placed between the mountainous northern coast (Rif Mountains) and interior (Atlas Mountains) bordered by large plateaus with intermontane valleys, and fertile coastal plains. Mean elevation of this area is 909 m, the lowest point, Sebkha Tah is -59 m and highest point is Jebel Toubkal, 4,165 m. Agricultural land covers 67.5%, arable land, 17.5%, with permanent crops of 2.9% and permanent pasture of 47.1%. Forests cover 11.5% (other: 21%).

Northern mountains are geologically unstable and subject to earthquakes; periodic droughts; windstorms; flash floods; landslides. This region characterise soil erosion resulting from farming of marginal areas, overgrazing, destruction of vegetation; but also some water and soil pollution due to dumping of industrial wastes into the ocean and inland water sources, and onto the land.

Oued El Abid watershed The study was conducted upstream the Bin El Ouidane dam and is with a total surface of 3119 km^2, in the high Atlas chain in the region of Beni Mellal Khenifra, Morroco. The drainage area of Oued El Abid is presented on the Figure 2. The Bin El Ouidane Dam accumulates the water comes from two rivers and the water is used for irrigation and hydraulic power production.

Figure 1. Details from the Studied area (Photo: Velibor Spalevic, 11/2017)

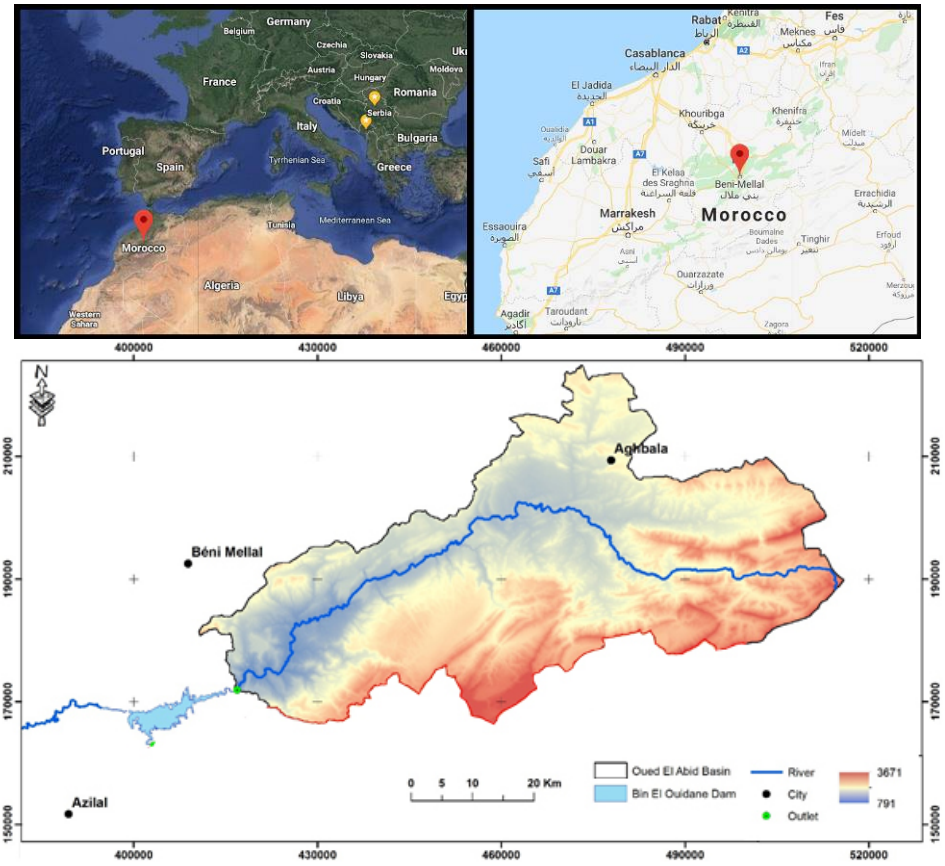

Figure 2. Study area of the Oued El Abid Watershed, Morocco

Soil Erosion IntErO model

Soil erosion models have been developed worldwide. Some of these are chemical runoff and erosion from agricultural management systems – CREAMS (Knisel, 1980), areal nonpoint source watershed environment response simulation – ANSWERS (Beasley *et al* 1980), agricultural nonpoint source pollution model – AGNPS (Young *et al*, 1987), water erosion prediction project – WEPP (Nearing *et al*, 1989), Universal Soil Loss Equation - USLE, Modified Universal Soil Loss Equation - MUSLE and Revised Universal Soil Loss Equation – RUSLE (Wischmeier and Smith, 1978: Williams, 1975; Renard, 1991) and MUSLT (Nicks *et al*, 1994; Sadeghi *et al*, 2013).

Sediment delivery ratio (SDR), transport limiting sediment delivery (TLSD), unit stream power erosion and deposition model (USPED), and sediment distributed delivery (SEDD) have been used to model the sediment removal, transportation, and delivery. Employing soil erosion models to estimate the soil erosion severity at a place is gaining popularity nowadays as field-based erosion studies are tedious, costly, and take a considerable amount of time.

