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## Fungal pathogens of vetch genotypes in Serbia

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### SUMMARY

(*Vicia sativa* L.)  
(*Fabaceae*),

Vetch (*Vicia sativa* L.) is annual plant from the legume family (*Fabaceae*) and originates from the temperate zone of Europe and Asia. It has a special place in the provision of animal fodder in the zone of moderate climate. It belongs to high-quality protein fodder plants.

Diseases caused by phytopathogenic fungi every year have a significant impact on yields and quality of the final product to a greater or lesser degree. They can also affect trade plant material and cause the expansion of the disease in new areas where legumes are grown.

There has not been a systematic research of vetch mycoflora in Serbia. This research aims to present the results of preliminary research of mycopopulation of 20 different genotypes of vetch. Total of 800 plant parts and 400 seeds have been examined and 8 genera of fungi were isolated: *Fusarium*, *Phytophthora*, *Rhizoctonia*, *Phoma*, *Verticillium*, *Alternaria*, *Sclerotinia*, and *Penicillium*. On the plants from which the fungi were isolated, there were macroscopically clearly visible symptoms of infection.

**Key words:** pathogens, vetch

800 400  
8 : *Fusarium*,  
*Phytophthora*, *Rhizoctonia*, *Phoma*,  
*Verticillium*, *Alternaria*, *Sclerotinia*  
*Penicillium*.

## INTRODUCTION

(*Vicia sativa*)  
*Vicia sativa*

Leguminosae (  
Fabaceae)

(Miškovi , 1986).

Aragona, 1997).

*Ascochyta fabae* Speg. (Teleomorph *Didymella fabae* G. J. Jellis & Punith.) (Tivoli et al., 2006). *Uromyces viciae-fabae* (Pers.) J. Schröt.

*Pisum*, *Lathyrus* and *Lens* (Sillero and Rubiales, 2014).

*Fusarium*, *F. verticillioides* *F. proliferatum*

(Mili evi et al., 2013).

*Erysiphe pisi*, *Botrytis cinerea*, *Rhizoctonia solani*, *Cercospora medicaginis*, *Pseudopeziza medicaginis*, *Sclerotinia trifoliorum*, *Stemphylium botryosum*, *Verticillium albo-atrum*, *Aphanomyces euteiches*,

Common vetch (*Vicia sativa*) is widely used as green manure, pasture, silage, hay and for grain for livestock feed. *Vicia sativa* is a widely grown forage crop, although outside cultivation it is often considered to be a weed.

It belongs in the legume family, Leguminosae (also known as Fabaceae), and, like many other legumes, it has the ability to fix nitrogen from the air due to a symbiotic relationship with bacteria housed in root nodules.

As a result, common vetch is high in protein (Miškovi , 1986.).

The diseases of legumes caused by phytopathogenic fungi occur, in a stronger or weaker intensity, regularly every year in all areas of the world. They have a significant impact on the reduction of potential yield of these cultures, but also the quality of the final product, trade of plant material and expansion of legumes into new areas (Porta-Puglia and Aragona, 1997).

Vetch and bean anthracnose is caused by the fungus *Ascochyta fabae* Speg. (teleomorph *Didymella fabae* G.J. Jellis & Punith.) (Tivoli et al., 2006). *Uromyces viciae-fabae* (Pers.) J. Schröt. is the cause of rust in vetch and bean and has a wide range of hosts, which it can infect, and those are plant species from the genera *Pisum*, *Lathyrus* and *Lens* (Sillero and Rubiales, 2014). Two species of the genus *Fusarium*, *F. verticillioides* and *F. proliferatum* were determined in the vetch seeds (Mili evi et al., 2013).

Similarly, inducers of diseases such as *Erysiphe pisi*, *Botrytis cinerea*, *Rhizoctonia solani*, *Cercospora medicaginis*, *Pseudopeziza medicaginis*, *Sclerotinia trifoliorum*, *Stemphylium botryosum*, *Verticillium albo-atrum*, *Aphanomyces euteiches* as well as

*Phytium*, *Leptosphaerulina*,  
*Phoma*, *Phytophthora* *Alternaria*

(Morgan and Johnson, 1965; Stovold and Walker, 1980; Hughes and Grau, 2007; Villegas-Fernández and Rubiales, 2011; Salam et al., 2011; Sillero et al., 2014).

(*Vicia sativa* L.),  
*sativa* L.)

(*Vicia villosa* Roth.)

2015 .,

0,5-1 cm.

96%

10

1%

1

(NaOCl)

(PDA)

( )

species of genus *Phytium*, *Leptosphaerulina*, *Phoma*, *Phytophthora* and *Alternaria* are significant disease agents in vetch, spread in all the areas of its production (Morgan and Johnson, 1965; Stovold and Walker, 1980; Hughes and Grau, 2007; Villegas-Fernández and Rubiales, 2011; Salam et. al., 2011; Sillero et al., 2014).

Given the importance of vetch as a fodder crop in Serbia, the aim of this paper is the determination of phytopathogenic fungi that cause diseases in vetch for a clearer perception of problems (the extinction of plants, reducing yields, deterioration of the quality of feed and other) arising as a result of the presence those fungi.

## MATERIAL AND METHODS

For the study of mycopopulations, samples were collected from the sixteen experimental plant genotypes of vetch (*Vicia sativa* L.) originating from Australia, two wild-genotype (*Vicia sativa* L.) originating from Serbia, from the Rasina region, and two wild genotypes (*Vicia villosa* Roth.) originating from Australia. The samples were collected between May and June 2015 at the location of the Institute for forage crops in Globoder. Parts of plants as well as seeds from immature pods are carefully washed under running water. After washing, the parts of stem and roots are cut to piece of 0.5-1 cm in size. Prepared samples of roots, stems, and seeds were disinfected with 96% ethanol for 10 seconds and with 1% sodium hypochlorite (NaOCl) for 1 min. and then washed three times in sterile distilled water.

They were then dried on sterile filter paper and placed on potato dextrose agar (PDA) with streptomycin. Five pieces of the plant parts (roots and tree) and five seeds were placed in each Petri dish in four replications. They were kept in a thermostat at 25 °C in 12 h light / 12 h

12 / 12 25 ° ,  
 14 PDA  
 PDA.  
 Olympus CX31.  
 %  
 Vrande i i sar., (2011):

night regime. The observations were performed every 3 days, and the majority of mycelium samples were developed up to 14 days. Developed mycelia were screened to a new PDA substrate and, after an initial grow, the peak part of the mycelium was reseeded on PDA again.

Microscopic examination was performed using microscopes Olympus CX31. Morphological identification of fungi to the genus was carried out using a standard key. Calculated by the frequency of isolation in % according to the formula Vrande i i sar., (2011):

$$\text{The isolation percentage} = \frac{\text{Number of segments containing the fungal species}}{\text{Total number of segments used in the isolation}} \times 100$$

## RESULTS

800  
 400  
*Fusarium, Phytophthora Alternaria.*  
*Sclerotinia.*  
*Phoma* ( 1).  
*Fusarium, Phytophthora, Phoma Rhizoctonia.*  
*Verticillium.*  
*Fusarium, Sclerotinia*  
*Penicillium*  
 ( 1).

In this study of mycopopulations of vetch genotypes, total of 800 parts of the plant and 400 seeds were observed. On all plants from which fungi were isolated, there were clear symptoms on stems in the form of spots and necrotic lesions. From these plants, fungi from genus *Fusarium*, *Phytophthora* and *Alternaria* were isolated. Also, in large number of plants, there were necroses with white airy mycelium in the lower third of stems and fungi from the genus *Sclerotinia* were isolated from those plants. In some plants, black fruiting bodies (pycnidia) were observed on stems, which have been found to belong to the genus *Phoma* (Table 1).

There were symptoms as a light to dark brown necroses on the root system of the plants and from these plants fungi of the genera *Fusarium*, *Phytophthora*, *Phoma* and *Rhizoctonia* were isolated. Also in a large number of plants decolorisation of the conduction tissues on root system was observed and from these plants fungus of the genus *Verticillium* was isolated.

Only at eight genotypes of vetch, fungi of the genus *Fusarium*, *Sclerotinia* and *Penicillium* have been isolated from seeds. (Table 1).

## 1.

Table 1. Frequency of fungal isolation on vetch

/Species	/Number of samples			/Fungi species-stem	(%) /Isolation Frequency	/Fungi species-root	(%) /Isolation Frequency	Fungi species-seed	(%) /Isolation Frequency
	/Plant part-stem	Plant part-root	Plant part-seed						
<i>Vicia sativa</i> (A)	20	20	20	<i>Phytophthora</i> sp. <i>Fusarium</i> sp.	15 10	<i>Fusarium</i> sp. <i>Verticillium</i> sp.	10 15	-	-
<i>Vicia sativa</i> (A)	20	20	20	<i>Phytophthora</i> sp. <i>Fusarium</i> sp.	15 10	<i>Phytophthora</i> sp. <i>Fusarium</i> sp. <i>Verticillium</i> sp.	20 15 15	<i>Fusarium</i> sp. <i>Sclerotinia</i> sp.	5 10
<i>Vicia sativa</i> (A)	20	20	20	<i>Phytophthora</i> sp. <i>Phoma</i> sp.	15 15	<i>Phytophthora</i> sp. <i>Phoma</i> sp. <i>Alternaria</i> sp.	40 5 5	<i>Sclerotinia</i> sp.	5
<i>Vicia sativa</i> (A)	20	20	20	<i>Phytophthora</i> sp.	25	<i>Alternaria</i> sp. <i>Rhizoctonia</i> sp.	15 35	<i>Fusarium</i> sp. <i>Sclerotinia</i> sp.	35 15
<i>Vicia sativa</i> (A)	20	20	20	<i>Sclerotinia</i> sp. <i>Phytophthora</i> sp.	15 20	<i>Phytophthora</i> sp. <i>Fusarium</i> sp. <i>Alternaria</i> sp.	15 10 5	<i>Penicillium</i> sp.	10
<i>Vicia sativa</i> (A)	20	20	20	<i>Rhizoctonia</i> sp.	25	<i>Phytophthora</i> sp. <i>Phoma</i> sp. <i>Fusarium</i> sp.	20 5 25	-	-
<i>Vicia sativa</i> (A)	20	20	20	<i>Phoma</i> sp. <i>Fusarium</i> sp. <i>Alternaria</i> sp.	10 15 5	<i>Phytophthora</i> sp.	25	-	-
<i>Vicia sativa</i> (A)	20	20	20	<i>Sclerotinia</i> sp. <i>Phytophthora</i> sp. <i>Alternaria</i> sp.	10 15 5	<i>Phytophthora</i> sp. <i>Phoma</i> sp.	10 5	-	-
<i>Vicia sativa</i> (A)	20	20	20	<i>Rhizoctonia</i> sp.	25	<i>Rhizoctonia</i> sp. <i>Rhizoctonia</i> sp. <i>Fusarium</i> sp. <i>Alternaria</i> sp.	35 15 20 15	-	-
<i>Vicia sativa</i> (A)	20	20	20	<i>Phytophthora</i> sp. <i>Rhizoctonia</i> sp.	15 10	<i>Phytophthora</i> sp. <i>Phoma</i> sp. <i>Fusarium</i> sp.	10 25 15	<i>Fusarium</i> sp.	10
<i>Vicia sativa</i> (A)	20	20	20	<i>Fusarium</i> sp. <i>Alternaria</i> sp.	15 10	<i>Verticillium</i> sp. <i>Phytophthora</i> sp. <i>Rhizoctonia</i> sp.	10 20 30	<i>Sclerotinia</i> sp.	15
<i>Vicia sativa</i> (A)	20	20	20	<i>Phytophthora</i> sp. <i>Rhizoctonia</i> sp.	5 20	<i>Phytophthora</i> sp. <i>Rhizoctonia</i> sp. <i>Phoma</i> sp.	15 10 25	-	-
<i>Vicia sativa</i> (A)	20	20	20	<i>Phytophthora</i> sp. <i>Phoma</i> sp.	20 5	<i>Rhizoctonia</i> sp. <i>Phytophthora</i> sp. <i>Alternaria</i> sp.	25 5 10	<i>Fusarium</i> sp.	15
<i>Vicia sativa</i> (A)	20	20	20	<i>Phytophthora</i> sp.	20	<i>Phytophthora</i> sp. <i>Phoma</i> sp.	15 5	-	-

<i>Vicia sativa</i> (A)	20	20	20	<i>Fusarium</i> sp.	15	<i>Phytophthora</i> sp.	10	-	-
				<i>Alternaria</i> sp.	10	<i>Fusarium</i> sp.	10		
						<i>Phoma</i> sp.	5		
<i>Vicia sativa</i> (A)	20	20	20	<i>Fusarium</i> sp.	15	<i>Fusarium</i> sp.	10	-	-
						<i>Rhizoctonia</i> sp.	15		
<i>Vicia sativa</i> (SR)	20	20	20	<i>Verticillium</i> sp.	25	<i>Phytophthora</i> sp.	10	-	-
						<i>Verticillium</i> sp.	30		
<i>Vicia sativa</i> (SR)	20	20	20	<i>Verticillium</i> sp.	15	<i>Fusarium</i> sp.	5	-	-
				<i>Fusarium</i> sp.	10	<i>Verticillium</i> sp.	20		
						<i>Phytophthora</i> sp.	25		
<i>Vicia villosa</i> (A)	20	20	20	<i>Rhizoctonia</i> sp.	15	<i>Fusarium</i> sp.	15	-	-
				<i>Fusarium</i> sp.	10	<i>Phytophthora</i> sp.	30		
<i>Vicia villosa</i> (A)	20	20	20	<i>Fusarium</i> sp.	15	<i>Rhizoctonia</i> sp.	20	<i>Sclerotinia</i> sp.	10

The results indicate that vetch is vulnerable to the attack of a large number of phytopathogenic fungi that can have a significant impact on reducing its yield and quality. Also from the obtained results we can conclude that seed of vetch becomes infected in warehouses and not on the field.

