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Editorial

Cultivate our gardens

The Enlightenment was an intellectual and philosophical movement in the Baroque era of Europe during the 18th century. François-Marie Arouet – Voltaire, a French writer, historian, and philosopher was a key person of that period. His marvellous novel “Candide” was published first in 1759. The story is about a hopelessly optimistic young gentleman, Candide, who is exposed to all troublesome impacts human beings may experience. Searching for love, career and happiness, he is turned into a miserable, exploited person committing crime, and suffering from all disgusting pressures of the society. Finally, he arrives at the solution and defines the purpose of life.



Source: *Candide: or, The Optimist* (1762) by Voltaire, second English edition.

Candide is the illegitimate nephew of baron Thunder-ten-tronckh . He grows up in the nobleman’s castle in Westphalia under the tutelage of the scholar Pangloss who teaches him that this world is “the best of all possible worlds.” Candide falls in love with the baron’s young daughter, Cunégonde. The baron expels Candide brutally from his home. Exile from Eden causes Candide to live on his own for the first time.

His life turns to be more than an Odyssey. Temporally, he is wandering over a period of two decades. Spatially, he happens to be in Holland, Portugal, various South American lands like Argentina, Surinam and Eldorado. He then returns to Europe, roaming in France and Italy until he settles in Turkey. His activities and occupations are as variable as his soul and passion. First he enrolls in the Bulgar army, from where he escapes as a deserter. Later he will be arrested several times, jailed, punished and almost executed twice. He serves as a slave, a servant, a sailor and merchant. Killing people in war and murdering his occasional enemies, as well as hunting for innocent apes while misunderstanding their strange sexual patterns. Also, he is a liberator and a Maecenas of many people in need. His fortune is fluid. Often he remains with no money, or even sunk in debt, while in unexpected situations he gains power and wealth.

All his personal contacts are labelling this unbelievable life record. The most crucial persons in his life are intellectually diverse. His Panglossian naïve goodwill has to face the kind Anabaptists, the cruel Inquisitors, the militant Jesuits, the idealistic natives of Eldorado, the proud nobles,

the bargaining Jews, the viperous scrounging Dutch, the degenerated rich, the greedy poor, and continuously the believers of all sort of philosophies.

Candide is dedicated by his optimism to be good and to render services of peace and love to all he meets, however he often fails during his mission. Meeting Cunégonde, his friends, teachers and humble companions again, he decides not to fight, move and chase fortune anymore, but settle and work. The conclusion of his adventures can be formulated in one sentence: happiness in life can only be achieved by work. So, - cultivate our gardens!

What can be the message of Voltaire to us, to the people of science and education? The editors believe our activities do not differ from any other activities. Happiness depends on satisfaction, and the only way to that is through work. This idea is similar to that of the basic principle written in the Bible - “By the sweat of your brow you will eat your food until you return to the ground, since from it you were taken; for dust you are and to dust you will return”.

Márton Jolánkai

Response of soybean and barley to Fertdolomite application on acid soil

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Abstract: The stationary field experiment with the application of granulated dolomite ($\text{MgCO}_3 \times \text{CaCO}_3$) enriched with nitrogen, phosphorus and potassium (Fertdolomite: 24.0 % CaO + 16.0 % MgO + 3.0 % N + 2.5 % P_2O_5 + 3.0 % K_2O) in rates 0, 5, 10, 20, 30 and 40 t ha⁻¹ on standard fertilization was started on 13th November 2007 on the acid soil (pH in 1n KCl: 3.90). The trial was conducted by randomized block design in four replicates (basic plot 40 m²). Standard fertilization of trial was applied in the next years for crops in rotation. In this study the response of soybean (2010) and winter barley (2012/2013) was shown. The average grain yield of soybean was 4830 kg ha⁻¹ with variation among the treatments ranging from 4341 to 5361 kg ha⁻¹. At the rates 10 t ha⁻¹ and 20 t ha⁻¹ soybean yields were significant increased for 8% and 16%, respectively and additionally by 6% at the highest rate of fertolomite. Fertdolomite had a moderate positive effect on protein contents in grain, while oil content was independent on the treatments. The average grain yield of barley was quite low (3630 kg ha⁻¹), mainly due to low ears density (average 493 per m²) which was affected by oversupplies of precipitation in winter period under less permeable soil conditions. Extreme variations of precipitation regime are in connection with climatic change. Due to the application of 10 and 20 t ha⁻¹ of Fertdolomite, yields of barley were significantly increased by 20% and 34%, respectively. However, the rates of 40 t ha⁻¹ showed a non-significant difference of the barley yield as compared to the control level. The ear densities were significantly increased by application ≥ 10 t ha⁻¹ Fertdolomite rates. Improvement of soil status by liming, adequate fertilization and similar managements contribute to the alleviation of detrimental effects of soil limitations and recent climate change on field crop yields.

Keywords: soybean, barley, Fertdolomite, grain yield, climate change

Introduction

Soil acidity considerably limits crop yields worldwide. The aluminium (Al) toxicity and phosphorus (P) deficiency are considered to be two main constraints for crop production in acid soils (von Uexkull and Mutert, 1995, Sumner and Noble, 2003; Sarkar and Sharma, 2005). Acid soil in Croatia account for 1.6 mill. ha (Bogunovic et al., 1997), while Mesic et al. (2009) noted that in Croatia, 831704 ha or 32% of agricultural soil is acid. Also, with more than 50% of acid soils in all agricultural land in Croatia, soil acidity is recognized as a big problem (Bogunovic et al., 2016). Excessive soil acidity can be alleviated or neutralized by the addition of different lime materials containing calcium (Ca) and magnesium (Mg) ions. The correction of acid soil pH close to neutrality and improvement of nutrient availability by adequate fertilization, could be contribute to more favorable environment conditions for crop growth and for this reason to alleviate negative effects of soil properties (Stojic et al., 2012;

Jolankai and Birkas, 2013). Antunovic et al., (2014) and Tang et al. (2003) also showed that lime applied on the soil surface has brought about a short-term and long-term decrease in soil acidity. Low soil pH often associated with P deficit cause the soil infertility problem and for a sustainable soybean and barley production both lime and P fertilization is recommended. Temesgen et al. (2017) reported a yield increase in barley by 58% due to liming and by 44% due to P fertilization. Lokia et al. (2017) found in the pot experiment in acid soil that liming resulted in an increase total biomass of maize by 45%, root volume by 50% and plant height by 36% in comparison to the control.

Recent climate change characterized by global warming and frequently extreme variation of the precipitation regime has additional detrimental effect on productivity of acid and other soil types that are reflected in decreased yields of the main field crops (Rosenzweig et al., 2002; Cindric et al., 2009; Parry et al., 2005; Lobell and Field 2007; Lobell et al., 2007; Magas, 2013; Osborne et al., 2013; Lesk et al., 2016; Bootsma et al.,

2015; Zipper et al., 2016). According to UN data (2017) the average global temperature increased by 0.85 °C from 1880 to 2012. To put this into perspective, for each degree of temperature increase grain yields decline about 5%. Maize, wheat and other crops have experienced significant yield reductions at global level of 40 megatons per year between 1981 and 2001 due to warmer climate. Schenkler and Roberts (2008) estimated that in the United States yield increase under the temperature of up to 29 °C for maize and 30 °C for soybeans, but temperatures above these thresholds become very harmful. Appropriate soil management including liming of acid soil combined by adequate fertilization could contribute to the alleviation of negative effects of climate change on crop production.

The aim of this study was to test subsequent effects of granulated dolomite enriched with nitrogen, phosphorus and potassium (trade name Ferdolomite) applied in autumn of 2007 on soybean in 2010 and winter barley in 2013 growing seasons under acid soil conditions in central Croatia. Both growing seasons were characterized by unfavorable monthly distribution and considerable excess of precipitation in comparison to the long-term averages in the period 1961-1990.

Material and methods

The field experiment

The stationary field experiment with the application of granulated dolomite enriched with nitrogen, phosphorus and potassium (trade name Ferdolomite: 24.0 % CaO + 16.0 % MgO + 3.0 % N + 2.5 % P₂O₅ + 3.0 % K₂O) started on 13th November 2007 on the Kolar Family Farm in Pavlovac (municipality Veliki Grdjevac, Bjelovar-Bilogora County). Ferdolomite was applied on the standard fertilization in the amounts of 0 (the control), 5 t ha⁻¹, 10 t ha⁻¹, 20 t ha⁻¹, 30 t ha⁻¹ and 40 t ha⁻¹.

The experiment was conducted by randomized block design in four replicates (basic plot 40 m²). After the distribution of NPK 7:20:30 fertilizer (500 kg ha⁻¹) and Ferdolomite the soil was

ploughed to 25 cm depth. In the next years (from 2009 to 2015) only standard fertilization of the experiment was applied and subsequent effects of Ferdolomite were tested. The crop rotation on the experiment was as follows: maize (2008 and 2009) - soybean (2010) – winter wheat (2010/2011) – maize (2012) — winter barley (2012/2013) –maize (2014) – winter wheat (2014/2015). Standard fertilization of the experiment (kg ha⁻¹) for soybean was 80 N + 60 P₂O₅ + 80 K₂O and for barley 120 N + 53 P₂O₅ + 70 K₂O. This study shows the response of soybean and winter barley to the applied fertilization.

Soybean (cultivar *Lucija* developed at the Agricultural Institute Osijek) was sown on 26th April 2010 by pneumatic sowing machine and harvested on 24th September 2010. From each basic plot an area of 2.0 m² of soybean crop was manually harvested, plants enumerated and pods separated from stem. Soybean plants were trashed by special combine. Grain of soybean was calculated on 13% grain moisture basis and realized plant density.

Winter barley (cultivar *Barun* developed at the Agricultural Institute Osijek) was sown by pneumatic sowing machine on 21th October 2012 and harvested on 22nd June 2013. From each basic plot barley ears were manually harvested from 4 x 0.25 m² (total 1.0 m²) area. The ears were enumerated and trashed by special combine. Grain of soybean and barley were calculated on a 13% grain moisture basis.

The sampling, chemical and statistical analysis

Selection of soil for the experiment was made based on the previous soil test. The average soil sample was taken by the auger to 30 cm depth on October 27, 2007. The second soil sampling was made on September 24, 2010 after harvest of soybean from each basic plot. Protein and oil contents in soybean grain were determined by Near Infrared Transmittance spectroscopic method on Grain Analyzer (Infratec 1241, Foss Tecator) at the Agrochemical laboratory of the Agricultural Institute Osijek.

Data were statistically analyzed by ANOVA and treatment means were compared using t-test and

LSD at 5% and 1% probability levels.

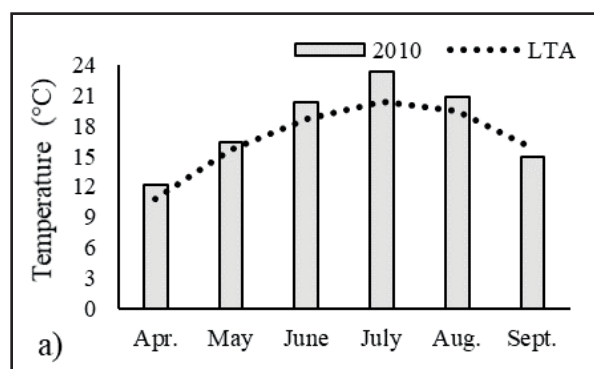
Soil reaction and organic matter were determined according to ISO (1994, 1998). Mobile fraction P and K were extracted by AL-method (Egner et al., 1960), while Ca and Mg in the soil were extracted by acid solution (pH 4.65) of NH_4 -acetate + EDTA (Lakanen and Ervio, 1971) and determined by ICP-AES.

Besides, another four stationary field trials were carried out with Fertdolomite application and the results were published in the previous studies. The first was identical as the above mentioned trial but with different crop rotation and was situated in Badljevina (25 km from Pavlovac in SE direction), site of Pakrac municipality (Kovacevic et al., 2015a, 2015b). The second trial was about 2 km away from the first experiment (Kovacevic et al., 2012) and the third trial was carried out in Gorjani, site of Osijek-Barannya County (Kovacevic et al., 2014b). The fourth trial was carried also carried out on the Kolar family farm in Pavlovac as a supplement to the trial with increasing rates of PK-fertilization because its 4-year effect was below expected (Rastija et al., 2006; Kovacevic et al., 2009): two replicates of the trial were limed with 10 t ha^{-1} of Fertdolomite and results of 5-year study were presented by Kovacevic et al., 2014a.

The soil and weather conditions

Table 1. Chemical properties of the soil surface layer to 30 cm depth

pH		%	mg 100 g ⁻¹		mg kg ⁻¹		cmol ⁺ kg ⁻¹ Hydrolytical acidity
H ₂ O	KCl		P ₂ O ₅	K ₂ O	Ca	Mg	
4.73	3.90	2.31	12.6	34.1	629	111	5.12



The experimental plot was selected based on the previous soil test. The soil is classified as stagnosol. Very acid reaction, moderate levels of available phosphorus, calcium and magnesium, averagely supply with potassium are main chemical properties of the soil (Table 1). Also, the high hydrolytical acidity was indication for liming.