Instead, soil erosion models can assess the soil loss within a short time, provided data are available handy (Chalise *et al*, 2019).

The Erosion Potential Method - EPM (Gavrilovic, 1972) describe the annual sediment yield and transport, the forms and intensity of erosion, from the physical-geographic and hydrological characteristics of the river basin. It is most often used in Europe, North Africa and the Middle East. In recent times the EPM is repeatedly applied in several watersheds in the Mediterranean area, especially in the South East Europe - Balkan region (Blinkov and Kostadinov, 2010; Kostadinov *et al*., 2006, 2014; Milevski *et al*., 2008; Spalevic *et al*. 2013, Spalevic *et al*. 2014; Spalevic *et al*. 2015; Spalevic *et al*. 2016; Stefanovic, 2004; Tazioli, 2009; Tavares *et al*., 2019), and also in arid and semi-arid areas of the south-western USA (Gavrilovic Z., 1988), Saudi Arabia (Aburas Al-Ghamdi, 2010). The method was based on the factors affecting erosion in a watershed; its parameters were dependent on the temperature, the mean annual rainfall, the soil use, the geological properties and some other factors in the watershed scale (Gholami *et al*, 2016; Khaledi Darvishan *et al*., 2016, 2017). The synergic influences of climate and human abandonment could have triggered erosion processes.

The Intensity of Erosion and Outflow - IntErO program package (Spalevic, 2011) with the EPM embedded in the algorithm of this model, was developed to predict the runoff peak discharge and the intensity of soil erosion in a watershed scale. The use of this model has been reported in various countries all around the world including Bosnia & Herzegovina, Bulgaria, Croatia, Czech Republic, Italy, Iran, Montenegro, Macedonia, Serbia, Slovenia, Morocco, Brazil and Nepal. The efficiency of IntErO model to predict peak outflow, soil erosion and sediment yield was also assessed in some cases and the results showed that this model can be use in variety of watershed sizes with various land uses (Spalevic, 2011).

The present study was conducted to use the IntErO for modelling of soil erosion processes and runoff for sustainable watershed management to predict peak outflow, soil erosion and sediment yield for the Oued El Abid Watershed in Morocco.

The IntErO model estimates the total erosion yield on the catchment level and quantifies the hydro-sedimentological parameters using the equations 1-5:

$$W_{yr} = T_i \cdot H_{yr} \cdot \pi \cdot \sqrt[3]{Z^3} \cdot F \qquad \text{Equation 1}$$