## DISCUSSION

In all the plants from which isolations were conducted there were clearly visible symptoms of the disease present. In these studies, there was difference in frequency of isolation of some genera of phytopathogenic fungi in both Australian and Serbian populations. It was observed that in the genotypes that originated in Serbia fungi of genus *Verticillium*, *Fusarium* and *Phytophthora* were frequently isolated. Also genera *Alternaria*, *Rhizoctonia*, *Phoma* and *Sclerotinia* were frequently isolated from Australian genotypes.

Genera *Fusarium*, *Phytophthora*, *Rhizoctonia*, *Phoma*, *Verticillium*, *Alternaria*, *Sclerotinia*, *Botrytis* and *Ascochyta* were dominant in annual and perennial legumes worldwide (Tivoli et al., 2006; Villegas-Fernández and Rubiales, 2011; Salam et al., 2011; Sillero et al., 2014, Vasi et al., 2015). Mili evi et al. (2013) determined two *Fusarium* species *F. verticillioides* and *F. proliferatum* on vetch seed in Croatia. While Salam et al. (2011) cited genera *Ascochyta* and *Botrytis*, especially species *Ascochyta fabae* Speg. (teleomorph: *Didymella fabae*) and *Botrytis fabae*, *Botrytis cinerea* as significant pathogens on faba bean in Australia.

It is important to mention that the parasites of the genus *Botrytis* overwinter in the form of sclerotia or mycelium into plant residues in the soil (Davidson et al., 2004). So, for these reasons, it is recommended to utilize crop rotation of four years, when it comes to the sowing of vetch after the faba bean and pea (Salam et al., 2011). Salam et al. (2011) also cited *Phoma medicaginis* var. *pinodella* and *Ascochyta pisi* as significant pathogens in pea.

*Phytophthora medicaginis* was recorded on chickpea in Australia and it was also found that this parasite can infect other

*Verticillium*, *Fusarium*  
*Phytophthora*

*Alternaria*, *Rhizoctonia*, *Phoma*  
*Sclerotinia*

*Fusarium*, *Phytophthora*,  
*Rhizoctonia*, *Phoma*, *Verticillium*,  
*Alternaria*, *Sclerotinia*, *Botrytis*  
*Ascochyta*

(Tivoli et al., 2006; Villegas-Fernández and Rubiales, 2011; Salam et al. Sillero et al., 2014, Vasi et al., 2015). Mili evi et al. (2013 .)

*Fusarium* *F. verticillioides* *F. proliferatum*

Salam et al. (2011)  
*Ascochyta* *Botrytis*,  
*Ascochyta fabae* Speg.  
(Teleomorph: *Didymella fabae*) *Botrytis fabae*, *Botrytis cinerea*

*Botrytis*

(Davidson et al., 2004).

(Salam et al., 2011). Salam et al. (2011)  
*Phoma medicaginis* var.  
*pinodella* *Ascochyta pisi*

*Phytophthora medicaginis*

(Salam et al., 2011). *Sclerotinia trifoliorums* Eriks.

(Lithourgidis, 2005). *Rhizoctonia solani* Kühn

(Assunção, 2011). 304

*R. solani*

(Rashid and Bernier, 1993). Ligoxigakis et al. (2002) *V. dahliae*

20

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types of legumes (Salam et al., 2011). *Sclerotinia trifoliorums* Eriks. often causes serious problems in many legumes in Greece (Lithourgidis, 2005). *Rhizoctonia solani* Kühn is soil parasite that can cause serious problems in many legumes, especially on faba bean (Assunção, 2011).

- In Canada, 304 faba bean genotypes were tested on the resistance to *R. solani* and only five of them were identified with high resistance (Rashid and Bernier, 1993). Ligoxigakis et al. (2002) determined the *V. dahliae* as a parasite on vetch and other legumes in Greece.

- This paper presents the preliminary results of mycopopulations of 20 experimental vetch genotypes. Vetch is very important forage crop and its importance as animal feed is growing within our country. This work is the beginning of a more comprehensive study of phytopatogenic fungi on vetch. So far, there were no significant researches in this direction in Serbia, so the future researches related to vetch will go in the direction of selection of genotypes with increased tolerance to diseases.

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## Variability of some traits of vetch genotypes originating from different regions

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### SUMMARY

10 :  
(*Vicia sativa* L.)  
(*Vicia villosa* Rooth.).  
-  
(2013, 2014 2015 .)  
, 1000 .  
( )

The objective of this research was ten genotypes of spring vetch: five genotypes of common vetch (*Vicia sativa* L.) and five genotypes of hairy vetch (*Vicia villosa* Rooth.). New genetic material in the selection process would contribute to the obtaining of new varieties with better qualitative and quantitative characteristics. The tested genotypes were taken from the collection of Institute for forage crops, and they have different geographic origin. The small plot trials, was conducted in three years (2013, 2014, and 2015) at the experimental field of the Institute for forage crops Krusevac.

- The following quantitative traits were investigated: number of pods per plant, seed number per pod, 1000 seed weight.
- Total protein content, insoluble proteins and soluble proteins were determined from the forage (whole plant) in the phase of pod filling. All studied chemical

(P 0.01).  
 ( ,  
 , 1000 )  
 ,  
 : , *Vicia*  
*sativa* L., *Vicia vilosa* Roth.,

- parameters revealed significant difference  
 - between the genotypes, and years  
 - (P 0.01). Quantitative parameters  
 (number of pods per plant, seed number  
 per pod and 1000 seed weight) varied  
 between genotypes, but there were no  
 statistically significant differences  
 between years.

**Key words:** quality, *Vicia sativa* L.,  
*Vicia vilosa* Roth., total protein content,  
 insoluble protein

## INTRODUCTION

(Tenopala et al.,  
 2012). *Vicia*  
 (Maxted,  
 1995).  
 , - ,  
 . -  
 , " -  
 "  
 (*V. sativa* L.),  
 (*V. vilosa* Roth.)  
 (*V. pannonica*).

Vetch is widely used for forage and  
 grain production for animal feeding. As a  
 nitrogen fixing legume, it can be used as  
 plant fertilizer. Due to the high quality of  
 seeds, and only seeds can be used as  
 animal feed. Vetch is cultivated for forage,  
 grain production and as a green fertilizer  
 plant (Tenopala et al., 2012). Within many  
 species of the genus *Vicia* there are many  
 subspecies and varieties (Maxted, 1995).  
 In the literature there are numerous  
 references to studies on a common vetch,  
 more often than on hairy vetch. In the  
 agronomic practices in Serbia is  
 commonly used common vetch, while  
 hairy vetch is used to a lesser extent.  
 Also, on the "List of recognized varieties  
 of agricultural plants" of the Republic of  
 Serbia, there are five varieties of common  
 vetches (*V. sativa* L.), two varieties of  
 hairy vetch (*V. vilosa* Roth.) and one  
 variety of Pannonian vetch (*V.*  
*pannonica*).

(Buykkartal et al, 2013).

Whole plant is high quality feed for  
 use in forage production. The seeds are  
 with high protein content and may be  
 used as animal feed, too (Buykkartal et al,  
 2013).

(Hintz et al., 1992; Caballero et  
 al., 1996).

Seeds of annual legumes, used as  
 forage, contribute to an essential  
 proportion of the biomass (Hintz et al.,  
 1992; Caballero et al., 1996). If we  
 consider that most of the protein, as the  
 one of the main quality indicators,

contained in the seed, that the some important properties (the number of seed per pod, number of pods per plant, and the mass of 1000 seeds) contributes to a higher proportion of protein in the biomass. It is very important to find the balance between increasing protein content in pod filling and decreasing the quality of vegetative plant parts (Alzueta et al., 2001).

Results of Samarah and Ereifej (2009) suggest that the majority of seed chemical composition was accumulated by the greenish-yellow pods stage which is optimal time for harvest of common vetch seed without reducing seed mass and nutrient loss.

Besides crude protein, an important indicator of quality is the ratio of soluble and insoluble protein.

Soluble protein is defined as true protein that is soluble in buffer at rumen pH (Licitra et al., 1996). Same author stated that most soluble nitrogen components were rapidly degraded in the rumen, and therefore reduced protein that could be passed to the lower tract.

Earlier procedures (Wohlt et al., 1973; Crooker et al., 1978; Waldo and Goering, 1979) were applied from a biological point of view, which reduces the impact of rumen environment. A better indicator of the feed quality is the content of insoluble protein, because that fraction mostly passed to the lower tract and can be used for physiological processes.

Quantification of crude protein fraction on vetch forage was not investigated to a greater extent (Alzueta et al., 2001).

Genetic variability within tested genotypes and knowledge about it offer a basis for improvement and developing new cultivars (Miki et al., 2013). The ten

genotypes from the collection of the Institute for forage crops Kruševac were selected for testing.

The aim of this study was to investigate protein content (especially insoluble proteins) of vetch genotypes from different geographical areas. Based on this, select the best genotypes for further selection in order to produce high-value animal feed.

## MATERIAL AND METHODS

The material for this study was ten spring vetch genotypes of species *V. sativa* and *V. vilosa*. The tested genotypes were taken from the landrace collection of Institute for forage crops, and they have different geographic origin (Table 1).

**Table 1. Investigated genotypes and their origin**

/Genotype	/Species	/Origin
VSA1	<i>V. sativa</i>	Australia/
VSA2	<i>V. sativa</i>	Australia/
VST2	<i>V. sativa</i>	Eastern Serbia/
VSA4	<i>V. sativa</i>	Australia/
VSAC3	<i>V. sativa</i>	Central Serbia/
VVA1	<i>V. vilosa</i>	Australia/
VVA2	<i>V. vilosa</i>	Australia/
VVT3	<i>V. vilosa</i>	Eastern Serbia/
VVAC1	<i>V. vilosa</i>	Central Serbia/
VVAC2	<i>V. vilosa</i>	Central Serbia/

The small plot trials, was conducted in three years (2013, 2014, and 2015) at the experimental field of the Institute for forage crops Kruševac. Experimental area was located at 137m elevation; 43° 34' 60" N and 21° 19' 36" E. Soil type of the experimental field was relegated alluvium. Standard agricultural technology was applied.

The following traits were investigated: number of pods per plant, seed number per pod, 1000 seed weight, total protein content, soluble proteins and

insoluble proteins. The experiment was planted in a randomized complete block design with three replications.

Counting of number of pods per plant, number of seeds per pod, and 1000 seed weight was done in full maturity stage.

Samples were obtained at five plants from each replication on which number of pods per plant and number of seeds per pod were counted. The 1000 seed weight was counted from the unique pattern of seed for each replication.

Determination of the protein content was done at the five plants from each replication in a physiological stage of pod filling. Cutting was done in the same day for all genotypes. Whole plants are chopped, dried and a unique pattern for the chemical analysis was made. Total crude protein content was determinate according to AOAC 2011.11. Soluble protein content (true soluble protein) was determined by Licitra et al. (1996). Values of soluble protein are given in percent of crude protein. Insoluble protein was calculated as the difference between the total crude protein and the value of soluble protein.

Average monthly temperatures ( $^{\circ}\text{C}$ ) and total precipitations (mm) data recorded during study period are presented in Table 2.

2011.11. (Licitra et al. (1996).)