For characterization of the weather condition during soybean and winter barley growing seasons, the meteorological data of Bjelovar Weather Bureau were used (SHS, 2013). Bjelovar is located 25 km as the crow flies northwest from the experiment site Pavlovac.

The 2010 growing season was characterized by excessive precipitation. Total precipitation in April-September period in Bjelovar was 834 mm or 80% higher compared to the 1961-1990 average. With that regard, precipitation in April and July were at the level of averages, while in May-June and August-September they were higher by 79% and 156%, respectively. Mean air temperature in the above mentioned period of 2010 was 18.0 °C or by 1.2 °C higher. With that regard, the highest difference in temperature level of 2.9 °C was recorded in July, while in April, August and September it was close to the long-term average (Fig. 1 and 2). Weather characteristics in 2010 were mainly favourable for soybean growth (Vrataric and Sudaric, 2008).

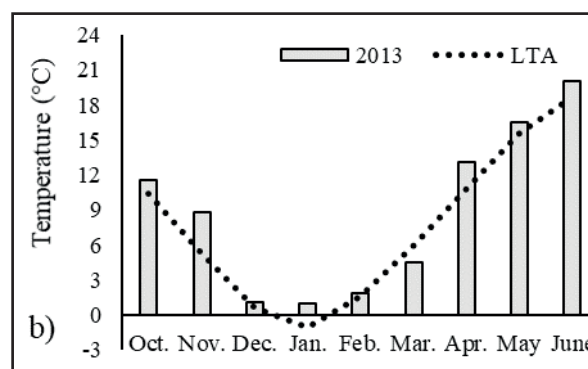


Figure 1. Average monthly air temperature (°C) in soybean growing season from April to September 2010 (a = left) in winter barley growing season from October 2012 to June 2013 (b = right) as compared to the long term average (LTA, 1961-1990) for Bjelovar (SHS, 2013)

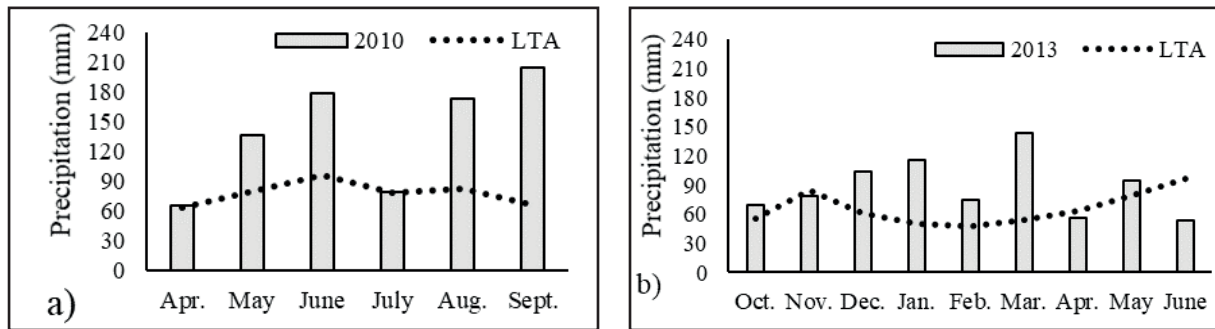


Figure 2. Average monthly precipitation (mm) in soybean growing season from April to September 2010 (a = left) in winter barley growing season from October 2012 to June 2013 (b = right) as compared to the long term average (LTA, 1961-1990) for Bjelovar (SHS, 2013)

Precipitation amount in the 2012/2013 growing season from October to June in Bjelovar was 790 mm or by one third higher than the average of 1961-1990 period. In the same period mean air temperature was 1.2 °C higher. Monthly distribution of precipitation was characterized by considerably higher values (total 439 mm) in December-March period or by 107% higher compared to the long-term average. In the remaining months these values were above but still close to averages with the exception of June when precipitation was lower by nearly 50% (Fig.1 and 2). Excessive precipitation during winter period was less favorable for barley growth, particularly under the less permeable and unreclaim soil conditions (Paunovic and Madic, 2011), for example in the soil of the experimental site. In general, the precipitation and temperature trends in the recent period are in accordance with the above mentioned global climate change.

Results and discussion

Table 2. Impact of Fertolomite application on soybean properties

Property*	Fertdolomite (13 th November 2007) amount (t ha ⁻¹)						LSD		
	0	5	10	20	30	40	Mean	5%	1%
Soybean properties (the 2010 growing season)									
Grain yield (kg ha ⁻¹)	4341 (100)	4352 (100)	4708 (108)	5040 (116)	5179 (119)	5361 (123)	4830	330	456
Plant density (thousand plants ha ⁻¹)	655.0 (100)	635.0 (97)	625.0 (95)	612.5 (93)	602.5 (92)	565.0 (86)	615.8	59.1	73,9
Thousand grain weight (g)	192.6 (100)	188.0 (98)	187.8 (98)	193.5 (100)	193.5 (100)	192.1 (100)	191.3	ns	
Protein in grain (%)	36.98 (100)	38.42 (104)	38.46 (104)	38.92 (105)	38.72 (105)	38.23 (103)	38.29	1.44	ns
Oil in grain (%)	23.59 (100)	23.29 (99)	23.38 (99)	23.42 (99)	23.25 (99)	23.47 (99)	23.40	ns	

*in the brackets: index (the control = 100)

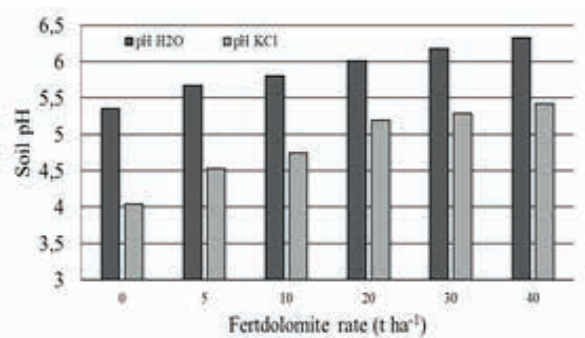


Figure 3. The impact of fertdolomite (t ha⁻¹) on soil pH (0-30 cm depth, in October 2010). Error bars that represent the 95% confidence interval of a mean

Liming with Fertdolomite considerably affected on soil pH from 5.35 (pH in H₂O) and 4.04 (pH in KCl) on the control treatment to 6.33 and 5.42, respectively, after application the highest rate of lime (Fig. 3).

Average grain yield of soybean in the experiment was 4830 kg ha⁻¹ with variation among the treatments from 4341 to 5361 kg ha⁻¹, for the control and application the highest rate

Table 3. Impact of Fertolomite application on winter barley status

Property	Fertdolomite (13 th November 2007) amount (t ha ⁻¹)						Mean	LSD	
	0	5	10	20	30	40		5%	1%
	Winter barley status (the 2013 growing season)								
Grain yield kg ha ⁻¹	3190 (100)	3420 (107)	3840 (120)	4280 (134)	3490 (109)	3550 (111)	3630	640	832
ED per square m	408 (100)	442 (108)	488 (120)	590 (145)	530 (130)	502 (123)	493	74	93
TGW g	44.5 (100)	45.0 (101)	43.1 (97)	41.8 (94)	42.1 (95)	41.0 (92)	42.9	2.8	ns
HM kg	65.2 (100)	66.3 (102)	65.4 (100)	65.5 (100)	65.8 (101)	65.1 (100)	65.5	ns	

* ED (ears density),. TGW (thousand grain weight), HM (hectoliter mass)

of Fertdolomite, respectively (Table 2). The application of Fertdolomite in autumn 2007 resulted in a significant increase in grain yields of soybean in the 2010 growing season up to 23% compared to the control. With that regard, for a significant increase in yield at the levels of 95% and 99%, rates of 10 t ha⁻¹ and 20 t ha⁻¹ of Fertdolomite, respectively, were needed. Yield increases by the application of these two rates of Fertdolomite were 8% and 16%, respectively.

By increasing the rate of Fertolomite to 30 and 40 t ha⁻¹ the yields of soybeans were additionally increased compared to the level of 20 t ha⁻¹ of applied Fertdolomite but these increases were non-significant (Table 3). Increasing rates of Fertdolomite had a negative effect on plant density realization (PDR) but significant difference and PDR decrease by 14% compared to the control were found only in the application of the highest Fertdolomite rate. Fertdolomite application had a moderate effect on protein contents in soybean grain. With that regard, the application of Fertdolomite in the amounts of 20 and 30 t ha⁻¹ caused a significant increase in protein content amounting 1.94% and 1.74% compared to the control, while the application of the highest rate of Fertdolomite caused a decrease in protein content to non-significant level relative to the control. Thousand grain weight and oil content in soybean grain were independent of Fertdolomite application (Table 2).

Grain yields of winter barley in the experiment was 3630 kg ha⁻¹ and it is low compared to yield potential of high-yielding *Barun* cultivar.

Yield variation among the applied treatments ranged from 3190 (the control) to 3840 (20 t ha⁻¹ Fertdolomite) kg ha⁻¹ (Table 3). Main reasons of low yield were too low plant density and ears density (average 493 ears per square meter) of barley crop. This barley status is probably a result of excessive precipitation during the winter period (Fig. 2) under less permeable and unreclaim soil conditions of the experiment site.

As in case with soybean three years ago, for a significant increase in yields of barley in the 2013 growing season to the level of 95% and 99% probabilities, the rates of 10 t ha⁻¹ and 20 t ha⁻¹ of Fertdolomite, respectively, were needed.

Yield increases of barley by the application these two rates of Fertdolomite were considerably higher than in soybean, 20% and 34%, respectively. By increasing the rates of Fertolomite to 30 and 40 t ha⁻¹ the yields of barley decreased to the level of non-significant differences related to the control. Ear densities per unit area were significantly increased to a maximum of 590 ears per m² by the application 10 and more tones of Fertdolomite ha⁻¹. With that regard, increases compared to the control were 20%, 45%, 30% and 23%, for the treatments with 10, 20, 30 and 40 t ha⁻¹, respectively.

Regarding thousand grain weight (TGW), a decreasing trend from 44.5 g to 41.0 g with increased Fertdolomite rates was found, but only with significant difference and by 8% lower value in the highest rate of applied Fertdolomite. Values of hectoliter mass were 65.5 kg on the average and independent of applied treatments.

In the study of the identical field experiment on Badljevina, acid soil crop rotation was as follows: maize (2008) – spring barley (2009) – maize (2010 and 2011) – wheat (2012) – maize (2013). Response of maize was specific in the tested four growing seasons. Significant yield reductions were found in the application of the highest rate of Fertilizer (for 7%, 10% and 14%, for 2008, 2010 and 2011, respectively), while in 2013 in the application of 20 – 40 t ha⁻¹ rates, yields of maize were increased by up to 16% compared to the control. Yield increases by Fertilizer were 10% on the average in 2010 and 2011 growing seasons with the application of 5 - 30 t ha⁻¹ (2010) and 5 - 10 t ha⁻¹ rates (2011), respectively. The lower rates of Fertilizer in the amount of 5 and 10 t ha⁻¹ were sufficient for the increase in yields of spring barley in 2009 by 22% and 14%, respectively, while the application of the higher rates caused yields to reduce to the level of the control. However, yields of wheat in 2012 were similar in all treatments with the exception of a slight reduction by 7% under the application of the highest rate of Fertilizer (Kovacevic et al., 2015a, 2015b).

In the second experiment in Badljevina, rates of 5, 10, 20 and 40 t ha⁻¹ of Fertilizer and crop sequence maize (2009) – wheat (2010) – winter barley (2011) were applied. With that regard, yield reduction by 10% by using the highest rate (maize) and yield increase by 10% by using the 5 and 10 t ha⁻¹ rates (wheat) were found. However, yields of barley were increased by 25% and 50% by using the 20 and 40 t ha⁻¹ rates, respectively (Kovacevic et al., 2012).

By the second experiment with Fertilizer in the amount of 10 t ha⁻¹ on the Kolar Family Farm in a 5-year period from 2008 to 2012, a moderate response of maize in three growing seasons (yield increases less than 10%) was recorded, yields of wheat were similar to the control, while only soybean responded by a considerable yield increase of 17%. Response of maize to liming was mainly moderate probably because of the other limitations of the soil fertility, for example unregulated air-water relations and low humus contents (Kovacevic et al., 2014a).

The field experiment with application of three rates of Fertilizer (3.5, 7.0 and 14.0 t ha⁻¹) and the control started in the autumn of 2011 in Gorjani (Osijek-Baranya County) on acid soil for maize (2012) – wheat (2013) rotation. Yield of maize on the control was only 1.41 t ha⁻¹ and barren plants 73% as affected by drought and high temperature stress in flowering stage, while on the 14 t ha⁻¹ treatments yield increased to the level of 4.51 t ha⁻¹ and barren plants reduced to the 60% level. Influenced by Fertilizer yields of wheat were significantly increased by 22% (8.47 t ha⁻¹) and 14% by using 7.0 and 14.0 t ha⁻¹ rates (Kovacevic et al., 2014b).