$$T_i = \sqrt[3]{\frac{t_0}{10}} + 0.1 \qquad \text{Equation 2}$$

$$Z = Y \cdot X_a \cdot (\varphi + \sqrt[3]{I_{sr}}) \qquad \text{Equation 3}$$

$$G_{yr} = W_{yr} \cdot R_u \qquad \text{Equation 4}$$

$$R_u = \frac{(O - D)^{0.5}}{0.25 - (Lv - 10)} \qquad \text{Equation 5}$$

where: W_{yr} = Annual erosion (m^3 km^{-2} yr^{-1}); T_i = Coefficient of temperature (dimensionless); H_{yr} = Mean annual rainfall (mm yr^{-1}); Z = Coefficient of erosion (dimensionless); F = Basin area (km^2); t_0 = Mean air temperature (°C yr^{-1}); Y = Soil resistance to erosion (dimensionless); X_a = Coefficient of soil use and management (dimensionless); φ = Coefficient of visible erosion features (dimensionless); I_{sr} = Mean slope (%); G_{yr} = Sediment production (m^3 km^{-2} yr^{-1}); R_u = Coefficient of retention (dimensionless); O = Basin length (km); D = Difference in basin elevation (m) and; Lv = Length of main stream (km).

The analysis of the Geological and Physical and chemical soil properties were done at laboratory based on soil survey and geological maps (scale 1:500.000). To obtain accurate analytical results, each of the 143 soil samples was reported based on the mean of five subsamples taken within a 1000 m^2 area.

The meteorological data obtained from the measurements of two meteorological stations in the last 50 years (1970-2019), the physical characteristics were extracted from a Digital Elevation Model using Gis tools. The land cover data has obtained after a radiometric- atmospheric treatments and classification of satellite images.

After the field visit, but also using all the available data from the Soil and Geological surveys, receiving the relevant data from the local Hydro meteorological stations, and analysing the satellite images we completed the table with the inputs needed for calculations with the IntErO model. The input data needed for modelling of erosion processes are presented in the Table 1.

Table 1. Input data needed for modelling of soil erosion processes and runoff

Inputs	Symbol	Value	Unit
River basin area	F	3119.14	km²
The length of the watershed	O	484.16	km
Natural length of the main watercourse	Lv	175.85	km
The shortest distance between the fountainhead and mouth	Lm	93.79	km
The length of the main watercourse with tributaries	ΣL	58984.36	km
River basin length measured by a series of parallel lines	Lb	130	km
The area of the bigger river basin part	Fv	2272.15	km²
The area of the smaller river basin part	Fm	846.99	km²
Altitude of the first contour line	h0	900	m
The lowest river basin elevation	Hmin	791	m
The highest river basin elevation	Hmax	3699	m
A part of the basin with very permeable product from rocks	fp	0.2	
A part of the river basin area consisted of medium permeable rocks	fpp	0.32	
A part of the basin consisted of poor water permeability rocks	fo	0.48	
A part of the river basin under forests	fš	0.49	
A part of the basin under grass, pastures and orchards	ft	0.32	
A part under bare land, plough-land and ground without grass	fg	0.19	
The volume of the torrent rain	hb	112.8	mm
Average annual air temperature	t0	16.8	°C
Average annual precipitation	Hyr	347.1	mm
Types of soil products and related types	Y	1.4	
coefficient of the river basin planning	Xa	0.91	
Numeral equivalents of visible erosion process	φ	0.5	

RESULTS AND DISCUSSIONS

After preparing the inputs required for IntErO model, the model was ran and all the model outputs were obtained and shown in Table 2.

Table 2. Modeling results for the Oued El Abid Watershed, Morocco

Results	Symbol	Value	Unit
Coefficient of the river basin form	A	0.58	-
Coefficient of the watershed development	m	0.8	-
Average river basin width	B	24.62	km
(A)symmetry of the river basin	a	0.17	-
Density of the river network of the basin	G	18.82	-
Coefficient of the river basin tortuousness	K	1.55	-
Average river basin altitude	Hsr	1975.12	m
Average elevation difference of the river basin	D	1325.12	m
Average river basin decline	Isr	2.17	%
The height of the local erosion base of the river basin	Hleb	2576	m
Coefficient of the erosion energy of the basin's relief	Er	111.22	-
Coefficient of the region's permeability	S1	0.66	-
Coefficient of the vegetation cover	S2	0.74	-
water retention in inflow	W	8763.61	m
Energetic potential of water flow during torrent rains	2gDF^½	2845.94	m km s
Temperature coefficient of the region	T	1.33	
Coefficient of the river basin erosion	Z	0.796	
Production of erosion material in the river basin	Wyr	3960115	m^3 yr-1
Coefficient of the deposit retention	Ru	0.3	
Real soil losses	Gyr	1188657	m^3 yr-1
Real soil losses per km^2	Gyr (km^2)	402.39	$m^3km^2yr^{-1}$