( $^{\circ}\text{C}$ ) (mm),

2. ( $^{\circ}\text{C}$ ) (mm)

**Table 2. Average monthly temperatures ( $^{\circ}\text{C}$ ) and total precipitations (mm) in investigated years**

		March	April	May	June	July	Aug.
2013	T ( $^{\circ}\text{C}$ )	6.4	13.5	18.1	20.0	22.2	23.9
	/Precip. (mm)	78.4	54.5	96.8	44.0	6.1	14.4
2014	T ( $^{\circ}\text{C}$ )	9.4	11.4	14.9	19.4	21.3	21.0
	/Precip. (mm)	80.1	169.9	111.0	138.5	75.3	61.7
2015	T ( $^{\circ}\text{C}$ )	6.3	11.4	17.7	19.7	24.2	23.7
	/Precip. (mm)	105.8	55.2	62.6	101.7	2.8	22.4

Fisher  
0.01.

(ANOVA).  
-  
-  
STATISTICA.

( 499.5 mm)  
-  
( 2),  
-  
2014 .  
(2013 .)  
, -  
-  
3.  
-  
(9.31), - VSAC3  
VSA4 (16.71).  
-  
(43,90), VVA1  
(46,32 ) VVA2  
(VVA1 – ) 3.72  
(VSAC3 – ) 5.52  
-  
- VVA1 (26.37g)  
- VVAC1 (23.59g).  
1000  
VST2 (66.43g).  
, , -  
, -  
1000 .  
(VST2).  
-  
Georgieva et  
al. (2016 .), .

The results were processed by the analysis of variance (ANOVA). Fisher test at the 0.01 probability level was used. For the statistical analysis, the STATISTICA software was used.

## RESULTS AND DISCUSSION

Very high total precipitation (from March to June 499.5 mm) with lower average monthly air temperatures (Table 2) have caused the extension of vegetation and late of flowering phase during 2014. First year of investigation (2013) had the lowest amount of rainfall with the highest average monthly temperatures in the examined period. Third year of investigation had favourable conditions for the development of the vetch.

Results of investigating are presented in the Table 3. Between common vetches, the smallest number of pods per plant had VSAC3 (9.31), and the highest number was at VSA4 (16,71). The minimum number of pods in hairy vetch was at VVA1 (43,90) and the maximum was at VVA2 (46.32 pods per plant). Seed number per pod was in the interval 3.72 (VVA1 – hairy vetch) to 5.52 (VSAC3 – common vetch). The largest seed of hairy vetch had genotype VVA1 (26.37g), and the smallest genotype VVAC1 (23.59g). The highest 1000 seed weight in experiment had common vetch genotype VST2 (66.43g). These results indicate that genotypes originating from Australia have a greater number of pods per plant and increasing number of seeds per pod. The highest 1000-seed weight had genotype from eastern Serbia (VST2). Obtained results of these features were slightly higher than those from Georgieva et al. (2016), for both types of vetch.

## 3.

1000

(g),

(%),

(%),

**Table 3. Variability of ten vetch genotypes for average values of number of pods per plant, seed number per pod, 1000 seed weight (g), crude proteins (%), soluble proteins (%), and insoluble proteins (%)**

	Years	VSA1	VSA2	VST2	VSA4	VSAC3	VVA1	VVA2	VVT3	VVAC1	VVAC2	X <sub>B</sub>
Number of pods per plant	2013	10.81 <sup>f</sup>	12.83 <sup>def</sup>	11.30 <sup>f</sup>	18.2 <sup>c</sup>	9.47 <sup>f</sup>	43.3 <sup>b</sup>	45.00 <sup>ab</sup>	44.73 <sup>ab</sup>	45.60 <sup>ab</sup>	44.60 <sup>ab</sup>	28.59 <sup>ns</sup>
	2014	10.93 <sup>f</sup>	12.63 <sup>ef</sup>	10.73 <sup>f</sup>	16.37 <sup>cd</sup>	9.30 <sup>f</sup>	44.43 <sup>ab</sup>	47.73 <sup>a</sup>	46.73 <sup>ab</sup>	44.07 <sup>ab</sup>	44.60 <sup>ab</sup>	28.75 <sup>ns</sup>
	2015	10.93 <sup>f</sup>	11.77 <sup>f</sup>	10.20 <sup>f</sup>	15.57 <sup>cde</sup>	9.17 <sup>f</sup>	44.20 <sup>ab</sup>	46.23 <sup>ab</sup>	44.70 <sup>ab</sup>	46.27 <sup>ab</sup>	45.47 <sup>ab</sup>	28.45 <sup>ns</sup>
	X <sub>A</sub>	10.89 <sup>bc</sup>	12.41 <sup>b</sup>	10.74 <sup>bc</sup>	16.71 <sup>a</sup>	9.31 <sup>c</sup>	43.90 <sup>ge</sup>	46.32 <sup>d</sup>	45.40 <sup>de</sup>	45.31 <sup>de</sup>	44.89 <sup>de</sup>	
Seed number per pod	2013	5.17 <sup>b</sup>	5.24 <sup>ab</sup>	5.27 <sup>ab</sup>	5.33 <sup>ab</sup>	5.78 <sup>a</sup>	3.67 <sup>c</sup>	3.93 <sup>c</sup>	3.96 <sup>c</sup>	3.61 <sup>c</sup>	4.14 <sup>c</sup>	4.6 <sup>ns</sup>
	2014	5.40 <sup>ab</sup>	5.30 <sup>ab</sup>	5.23 <sup>ab</sup>	5.31 <sup>ab</sup>	5.53 <sup>ab</sup>	3.73 <sup>c</sup>	3.97 <sup>c</sup>	3.70 <sup>c</sup>	3.84 <sup>c</sup>	3.92 <sup>c</sup>	4.59 <sup>ns</sup>
	2015	5.33 <sup>ab</sup>	5.32 <sup>ab</sup>	5.12 <sup>b</sup>	5.40 <sup>ab</sup>	5.27 <sup>ab</sup>	3.77 <sup>c</sup>	4.07 <sup>c</sup>	3.77 <sup>c</sup>	3.82 <sup>c</sup>	3.72 <sup>c</sup>	4.57 <sup>ns</sup>
	X <sub>A</sub>	5.30 <sup>a</sup>	5.29 <sup>a</sup>	5.23 <sup>a</sup>	5.35 <sup>a</sup>	5.52 <sup>a</sup>	3.72 <sup>b</sup>	3.99 <sup>b</sup>	3.81 <sup>b</sup>	3.76 <sup>b</sup>	3.94 <sup>b</sup>	
1000 seed weight (g)	2013	55.53 <sup>b</sup>	63.77 <sup>a</sup>	66.77 <sup>a</sup>	66.40 <sup>a</sup>	65.10 <sup>a</sup>	25.33 <sup>cde</sup>	25.03 <sup>cde</sup>	27.00 <sup>cd</sup>	23.53 <sup>cde</sup>	23.60 <sup>cde</sup>	44.19 <sup>ns</sup>
	2014	58.27 <sup>b</sup>	65.33 <sup>a</sup>	66.60 <sup>a</sup>	65.97 <sup>a</sup>	66.60 <sup>a</sup>	27.07 <sup>cd</sup>	25.00 <sup>cde</sup>	27.40 <sup>c</sup>	23.87 <sup>cde</sup>	23.43 <sup>cde</sup>	44.95 <sup>ns</sup>
	2015	55.97 <sup>b</sup>	65.70 <sup>a</sup>	65.93 <sup>a</sup>	66.73 <sup>a</sup>	65.23 <sup>a</sup>	26.70 <sup>cd</sup>	21.70 <sup>e</sup>	23.30 <sup>de</sup>	23.37 <sup>de</sup>	23.67 <sup>cde</sup>	43.83 <sup>ns</sup>
	X <sub>A</sub>	56.52 <sup>a</sup>	64.93 <sup>e</sup>	66.43 <sup>e</sup>	66.37 <sup>e</sup>	65.64 <sup>e</sup>	26.37 <sup>b</sup>	23.91 <sup>cd</sup>	25.90 <sup>bc</sup>	23.59 <sup>d</sup>	23.57 <sup>d</sup>	
Crude proteins (%)	2013	21.67 <sup>cd</sup>	15.09 <sup>m</sup>	21.88 <sup>bcd</sup>	18.33 <sup>ghij</sup>	19.00 <sup>igh</sup>	22.77 <sup>abc</sup>	16.53 <sup>kl</sup>	19.31 <sup>fg</sup>	14.43 <sup>kl</sup>	23.53 <sup>a</sup>	19.55 <sup>b</sup>
	2014	22.37 <sup>abcd</sup>	16.20 <sup>lm</sup>	22.00 <sup>bcd</sup>	19.61 <sup>fg</sup>	18.73 <sup>ghi</sup>	23.17 <sup>ab</sup>	17.67 <sup>hijk</sup>	20.20 <sup>ef</sup>	18.83 <sup>fghi</sup>	22.10 <sup>bcd</sup>	20.09 <sup>a</sup>
	2015	22.20 <sup>abcd</sup>	16.03 <sup>lm</sup>	21.17 <sup>de</sup>	18.83 <sup>fghi</sup>	18.20 <sup>ghij</sup>	21.70 <sup>cd</sup>	17.07 <sup>kl</sup>	19.60 <sup>fg</sup>	17.87 <sup>hijk</sup>	21.67 <sup>cd</sup>	19.43 <sup>b</sup>
	X <sub>A</sub>	22.08 <sup>fg</sup>	15.77 <sup>b</sup>	21.68 <sup>g</sup>	18.92 <sup>cd</sup>	18.64 <sup>de</sup>	22.54 <sup>f</sup>	17.09 <sup>a</sup>	19.70 <sup>c</sup>	18.04 <sup>e</sup>	22.43 <sup>fg</sup>	
Soluble proteins (%)	2013	43.86 <sup>gh</sup>	46.90 <sup>def</sup>	34.71 <sup>m</sup>	51.27 <sup>bc</sup>	40.23 <sup>ijk</sup>	34.09 <sup>m</sup>	47.80 <sup>de</sup>	35.87 <sup>lm</sup>	46.30 <sup>efg</sup>	38.17 <sup>kl</sup>	41.92 <sup>a</sup>
	2014	46.13 <sup>efg</sup>	49.33 <sup>cd</sup>	34.53 <sup>m</sup>	52.40 <sup>ab</sup>	41.23 <sup>ij</sup>	35.40 <sup>m</sup>	48.47 <sup>de</sup>	36.30 <sup>lm</sup>	46.17 <sup>efg</sup>	39.60 <sup>ijk</sup>	42.96 <sup>b</sup>
	2015	44.03 <sup>gh</sup>	47.77 <sup>de</sup>	35.43 <sup>m</sup>	54.20 <sup>a</sup>	42.03 <sup>hi</sup>	35.63 <sup>m</sup>	51.33 <sup>bc</sup>	35.57 <sup>m</sup>	45.27 <sup>fg</sup>	38.87 <sup>jk</sup>	43.01 <sup>b</sup>
	X <sub>A</sub>	44.67 <sup>e</sup>	48.00 <sup>d</sup>	34.89 <sup>f</sup>	52.62 <sup>a</sup>	41.16 <sup>b</sup>	35.04 <sup>f</sup>	49.20 <sup>d</sup>	35.91 <sup>f</sup>	45.91 <sup>e</sup>	38.88 <sup>c</sup>	
Insoluble proteins (%)	2013	56.14 <sup>fg</sup>	52.80 <sup>hij</sup>	65.29 <sup>a</sup>	48.73 <sup>kl</sup>	59.77 <sup>cde</sup>	65.91 <sup>a</sup>	52.20 <sup>ij</sup>	64.13 <sup>ab</sup>	53.70 <sup>ghi</sup>	61.83 <sup>bc</sup>	58.05 <sup>a</sup>
	2014	53.87 <sup>ghi</sup>	50.67 <sup>jk</sup>	65.47 <sup>a</sup>	47.60 <sup>m</sup>	58.77 <sup>de</sup>	64.6 <sup>a</sup>	51.53 <sup>ij</sup>	63.70 <sup>ab</sup>	53.83 <sup>ghi</sup>	60.40 <sup>cde</sup>	57.04 <sup>b</sup>
	2015	55.97 <sup>fg</sup>	52.23 <sup>ij</sup>	64.56 <sup>a</sup>	45.80 <sup>m</sup>	57.97 <sup>ef</sup>	64.37 <sup>a</sup>	48.67 <sup>kl</sup>	64.43 <sup>a</sup>	54.73 <sup>gh</sup>	61.13 <sup>cd</sup>	56.99 <sup>b</sup>
	X <sub>A</sub>	55.32 <sup>d</sup>	51.90 <sup>e</sup>	65.11 <sup>f</sup>	47.37 <sup>c</sup>	58.83 <sup>b</sup>	64.96 <sup>f</sup>	50.80 <sup>e</sup>	64.09 <sup>f</sup>	54.09 <sup>d</sup>	61.12 <sup>a</sup>	

\* Fisher LSD (p 0.01). B -  
 \*The same letters indicate the absence of statistically significant differences according Fisher LSD test (p 0.01). Factor A - genotype, factor B - year



Alzueta et al. (2001),

2014 .  
(2014 .),  
22%  
(VSA1 – 22,08%)  
22,54% VVAC2 – 22,43%).  
VSA2 (15,77%)  
VVA2 (17,09%).