Increases in yields of both soybean and barley in our investigations could be explained primarily by the correction of soil acidity by liming. In acidic soil areas especially, when the pH drops below 4.5 the highly soluble toxic metals like aluminum (Al) are predominant in a soil solution. As a result, the concentration and supply of most basic plant nutrients become limited (Eduardo et al., 2005). Liming of acid soils may often increase the plant uptake of P by reducing the amounts of soluble Al rather than any direct effects on P availability (Curtin and Syers, 2001).

To reach their full potential, soybean grows best on soils of medium to high fertility with favorable soil pH. Besides that, soybean as a legume crop needs neutral pH reaction for nodules development. The optimum soil pH for soybean growth is 6.8, and the critical value ranges from 4.0 to 5.5 (Follet et al., 1981). Economic yield reductions due to soil acidity generally occur on sandy and silt loam soils at pH values less than 5.5. If soil pH values are 5.0 or less, liming should take priority over P and K fertilization. Soybeans may tolerate pH values as low as 5.2 on many alluvial clayey soils without significant yield loss (Slaton et al., 2017).

Tolerance to acid soils differs greatly among cereal species, and barley is usually considered the most susceptible crop (Garvin and Carver, 2003; Zhu et al., 2003; Wang et al., 2006; Paunovic and Madic, 2011). This fact could be used as an explanation for the very friendly response of barely to the applied treatment in our study. Komljenovic et

al., (2015) tested effects of liming with 10 t ha⁻¹ of hydrated lime and phosphorus fertilization by monoammonium phosphate to 1500 kg P₂O₅ ha⁻¹ on maize yield in northern Bosnia for maize in monoculture. Influenced by liming, yield (4-year mean) of maize was increased by 31%, while P effect was considerably lower (6.14 and 6.65 t ha⁻¹, for the control and average of ameliorative P treatments, respectively).

Conclusion

In our study, application of Fertdolomite resulted in a considerable yield increase of soybean and barley and these responses were mainly more expressed compared to the response of maize and wheat in our other similar investigations. Response of soybean and barley to Fertdolomite

was specific because the yield of soybean continuously increased to 23% under the maximum applied rate, while for the increase in barley yield by 34% the rate of 20 t ha⁻¹ was appropriate. By additionally increasing Fertdolomite rates to 30 and 40 t ha⁻¹, yields of barley were reduced to the control level.

Weather conditions also considerably affected the level of yields. Particularly low yields of barley were found as affected by plant density reduction due to extreme oversupply of precipitation in the winter and early spring periods. Extreme weather conditions are more frequent in the recent period as a result of climate change. With that regard, Fertdolomite application contributed to the alleviation of biotic stress induced by unfavorable weather conditions.

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Effects of iron, manganese and zinc enriched coffee and tea wastes on lettuce – a field trial

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Abstract: Ensuring proper microelement supply under alkaline soil conditions could be a challenge even with the application of synthetic chelates. In this study, the application of coffee and tea wastes enriched with water soluble inorganic iron, manganese and zinc compounds was compared to water solution application of the same compounds at the same amount on a field with an alkaline Calcaric Arenosol. One butterhead and two iceberg lettuce cultivars were used as test plants. The effects of microelement enriched wastes on microelement availability in the soil, measured by DTPA-TEA method, was not clear-cut. However, the soil application of those microelement enriched wastes increased the nutritional value of lettuce by resulting in significantly higher concentration in cores for all the three investigated microelements. The highest rate of increase was observed for iron. As a consequence, lettuce heads accumulated significantly higher amount of iron, while this was not the case for manganese and zinc. There were comprehensive differences in the microelement concentration of the cores of the three investigated cultivars, with the butterhead type having especially high iron concentration. Head weights were not affected by the treatments. Hence, under the field conditions of this study, higher microelement concentration and uptake in the lettuce heads was not a prerequisite for good lettuce yield, as it was proved by the results of a zero control. However, the soil application of microelement enriched coffee and tea wastes for supplying microelements for lettuce in alkaline soil proved to be promising, especially for iron.

Keywords: iron, zinc, manganese, butterhead lettuce, iceberg lettuce

Introduction

Soil or irrigation water alkalinity limits production of vegetable crops in many parts of the World (Roosta, 2011). Alkaline soil and alkaline irrigation water are also quite common in Hungary (Jones et al., 2005; Rácz, 2007). Uptake of some microelements, for example iron, manganese and zinc, are especially restricted under alkaline conditions (Marschner, 1998; Füleky and Rajkainé Végh, 1999). In order to avoid yield loss and reduced nutritional value under alkaline conditions synthetic chelates are applied to provide available microelements for the plants. Several different carriers (EDTA, HEEDTA, DTPA, EDDHA, EDDHHA and NTA) are used to produce microfertilizer chelates (Hoffmann and Górecki, 2000 cited in Tyksinski and Komosa, 2008). Compared to a conventional, water soluble inorganic microelement compound (ferrous sulphate), application of chelates gave good results in soilless lettuce production (Roosta et al., 2015). However, these compounds can be

very expensive; hence their use is often not cost effective (Roosta et al., 2015). Moreover, the application of these synthetic chelates is often less effective in soil culture. Even significantly decreased head weights and chelate excess symptoms as a result of Fe-EDTA+DTPA, Fe-DTPA and Fe-AM-4 applications were reported (Tyksinski and Komosa, 2007 cited in Tyksinski and Komosa, 2008; Kozik et al., 2011). Thus, there is a need for cheaper chelating agents which can be used effectively and economically even in open field production.

The use of agricultural waste products is a logical answer to the problem outlined above. Their volume is constantly increasing with growing population (Sönmez et al., 2017) and their organic matter content offers the possibility of containing potentially metal-chelating substances (Morikawa and Saigusa, 2008). Sönmez et al. (2017) applied greenhouse wastes, used coco peat and spent mushroom compost at different ratios for greenhouse-grown lettuce. Marketable yield

increased compared to the control while iron and manganese concentration did not decrease. However, zinc concentration in the lettuce heads fertilized by compost became lower.

Coffee and tea are among the most popular beverages worldwide. Large amounts of coffee and tea wastes are produced by companies manufacturing coffee and tea beverages. These wastes should be reused on a sustainable way (Morikawa and Saigusa, 2008). It was found that coffee waste application to an Arenosol in the Democratic Republic of Congo promoted nutrient retention of this sandy soil besides of several other favourable physico-chemical changes (Kasongo et al., 2011). Coffee and tea are very rich in phenolic compounds which can act as metal chelating agents (Brown et al., 1998). Morikawa and Saigusa (2008) composted coffee grounds and tea leaf wastes together with ferrous-sulphate. Application of the resulting compost increased plant-available iron concentration in neutral and alkaline soils, and significantly enhanced iron content of Japanese leaf radish. Top-dressing application of microelement enriched coffee and tea waste materials was suitable to increase iron, manganese and zinc content of rice grains and to enhance grain yield (Morikawa and Saigusa, 2011).

Lettuce is rich in mineral nutrients (Rubatzky and Yamaguchi, 1997). Calcium, iron and phosphorus content of lettuce is especially high, and its manganese and zinc concentration is also considerable (Hartz et al., 2007). Bosiacki and Tyksinski (2009) found the highest manganese and zinc content in lettuce, out of nine investigated vegetable crops. Hence, effectiveness of microelement fertilisation has special importance in lettuce production, both in soil and soilless cultures. Accordingly, microelement fertilisation of lettuce has being extensively studied recently (Tiksinski and Comosa, 2008; Kozik et al., 2011; Roosta, 2011; Roosta et al. 2015; Sönmez et al., 2017).

The objective of this study was to investigate the effects of tea and coffee waste products enriched with water soluble inorganic microelement compounds, on iron, manganese and zinc

concentration and uptake of lettuce heads under field conditions on an alkaline sandy soil in the central region of Hungary.

Material and methods

Climatic and edaphic conditions

The field trial was carried out in the horticultural experimental field of Szent István University (NL 47°35', EL 19°21') in 2013. The average air temperature of the cultivation period (22nd April – 14th June) was 16.8 °C, while total precipitation was measured 134 mm. The used field was under constant intensive vegetable cultivation for almost six decades. The loamy sand soil of the field was classified as Calcaric Arenosol. According to measurements made in a soil suspension using a soil/deionised water ratio of 1:5, the soil of the exact site showed the following characteristics: pH 8.0, EC 0.61 mS cm⁻¹, organic matter content 1.1%. Iron, manganese and zinc concentrations of the soil were measured from both 0.1 mol L⁻¹ HCl (Fe 25.2, Mn 116, Zn 29.0 mg kg⁻¹) and DTPA-TEA (diethylene-triamine-pentaacetic acid – triethanol amine) (Fe 16.3, Mn 16.6, Zn 9.0 mg kg⁻¹) extracts by an inductively coupled plasma (ICP-OES) instrument (Thermo Scientific iCAP 6000, Tokyo, Japan) following the methods of Jones & Case (1990) and Provin & Zhang (2014), respectively. Some chemical parameters of the irrigation water were the following: pH 7.25, EC 0.55 mS cm⁻¹, HCO₃⁻ 372 mg L⁻¹, Fe 0.3 mg L⁻¹, Mn and Zn under 0.01 mg L⁻¹.

Cultivation methods

Three lettuce cultivars were selected for the trial. A buterrhead type lettuce, 'Jolito RZ' (Rijk Zwaan Zaadteelt en Zaadhandel B.V.), a European iceberg type lettuce, 'Diamanthinas RZ' (Rijk Zwaan Zaadteelt en Zaadhandel B.V.) and a Japanese iceberg type lettuce, 'V lettuce' (Kaneko Seeds Co. Ltd). Unlike iceberg (or crisphead) lettuce, butterhead type does not form a firm, cabbage like core; hence the central part of the head is not separated from the outer leaves during harvest and selling. Seeds were sown into peat mixture filled plug seedling trays, having

61 cm³ plug volume, on 22nd March and raised in an unheated greenhouse. Basal fertilization was carried out by broadcasting on 21st April, providing nitrogen (N), phosphorus (P₂O₅) and potassium (K₂O) at 10, 11.8 and 13.9 g m⁻² rate, respectively. Raised beds having 0.6 m width and 0.1 m height were formed on the same day. Distance between the centres of two neighbouring beds was 1.4 m. Beds were covered with black polyethylene mulch. Seedlings having 5 true leaves were transplanted on 22nd April into double rows. Distance between the rows and also distance between the plants in a row were 0.3 m. The field was equipped both with micro sprinkler and drip irrigation (20 mm i.d., 30 cm emitter spacing, 1.7 L·h⁻¹ emitter discharge) systems. Irrigation was performed based on tensiometer readings. The amount of supplied irrigation water was 70 mm altogether. Nitrogen fertigation was applied on 17th and 25th of May through the drip irrigation system at 2.5 g m⁻² rate at each occasion, using ammonium-nitrate as fertilizer. In accordance with their growing periods, the three cultivars were harvested at different dates, 'Jolito RZ' on 27th May, 'V Lettuce' on 6th June and Diamantinas on 10th June.

Treatments

Methods of microelement (Fe, Mn, Zn) supply meant the treatments. Three treatments and a zero control (CNT) were applied on 21st April. Plants of the CNT were not supplied by any microelement fertilizers. Microelement enriched coffee waste (MCW) and tea waste (MTW) were prepared from coffee grounds or from tea drags, respectively, following the method described by Morikawa & Shinohara (2013). Water soluble inorganic microelement compounds, 116 g iron-chloride (FeCl₃), 44 g manganese-sulphate (MnSO₄*5H₂O) and 44 g zinc-sulphate (ZnSO₄*7H₂O), were mixed to 796 g coffee or tea waste and used as microelement sources. MCW and MTW were mixed at 10 cm depth into the soil at 100 g m⁻² rate simultaneously with bed formation. For an other treatment the same amount of inorganic microelement compounds (IMC) (FeCl₃ 11.6 g m⁻², MnSO₄*5H₂O 4.4 g m⁻², ZnSO₄*7H₂O 4.4 g m⁻², representing 4.00

g m⁻² iron, 1.00 g m⁻² manganese and 1.00 g m⁻² zinc concentrations, respectively) were supplied alike for the microelement enriched waste treatments. Microelements were dissolved in water, distributed onto the surface of the plots and mixed into the soil thereafter at 10 cm depth.

A randomised split plot design with three replications was used. Each plot contained 42 plants arranged in double rows. Accordingly, width and length of a plot was 0.6 m and 6.3 m, respectively, and consisted of three 2.1 m long subplots each containing 14 plants, representing the three cultivars. Hence, area of a plot was 3.8 m².

Measurements

The six central plants were sampled from every subplot. Stems were cut at the soil level, and fresh weight of the whole heads, including all the outer leaves, was measured immediately. In the case of the two iceberg type cultivars the core and the outer leaves were divided, also weighed separately and handled as two samples. The samples were dried in an oven at 65°C until reaching constant weight, then dry weight was determined. Iron, manganese and zinc concentration of lettuce cores and outer leaves were measured from dry grounded samples by an ICP-OES instrument (Thermo Scientific iCAP 6000, Tokyo, Japan) using the method of Provin & Zhang (2014). Microelement uptake of above ground parts of lettuce plants were calculated based on these concentrations and dry weight data. An average soil sample was formed from six subsamples for every subplot. Samples were taken from the 0 to 0.1 m depth on 14th June. Soil samples were air dried for four days on room temperature and passed through a 2 mm sieve. Plant-available microelement concentration of these samples was determined by the method of Provin & Zhang (2014).