With the modelling of the erosion processes at the Oued El Abid Watershed, in Morocco we concluded that there is a possibility for large flood waves to appear in the studied river basin. The value of G coefficient of 18.82 indicates that there is very high density of the hydrographic network in the studied river basin. Calculations showed that in the river basin prevail mild slopes. The strength of the erosion process is high, and according to the erosion type, it is surface erosion.

In 2015 the team led by Sabri el Mouatassime in the watershed of Oued El Abid, using the USLE model came out with a result of sediment yield which shows that an annual amount of soil loss of 5.2 million m^3 reaches the dam of Bin El Ouidane downstream the watershed. Our research showed that annual real soil losses for the Oued El Abid Watershed in Morocco are 1.2 million m^3. The other, unpublished result from the same team of researchers, calculated annual real soil losses for both river basins: Oued El Abid and the Tillouguite basin, that all together counts on 4.1 million m^3. Analysing the bathymetric measurement for the period 1953-2008, sediment yield rate resulted to 5 million m^3 $year^{-1}$. The tributaries are inflowing from the northeast and southeast and that most but not all sediments are depositing from those two rivers, the result of modelling by using the IntErO is quite satisfactory.

CONCLUSION

Different geographical factors and hydrological processes govern sediment dynamics in the studied river basin, which are highly variable in spatial and temporal scales. The IntErO is an appropriate technique for modelling of soil erosion processes to estimate the soil losses by water erosion in the conditions similar to the study area of Oued El Abid Watershed, Morocco. For the conditions of the studied area, the USLE model is providing slightly higher results (4% higher than the bathymetric measurements). The IntErO modelling provided about 10% less than measurements for this specific case. Both models proof to be useful for modelling of soil erosion processes and runoff for the needs of sustainable watershed management.

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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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TITLE - Harmonization and Innovation In PhD Study Program for Plant Health in Sustainable Agriculture
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TOTAL GRANT - 967.614,00 EUR
PROJECT LEADER - Prof. Renata Bažok, Ph.D.
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PROJECT PARTNERS

Country	University
Croatia	J.J. Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek
Italia	University of Bari Aldo Moro
Bulgaria	Agriculture University Plovdiv
Greece	Agriculture University of Athens
Albania	Agriculture University of Tirana "Fan S. Noli" University
Bosnia and Herzegovina	University of Sarajevo University of Mostar
Serbia	University of Belgrade Faculty of Agriculture University of Novi Sad Faculty of Agriculture
Montenegro	University of Montenegro

In today's sustainable agricultural production, comprehensive care for plant health is essential. Apart from modern diagnostic methods, it includes the application of sophisticated plant protection methods based on the latest scientific. knowledge . Without the highly educated experts who are well acquainted with the issue of all aspects of plant health in sustainable agricultural production, it is impossible to achieve required goals. The HarlSA project (Harmonization and Innovation in PhD Study Programs for Plant Health in Sustainable Agriculture) was approved under the Erasmus+ program in the field of Strengthening Capacities in Higher Education on the European Movement politics and culture (EACEA) . This project has the ambition to harmonize and modernize doctoral education in the Western Balkans countries and to create a network of researchers capable of responding to current challenges in plant health. The project aims to provide support for modernization and internationalization of higher education in the field of plant health and to contribute to co-operation between the European Union and the countries of Western Balkans in implementing the European Union's policy on plant health. It also seeks to ensure the acquisition of scientific knowledge and the transfer of skills and competences to achieve sustainable use of pesticides and the application of the major principles of integrated plant protection in agricultural production. The project was submitted by the University of Zagreb, Faculty of Agriculture, in cooperation with partners from the EU countries (Croatia, Italy, Bulgaria and Greece) and countries of the so-called Western Balkans (WBC) (Bosnia and Herzegovina, Serbia, Montenegro and

Albania). The project will achieve specific goals such as: harmonize, improve and modernize doctoral programs in the area of plant health among partner universities, to increase the ability of the PhD to respond to global health problems; to develop a common framework and a draft curriculum for an international joint doctoral study in plant health; to foster regional integration in the field of research and education of plant health within the Western Balkan countries and between the countries of the Western Balkans and the EU countries.