(Alzueta et al., 2001).

(2009) . Samarah Ereifej

( )  
( )  
( 2).

Based on the research of Alzueta et al. (2001), crude protein yield of common vetch were affected by growing season but not by plant maturity. In our investigation there was variability between years for this feature, too. Higher temperatures during first growing season caused advanced flowering stage of plants. High amount of precipitations during 2014 caused extension of the growing season. The crude protein content was higher in the second year (2014), than in the first and the third.

Protein content was higher than 22% in three investigated genotypes. One of common vetch (VSA1 – 22,08%), and two of hairy vetch genotypes (VVA1 – 22,54%, and VVAC2 – 22,43%). The minimum protein content had common vetch VSA2 (15,77%) and hairy vetch VVA2 (17,09%).

These results of crude protein content was higher than results obtained in investigation of crude protein fractions of common vetch forage at three harvesting stages during two years (Alzueta et al., 2001). But, also in our investigation, we have genotypes with slightly lower crude protein content.

It is important to choose the right time of cutting to utilize the maximum genetic potential for desirable traits. There are differences in the chemical composition in relation to the developmental stage. Samarah and Ereifej (2009) founded that ash, fat, fiber, protein, and carbohydrate content increased between the FS (full-size seed) and greenish-yellow pods (GY) stage. Same author stated that during the desiccation phase, there was an increase in carbohydrate and reducing sugars and a decrease in proteins.

Soluble protein fraction differs significantly in investigated years (Table 2). The highest content of soluble proteins was at genotype VSA4

VSA4 (52.62%), VST2 (34.89%).

1,

Alzueta et al. (2001).

( 3).

60%,

VST2 (65.11%) : VVA1 (64.96%), VVT3 (64.09%) VVAC2 (61.12%).

(52.62%), and the lowest was at VST2 (34.89%). These results are in accordance with the sum of fractions A and B<sub>1</sub> obtained in investigation of crude protein fractions in investigation of Alzueta et al. (2001).

Average values of insoluble proteins also varied by years (Table 3). Even four genotypes had insoluble protein content greater than 60% which could be very interesting for further research. These were one common vetch genotype VST2 (65.11%), and three hairy vetch genotypes: VVA1 (64.96%), VVT3 (64.09%), and VVAC2 (61.12%). The insoluble proteins are better exploited in the digestive tract of animals, and this fraction is significantly more interesting for further investigations.

## CONCLUSIONS

Number of pods per plant, seed number per pod and 1000 seed weight did not varied by year, but significantly varied by genotype. Crude protein content, soluble proteins and insoluble proteins were affected by year and by genotype. Four of the studied genotypes had the insoluble protein content higher than 60%.

Of these, three were hairy vetch genotypes and one was common vetch genotype. Also, crude protein content was higher in two of these three hairy vetch genotypes (VVA1 and VVAC2). These results indicate that hairy vetch, due to its quality features, could have more significant share in forage production. Also, the introduction of new genetic material from other areas in the selection would contribute to improving the quality and genetic variability.

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## Weed abundance in alfalfa crop and alfalfa mixtures under nitrogen fertilization

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### SUMMARY

The aim of the research was to determine the incidence of weeds in pure alfalfa crop and its mixtures, whether growing in mixtures can significantly reduce the proportion of weeds. Also, it should be determined how N fertilization affects the expansion of weed species.

The experiment included two legume (alfalfa and sainfoin) and two grass species (orchardgrass and tall fescue). Alfalfa was sown in pure crop and in mixtures with grasses and sainfoin: alfalfa+orchardgrass (50:50), alfalfa + orchardgrass + tall fescue (33.3:33.3:33.3) and alfalfa + orchardgrass + tall fescue + sainfoin (25:25:25:25).

The plots were fertilized with 0, 70, 140 and 210 kgN ha<sup>-1</sup> per year. Weed proportion in the mixtures, compared to pure alfalfa, was reduced by 46.8 to 55.6%. There was no significant variation among mixtures in the proportion of weeds. Nitrogen fertilization significantly increased weed pressure in canopy structure. N fertilization increased weed

proportion more in pure alfalfa than in their mixtures with grasses and sainfoin.

**Key words:** weed abundance, grass-legume mixtures, nitrogen fertilization

## INTRODUCTION

Weeds, as a very aggressive plant species, are great competitors for light, nutrients and water and their presence reduces forage yield, forage quality and persistence of the crop.

Alfalfa production, particularly in the period after planting, is almost impossible without herbicides otherwise proportion of weeds in the biomass of first cut can be very high (Spandl et al., 1999) and survival of alfalfa is uncertain.

According to Hoy et al. (2002), high proportion of weeds in alfalfa fields reduces alfalfa plant densities even by > 50%. Considering that due to the high pollution of soil and water, the main task of agriculture today is successful production while reducing the use of herbicides, many scientists were trying to substitute sowing of pure crops of grass and legume with their mixtures and therefore create a highly competitive environment that will effectively reduce the proportion of weeds.

Multispecies communities may express greater niche complementarity and make better use of soil, water, and light resources, thus reducing the opportunity for weeds to establish (Wilsey and Polley, 2002).

In the study of Surault et al. (2014) the proportion of weeds in grass-legume mixtures is reduced by 75-90% compared to pure alfalfa. When compiling mixtures it is very important to choose right species depending on the sward purpose.

(Spandl et al., 1999)

Hoy et al. (2002),

> 50%.

(Wilsey and Surault

Polley, 2002).  
et al. (2014),

75-90%,

(Emery and Gross, 2007; Sanderson et al., 2012). Sanderson et al. (2013)

Surault et al. (2014)

Roscher et al. (2009)

Picasso et al. (2008),

6.4

и

20°17'E, 96 masl).

°C,  
640.9 mm.

7.08.  
12.8

(44°49'N,

However, species characteristics can influence weeds invasiveness. Some scientists state that identity of the species is more important than the number of species in the mixture (Emery and Gross, 2007; Sanderson et al., 2012). Sanderson et al. (2013) claim that orchardgrass has a very negative impact on the spread of weeds.

Also, Surault et al. (2014) have concluded that perennial ryegrass and festulolium in mixtures are the two grass species that most limit the growth of weeds, while the highest proportion of weeds is observed in mixtures with brome and timothy. From the perspective of functional groups, Roscher et al. (2009) have concluded that the presence of grass has a negative impact, whereas the presence of legumes has a positive impact on the number and density of weed plants. In studies of Picasso et al. (2008), mixtures without the most weed suppressive species had by 6.4 times more weed biomass than the mixtures with the most weed suppressive species.

The aim of the research was to determine the incidence of weeds in pure alfalfa crop and its mixtures with grasses and legumes, whether growing in mixtures can significantly reduce the proportion of weeds. Since nitrogen fertilization leads to changes in the mixture structure, it is necessary to determine how it affects the expansion of weed species.

## MATERIAL AND METHODS

The experiment was performed at the experimental field in Institute for Animal Husbandry, Zemun, Belgrade (44°49'N, 20°17'E, elevation 96 masl). The study soil was deep silty clay loam of pH of 7.08. Mean annual temperature was 12.8°C and mean annual precipitation 640.9 mm. Prior to the establishment of trial, the site had been used as arable land. The experimental design was a

randomized block with four replications and plot size of 10 m<sup>2</sup>. The experiment included two legume (alfalfa and sainfoin) and two grass species (orchardgrass and tall fescue). Alfalfa was sown in pure crop and in mixtures with grasses and sainfoin.

Proportion of all species in the mixtures were even: alfalfa+orchardgrass (50:50), alfalfa+orchardgrass+tall fescue (33.3:33.3:33.3) and alfalfa+orchardgrass+tall fescue+sainfoin (25:25:25:25). Sowing density for alfalfa, orchardgrass, tall fescue and sainfoin were recommended for existing conditions: 25kg ha<sup>-1</sup> for alfalfa, 35 kg ha<sup>-1</sup> for grasses and 180 kg ha<sup>-1</sup> for sainfoin.

The plots were fertilized with 0, 70, 140 and 210 kgN ha<sup>-1</sup> per year as NH<sub>4</sub>NO<sub>3</sub>. One half of N doses were applied at the beginning of vegetation and second half after the first cut.

Plots were cut each time the canopy maturity reached one third inflorescence of alfalfa plants to determine herbage production. Prior to each cut, biomass was sampled by hand – cutting from the surface of 1m<sup>2</sup>. Samples were separated into different plant species in the laboratory, dried at 60° for 72 hours, and weighed. To assess the weed proportion, relative proportions of each species were calculated.

Analyses of variance were performed using General linear model in the SPSS statistical software package (SPSS 20.0). Shapiro-Wilk test was used to determine whether or not the observations were normally distributed and Levene's test for testing homogeneity of variances. Pairwise comparisons were conducted using LSD test at the probability level 0.05.

10 m<sup>2</sup>.

( )

( )

(50:50),

33,3: 33,3)

(25:25:25:25),

kg ha<sup>-1</sup>

180 kg ha<sup>-1</sup>, 35 kg ha<sup>-1</sup>

ha<sup>-1</sup>

N

0, 70, 140 210 kgN

NH<sub>4</sub>NO<sub>3</sub>.

1/3

1 m<sup>2</sup>.

60 ,

72

SPSS (SPSS 20.0).

Wilk

Shapiro-

Levene -

LSD

0.05.

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**RESULTS AND DISCUSSION**

The average weed proportions in pure alfalfa and their mixtures with grasses and sainfoin under different level

1.9 8.0%.

46.8 55.6%.

Surault et al. (2014),

Somme-Vesle 44

90% Lusignan 79 98%.

(Kirwan et al., 2007; Sanderson et al., 2012).

(Emery and Gross, 2007).

of N fertilization are presented in Table 1.

1. The proportion of weeds in the harvested biomass averaged from 1.9 to 8.0 %.

Mixtures significantly reduced presence of weeds in comparison with pure alfalfa by 46.8 to 55.6%. In research of Surault et al. (2014), frequencies of weeds, in binary alfalfa grass mixtures, were also reduced but in greater percentage, in Somme-Vesle 44 to 90% and in Lusignan 79 to 98%.

Also, other studies have shown similar results (Kirwan et al., 2007; Sanderson et al., 2012). There was no significant variation among mixtures in the proportion of weeds. In some results, it can be found that species richness and evenness as a very important component of species diversity could reduce the weed invasion (Emery and Gross, 2007).

In our findings this fact is evident only between monoculture and mixtures, regardless of the species number and contribution.

1.

**Table 1. Weed proportion in alfalfa monoculture and alfalfa mixtures with and without nitrogen fertilization (average over five years)**

/ Variants	N / N fertilization				Average canopy
	0	70	140	210	
/A	3.93	4.87	6.80	8.00	5.90 <sup>a</sup>
+ / A+O	2.33	2.60	3.62	3.71	3.10 <sup>b</sup>
+ + / A+O+TF	2.11	2.54	3.77	4.14	3.14 <sup>b</sup>
+ + + / A+O+TF+S	1.90	2.48	2.62	3.50	2.62 <sup>b</sup>
/Average fertilization	2.56 <sup>b</sup>	3.12 <sup>b</sup>	4.20 <sup>a</sup>	4.83 <sup>a</sup>	
/Level of significance					
/Mixture					**
/Fertilization					**
/Interaction					ns
- /A-alfalfa; - /O-orchardgrass; - /TF-tall fescue;					
- /S-sainfoin; /ns- non significant; **					p 0.01/**-significant at p 0.01

N

N fertilization significantly increased proportion of weeds in pure alfalfa and their mixtures. Treatment without nitrogen had the least weeds in



Blackshaw et al. (2003)

biomass. The high N fertilization showed significant increase of weed abundance in canopy structure. Likewise, Blackshaw et al. (2003) have shown that added nitrogen promotes weed growth same as crop growth. N fertilization increases weed proportion more in pure alfalfa than in its mixtures with grasses and sainfoin (Figure 1).