Statistical analysis

Differences in means were tested by a two-way ANOVA (with microelement fertilizer treatment and cultivar regarded as factors) and subsequent post-hoc comparisons of means (Fisher's protected least significant difference (LSD) test at $P=0.05$).

Results and Discussion

Concentration of iron, manganese and zinc in the soil

The method of microelement supply significantly affected the plant-available microelement concentration in the soil, while lettuce cultivar and fertilizer x cultivar interaction did not reveal a pronounced effect (Table 1).

effective, significantly increasing its plant-available concentration in the soil compared to MTW and IMC treatments. This result is in good agreement with the findings of Kasongo et al. (2011). MTW resulted in higher manganese concentration compared to MCW application, while significant differences in manganese level could not be detected neither between the IMC and MCW, and the IMC and MTW treatments.

Table 1. Effect of fertilizer type on plant available concentration of iron, manganese, zinc in the soil after lettuce cultivation, measured by DTPA-TEA method

Cultivar	Fertilizer	Iron (Fe)	Manganese (Mn) (mg kg ⁻¹)	Zinc (Zn)
'Jolito RZ'	CNT	16.7	18.1	7.7
	IMC	18.5	23.1	11.6
	MCW	20.4	19.7	12.2
	MTW	19.1	22.7	12.3
'V Lettuce'	CNT	16.7	18.4	8.0
	IMC	18.4	21.1	10.7
	MCW	21.3	21.1	12.1
	MTW	20.2	21.1	12.0
'Diamantinas RZ'	CNT	17.1	18.8	7.8
	IMC	20.0	22.0	9.9
	MCW	20.9	21.0	11.8
	MTW	19.6	25.7	11.4
LSD ($P < 0.05$)		2.2	3.0	1.6
P value by factors				
fertilizer		9.96*10 ⁻⁶	4.00*10 ⁻⁵	1.84*10 ⁻⁹
cultivar		0.4395	0.1316	0.1859
fertilizer x cultivar interaction		0.8184	0.1867	0.7834
Average by fertilizer treatments				
CNT		16.8	18.4	7.8
IMC		19.0	22.1	10.7
MCW		20.9	20.6	12.0
MTW		19.6	23.2	11.9
LSD 5%		1.3	1.7	0.9
Average by cultivars				
'Jolito RZ'		18.7	20.9	10.9
'V Lettuce'		19.2	20.4	10.7
'Diamantinas RZ'		19.4	21.9	10.2

(CNT = Zero control; IMC = Inorganic microelement compounds; MCW = Microelement enriched coffee waste; MTW = Microelement enriched tea waste)

In the average of the three cultivars, all the three treatments increased plant-available iron, manganese and zinc concentration in the soil compared to the CNT. However, in regard of concentration, order of the treatments was different for the three investigated microelements. For iron, MCW application was the most

Zinc concentration in the IMC treatment was significantly lower compared to the two waste product treatments, while MCW and MTW produced practically equivalent results. The measured values represent a high level of iron, manganese and zinc supply, even in the CNT plots (Stevens; Mahashabde and Patel, 2012).

This could be the result of the long-time intensive farming and thus intensive fertilization in the experimental field.

Concentration of iron, manganese and zinc in the core

Both investigated factors and also their interaction significantly affected microelement concentration in the core (Table 2).

IMC treatment. Differences between the results of MCW and MTW treatments could not be proved statistically. IMC treatment significantly enhanced manganese and zinc concentration compared to the CNT, but this was not the case for iron. Rate of difference between the results of the CNT and the three treatments was bigger for manganese and zinc than for iron. Iron concentration in the butterhead lettuce was about eight times higher

Table 2. Effect of fertilizer type on iron, manganese and zinc concentration (mg kg⁻¹ fresh weight) in cores of three lettuce cultivars

Cultivar	Fertilizer	Iron (Fe)	Manganese (Mn) (mg kg ⁻¹ fresh weight)	Zinc (Zn)
'Jolito RZ'	CNT	22.88	3.04	1.82
	IMC	23.95	2.94	1.88
	MCW	27.47	3.53	2.14
	MTW	28.43	3.67	2.16
'V Lettuce'	CNT	2.47	0.94	1.57
	IMC	3.01	1.06	1.96
	MCW	3.77	1.18	2.08
	MTW	2.85	1.02	1.86
'Diamantinas RZ'	CNT	2.79	0.90	1.61
	IMC	2.82	8.61	6.53
	MCW	3.42	8.93	7.06
	MTW	3.61	8.92	7.19
LSD (<i>P</i> < 0.05)		2.23	0.58	0.37
<i>P</i> value by factors				
fertilizer		0.0019	1.19*10 ⁻¹⁵	7.83*10 ⁻¹⁷
cultivar		1.24*10 ⁻²⁴	5.03*10 ⁻²³	1.94*10 ⁻²⁴
fertilizer x cultivar interaction		0.0182	7.65*10 ⁻¹⁷	3.56*10 ⁻¹⁷
Average by fertilizer treatments				
CNT		9.38	1.63	1.67
IMC		9.93	4.20	3.46
MCW		11.56	4.54	3.76
MTW		11.29	4.54	3.74
LSD 5%		1.29	0.34	0.21
Average by cultivars				
'Jolito RZ'		25.68	3.29	2.00
'V Lettuce'		3.02	1.04	1.87
'Diamantinas RZ'		3.16	6.84	5.60
LSD 5%		1.11	0.29	0.18

(CNT = Zero control; IMC = Inorganic microelement compounds; MCW = Microelement enriched coffee waste; MTW = Microelement enriched tea waste)

Core of the lettuce is the consumed part; hence its microelement concentration has nutritional importance. Therefore it is of great importance that in the average of the three cultivars, application of both waste materials resulted in significantly higher concentration of all the three investigated microelements, compared to the

compared to the two iceberg cultivars (Table 2). This result is in agreement with the Japanese Food Composition Database. The USDA nutritional database (U.S. Department of Agriculture, 2015) also indicates higher iron content for butterhead (12.4 mg kg⁻¹) than for iceberg lettuce (4.1 mg kg⁻¹). Mou (2009) explained the lower nutrient

content of the core of iceberg type lettuce compared to more open-headed lettuces with its closed structure, as the synthesis or absorption of many nutrients is light dependent. On the contrary Rubatzky and Yamaguchi (1997) listed very similar iron content for iceberg and butterhead lettuces. In their study Roosta et al. (2015) did not find significant differences in iron and zinc concentration of butterhead and iceberg lettuces either.

There were also comprehensive differences among the manganese and zinc concentration of the three cultivars, with 'Diamantinas RZ' having 4-6 times higher values compared to 'V lettuce', and three times higher values compared to the butterhead type 'Jolito RZ' (Table 2). It is supposed that these high concentrations were the main reason for that the extent of difference between the results of the treatments and the CNT was by far the biggest for 'Diamantinas RZ' manganese and zinc concentrations. Due

to its higher manganese and zinc accumulating capacity this cultivar could exploit the higher plant-available microelement level of the soil ensured by the treatments (Table 1). Zinc concentration results for 'Jolito RZ' and 'V lettuce' were in good agreement with data of the USDA nutritional database (U.S. Department of Agriculture, 2015), which indicates 1.5 mg kg⁻¹ value for iceberg and 2.0 mg kg⁻¹ for butterhead lettuce.

Concentration of iron, manganese and zinc in the outer leaves

Outer leaves were investigated separately for the two iceberg type cultivars, as their weight equals the core weight. MTW treatment resulted in significantly the highest iron and manganese concentration in the outer leaves, while MCW did not increase them compared to the CNT (Table 3). All the three treatments increased zinc concentration compared to the CNT, with IMC treatment producing the highest value. In the

Table 3. Effect of fertilizer type on iron, manganese and zinc concentration in outer leaves of two iceberg type lettuce cultivars

Cultivar	Fertilizer	Iron (Fe)	Manganese (Mn) (mg kg ⁻¹ fresh weight)	Zinc (Zn)
'V Lettuce'	CNT	12.25	2.54	0.97
	IMC	9.45	2.54	1.73
	MCW	12.26	2.81	1.38
	MTW	19.68	3.60	1.62
'Diamantinas RZ'	CNT	12.27	3.59	1.41
	IMC	11.24	3.31	1.83
	MCW	14.06	3.84	1.60
	MTW	15.67	4.55	1.65
LSD ($P < 0.05$)		2.79	0.81	0.42
P value by factors				
fertilizer		5.96*10 ⁻⁶	0.0027	0.0046
cultivar		0.8820	1.30*10 ⁻⁴	0.0674
fertilizer x cultivar interaction		0.0205	0.9514	0.4953
Average by fertilizer treatments				
CNT		12.26	3.06	1.19
IMC		10.35	2.93	1.80
MCW		13.16	3.30	1.49
MTW		17.68	4.07	1.63
LSD 5%		1.98	0.57	0.30
Average by cultivars				
'V Lettuce'		13.41	2.87	1.43
'Diamantinas RZ'		13.31	3.82	1.62
LSD 5%			0.40	0.21

(CNT = Zero control; IMC = Inorganic microelement compounds; MCW = Microelement enriched coffee waste; MTW = Microelement enriched tea waste)

average of the two iceberg cultivars, iron content of the outer leaves was four times higher than that of the core (Table 2). This result is in good agreement with the findings of Mou and Ryder (2002). On the other hand zinc and manganese concentration in outer leaves of ‘Diamantinas RZ’ was much smaller than that of the core, while this was not the case with ‘V lettuce’.

Head weight

Core and head fresh weights and head dry weight were not affected by the fertilizer treatment and by treatment x cultivar interaction (Table 4). Kozik et al. (2011) did not find any significant differences either between their microelement fertilizer treatments, except for a yield decreasing effect of Fe-DTPA at the highest rate. However, our result is not in agreement with the findings of Morikawa and Saigusa (2008, 2011), who

discovered higher leaf radish fresh weight and rice grain yield as a result of application of microelement enriched coffee and tea waste materials. As it was expected prior to the trial, the highest head weights were produced by ‘Diamantinas RZ’ and the lowest ones by the butterhead type ‘Jolito RZ’.

Iron, manganese and zinc uptake of lettuce heads

As fertilizer treatments did not affect head weight results significantly, microelement uptake results showed similar tendency to the concentration data. Microelement uptake of lettuce heads was significantly affected not only by the two investigated factors but also by their interaction (Table 5). In the average of the three cultivars the two waste treatments resulted in significantly higher iron uptake compared to the CNT and to the IMC treatment. This latter treatment

Table 4. Effect of fertilizer type on head weight of three lettuce cultivars

Cultivar	Fertilizer	Core fresh weight (g)	Head fresh weight (g)	Head dry weight (g)
‘Jolito RZ’	CNT	683	683	29.8
	IMC	742	742	30.0
	MCW	749	749	30.3
	MTW	742	742	31.2
‘V Lettuce’	CNT	498	1064	48.7
	IMC	483	1065	49.2
	MCW	525	1112	47.0
	MTW	549	1131	53.0
‘Diamantinas RZ’	CNT	857	1591	74.4
	IMC	877	1660	71.4
	MCW	849	1608	66.1
	MTW	869	1614	68.0
LSD ($P < 0.05$)		117	174	8.2
P value by factors				
fertilizer		0.6258	0.2566	0.4994
cultivar		3.23×10^{-11}	5.09×10^{-16}	1.00×10^{-15}
fertilizer x cultivar interaction		0.9193	0.4663	0.5243
Average by fertilizer treatments				
CNT		679	1124	50.9
IMC		701	1194	50.2
MCW		705	1102	47.8
MTW		722	1166	50.7
LSD 5%		58	87	4.7
Average by cultivars				
‘Jolito RZ’		729	729	30.3
‘V Lettuce’		514	1114	49.5
‘Diamantinas RZ’		863	1597	70.0
LSD 5%		58	87	4.1

(CNT = Zero control; IMC = Inorganic microelement compounds; MCW = Microelement enriched coffee waste; MTW = Microelement enriched tea waste)

Table 5. Effect of fertilizer type on iron, manganese and zinc accumulation in three lettuce cultivars

Cultivar	Fertilizer	Iron (Fe)	Manganese (Mn) (mg plant ⁻¹)	Zinc (Zn)
'Jolito RZ'	CNT	15.62	2.08	1.25
	IMC	17.73	2.18	1.40
	MCW	21.10	2.72	1.60
	MTW	20.65	2.66	1.61
'V Lettuce'	CNT	8.16	1.91	1.33
	IMC	6.93	1.98	1.96
	MCW	13.01	2.65	1.96
	MTW	9.15	2.27	1.91
'Diamantinas RZ'	CNT	11.40	3.40	2.43
	IMC	11.35	10.17	7.16
	MCW	14.85	11.17	7.50
	MTW	13.53	10.46	7.21
LSD ($P < 0.05$)		2.99	1.15	0.74
P values by factors				
fertilizer		2.36*10 ⁻⁵	4.53*10 ⁻⁹	8.21*10 ⁻¹⁰
cultivar		9.94*10 ⁻¹²	1.24*10 ⁻¹⁸	2.93*10 ⁻¹⁹
fertilizer x cultivar interaction		0.3891	3.67*10 ⁻⁹	2.98*10 ⁻⁹
Averages by fertilizer treatments				
CNT		11.73	2.46	1.67
IMC		12.00	4.78	3.51
MCW		16.32	5.51	3.69
MTW		14.44	5.13	3.58
LSD 5%		1.73	0.67	0.43
Averages by cultivars				
'Jolito RZ'		18.78	2.41	1.47
'V Lettuce'		9.31	2.20	1.79
'Diamantinas RZ'		12.78	8.80	6.07
LSD 5%		1.50	0.58	0.37

(CNT = Zero control; IMC = Inorganic microelement compounds; MCW = Microelement enriched coffee waste; MTW = Microelement enriched tea waste)

did not enhance iron uptake. In average of the three cultivars, manganese and zinc uptake was significantly increased by all the three treatments. This latter result was mainly due to the three-fold increase in manganese and zinc uptake of 'Diamantinas RZ' as the effect of the treatments.