PROJECT BACKGROUND

Consumers in the EU and beyond are increasingly concerned about the impact of pesticides on the environment and human health. These concerns are reflected in EU's 6th Environment Action Program, and Thematic Strategy on the Sustainable Use of Pesticides, whose key objectives include substitution of harmful substances by safer alternatives, encouragement of low input/low-pesticide crop cultivation and re-inforce integrated pest management applications. Increasing concerns of consumers about chemical pesticides have resulted in " EU pesticides package" (Regulations 1107/ 2009/ EEC and 1185/ 2009,Directives 2009/128/EC and 2009/127) which regarded stress upon the environment, residuals in the food chain, human health issues and impact on evolutionary pressure. Moreover, the 7th Environmental Action Program includes an "enabling framework" with four priority objectives to help EU deliver on these goals: better implementation of legislation, better information by improving the knowledge base, more and wiser investment for the environment, and full integration of environmental requirements into other policies.

Consortium members have special interest in taking part in the project as it is a unique opportunity to bring together a wide expertise in the field of plant health doctoral education and research. The project provides a great potential to generate synergies to help overcome the current challenges and build human capacities in plant health in the Western Balkan countries (WBC). All HarlSA partner countries are candidates for entering EU. The common challenges for them is to implement EU policy in plant health, and develop and establish all necessary conditions for the application of innovative plant health measures that fits with the principles of sustainable agriculture. The implementation of "EU pesticides package" requires highly educated professionals with deep understanding on this matter. They must be capable to transfer knowledge, skills and competences in the biological, biotechnological and agronomic control means and in applying strategies at pesticide application which can reduce environmental and human risk. This project has the ambition of harmonizing and modernizing doctoral education at Pls and crating a network of researchers that is capable to respond to the above mentioned challenges. HEIs will benefit by improving human potential and its success for conduction of competitive research in the field of plant health. At the same time this will ensure preconditions in partner countries to improve productivity and resilience of sustainable agriculture in the context of evolving climate. Finally, it will impact on a range of agricultural production (in chains context) and risk management practices, and will result with safeguarding of biodiversity (particularly near NATURA 2000 natural areas). Regional and partner country national priority is to improve quality of education and teaching by developing learning and teaching tools, methodologies and pedagogical approaches including learning outcomes and ICT- based practices. HarlSA project proposes the modernization of curriculum by developing new and innovative courses and methodologies. Therefore, the aims of the HarlSA project are entirely compatible with regional and partner national priorities in the field of education. The same time HarlSA

covers the field of agriculture which is also defined as national priority by all partner countries as well as for the region of WBCs. The strategic research agenda and project results of the “C-IPM - Coordinated Integrated Pest management in Europe" ERA- NET was activated to favor exchange and identification of IPM research and development priorities in order to face European challenges of responding to the mandatory implementation of the principles of integrated pest management (IPM). Proposed project is in line with numerous EU founded projects as are PURE-IPM, ENDURE, BIOFECTOR, BIOCOMES, BIOGUARD, Best Pass, XF-ACTORS, Al-Tree, EUFRUIT, EMPHASIS, BIOCOMES, etc. All these projects are (or were) research projects related to plant health issues as emerging pest and diseases, sustainable use of pesticides, developing new products and methodologies for pest control .

Until now, all completed and ongoing curricula reform EU projects on plant health topic were focused on undergraduate and graduate programs. The aims were to develop modern teaching contents at the agricultural faculties, to improve the quality and modularization of their curricula to improve the teaching skills of staff or to develop strategic planning management. Some of them aimed to develop and improve Master study programs in plant health (International Joint Master Degree in Plant Medicine and Plant Health project) but no projects and activities are carried out to harmonize the doctoral education neither in agriculture nor in plant health