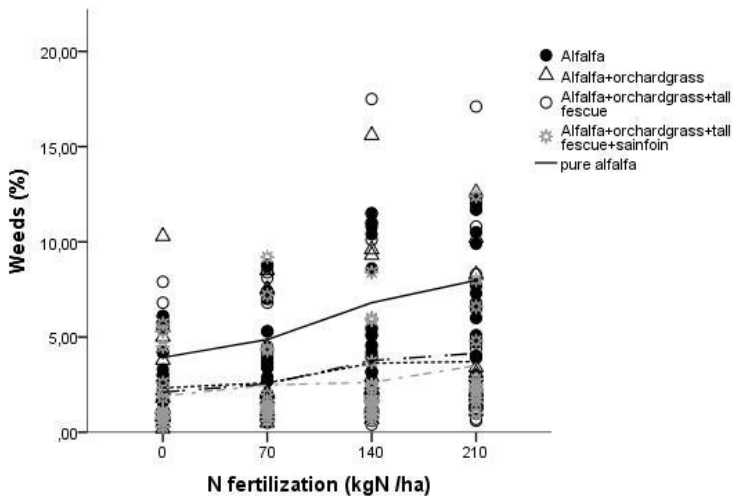


Fig. 1. Weeds proportion in pure alfalfa and their mixtures vs. nitrogen fertilization

Comakli et al. (2005)

This could be explained by the fact that N fertilization, in mixtures, favours the growth of grasses that are great competitors for light, water and soil and they occupy scope preventing weeds to invade. Comakli et al. (2005) state similar findings in their research.

During the years, weed abundance changed between crops. In the first year after sowing, weed proportion was higher in mixtures than in pure crop. In the second year, weed proportion in mixtures decreased significantly under proportion in pure alfalfa and remained low throughout the years. In pure alfalfa crop, weed proportion was the least in second production year. In each subsequent year

al. (2016)

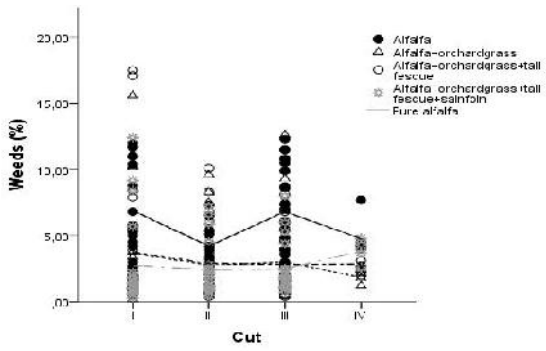
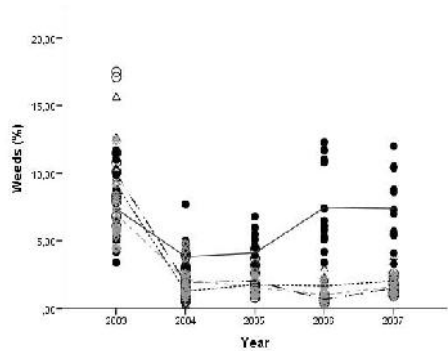
DM ( )

al. (2014)

2).  
Ergon et  
,  
,  
Surault et

it was higher (Figure 2). Contrary to our findings, Ergon et al. (2016) claim that weeds in the first year made up very small proportion of the DM yield, while in the second and third year they increased their contribution at the expense of all the sown species.

Share of weeds in mixtures' biomass had been constant per cuts. In pure alfalfa it showed significant differences, the first and third cut had higher proportion of weeds in comparison with the second and fourth cut. Similar as in our results, Surault et al. (2014) have concluded that there is no difference in weed abundance in mixture biomass between cuts. Pure alfalfa had significantly higher share of weed in biomass and depending on the year it fluctuated per cuts.



. 2.

Fig. 2. Weeds proportion in pure alfalfa and their mixture per years and per cuts

**CONCLUSIONS**

According to analysed data we can conclude that alfalfa-grass mixtures significantly reduced presence of weeds in comparison with pure alfalfa by 46.8 to 55.6%. There was no difference between mixtures in weed abundance regardless the species characteristics, number and contribution.

55.6%.

46.8

Nitrogen fertilization increased weeds pressure more in alfalfa monoculture than in their mixtures, what could be explained

by the fact that N fertilization favours competitive ability of grasses for resources. Our study shows that use of grass-legume mixtures is useful way to overcome weed invasion and maintain mixture structure.

## ACKNOWLEDGEMENTS

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TR 31053.

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***Triticum aestivum***  
***Pisum sativum* var. *Speciosum***

1\*, 2, 1  
2 1  
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**The potential for improvement of soil fertility: Cultivation of mixed legume-cereal cropping system in combination of *Triticum aestivum* and *Pisum sativum* var. *speciosum***

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**SUMMARY**

The aim of this study is to describe potential differences in concentration of TC, TN, amount of microbial biomass and microbial activity in soil sample taken from root zone of Winter Wheat – *Triticum aestivum* (Sole crops; SC-WW), Winter Wheat (Intercrops; IC-WW) with Winter Pea – *Pisum sativum* var. *Speciosum* (IC-WP).

The samples of rhizosphere soil were taken during flowering growth stages of Winter Wheat (GS 61-69) and Winter Pea (GS 61-69). The significant highest content of TC was found in soil samples from IC, about 200 % in comparison with the soil samples from SC-WW.

Cultivation of IC also supported microbial activity and development of microbial communities in rhizosphere zone documented by the highest soil respiration

– *Triticum aestivum* (SC-WW),  
; IC-WW) – *Pisum sativum* var. *Speciosum* (IC-WP).  
(GS 61-69)  
(GS 61-69).  
TC  
IC, 200%  
SC-WW.  
(IC)

IC.

(Hauggaard-Nielsen et al., 2008; Ofori and Stern, 1987; Brooker et al., 2015).

(Malezieux et al., 2009).

(Peoples et al., 1995).

(Kessel and Hartley, 2000).

$N_2$  (Hauggaard-Nielsen et al., 2001; Trenbath, 1976)

(Jensen, 1996).

and amount of microbial biomass in IC variants. Measured values indicate a positive effect of mixed culture on deposition of TC in rhizosphere soil and state of microbial communities in rhizosphere.

**Key words:** winter wheat, winter pea, mixed culture, roots exudates, rhizosphere, microbial communities

## INTRODUCTION

Intercropping can be broadly defined as a system where two or more crop species are grown in the same field at the same time during a growing season (Hauggaard-Nielsen et al., 2008; Ofori and Stern, 1987; Brooker et al., 2015). Mixing species in cropping systems may lead to a range of benefits that are expressed on various space and time scales from a short-term increase in crop yield and quality to longer-term agroecosystem sustainability (Malezieux et al., 2009).

For farming systems to remain productive, it will be necessary to replenish the reserves of nutrients which are removed or lost from the soil. In the case of nitrogen (N), inputs into agricultural systems may be derived from atmospheric  $N_2$  via biological  $N_2$  fixation (Peoples et al., 1995). Biological nitrogen fixation is an important aspect of sustainable and environmentally friendly food production and long-term crop productivity (Kessel and Hartley, 2000).

Grain leguminous can cover their nitrogen demand from biological fixation of atmospheric  $N_2$  (Hauggaard-Nielsen et al., 2001; Trenbath, 1976) and therefore, they compete less for soil  $N_{min}$  in intercropping with cereals (Jensen, 1996).

The success of intercrop farming systems depends initially on effective nitrogen fixation and more importantly, on

<p>-</p> <p>(Stern, 1993).</p> <p>-</p> <p>N</p> <p>-</p> <p>N,</p> <p>(Carlsson and Huss-Dannell, 2014).</p> <p>,</p> <p>(Bais, 2006).</p> <p>(Gregory, 2006).</p> <p>,</p> <p>0.4%</p> <p>(Fornara, 2013),</p> <p>,</p> <p>,</p> <p>,</p> <p>,</p> <p>,</p> <p>HCO<sub>3</sub>, OH<sup>-</sup> H<sup>+</sup></p> <p>(Dakora and Phillips, 2002).</p> <p>(Fornara, 2013).</p> <p>,</p> <p>(BGP)</p> <p>(Wichern, 2007).</p> <p>,</p> <p>(TC) (TN)</p>	<p>- subsequent transfer of nitrogen to the non-legume (Stern, 1993).</p> <p>- Nitrogen transfer is carried out from plants with higher content of N compounds such as leguminous plants into plants with a lower N content and thus with increased demand for it. (Carlsson and Huss-Dannell, 2014). The rhizosphere encompasses the millimetres of soil surrounding plants roots where complex biological and ecological processes occur (Bais, 2006).</p> <p>Root exudates are secreted by active roots during plant growth, and they have multiple effects in the plant-soil system (Gregory, 2006). Although the quantities of organic compounds exuding from roots are not large, seldom exceeding 0.4% of the photosynthesized carbon (Fornara, 2013), because root exudates are complex mixtures of carbon-containing compounds including carbohydrates, amino acids, organic acids, phenolic compounds, fatty acids, sterols, vitamins, enzymes, purines/nucleosides as well as inorganic molecules, such as HCO<sub>3</sub>, OH<sup>-</sup>, and H<sup>+</sup> (Dakora and Phillips, 2002).</p> <p>They do exert a very strong influence on the soil microorganisms and may be significant in affecting plant nutrient availability (Fornara, 2013).</p> <p>- Compounds released by plant roots during growth can make up a high proportion of below-ground plant (BGP) carbon and nitrogen, and therefore they influence soil organic matter turnover and plant nutrient availability by stimulating the soil microorganisms (Wichern, 2007).</p> <p>- Root exudes, which are released in to rhizosphere zone by plant roots, affect content of TC and TN in soil.</p>
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(Hauggaard-Nielsen et al., 2008; Ofori and Stern, 1987 and Brooker et al., 2015).

(Berendsen, 2012).

(Cheng, 1996).

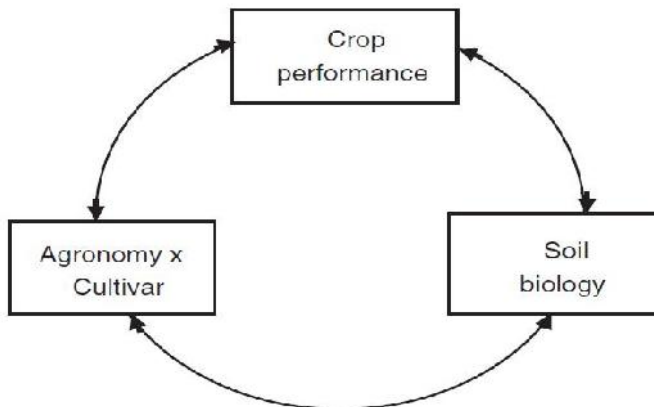
(Carvalhais, 2011).

(Bengough, 2009).

(Watt, 2006).

- These nutrients are necessary for soil microbes because they represent source of energy and basic compound of their biomass. The root exudate composition is variable (complex mixture of sugars, vitamins, amino acids etc.) depending on the plant species.

- Therefore, different species of plants have different influence on microbial communities in rhizosphere soil and thus on soil fertility (Hauggaard-Nielsen et al., 2008; Ofori and Stern, 1987 and Brooker et al., 2015). The diversity of microbes associated with plant roots is enormous, in the order of tens of thousands of species (Berendsen, 2012). It is widely known that the carbon availability in the rhizosphere is much higher than in the bulk soil (Cheng, 1996). Root exudates play a major role in the mobilization of sparingly soluble nutrients in the rhizosphere (Carvalhais, 2011). Crop yield depends on extracting sufficient nutrients and water from the soil (Bengough, 2009). What is important agriculturally is how the interactions between management and soil biology affect the performance of crops (Figure 1). Roots are thus an integral component of the soil biology (Watt, 2006).



1. (Watt, 2006)  
 Fig. 1. Interactions among management, crop performance, and soil biology can be used to improve farming systems (Watt, 2006)



Soil microbial biomass comprises a small proportion of total soil organic matter but it is more dynamic than total soil organic matter (Lupwayi, 1998). Plant-associated microorganisms fulfil important functions for plant growth and health. Diverse mechanisms are involved in the suppression of plant pathogens which is often indirectly connected with plant growth (Berg, 2009). Utilization of rhizodeposition products induces at least a transient increase in soil biomass but a sustained increase depends on the state of the native soil biomass, the flow of other metabolites from the soil to the rhizosphere and on the water relations in the soil (Lynch, 1991). Roots are also responsible for mitigation of greenhouse Gases by storing a large amount of C in the soils (Fageria, 2013). Soil C is predominantly derived from plants, directly or indirectly. Whilst weathering may be due to physical and chemical influences, most weathering processes involve plants, primarily roots, or microbial activities that depend on root-derived C (Raven, 2001). Oelbermann and Echarte (2010) confirmed that the cultivation of mixed culture had positive effect on biological and chemical properties of soil after the one year of use.