There were comprehensive differences among the cultivars in the accumulation of microelements (Table 5). Despite its inferior weight 'Jolito RZ' had significantly the highest iron accumulation, and hence nutritional value, due to its very high iron concentration (Table 2). Manganese and zinc uptake was significantly the highest for 'Diamantinas RZ' due both to its higher head weight and to its higher manganese and zinc concentration. No significant differences in the

uptake of these two microelements between 'Jolito RZ' and 'V lettuce' were found.

Conclusions

Application of microelement enriched coffee and tea wastes increased the nutritional value of lettuce by resulting in significantly higher concentration in cores for all the three investigated microelements (Fe, Mn, Zn). The highest rate of increase was observed for iron. There were comprehensive differences in the microelement concentration in the cores of the investigated cultivars, high iron content of 'Jolito RZ' and high manganese and zinc content of 'Diamantinas RZ' are worth noting. Although head weights were not affected by the treatments, due to the higher concentration, iron uptake was

significantly higher in the microelement enriched waste treatments, in some agreement with the soil extract measurements. In contrast with some previous experiments conducted with the same waste materials the yield could not be increased by the investigated microelement fertilization method. Hence, under the field conditions of this study, higher microelement concentration and uptake in the lettuce heads was not a prerequisite for good lettuce yield, as it was proved by the results of a zero control. However, even under the high microelement level condition of the soil of the experimental field, the application of

microelement enriched coffee and tea wastes for supplying microelements for lettuce in alkaline soil proved to be promising, especially for iron.

Newly developed techniques for recycle organic wastes like coffee and tea wastes will certainly increase the add-value of those wastes, whose disposal is currently an environmental concern, because presently they have few uses. These wastes are rich in polyphenols that can bind metals like iron, manganese and zinc, serving as a carrier for plant absorption, increasing the nutritional value of crops for human health.

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Study of yellow rust infection on various winter wheat genotypes

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Abstract: Winter wheat (*Triticum aestivum* L.) is our most important cereal. During its production several pathogens can infect the population. In our two-year study susceptibility of 9 winter wheat genotypes against the pathogen *Puccinia striiformis* var. *striiformis* was investigated by three different nitrogen supply levels on a humous sandy soil type. Percentage frequency of yellow rust infection was lower in case of the genotypes Hystar, Mv. Csárdás, Mv. Magdaléna than in case of HK1307, 1304KV, Antonius or Hywin. Based on our results it can be stated that in the crop year 2016 the highest susceptibility towards the pathogen yellow rust was observed for the winter wheat variety GK Csillag. No consequent effect of nitrogen active substance treatments on yellow rust infection could be stated, different applied nitrogen dosages did not affect the infection rate of different genotypes to a statistically verifiable extent.

Keywords: wheat, yellow rust, infection, susceptibility, nitrogen

Introduction

As a result of the production area of winter wheat the extent of yield loss due to pathogens is rather significant, which is combined with both weather conditions favourable for the infection of some pathogens, and the susceptibility of different genotypes towards pathogens. Yellow rust infection occurs in Hungary quite seldom, but in outbreaks (1977, 1985, 1994, 1995, 2000, 2001, 2014) (Békési and Viola 2000, Csősz et al. 1993, Csősz 2005, Limpert et al. 1994, Szunics et al. 1989) that is a consequence of the low temperature demand of the infectious pathogen (Szepessy 1977, Barabás 1987). Leaf rust, occurs worldwide wherever wheat is grown. Among the rusts, yellow (stripe) rust is the most serious in globally. Pustules are light yellow and occur on leaves in distinct straight-sided stripes about 1/16 inches wide and of regular length. The spores are yellow to orange in colour (Wellings 2011). Yellow rust is a highly destructive disease threatening wheat production and quality worldwide. This is mainly due to the pathogen's ability to mutate and multiply rapidly as well as to use its air borne dispersal mechanism from one field to another (Brown and Hovmöller 2002). In case weather conditions are favourable for the pathogen and the applied genotype is susceptible, the extent of yield loss may even range between 40 and 70% (I2). A number of genes controlling yellow or

stripe rust resistance in wheat has been identified (McIntosh et al. 2011, (I1)), but need to know more about the effect of several conditions.

Nitrogen (N) nutrition is thought to be an important environmental factor affecting quantitative resistance: high N is associated with increased severities of some foliar diseases such as cereal rusts and powdery mildew. Severe epidemics of yellow rust and substantial effects of N treatment on disease severity were observed by Bryson et al. (1995) and Paveley et al. (2005). Different mechanisms have been suggested to be involved in this response. Some studies suggest that increased crop density and canopy density associated with N fertilisation creates a more favourable microclimate for stripe rust development (Ash and Brown, 1991; Danial and Parlevliet, 1995)

Timing of nitrogen supply in wheat production is important, because in the early development stages (BBCH 30-50) wheat plants utilize the applied nitrogen, but their resistance against leaf diseases decreases in this stage (Nelson, 1982). Hornok and Pepó (2005) studied the resistance and yield of a winter wheat genotype in their experiment in case of the application of different pre-crops and plant protection technologies. Regarding the three studied crop years higher leaf rust infection rate was observed after wheat pre-crop. Unfavourable effects of different pre-crops (winter wheat, maize or pea) could be reduced

using intensive crop protection technologies. The studied genotypes showed susceptibility towards leaf rust (16.4%) in his experiment. According to the results of Vári and Pepó (2012) it can be stated that weather conditions that are more humid and warmer than the average are favourable for the occurrence and spread of the leaf rust pathogen. In case of an extensive plant protection experiment they found an infection rate of 24% for the pre-crop maize, and 31% for pea in case of the nutrient supply level of $N_{200} + PK$. Furthermore they concluded that pathogen infection parameters increased parallel to increasing nutrient supply levels. In the present study following objectives were set:

- Parameterisation of 9 studied winter wheat genotypes yellow rust (*Puccinia striiformis* var. *striiformis*) infection rates.
- Quantification of the effect of different nitrogen active substance dosage applications with regard to yellow rust infection rate in case of different winter wheat genotypes.
- Evaluation of the effect of different nitrogen supply levels on the yellow rust infected leaf area in case of different winter wheat genotypes.

Materials and methods

Small plot field experiments were set up in October 2014 and 2015 with 9 winter wheat genotypes (1304 KV, HK1307, HB0304, Mv Csárdás, GK Csillag, Mv Magdaléna, Antonius, Hywin, Hystar) and 3 nitrogen supply levels ($N_0 \text{ kg} \cdot \text{ha}^{-1}$, $N_{30} \text{ kg} \cdot \text{ha}^{-1}$, $N_{120} \text{ kg} \cdot \text{ha}^{-1}$) with 4 replications on humous sandy soil at the Nyíregyháza Research Institute of the University of Debrecen, Institute of Agricultural Research and Educational Farm. The studied 9 genotypes consist of landraces (1304 KV, HK1307, HB0304), widespread Hungarian varieties (Mv Csárdás, GK Csillag, Mv Magdaléna), hybrids (Hywin, Hystar), and an Austrian winter wheat variety (Antonius).

Before the evaluation leaf samples with visible symptoms were collected – 3 leaves per pot – and pathogens were identified with a microscope

type MOTIC SMZ 168. Disease scoring was executed in four replications. Percentage of yellow rust infected leaf area was determined. Degrees of yellow rust (*Puccinia striiformis* var. *striiformis*) severity was determined according to the modified Cobb-scale (Figure 1).

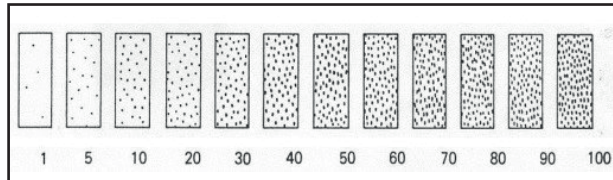


Figure 1. The modified Cobb-scale: severity of rust infection

Monitoring of yellow rust (*Puccinia striiformis* var. *striiformis*) was executed on the following dates:

- 9.06.2015. (on the figures marked as 2015.)
- 06.06.2016. (on the figures marked as 2016.)

During the experiment daily average temperature and daily amount of precipitation were recorded as well. Daily average temperature was over $10 \text{ }^\circ\text{C}$ from the end of March in both studied crop years that was favourable for the pathogen development. In case of yellow rust germination and infection optimum of uredospores is between $10\text{-}15 \text{ }^\circ\text{C}$ (I2), thus temperature was favourable for the infection of the pathogen already in the beginning – middle of April.

Regarding precipitation it can be stated that May was rather wet in both crop years (2015: 52.5 mm; 2016: 66.8 mm), that was favourable for the multiplication and spread of the pathogen.

For data analyses ANOVA tests were conducted using the Tukey's Multiple Range Test for mean separation. The analysis of all data was conducted using SigmaPlot for Windows Version 12.0 (Systat Software Inc., Germany) with the significant level determined at 95% confidence limit ($p \leq 0.05$).

Results

The effect of genotypes and nitrogen supply on the severity of winter wheat (*Triticum aestivum* L.) yellow rust (*Puccinia striiformis* var. *striiformis*) infection was studied on

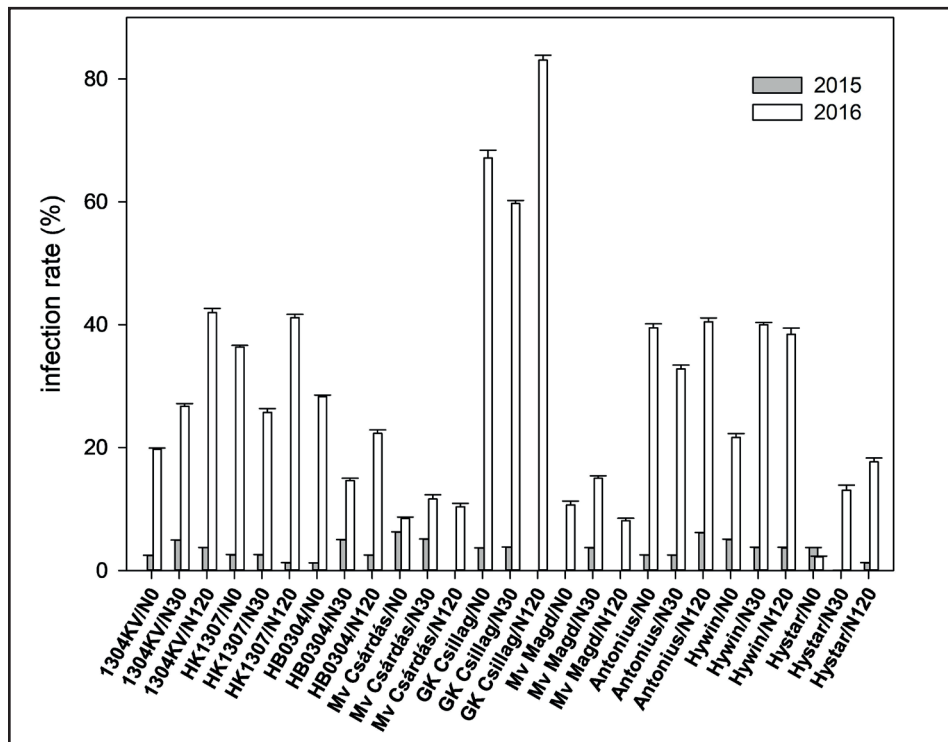


Figure 2. The effect of genotypes and nitrogen supply on the development of yellow rust (*Puccinia striiformis* var. *striiformis*) infection rate of winter wheat (*Triticum aestivum* L.) on humous sandy soil (Nyíregyháza, 2015-2016) n=4, \pm s.e. No significant differences between treatment and varieties at $p \leq 0.05$ level