The main objective of this study was to find and quantify the potential effects of inter (mixed) crop cultivation on content of basic soil nutrients (C and N), the amount of microbial biomass and on microbial activity in soil.

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**MATERIAL AND METHODS**

*Field Experiment*

The studied area is located in the Olomouc region, in the east of Czech Republic, 8 km north from the city Prost jov, within agricultural region. Experimental sites are situated in the protective zone of drinking water source

"Kvartér eky Moravy".  
 Quitt (1975)  
 2,  
 350-400 mm  
 , 200-300 mm  
 8-9  
 e  
 , , .  
 2012 . (2  
 10 m)  
 • WW (SC):  
 (*Triticum aestivum*) –  
 (SC), 140 kg of N  
 $\text{ha}^{-1}\text{yr}^{-1}$ . (100 %  
 ).  
 2 cm 10-  
 2012.  
 • WW (IC):  
 (*Triticum aestivum*) – (  
 (IC) (1:1)  
 (*Pisum sativum* var. *speciosum*:  
 2 cm  
 10- 2012.  
*Pisum sativum* var. *speciosum*  
 5 cm.  
*Triticum aestivum* 2 cm.  
 • WW (IC – N50):  
 (*Triticum aestivum*) – (  
 (IC) (1:1)  
 (*Pisum sativum* var. *speciosum*): 70 kg N  
 $\text{ha}^{-1}\text{yr}^{-1}$  50%  
 N  
 • WW (IC – N80):  
 (*Triticum aestivum*) – (  
 (IC) (1:1)  
 (*Pisum sativum* var. *speciosum*): 112 kg N  
 $\text{ha}^{-1}\text{yr}^{-1}$  80%  
 N  
 IC SC  
 10<sup>th</sup> 2012 (  
 ), 5- 2013  
 ( ) 14<sup>th</sup>  
 2014 (

"Kvartér eky Moravy". According Quitt (1975), this is, the climatic region T2 where annual climatic averages are 350-400 mm of precipitation in growing season, 200-300 mm of precipitation in winter, and 8-9 °C of mean annual air temperature. The experiment was based on the black earth, moderate, loess without skeleton.

Field experiment was conducted in 2012 with four replicates (2 x 10 m) per treatment. Experimental sites were arrayed in blocked design. These variants were prepared:

• WW (SC): Winter Wheat (*Triticum aestivum*) – Sole crops (SC), application of 140 kg of  $\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ . (100 % of recommended dose). Seeds were sown in the rows into the same depth of 2 cm on the 10<sup>th</sup> of October 2012.

• WW (IC): Winter Wheat (*Triticum aestivum*) – (Inter crops (IC) in combination (1:1) with Winter Pea (*Pisum sativum* var. *speciosum*): without fertilizers. Seeds were sown mixed in the rows in the different depth on the 10<sup>th</sup> October 2012. The first one was *Pisum sativum* var. *speciosum* into depth of 5 cm. The second one was *Triticum aestivum* into depth of 2 cm.

• WW (IC – N50): Winter Wheat (*Triticum aestivum*) – (Inter crops (IC) in combination (1:1) with Winter Pea (*Pisum sativum* var. *speciosum*): 70 kg of  $\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  50 % of recommended doses of N for Winter Wheat.

• WW (IC – N80): Winter Wheat (*Triticum aestivum*) – (Inter crops (IC) in combination (1:1) with Winter Pea (*Pisum sativum* var. *speciosum*): 112 kg of  $\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  80 % of recommended doses of N for Winter Wheat.

Seeds of IC and SC were sown mixed in the rows into the different depth on the 10<sup>th</sup> October 2012 (the first year of experiment), 5<sup>th</sup> October 2013 (the second year of experiment) and 14<sup>th</sup> October 2014 (the third year of

).  
*Pisum sativum* var. *speciosum* -  
 5 cm.  
*Triticum aestivum* 2 cm.  
 WW (SC) WW (IC) -  
 .  
 (GS 61-69)  
 (GS 61-69).  


---

 (BR) -  
 CO<sub>2</sub> 24 h.  
 (15 )  
 120-ml .  
 24 0.5 ml 25 ° . 3  
 (Agilent  
 Technologies 7890 GC System,  
 ).  
 CO<sub>2</sub> 21-  
 (24-3 h).  
 CO<sub>2</sub>  
 (Šimek, 2011).  
 (SIR)  
 CO<sub>2</sub>  
 24 h (5 g)  
 BR  
 (4 mg C g<sup>-1</sup> 2 ml ).  
 25  
 ° . 2 4  
 0.5 ml -

experiment). The first one was *Pisum sativum* var. *speciosum* in 5 cm depth. The second one was *Triticum aestivum* into depth of 2 cm. The only results from WW (SC) and WW (IC) from first year of experiment are presented. The samples of rhizosphere soil were taken during flowering growth stages of Winter Wheat (GS 61-69) and Winter Pea (GS 61- 69).

Determination of basal and substrate induced respiration

Basal respiration (BR) was determined by measuring the CO<sub>2</sub> production from soils incubated in serum bottles for 24 h. Field moist soil (15 g) was weighed into each of three 120-ml serum bottles. Bottles were sealed with butyl rubber stoppers and incubated at 25 °C. After 3 and 24 h, a 0.5 ml sample of the internal atmosphere from each bottle was analysed by gas chromatography (Agilent Technologies 7890A GC System equipped with a thermal conductivity detector). Respiration was calculated from the increase in CO<sub>2</sub> during the 21 h incubation period (24–3 h). At the end of measurements, the total headspace volume for each replicate bottle was determined by measuring the volume of water required to fill the bottle. The measured amounts of CO<sub>2</sub> were corrected for the gas dissolved in the liquid phase. The results are expressed per gram of dry soil and hour (Šimek, 2011).

Substrate induced respiration (SIR) was determined by measuring the CO<sub>2</sub> production from soils incubated in serum bottles for 4 h after the addition of glucose. Field-moist soil (5 g) was added to three replicate serum bottles as described for the determination of BR in the previous paragraph, and 2 ml of a glucose solution was added to each bottle (4 mg C g<sup>-1</sup> of dry soil). Bottles were sealed with butyl rubber stoppers, and soils were incubated at 25 °C. After 2 and 4 h, a 0.5 ml sample of the internal atmosphere was analysed by gas chromatography (see the previous

4 h (4-2 h).  
BR.  
(  
SIR  
CO<sub>2</sub>  
(  
), (Šimek  
2011). BR SIR  
,  
WW (SC, IC) WP (IC).

paragraph). SIR was calculated from the CO<sub>2</sub> increase during the 4 h incubation period (4–2 h). The bottles were further processed as described for BR measurement. The amount of glucose amendment necessary for maximal respiratory response and linearity of CO<sub>2</sub> development during first 4 h were both checked in pilot experiments (data not shown), (Šimek 2011). BR and SIR were measured in soil sample which was collected from rhizosphere zone of WW (SC, IC) and WP (IC).

Determination of total nitrogen and total carbon in microbial biomass and soil samples

(2013).  
μm<sup>3</sup>.  
(N<sub>mic</sub> C<sub>mic</sub>)  
al., 2013).  
N<sub>mic</sub>  
(Elbl et al., 2013).  
C<sub>mic</sub> N<sub>mic</sub>  
(Friedel, 2002),  
(Turner, 2003) C<sub>mic</sub>  
(Brookes, 1985).

Process of determining total nitrogen and total carbon in microbial biomass and soil was described in Elbl et al. (2013). Microbial biomass has been defined as the part of the organic matter in soil that constitutes living microorganisms smaller than 5-10 μm<sup>3</sup>. Soil microbial biomass is considered to be a pool for subsequent delivery of nutrients – nitrogen, phosphorus etc. (Rustad, 2000). Therefore, we consider the content of nitrogen and carbon in microbial biomass (N<sub>mic</sub> and C<sub>mic</sub>) to be an important indicator of microbial activity in the soil (Elbl et al. 2013). Content of C<sub>mic</sub> and N<sub>mic</sub> was measured using fumigation-extraction method (Friedel, 2002), N<sub>mic</sub> was measured by (Turner, 2003) and C<sub>mic</sub> was measured by (Brookes, 1985).

Statistical Analysis

(  
)  
(ANOVA, P <0.05)  
LSD  
Statistica 10 CZ.  
Microsoft Excel 2010.

Potential differences in values of nitrogen and carbon concentration (in soil and microbial biomass) and soil respiration were analysed by the one-way analysis of variance (ANOVA, P<0.05) in combination with the Fischer’s LSD test. All analyses were performed using Statistica 10 CZ software. Graphic processing of measured data was performed in Microsoft Excel 2010.

## RESULTS AND DISCUSSION

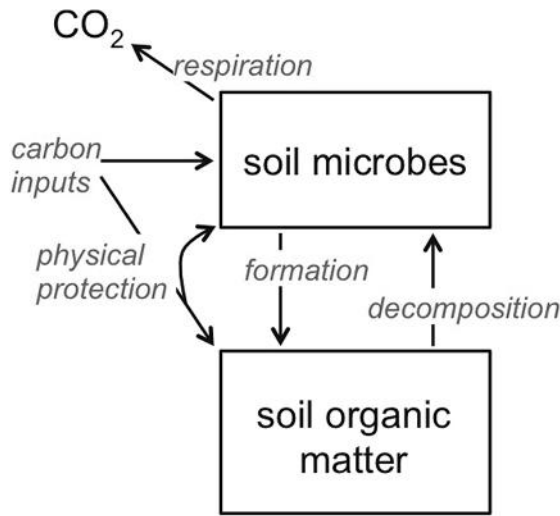
This work presents the first results from the field experiment which is focused on different quantity of nitrogen and carbon in rhizosphere of Winter Wheat (*Triticum aestivum*) grown as sole crops (SC) and inter crops (IC) with Winter Pea (*Pisum sativum* var. *speciosum*) in seed ratio (1:1).

The chloroform fumigation-extraction method was used to estimate  $C_{mic}$  and  $N_{mic}$  in soil samples, which were removed from rhizosphere zone, and the microbial activity in these samples was expressed by basal and substrate induced respiration.

The rhizosphere is the soil region that is influenced by plant roots and is characterized by a high microbial activity (Hiltner, 1904). It is well established that N transformations in the rhizosphere soil are related to C dynamics and release of available C from roots. For example, in the presence of maize roots, 67% more soil mineral N was immobilized into organic N than without plants, despite higher competition by plants for mineral N (Qian et al., 1997). Plant roots may be effective competitors with microorganisms for the nutrients released from decomposing organic patches buried in soil (Hodge, 2000).

Bacteria associated with plant roots are fundamentally important in plant nutrition (decomposition and formation of SOM, see the Figure 2), growth promotion, and disease interactions (Marschner, 2001).

(*Triticum aestivum*),  
 ( )  
 (IC)  
 (*Pisum sativum* var. *Speciosum*)  
 (1:1).  
 $C_{mic}$   $N_{mic}$   
 (Hiltner, 1904).  
 N  
 , 67%  
 N  
 N (Qian et al., 1997).  
 (Hodge, 2000).  
 (SOM), 2),  
 (Marschner, 2001).



. 2.  
(SOM),

(Bradford, 2013 )

**Fig. 2. Theoretical framework for soil organic matter (SOM) dynamics emphasizing the central role that soil microbes play in both SOM decomposition and formation – Relationship between activity of microbial community in soil, production of CO<sub>2</sub> and quality and quantity of SOM in soil (Bradford, 2013)**

\_\_\_\_\_

\_\_\_\_\_

( 30% ),

(Sutton, 2011; Zhang, 2015). (TC) (TN)

. TC

. TN (Wang et al., 2006; Muñoz and Kravchenko, 2011).