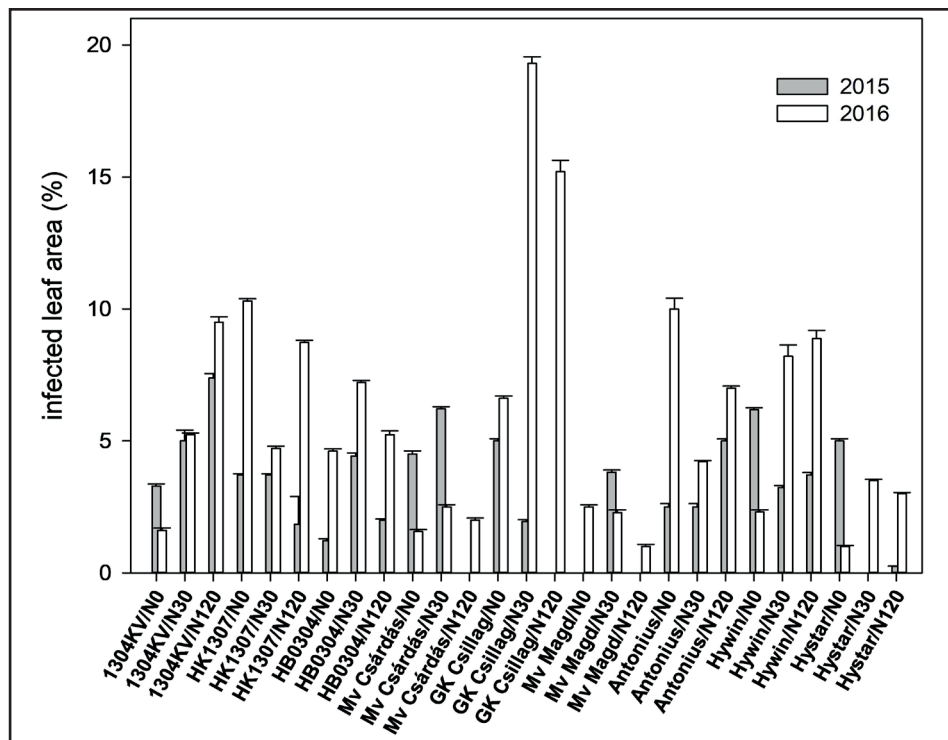


Figure 3. The effect of genotypes and nitrogen supply on the development of yellow rust (*Puccinia striiformis* var. *striiformis*) infected leaf area of winter wheat (*Triticum aestivum* L.) on humous sandy soil (Nyíregyháza, 2015-2016) n=4, \pm s.e. No significant differences between treatment and varieties at $p \leq 0.05$ level.

humous sandy soil (*Figure 2*). Regarding the results of 2015 it can be stated that there was no significant difference between the infection frequency of the pathogen in case of different genotypes and nitrogen treatments. In the 2016 vegetation period the infection rate (%) was generally 3-4 times more, than in 2015. In the crop year 2016 no stable statistically verifiable difference was observed regarding the average of the nitrogen supply treatments. Infection rate of the studied Hystar, Mv Csárdás, Mv Magdaléna winter wheat genotypes was lower, than that of genotypes Antonius, Hywin, HB0304, HK1307 and 1304KV. Independent from nitrogen supply levels significantly higher infection rates were observed for the variety than in case of other genotypes or the respective nitrogen supply levels. However, this tendency could not be revealed in the crop year of 2015.

The yellow rust infected winter wheat leaf area rates (%) were also determined in the different crop years (2015, 2016) (*Figure 3*). In the crop year 2015 the infected leaf area rate was higher in case of the genotypes 1304 KV, Antonius, Hywin and Hystar by the nutrient treatment N120, than in case of the supply level N30. In 2016 the severity (%) was much higher in HK1307 and GK Csillag, than in 2015. Regarding the results of 2016 it can be concluded that the genotype GK Csillag showed higher susceptibility towards yellow rust than any other studied genotype. However, in case of the other studied genotypes and nitrogen supply levels no significant difference was found between the rate of infected leaf areas.

Discussion

In the present study susceptibility of 9 winter wheat genotypes towards yellow rust (*Puccinia striiformis* var. *striiformis*) infection by the application of different nitrogen supply levels was studied under field conditions on a humous sandy soil type. The infection rate of the genotypes Hystar, Mv Csárdás, Mv Magdaléna was lower than that of the genotypes HK1307, 1304KV, Antonius and Hywin.

In the crop year 2016 the genotype GK Csillag showed the highest susceptibility towards yellow rust pathogen, but between other genotypes there were no statistically verifiable differences.

Some studies suggest that increased crop and canopy density due to N fertilisation means a more favourable microclimate for yellow rust development (Ash and Brown, 1991; Daniel and Parlevliet, 1995), thus increased nitrogen fertilization should increase the infection rate of yellow rust. In our study the increased nitrogen treatments did not affect the yellow rust infection frequency and infection rate, there was no statistically differences between the treatments. Other mechanisms suggested in the literature from studies on a range of pathosystems include changes in biochemical processes in the plant, such as a decrease in the content of phenol with an increase of nitrogen application (Király, 1964) and changes in crop canopy structure (Tompkins et al. 1992). Early studied declared, that increases in the severity of yellow rust (Huber and Watson, 1974) were observed with application of nitrate-N, but decreases were observed with ammonium-N, although both N forms would have been expected to increase canopy size.

Different sensitivity was found among the genotypes, breeding for resistance to yellow rust and developing new resistant cultivars became the main target in wheat breeding programs and considered as the most economical and effective way to eliminate the use of fungicides and reducing crop losses caused by the disease.

Acknowledgement

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The influence of different substrates on the reproduction traits of *Folsomia candida* (Collembola) in an insecticide test

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Abstract: The standard OECD tests for *Folsomia candida* should be performed on the standard OECD soil. However, if it is necessary to measure more parameters than the juvenile number and mortality, the use of different substrates is necessary. But these substrates can modify the results of the experiments comparing to the standard OECD soil, resulting in different toxicity values. In our research, we tested the toxicity of an insecticide, Trebon® 10F to *F. candida* (Collembola). We tested the dose-dependent effect of Trebon® 10F on the egg number and the egg size on the substrates of (i) plaster of Paris mixed with activated charcoal and (ii) in standard OECD soil on two generation of collembolas.

Similarly to our previous study, the insecticide had a dose-dependent negative effect on the parent's egg size in the OECD soil, but on plaster of Paris, no response was found. The egg number did not show any response to the treatments. On the contrary, in the offspring generation, the egg size increased both in OECD and plaster of Paris tests. In the soil test, parent collembolas laid significantly more eggs, while the offspring laid much fewer, compared to the plaster of Paris test. Similar results were found in the case of egg size. In the soil test parent collembolas laid larger, while the offspring laid smaller eggs, compared to the plaster of Paris test.

In multigenerational tests (i) Trebon 10F could affect the different generations in the opposite way. The experiment performed on the plaster of Paris (ii) could underestimate toxicity compared to the OECD soil test. This should be taken into account when Ecotoxicologists interpret their data.

Keywords: springtail, reproduction, OECD soil, plaster of Paris, etofenprox

Introduction

Folsomia candida Willem, 1902 (Collembola, Isotomidae) is one of the most often used model animal in soil ecotoxicological tests. This species is distributed all over the world in the organic matter-rich soils. *Folsomia candida* is easy to rear in the laboratory because it is a parthenogenetic species (Fountain and Hopkin, 2005). Consequently, it is the subject of standard soil ecotoxicology tests (ISO, 1999, 2011; Canada, 2007; OECD, 2009).

Different properties of the soil, such as organic material content or pH are usually taken into consideration in ecotoxicological experiments. According to pore-water hypothesis, toxicity of xenobiotics is mainly influenced by their availability in the soil pore water, which depends primarily on soil pH and organic matter content (Van Gestel, 1997). Different environmental parameters can modify the toxicity of the xenobiotics significantly through changing their bioavailability. Bioavailability can not be described with the pore-water hypothesis

in every case, while for example, springtails live in the air-filled pores and in the litter layer where they do not directly get into contact with pore-water (e.g., Pedersen *et al.*, 1997; Pedersen, 1999; Pedersen, Van Gestel and Elmegaard, 2000). A further problem may arise, namely that accurate measuring of pore-water concentration can be quite difficult (Pedersen, Van Gestel and Elmegaard, 2000). Organic matter influences toxicity not only by binding xenobiotics but through modifying microbial degradation (Shrestha *et al.*, 2015) and enhancing photodegradation (Amarathunga and Kazama, 2014).

Temperature could also affect toxicity in poikilothermic animals, like arthropods. The activity of animals decrease at a lower temperature, so they have reduced contact with the chemicals. Furthermore, because of the lower metabolism rate, detoxification rate will also decrease (Heimbach and Baloch, 1994; Martikainen and Rantalainen, 1999).

A standard method of keeping, rearing and testing

collembola was developed by Goto (1960). According to his method, collembola is kept on the plaster of Paris mixed with activated charcoal. If pesticide test is performed on this substrate, chemicals could be bound by the activated charcoal (Li *et al.*, 2016). Crommentuijn *et al.* (1997) found that increasing organic matter content of artificial soil (2-10%) can decrease the solvated cadmium content of the soil by 50%. Moreover, decreasing pH caused more available cadmium in the pore-water. Similar results were found in other studies as well (Van Gestel and Mol, 2003; Waalewijn-Kool *et al.*, 2014).

There are some important population parameters, that could not be measured in soil or artificial soil. For example, collembola eggs are not removable from the soil. Moreover, the effect of the xenobiotics is determined in standard tests at the end of the experiment (Fountain and Hopkin, 2001), so the measure of the exact time-response curve is problematic in the soil. Consequently, it is not possible involving egg parameters in the experiment if it is performed in the soil. This problem can be easily solved by using plaster of Paris instead of soil. Collembola can lay eggs on this flat surface, and the total number of eggs and any egg parameters are observable later on. One of the most studied parameters in ecotoxicological experiments with *F. candida* is the egg number, which is investigated from an ecotoxicological and evolution biological point of view. From the ecotoxicological point of view, the egg number decreases due to the effect of heavy metal contamination (Fountain and Hopkin, 2001; Smit *et al.*, 2004). If evolution biology of *F. candida* was in the focus of the study, difference between clonal lines (Stam, Leemkule and Ernsting, 1996), life-history traits (Tully and Ferrière, 2008; Hafer *et al.*, 2011), or *Wolbachia* symbiont presence (Timmermans and Ellers, 2009) were studied. Other parameters as the egg shape, the egg viability, and the egg size are rather involved in ecological and evolution biological studies not in ecotoxicological ones (Stam, Leemkule and Ernsting, 1996; Tully and Ferrière, 2008; Timmermans and Ellers, 2009; Hafer and Pike, 2010; Hafer *et al.*, 2011). For

example, Tully and Ferrière (2008) measured egg size, egg number, clutch size and clutch number to reveal the different life-history strategies of *F. candida* reacting to different food availability and overcrowding. Another parameter is the growth of animals which is not measurable in experiments carried out in soil only in plaster of Paris. The growth of animals were estimated by weight (Stam, Leemkule and Ernsting, 1996; Smit *et al.*, 2004) or length (Fountain and Hopkin, 2001) of the animals. Smit *et al.* (2004) measured the dose-dependent effect of dietary zinc on *F. candida* by measuring the fresh weight of the animals. The weight of the animals was significantly lower in the treated group than in the control. The clutch size was decreased by zinc, and the juvenile period also became longer, as the hatching time, as well as, the hatching success also decreased significantly.

The aim of the study was to reveal whether different substrates, as artificial soil and plaster of Paris, will cause a difference (i) in the egg number or (ii) in the egg size of *F. candida* in a dose-response test with the pesticide Trebon® 10F? Moreover, it was questioned, whether (iii) a different effect is observable on parent and offspring generation in a two-generation test, and if so, (iv) is the substrate strengthening or weakening the effect?

Materials and Methods

F. candida was obtained from the stock population reared in the laboratory of the Szent István University, Department of Zoology and Animal Ecology for the past 20 years. Collembolas were kept in Petri dishes with a diameter of 9 cm and height of 1 cm based on the method of Goto (1960), so the dish was poured with plaster of Paris mixed with activated charcoal (10:1 volume ratio). The animals were kept at a temperature of 20 ± 0.2 °C, with constant humidity (~100%) and in total darkness. Petri dishes were watered, and the collembolas were fed with dry baker's yeast once per week ad libitum. During the breeding phase, they were aerated weekly. All phases of the experiment were performed at a temperature of 20 ± 0.2

°C, with constant humidity (~100%) and in total darkness except when individuals were moved. Collembolas were transported with the aid of a small aspirator.

The Trebon® 10F (water soluble) is pyrethroid insecticide with the active ingredient, etofenprox. Etofenprox is an UV sensitive chemical. It degrades fast in homeotherm animals. It affects the sodium channels of the neurones inducing constant stimuli (FAO, 2006). It is not used in Europe anymore only in Asia, but it was applicable in orchards, cereals, maize, and forests. Nowadays Trebon® 30 EC (a.i. etofenprox) have been used instead, which contains aromatic hydrocarbons in order to enhance the dissolution. Recommended field concentration of Trebon® 10F in orchards and in forests is 0.883 ml/l. A reproduction test of *F. candida* was performed with the field concentration (0.883 ml/l) and its tenth diluted (0.0883 ml/l) and tenfold concentrated solution (8.83 ml/l), extended to parents and offspring.