N

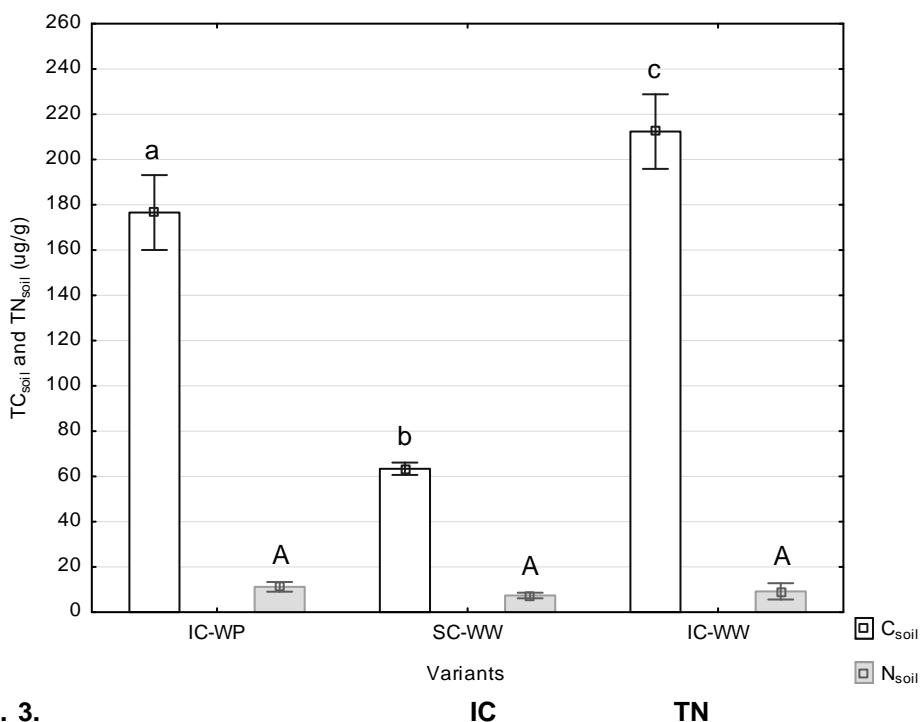
Content of total carbon and nitrogen in rhizosphere soil

- Soil carbon and nitrogen represent important reservoirs of carbon and nitrogen in arid and semi-arid regions (approximately 30% of the territory of the Czech Republic), thus they are important in the global carbon and nitrogen cycle, and climate change (Sutton, 2011; Zhang, 2015). Soil total carbon (TC) and total nitrogen (TN) both play critical roles in soil health and ecosystem dynamics. TC can improve soil fertility, quality, and water retention, and ultimately maintain and increase crop production. TN is necessary for soil fertility (Wang et al., 2006; Muñoz and Kravchenko, 2011).

- In legume-based leys knowledge about the below-ground N, pools are important for correct estimates of the total biological N<sub>2</sub>-fixation as well as for the potential N

$N_2$ , N  
 ,  
 ,  
 ,  
 ,  
 20 ± 50%  
 N  
 (Høgh-  
 Jensen and Schjoerring, 2001).

supply to the succeeding crops. Rhizodeposition may contain many different organic compounds including water-soluble exudates, sloughed-off root tissue and dead roots. The rhizodeposition of carbon can be very substantial, exceeding that present as fibrous roots at the end of the growing season by more than 20±50%. Additionally, substantial amounts of N can be deposited together with the carbon (Høgh-Jensen and Schjoerring, 2001).



. 3.  
 ( ± , n = 4,  
 0.05 - ANOVA, LSD , P <0.05)

**Fig. 3. Effect of IC cultivation on TC and TN in soil samples (mean values ± standard error, n = 4, different letters indicate significant differences at the level 0.05 – ANOVA, LSD Fischer test, P<0.05)**

3  
 ( <0.05)  
 TC.  
 TC IC

The above Figure 3 shows a significant difference (P<0.05) between individual variants only in content of TC. The significant highest amount of TC was found in IC variants (IC-WW), conversely

(IC-WW), SC (Høgh-Jensen Schjoerring, 2001; Wichern et al., 2007a 2007b, Song et al., 2007; Fustec et al., 2010) IC (C, N, P), SOM N). Mohsenabadi (2008) (2010) Rhizobium, N IC

the lowest content was detected in SC variant. Various scientific works (Høgh-Jensen and Schjoerring, 2001; Wichern et al., 2007a and 2007b; Song et al., 2007; Fustec et al., 2010) confirm that cultivation of IC (legumes) has a positive effect on content of nutrients in rhizosphere zone because these crops produce large amounts of compounds (with C, N, P), i.e. root exudates. Moreover, inclusion of this crop into crop rotation has a positive influence on increasing the content of SOM in soil, which may be subsequently mineralized – distributed to elementary nutrients (C and N).

Moreover, Mohsenabadi (2008) confirmed the cultivation of IC has positive effect on water use efficiency. Fustec et al. (2010) state legumes form a symbiosis with Rhizobium but release a substantial part of the biologically fixed N into the rhizosphere. Ecological functions of these rhizodeposits are still unknown but they may constitute a rapidly incorporating source of C and N for soil microorganisms and neighbouring plants.

Therefore, we assume long-term cultivation of IC will result in improvement of soil fertility (content of essential nutrients) and soil health (restoration of the natural functions of the soil).

Content of carbon and nitrogen in bacterial biomass

Although the quantities of organic compounds exuding from roots are not large, seldom exceeding 0.4% of the photosynthesized carbon, they do exert a very strong influence on the soil microorganisms and may be significant in affecting plant nutrient availability (Rovira, 1969). Lupwayi (1998), according to his results, indicates that legume-based crop rotations present more sustainable crop management systems.

Legumes are able to accumulate substantial quantities of nitrogen, and the

(Rovira, 1969). Lupwayi (1998),



(Jarvis et al., 1996).

(Hodge, 2000).

(Watt, 2006).

soil population of microbes has an enormous capacity to cycle this N in the right conditions (Jarvis et al., 1996).

As plants only take up inorganic N from the patch, our results indicate that microbes initially out-compete plants for the added N, but with time, plants capture more of the N originally added as they represent a slower turnover pool (Hodge, 2000).

With fumigants, caution is needed regarding confounding interactions with nutrients released from killed cells. Such treatments present gross disturbances of the soil organisms at best rather than complete sterilisation, nevertheless they remain our best method for assessing pervasive roles for soil organisms in cropping systems (Watt, 2006).

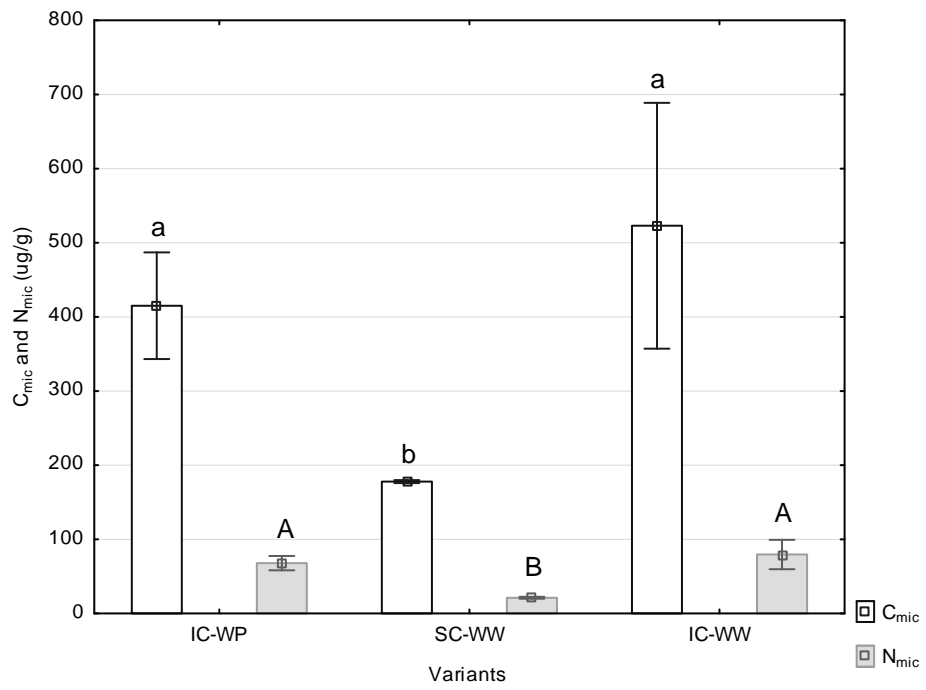


Fig. 4. Content of C and N in microbial biomass (mean values ± standard error, n = 4, different letters indicate significant differences at the level 0.05 – ANOVA, LSD Fischer test, P<0.05)

4, ( IC-WP  
 IC-WW) -  
 (WW) SC-WW IC-WP  
 (2006) Maul et al. (2014), Bloem et al. (2006) and Wenhao (2013).  
 (C<sub>org</sub>),  
 ( 3) IC.  
 -  
 (Bloem et al., 2006).  
 CO<sub>2</sub>,  
 (Schlesinger, 2000).  
 CO<sub>2</sub>  
 (Rustad et al., 2000).

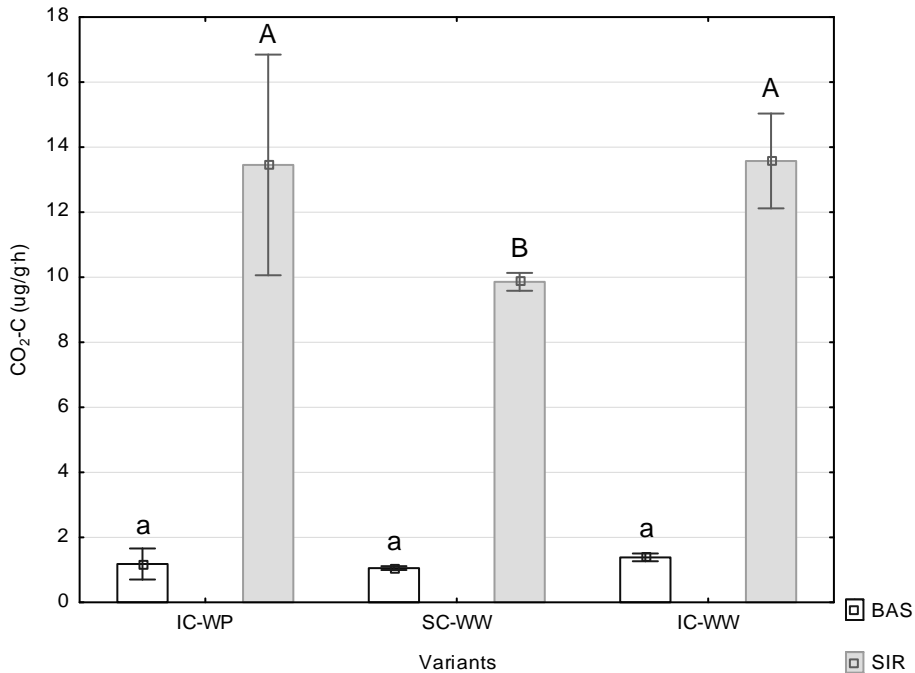
The data presented in Figure 4 indicated the cultivation of mixed culture (see variants IC-WP and IC-WW) had positive effect on development of microorganisms in soil. Significant difference between IC-WP (WW) and SC-WW was caused by root exudates having direct impact on soil microbial communities. Positive effect of root exudates on development of microbial community in rhizosphere soil based on its composition was confirmed by Maul et al. (2014), Bloem et al. (2006) and Wenhao (2013). The root exudates consist mainly of organic carbon (C<sub>org</sub>), which is useful as a source of nutrients for soil microorganisms. Therefore, microorganisms had enough energy to utilize nitrogen and carbon (see Figure 3) in variants with IC.

Microbial activity – basal and substrate induced respiration

Microbial communities in soil consist of a great diversity of species exploring their habitats by adjusting population abundance and activity rates to environmental factors.

Soil microbial activities lead to the release of nutrients available for plants, and are of crucial importance in biogeochemical cycling (Bloem et al., 2006). Soil respiration is the primary path where CO<sub>2</sub> fixed by land plants returns to the atmosphere (Schlesinger, 2000).

Efflux of CO<sub>2</sub> from soil respiration is a major contributor to net carbon exchange in terrestrial ecosystems, second only in magnitude to photosynthesis by plants (Rustad et al., 2000).



5. , n = 4, 0.05 - ANOVA, LSD , P <0.05) ±

**Fig. 5. Basal and substrate induced respiration (mean values ± standard error, n = 4, different letters indicate significant differences at the level 0.05 – ANOVA, LSD Fischer test, P <0.05)**

(BAS) -  
 (Bloem et al., 2006).  
 (SIR)  
 (.  
 (Bloem et al., 2006).  
 5, -  
 IC.  
 (2006) Bloem et al.

Basal respiration (BAS) is the steady rate of respiration in soil which originates from the turnover of organic matter (Bloem et al., 2006). The substrate-induced respiration (SIR) method is based on the detection of a respiratory response of soil microorganisms on supply of glucose (organic carbon compounds). Thus, only glucose-responsive and active organisms are measured. Based on this principle, the substrate-induced respiration method detects predominantly bacterial biomass (Bloem et al., 2006). Consider data presented in the Figure 5, the highest values of SIR were found in variants IC.