Two experiments were performed. For the first experiment standard OECD artificial soil was used (OECD, 2009), which contains 74% sand, 20% kaolin clay, 5% sphagnum peat, and 1 % calcium carbonate, and the pH 7.29. In the test with the artificial soil (soil test hereafter) 10-12 days old synchronised individuals (n = 90 per treatment) were placed in plastic boxes (5 cm high, the basic diameter was 5.3 cm, and upper diameter was 6.6 cm). At this age, the size of the animals was 0.90 ± 0.11 mm. The box was filled with 24.5 g of dry soil and mixed with 5.5 ml of pesticide solution or tap water (60% water holding capacity). Four boxes were used, three for the treatments plus one for the control. After 20 days all of the animals were carefully separated from the dry soil. Thereafter, 30 out of the 90 individuals were chosen randomly from each treatment and the collembolas were placed individually in Petri dishes (9 cm in diameter) prepared in 10:1 volume ratio of plaster of Paris and activated charcoal. The new place induced egg laying in most cases. The boxes were opened for aeration, feeding, and cleaning of the mould if needed once every week. So, each animal was

kept alone during the measurements and get a unique identification number (ID). The eggs were photographed on the 9th day, see in details later. At the start of the egg-laying phase, the animals were 1.99 ± 0.11 mm long. There were 30 replicate per concentration.

The second experiment in Petri dishes with plaster of Paris mixed with activated charcoal (plaster of Paris test hereafter) was performed. The collembolas were transferred to Petri dishes soaked with the concentrations mentioned before (30 dishes per concentration and 30 dishes as control). The animals were transferred to the Petri dishes at the age of 10-12 days, and they were left there for 20 days. The dishes were opened for aeration, feeding, and cleaning of the mould if needed once every week. Then egg-laying period began. The eggs were measured on the 9th day. Collembolas were kept individually during the experiment and get an ID. There were 30 replicate per concentration.

The egg clutches were spread carefully with a wet brush and a digital photo was taken of each (Olympus C7070 Wide zoom camera with Olympus C5060 ADL optic). The eggs were numbered in the photo manually. Thereafter, ten eggs were chosen randomly from each clutch with a random number generator. The first ten eggs with the smallest random number were chosen. The shortest and longest diameters of the eggs relative at a 90° angle to each other were measured with the aid of the ImageJ open source software (Schneider, Rasband and Eliceiri, 2012). A premade standard was used to calibrate the measurements at every magnification. The mean of five measurements was used as a final conversion rate from pixel to mm.

From the offspring generation, individuals were chosen randomly. In the soil test collembola was kept on artificial soil for 20 days as described above (different lineages were not mixed, the pedigree of the animals was recorded). In the plaster of Paris test, they get on Trebon® 10F soaked Petri dishes, and they were left there for 20 days. Then egg-laying period began. After 9 days the clutches were spread carefully with a wet brush and a digital photo was taken by each.

Thereafter, ten eggs were chosen randomly from each clutch with a random number generator as described above. The shortest and longest diameters of the eggs relative at a 90° angle to each other were measured. All of the statistical analyses were made with the R Statistical program 3.1.1 (R Core Team, 2012). The total number of laid eggs and the size of eggs were analysed with more type of models. The egg diameters were transformed so that the shortest and longest diameters were multiplied by each other and the square root of it was extracted. This kind of transformation results in a diameter as if the eggs were perfectly round. This is the geometrical mean of the two diameters which is less influenced by the shape of the egg than the mathematical mean. This measure is called egg size, hereafter. Respectively the logarithm of concentrations was inserted in the models to reduce the number of inner comparisons and the probability of the type I error.

The effect of concentration and test method on egg number were analysed with a linear model. The mixed effect model was applied for egg size analysis because the measurements on the eggs from the same clutch were not independent. Mixed effect model corrects the calculation accordingly with the so-called random effect (nlme package from R, (Pinheiro *et al.*, 2013). The identification numbers of the individuals were the random effect, which showed that the eggs had originated from the same dish. The total data set (which contains both tests) and concentrations were tested separately too. In models with total datasets including both tests interaction of the concentration and test type was included.

Results

Concentration does not have a significant effect on the total number of eggs either in soil or in the plaster of Paris test. In the soil test the egg size was significantly affected by concentration ($p < 0.001$, $t = -5.16$) (residual and standard deviation of the model were 0.018 and 0.0112, respectively). If Trebon® 10F concentration increase the egg size decreases, while there was

no effect on egg size in the plaster of Paris test.

In the offspring generation, egg number was affected neither in the soil nor in the plaster of Paris test. The egg size was increased with Trebon® 10F concentration in both test types. Pesticide effect was significant in the soil test $p < 0.001$, $t = 4.57$ (residual and standard deviation of the model were 0.022 and 0.010) and in the plaster of Paris test $p = 0.021$, $t = 2.38$ (residual and standard deviation of the model were 0.017 and 0.0096) as well.

Statistical data are summarised in Table 1. In the soil test, parent animals laid significantly more eggs than in the plaster of Paris test, but offspring animals laid much fewer in the tenth concentration and in the field concentration. Similar results occurred in the case of egg size. In the parent generation animals in the soil test laid larger eggs in the control (14.4% larger) and in the tenth concentration (9.7% larger). By contrast, the offspring in the soil test laid smaller eggs in the control (8.3% smaller) and in the tenth concentration (11.9% smaller).

Discussion

In the case of the soil experiment, parents invested less into their offspring. In the plaster of Paris experiment, no such observable effect was found. This is consistent with the results of Mousseau and Fox (1998), who showed that trade-off between the reproduction and production is a common phenomenon in insect species. So our result could refer to poor environmental conditions (Fox, Thakar and Mousseau, 1997; Fox and Czesak, 2000), which was resulted in insecticide treatment. In the case of offspring, in both tests, egg size is increasing as a reaction to the insecticide treatment. Because of the constant egg number, the investment into the next generation increases, too. According to Tully and Ferrière (2008), the increase of the egg size improves the viability of the eggs of *Folsomia candida*. Similar trends were found with many herbivore insect species (Awmack and Leather, 2002). According to our results, it is plausible, that the parents can transmit

Table 1. The statistical summary of Trebon 10F insecticide effects on *Folsomia candida*. The “model elements” refer to tested effects. The “difference” means the estimated difference to the effect of the model element to the total number of eggs (first column) and to the egg size (second column). The total number of eggs is the number of eggs laid during the experiments. The egg size is the geometrical mean of the shortest and longest diameter of the egg. The test type difference is the plaster of Paris test minus soil test. In the case of concentration model element is the continuous concentration effect. The difference between the different concentration levels (0.1; 1; 10) from the control are in the separate concentrations models. Total dataset models compare the plaster of Paris test to the soil test. The significant p-values are bolded.

Total number of eggs				Egg size			
Model elements	p-value	t-value	difference	Model elements	p-value	t-value	difference
Parents' total dataset				Parents' total dataset			
Test type	<0.001	4.731	51.93	Test type	0	4.618	0.0145
Concentration	0.245	-1.168	-1.95	Concentration	0.883	0.147	0.0008
Interaction	0.091	1.704	3.57	Interaction	0.004	-2.956	-0.0192
Parents' test type difference in separate concentrations				Parents' test type difference in separate concentrations			
Control	0.001	3.720	65.60	Control	<0.001	4.048	0.0187
0.1	0.033	2.242	41.88	0.1	0.005	3.056	0.0123
1	0.010	2.771	51.69	1	0.560	0.589	0.0035
10	<0.001	4.814	88.29	10	0.488	-0.706	-0.0039
Offspring's total dataset				Offspring's total dataset			
Test type	<0.001	-4.856	-107.90	Test type	0	-4.385	-0.0112
Concentration	0.001	-3.290	-12.00	Concentration	0.099	1.660	0.0064
Interaction	0.020	2.352	10.53	Interaction	0.134	1.506	0.0073
Offspring's test type difference in separate concentrations				Offspring's test type difference in separate concentrations			
Control	0.138	-1.520	-52.64	Control	0.001	-3.532	-0.0099
0.1	0.001	-3.683	-146.20	0.1	<0.001	-3.925	-0.0141
1	0.018	-2.480	-113.56	1	0.170	-1.400	-0.0063
10	0.984	-0.021	-0.53	10	0.389	-0.873	-0.0044

information about the unfavourable environment to their offspring. The transmission could happen through the egg content or through epigenetic variation. Further explanation of this hypothesis is to be found in Szabó and Bakonyi (2017).

The comparison of the two substrates shows, that parents lay more and larger eggs in a soil test, however offspring lay larger eggs and with twice more eggs in the plaster of Paris test. So in general in the soil test parents invest more in their offspring, but offspring invest much less in their eggs than in the plaster of Paris test. One explanation of this result is that probably in the soil the collembola has a contact with the pesticide on a much larger surface. So collembolas can take up Trebon® 10F not

only through the legs, but through the whole body surface, and this fact could cause higher toxicity. According to Smit *et al.* (2004), this toxicity is caused by the more intensive contact with the pore-water. The activated charcoal mixed in the plaster of Paris could bind the compound permanently (Lebo *et al.*, 2003; Yu *et al.*, 2011). That is why in experiments carried out with activated charcoal effective concentration of a pesticide is less than the nominal one. (Crommentuijn, Doornekamp and Van Gestel, 1997; Van Gestel and Mol, 2003; Waalewijn-Kool *et al.*, 2014; Li *et al.*, 2016). Crommentuijn *et al.* (1997) found that increasing organic matter content of artificial soil significantly decreased the cadmium content of the pore water. Kang *et al.* (2001) found similar

results as ours in the case of *Paronychiurus kimi* (Collembola), where the glufosinate-ammonium was less toxic to springtails on the plaster of Paris than in artificial soil.

We can assume, that the binding effect was not linear in the present experiment. While the organic matter content decreases the effect of the Trebon® 10F on the egg number approximately to the half in the artificial soil, but the effect is not halved in the case of the egg size. This fact supports the hypothesis that egg size is more sensitive or more strictly regulated parameter compared to the egg number.

Consequently, the substrate is a key factor in soil ecotoxicological studies with collembola.

In conclusion, the following hypothesis could be formulated: (i) Trebon 10F could affect different generations in the opposite way. The experiment performed on the plaster of Paris (ii) could underestimate toxicity compared to the OECD soil test. Further studies should be carried out with different materials in the plaster of Paris (e.g. graphite instead of activated charcoal), to find the substrate in which soil toxicity can be reliably estimated together with measuring egg and growth parameters.

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Determination of compost maturity using the Hot Water Percolation (HWP) method

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Abstract: Maturity is one of the parameters that needs to be examined when producing composts. The present paper aims to evaluate whether the Hot Water Percolation (HWP) method elaborated for soil analysis can be used to determine the maturity of composts. The method involves percolating boiling water through dry, ground compost samples, followed by the measurement of the dissolved carbon and mineral nitrogen contents and the spectral properties of the solution. Two groups of compost samples were examined. In one group the above parameters were recorded throughout the 42-day maturing process, while in the other group these parameters were measured on 7 samples judged to be mature and 5 judged to be fresh. The HWP-soluble $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$ ratio was less than 0.16 in mature composts; for both compost groups the HWP-C value was less than 100 mg L^{-1} , absorbance at 254 nm was $1.0\text{-}2.0 \text{ cm}^{-1}$, the E_4/E_6 ratio was generally less than 2.0 and the SUVA (specific UV absorption) value at least 1.4 but generally higher, depending on the initial materials. The extracts obtained using the HWP method provide a good demonstration of the maturity stages in the composting process in spite of the speed of extraction.

Keywords: compost maturity, E_4/E_6 ratio, HWP soluble carbon, $\text{NH}_4/\text{NO}_3\text{-N}$, spectral properties

Introduction

Compost maturity has been determined with different agents using a variety of indices based on the physical, chemical, biological, biochemical and humification characteristics of organic matter (Körner et al. 2003). Of all these indices, related to the water-soluble fraction of the organic matter because most of the biochemical transformation of organic matter take place in this fraction during composting. The water-soluble carbon content, the C/N ratio, the nitrate content, the pH and the temperature were found to be important factors affecting microbial community structure and metabolic pathways (Zhang et al. 2011; Sundberg et al. 2004). The intensity of organic matter degradation and the evolution of water-soluble compounds depend on the kind of material used in the starting mixture. Key indicators include water-soluble organic carbon (C_{ow}), and the ratios of water-soluble carbon to water-soluble organic nitrogen ($\text{C}_{\text{ow}}/\text{N}_{\text{ow}}$) and water-soluble organic carbon to total organic nitrogen ($\text{C}_{\text{ow}}/\text{N}_{\text{OT}}$). The values proposed for indicating maturity are the following: $\text{C}_{\text{ow}} < 0.5\text{-}1.7\%$, $\text{C}_{\text{ow}}/\text{N}_{\text{ow}} 5\text{-}6$, $\text{C}_{\text{ow}}/\text{N}_{\text{OT}} < 0.40$ (0.55), (Sanchez-Monedero et al. 2001; Bernal et al. 1998). The wide variation in recommendations for these parameters suggests that the value of

WSC should be established based on compost feedstock (Benito et al. 2009). The ratio of inorganic forms of nitrogen has been used as a criterion for assessing the maturity of compost. A NH_4/NO_3 ratio of < 1 at the end of the process suggests that the final compost has reached maturity (Bernal et al. 1998; Aparna et al. 2008).