These values, in accordance with Bloem et al. (2006), show that larger amount of microbial biomass was present in rhizosphere zone of intercrops. Moreover,

IC 3

( )

(SIR)

Jensen Schjoerring (2001),  
( N),

IC.

Høgh-

C<sub>org</sub>,

IC

the Figure 3 shows that higher content of organic carbon was found in variants IC.

Therefore, the cultivation of mixed culture contributes to the development of microbial activity (C represents energy for soil microbes) and thus to the development of soil organic-mineral complex. This complex is essential for uptake and utilization of soil water – for soil fertility.

The highest microbial activity (SIR) was found in variants where IC was cultivated. According Høgh-Jensen and Schjoerring (2001), the compounds (C and N) deposited by rhizodeposition have major effects on the density and activity of microorganisms in the rhizosphere and, hence, on the turnover and plant availability of nutrients in the root zone. After mineralisation, the nutrients contained in the rhizodeposited compounds will be subjected to plant or microbial uptake, adsorbed on soil particles, or lost from the plant soil system.

## CONCLUSIONS

This contribution presents the first results of a long-term field experiment. Therefore, these results must be interpreted with caution. Our objective was to characterize potential effect of intercrop cultivation on microbial activity in rhizosphere zone of individual crops. Modern agriculture faces to new challenges and problems. One of the greatest threats is depletion in soil fertility.

The rhizosphere is a living space for soil microorganisms which are necessary for soil health and fertility.

The above results indicate the legumes produce root exudates with a large amount of C<sub>org</sub> which have direct impact on soil microorganisms. We assume the crops in IC have the advantage as they can cooperate with each other.

IC

- Our research showed the potential of intercrops for retention and utilization of mineral nitrogen in soil. Cultivation of intercrops represents new opportunity to mitigate the negative influences of extensive agriculture – depletion in soil fertility, because cultivation of IC supports microbial life in rhizosphere zone.

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## Bacillus

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### The effect of bacteria strain Bacillus on the amount of sugar and total nitrogen in molasses made of rye grain

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#### SUMMARY

The impact of Bacillus subtilis strain No. 2-amylolytic on the amount of sugar and total nitrogen in the feed molasses from the grain of rye was conducted. It has been determined the highest total sugar content and minimum nitrogen content was in the sterile molasses and 85.4 g/kg and 1.9 g/kg, respectively. Less content of the total sugars was in a non-sterile molasses (85.1 g/kg). In the absence of substrate bacteria Bacillus subtilis No. 2-amylolytic bioconversion of carbohydrates is negligible, even in the non-sterile molasses «wild» species of microorganisms.

**Key words:** Culture of Bacillus subtilis strain No. 2-amylolytic, molasses, rye, sugar, total nitrogen

#### INTRODUCTION

In recent years, in farms with high milk yield animals, have widely spread diseases connected with metabolic

	<p>disorders. Their origin and development is associated with a deficiency or excess of energy, low digestibility of nutrients and biologically active substances in the diets of animals, imbalance sugar-protein ratio in the feed (Aksenov, 2014).</p>
<p>(Aksenov, 2014).</p>	<p>Using sugars in feeding farm animals has a positive effect on the process of digestion and increases the digestibility of the roughage. On the other hand, digestible carbohydrates are very necessary to the farm animals as the main source of energy.</p> <p>The most promising way to overcome the lack of sugars in the diet of animals, especially in Siberian climate region is grains of molasses obtained by processing different types of grain raw materials (Motovilov, 2015).</p>
<p>(Motovilov, 2015).</p>	<p>As a basis for the development treatment-and-prophylactic medicine, perspective for using in veterinary medicine, researchers pay attention to the ecologically clean alive of spore-forming aerobic bacteria of the genus <i>B. Subtilis</i></p>
<p><i>B. Subtilis</i> (Antipov and Ermakova, 1990; Koroljuk, 1996),</p>	<p>(Antipov and Ermakova, 1990; Koroljuk, 1996), which are the main species detected in the study of the intestinal microflora and are harmless to warm-blooded animals.</p>
<p>1976; Sattorov, 2012).</p>	<p>They exert their antagonistic activity to a wide spectrum of pathogenic and conditionally pathogenic microorganisms resistant to lytic enzymes of the digestive system and to antibiotics – penicillin, tetracycline, streptomycin, also considered as technological in production, stable at storage and environmentally safety (Kuvaeva, 1976; Sattorov, 2012).</p>
<p><i>Bacillus</i>, (Brotukhin,</p>	<p>The ability to grow on a simple in composition and inexpensive environments, high product yield, the storage stability of <i>Bacillus</i> strains allows creating highly efficient technologies (Brotukhin, 1999), and using the enzymes</p>

1999)  
(Antipov and Ermakova, 1990, Koroljuk, 1996; Sattorov, 2012).

(*Bacillus subtilis*) (Ivanova, 2006).

*Bacillus*,  
*Bacillus subtilis* 2-

*subtilis* 2-  
2013 . S. A Donkov,

(ARCIM),

2-

*Bacillus subtilis*

(Motovilov, 2015).

for the production of bio medicines (Antipov and Ermakova, 1990; Koroljuk, 1996; Sattorov, 2012). Milk replacers, vitamin, mineral, enzyme feed additives are enriched with them (*Bacillus subtilis*) (Ivanova, 2006).

Nowadays, it is not enough feed additives for farm animals, produced by a method of bioconversion of starch-containing plant material and containing probiotic microorganisms.

The aim of this work is to study the effect of strain of *Bacillus*, using *Bacillus subtilis* No. 2-amylolytic on the amount of sugar and total nitrogen in the feed molasses.

## MATERIAL AND METHODS

Bacterial strain *Bacillus subtilis* No. 2-amylolytic was secreted in 2013 by S. A. Donkov, a staff member of Krasnoyarsk Research Institute of Animal Husbandry and transferred to national patent deposit in All-Russia collection of industrial microorganisms (ARCIM), where was proved the strain produces an amylolytic enzyme.

The research was held in the laboratories of Krasnoyarsk Research Institute of Animal Husbandry and Biophysics Institute of Federal research center «Krasnoyarsk scientific center of Russian Academy of Sciences».

A culture of strain *Bacillus subtilis* No. 2-amylolytic was added to molasses of rye grain, produced in Breeding Farm Tazhny, Ltd, Sukhobuzimskiy region, Krasnoyarsk Krai which was used for feeding to milk cows. To prepare molasses was used a complex effect of cavitation, ionization, and fermentation in accordance with the methodical recommendations (Motovilov, 2015).

Breeding Farm Tazhny, Ltd prepared testing and experimental samples from grain molasses in

1. accordance with the scheme given in Table 1.

1.

**Table 1. Scheme experience**

/Example	/ Molasses
1	
1 <sup>st</sup> testing	Non-sterile molasses
1	+ 0,5 m/ml Bacillus subtilis 2-
1 <sup>st</sup> experimental	Non-sterile molasses + 0,5 m/ml Bacillus subtilis 2-amyloitic
2	+ 0,01 m/ml Bacillus subtilis 2-
2 <sup>nd</sup> experimental	Non-sterile molasses + 0,01 m/ml Bacillus subtilis 2-amyloitic
3	+ 0,05 m/ml Bacillus subtilis 2-
3 <sup>rd</sup> experimental	Non-sterile molasses + 0,05 m/ml Bacillus subtilis 2-amyloitic
2	
2 <sup>nd</sup> testing	Sterile molasses
4	+ 0,5 m/ml Bacillus subtilis 2-
4th experimental	Sterile molasses + 0,5 m/ml Bacillus subtilis 2-amyloitic
5	+ 0,01 m/ml Bacillus subtilis 2-
5th experimental	Sterile molasses + 0,01 m/ml Bacillus subtilis 2-amyloitic
6	+ 0,05 m/ml Bacillus subtilis 2-
6th experimental	Sterile molasses + 0,05 m/ml Bacillus subtilis 2-amyloitic

30 0,5 atm

Bacillus subtilis 2-  
200 ml

Bacillus subtilis 2-  
0,5; 0,01;  
0,05 m/ml.

Bacillus subtilis 2-  
0,5; 0,01; 0,05  
m/ml.

First testing sample submitted the original non-sterile molasses, the second one – molasses sterilized in autoclave at 0,5 atm within 30 min. Out of sterile and non-sterile samples of molasses were obtained experimental samples with different concentration added inoculums of Bacillus subtilis No. 2-amyloitic. For this, in one liter Erlenmeyer flasks was poured 200 ml of molasses and the appropriate amount of seed material. Each sample was prepared in three stage repetition.

Thus, First testing sample contained the non-sterile molasses, in the second, third and fourth experimental samples of non-sterile molasses was added Bacillus subtilis 2-amyloitic in the amount of 0,5; 0,01; 0,05 m/ml.

Second testing sample contained the sterile molasses and in the fifth, sixth and seventh experimental samples was added Bacillus subtilis 2-amyloitic in amount of 0,5; 0,01; 0,05 m/ml correspondingly.

37 ± 0,5 °C. 48

Duration of incubation was 48 hours at 37±0,5°C. In 48 hours after adding microorganisms the sugar content and nitrogen were determined.

## RESULTS AND DISCUSSION

After 48-hour incubation of the bacterial strain *Bacillus subtilis* No. 2-amylolytic using crop molasses is a partial hydrolysis of starch, thereby reducing starch content, at the same time increasing the content of oligo- and monosaccharides.

The latter, in turn, being a substrate for the growth of bacteria *Bacillus subtilis* No. 2-amylolytic, they can be disposed with them and as a result increasing biomass concentration of bacteria.

The content of the molasses before and after sterilization was in the limits of measurement error and almost identical. After adding inoculums in a different concentrations and the subsequent incubation period the content of the main components in the samples of the partial products of bioconversion was determined. In non-sterile molasses in addition to the bacterial strain *Bacillus subtilis* No. 2-amylolytic might also grow «wild» species of microorganisms.

In Table 2 presents the results of research of the effect of *Bacillus subtilis* No. 2-amylolytic on the amount of sugar and total nitrogen in molasses made from grain of rye.

According to the data in Table 2 the highest total sugar content and minimum nitrogen content was in the sterile molasses (2<sup>nd</sup> testing sample) – 85.4 g/kg and 1.9 g/kg, correspondingly. A little less of the total sugars was in a non-sterile molasses. It is suggested that in the absence of substrate bacteria *Bacillus subtilis* No. 2-amylolytic bioconversion of carbohydrates is negligible, even in the presence of non-sterile molasses «wild» species of microorganisms.

48-				
	<i>Bacillus subtilis</i>	2-		
	<i>Bacillus subtilis</i>	2-		
	<i>Bacillus subtilis</i>	2-	" "	
	<i>Bacillus subtilis</i>	2-		
			(2-	) -
	85.4 g/kg	1,9 g/kg.		
	<i>Bacillus subtilis</i> ,	2-		
			" "	

2.

**Table 2. The amount of sugar and total nitrogen in molasses made from grain of rye**

	/Samples	/Amount	
		/sugar g/kg	/total nitrogen g/kg
1	/1 <sup>st</sup> testing	85,1	1,90
1	/1 <sup>st</sup> experimental	54,2	2,56
2	/2 <sup>nd</sup> experimental	58,5	2,81
3	/3 <sup>rd</sup> experimental	59,0	3,85
2	/2 <sup>nd</sup> testing	85,4	1,90
4	/4 <sup>th</sup> experimental	57,2	2,82
5	/5 <sup>th</sup> experimental	57,5	2,92
6	/6 <sup>th</sup> experimental	61,5	3,86

(0,5 /ml).  
g  
2  
g/kg.  
2-  
" "

At the same time, the highest decrease in the content of total sugars was observed in case of adding the largest quantity of inoculum (0.5 million/ml). In this case the content of nitrogen increases at a maximum level. It indicates nitrogen inclusion in content of microbial protein when raising the concentration of biomass. It is obvious that the increase of biomass concentration of the microbial cells, the content of residual sugars will be reduced. Taking into account the most probable values of the economic factor of 0,4-0,5 per 1 g of biomass must be allocated up to 2 or more grams of the carbonaceous

**CONCLUSIONS**

The highest total sugar content and minimum nitrogen content was in the sterile molasses (2-testing sample) – 85.4 g/kg and 1.9 g/kg, respectively. A little less of the total sugars was in a non-sterile molasses (85.1 g/kg). This suggests that in the absence of substrate bacteria *Bacillus subtilis* No. 2-amylolytic bioconversion of carbohydrates is negligible, even in the presence of non-sterile molasses «wild» species of microorganisms.

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