E_4/E_6 has long been considered to reflect the degree of condensation of the aromatic nucleus of humus, thus indicating its maturity (Aparna et al. 2008). With an increase in composting time, the E_4/E_6 ratio decreases significantly, suggesting that carbohydrates and quinones have been oxidized and bound to methoxyl groups and/or aliphatic side chains in humic substances. A suitable degree of maturity and stability is denoted by a lower ratio of E_4 to E_6 at the end of the composting process (Sellami et al. 2008).

Usually UV absorption of bulk water-extractable organic matter samples was measured at 254 nm. The absorbance was normalized to the concentration of dissolved organic C, giving the specific UV absorption (SUVA_{254}), which serves as an indicator of the aromatic character of organic matter. The organic C content of water-extractable organic matter decreased significantly from 6.0 mg L^{-1} in the initial material to 1.5 mg L^{-1} during the 250-day composting period

towards the end of the process. The $SUVA_{254}$ values obtained for bulk water-extractable organic matter remained constant during the first 28 days of composting (with an average value of $0.97 \text{ L mg}^{-1} \text{ m}^{-1}$), but subsequently increased steadily to 1.77 and then to $3.02 \text{ L mg}^{-1} \text{ m}^{-1}$ by days 90 and 250, respectively (Said-Pollicino et al. 2007; Jaffrain et al. 2007).

The present paper aims to evaluate whether the Hot Water Percolation (HWP) method elaborated for soil analysis can be used to determine the maturity of composts. A further aim was to investigate whether the maturity values determined for German composts confirmed the results obtained using the HWP method.

Materials and methods

Compost experiment in Gödöllő

Two groups of composts were used in the experiments. In the first case the maturity of composts prepared at an open composting site at the Waste Management Unit in Gödöllő using the GORE™COVER technology was examined. This is a closed system measuring 1 heap $8 \times 35 \times 3.5 \text{ m}$ with forced aeration (blowers on for 5 min every 20 min). The cover used was a PTFE semipermeable membrane which helps to ensure the diffusion of gases but retains heat and steam.

Using this technology the composting process involves 4 weeks of intensive maturing, followed by a further 2 weeks without cover or aeration.

The composting windrow was built from garden, park and home wastes on 10th October and the process was terminated on 21st November. The outside temperature was originally above 10°C, dropping to about 5°C from 15th November onwards.

The sampling times were 0, 2, 8, 16, 21, 30, 37 and 42 days after the establishment of the compost windrow, with a sampling depth of 40 cm.

Investigation of German compost materials

The other group of composts consisted of samples kindly provided by BGK

(Bundesgütegemeinschaft Kompost e.V., Köln). Five compost samples were deemed to be “fresh” and seven samples were considered to be “mature” according to the German system of classification, which is based on the Dewar self-heating test (Table 1. and 2.).

Compost analysis

The organic carbon content and spectral properties of the HWP extracts were determined. Hot water percolation (HWP) is a new and easily applicable soil extraction method (Füleky and Czinkota 1993) which was adapted for compost analysis (Füleky et al. 2003). In the course of hot water extraction, 5 g dry, ground compost was placed in the sample holder, after then boiling water was poured onto the compost and the resulting solution was collected as five 100 cm³ samples. Approx. 1 min was required to obtain 100 cm³ extract.

The extractable, hydrolysable and readily soluble elements and compounds are extracted (102-105°C) at 120-150 KPa during hot water percolation (Takács and Füleky 2003). The organic carbon, nitrate-N and ammonium-N contents of each extract were determined by distillation. The chromic acid test was used to

Table 1. Fresh compost samples composition of German composts

Sample	Ratio v/v%	Composition
5/1	50:50	scb/gpw
5/2	70:30	scb/gpw
5/3	90:10	scb/gpw
5/4	70:20:10	scb/gpw and paper
5/5	50:50	scb/gpw

scb: separately collected biowaste; gpw: garden or park waste

Table 2. Mature compost samples composition of German composts

Sample	Ratio v/v%	Composition
7/1	15:85	ssw/ gpw
7/2	70:30	ssw/ gpw
7/3	80:20	ssw/ gpw
7/4	80:20	ssw/ gpw
7/5	70:30	ssw/ gpw
7/6	100	gpw
7/7	75:25	ssw/ gpw

ssw: sewage sludge, sawdust, wood shaving; scb: separately collected biowaste; gpw: garden or park waste

determine the carbon content, while the optical density was measured at 254, 465 and 665 nm. Except of Σ HWP-C content, the analysis of all parameter were determined from first 100 cm³ of extract. 2 repetitions were used in the experiments. The statistical method used was analysis of variance.

Results and Discussion

Gödöllő compost materials

A first-order kinetic function was fitted to the C content determined from hot water extracts of composts at various stages of maturity and to the quantity of water percolated. Then the

parameters of the function were determined, including the maximum extractable C content (Σ HWP-C), which declined substantially as composting proceeded (Figure 1. and Table 3.). Data in the table marked HWP-C represent the C concentration found in the first 100 cm³ portion.

In the case of the compost samples are shown in Table 3. the quantity of HWP-C was far higher in incompletely matured samples than in those judged to be mature. The amount of total organic carbon significantly decreased during the composting process (Table 3.). When the HWP-C content decreases during maturation, it means that the amount of fulvic acids also

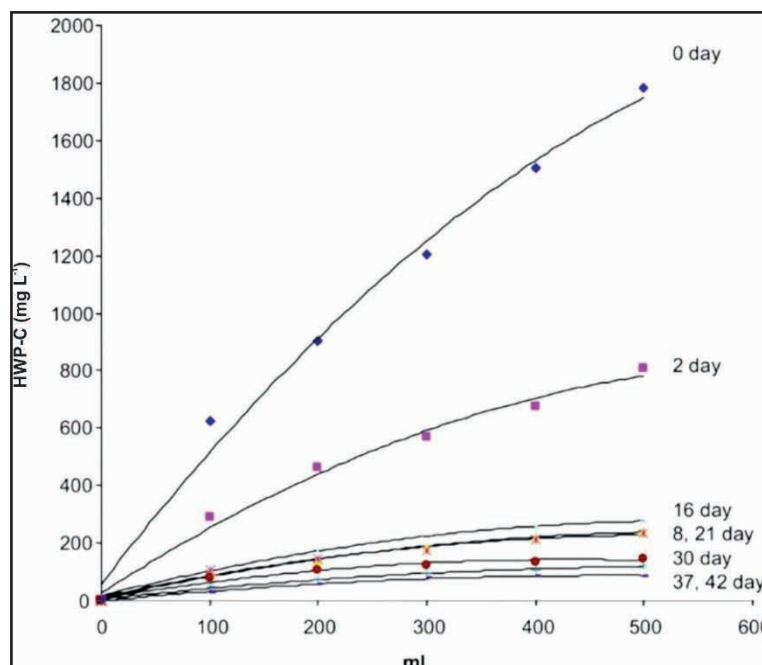


Figure. 1. First-order kinetics of organic carbon release during hot water percolation of compost samples during the composting process. (SzD5% (0 day): 127; SzD5% (2 day): 74; SzD5% (8 day): 95; SzD5% (16 day): 37; SzD5% (21 day): 68; SzD5% (30 day): 48; SzD5% (37 day): 25; SzD5% (42 day): 10.)

Table 3. Hot water extracted (HWP)-C and the spectral properties of Gödöllő compost materials

Days	Org.-C %	HWP-C mg L ⁻¹	Σ HWP-C mg L ⁻¹	254 nm cm ⁻¹	465 nm cm ⁻¹	665 nm cm ⁻¹	SUVA L mg ⁻¹ m ⁻¹	E4/E6
0	45.40	605.0	1910	6.71	0.310	0.062	1.11	5.11
2	41.02	289.0	807	3.05	0.210	0.056	1.06	3.79
8	20.89	97.6	240	1.52	0.096	0.026	1.56	3.62
16	21.51	133.0	285	1.43	0.144	0.035	1.34	3.21
21	18.78	108.0	233	1.39	0.120	0.044	1.28	2.70
30	20.47	75.7	145	1.15	0.083	0.030	1.53	2.73
37	11.26	49.3	121	1.59	0.075	0.026	3.24	2.80
42	11.83	27.9	86	0.92	0.044	0.025	3.46	1.74
SzD5%	2.13	17.54	29.94	0.29	0.05	0.05	0.44	0.88

decreases. At the same time the amount of humic acid increases (however the method is not able to measure that). Thus the decrease of the fulvic acid suggests its transformation, which means the increase of humic acid, and this process goes with maturity (Sugahara & Inoko, 1981; Inbar et al. 1990). Additionally, the hot water-soluble HWP-C content decreased from 605 mg L⁻¹ on the day 0 of composting to 27.9 mg L⁻¹ on the 42nd day (Table 3.). There was a strong positive correlation between the HWP-C content of the first 100 cm³ fraction and the sum of the 5 x 100 cm³ fractions (R=0.99), which means that it was sufficient to measure the carbon content and spectral properties of the first HWP fraction only. The measured HWP-C content is naturally less than the values reported by other authors (0.5-1.7%) (Benito et al. 2009; Sellami et al. 2008), because hot water percolation is a rapid method taking only one or two minutes. There was a very strong linear correlation between absorbance at 254 nm and the HWP-C content of the first 100 cm³ extract and the sum of the 5 x 100 cm³ fractions (R=0.97, R=0.96), and also between absorbance at 465 and 665 nm and the HWP-C content of the extracts (R=0.95, R=0.79).

First of all, organic molecules degrade as a result of enzymatic breakdown, leading to a large volume of water-soluble C. Then, as composting progresses, the smaller molecules synthesize into macromolecules and become water-insoluble. The absorbance was measured at 254 nm is proportionate to the amount of fulvic acid. Both the HWP-C content was measured in the first 100 cm³ and the 5 x 100 cm³ extracts and the optical density was measured at 254 nm, 465 nm and 665 nm indicate a decrease in fulvic acid during the process of maturation (Table 3.).

The SUVA values proved the presence of larger aromatic molecules. The trend seen in Table 3. is very similar to that reported by Said-Pollicino et al. (2007), who noted a sudden increase in SUVA after the 28th day of composting, indicating the maturity of the compost (Table 3.).

E4/E6 indicates the proportion of fulvic acid/humic acid. As the process progresses, lower values are obtained. The E4/E6 trend in Table 3.

agrees with the findings of Sellami et al. (2008): E4/E6 significantly decreases with the stage of maturity (Figure 2.).

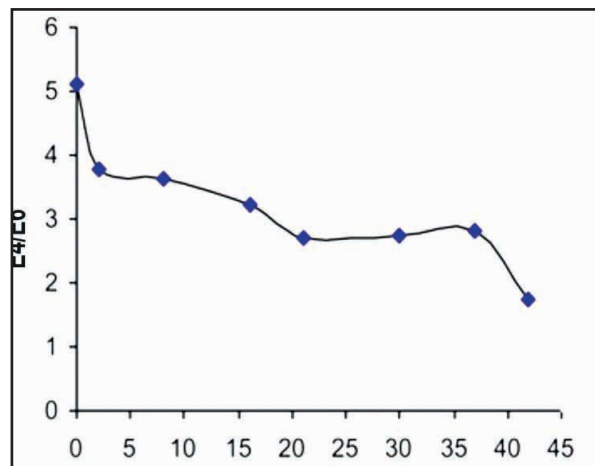


Figure 2. The E₄/E₆ values of HWP extracts at different stages of maturity

German compost materials

The analytical results obtained for composts judged to be mature or fresh on the basis of the Dewar test are presented in Table 4. Many parameters examined for these samples were in good agreement with the results of the Dewar test. For instance, the pH was generally higher in fresh compost samples. However, the sample 7/7 is an exception (Table 4). Although it was judged to be almost completely mature, it had very high values of HWP-C content (422 mg L⁻¹) and absorbance at 254 nm (7.844). The NH₄-N content, which is generally much lower in mature than fresh composts, was also high, suggesting that the sample was not fully mature, with a maturity index of 4. On the other hand the NH₄/NO₃-N ratio was less than the values reported by Aparna et al. (2008) and Bernal et al. (1998) (1.0 and 0.1, respectively) in mature samples, but was well above these values in fresh samples. Both the hot water-soluble carbon content and absorbance at 254 nm, which are closely correlated, were higher in fresh samples than in mature composts. However, the SUVA index did not fit the pattern expected from previous experience, as the values recorded in mature samples were only higher than those found for fresh samples in a few cases.

Table 4. Selected characteristics (hot water extracted (HWP)-C and -N) and spectral properties of German compost materials

Sample	Dewar test		pH	HWP- NH ₄ -N/			HWP-C			Abs.254
	Maturity	C°		value	NH ₄ -N mg L ⁻¹	NO ₃ -N mg L ⁻¹	NO ₃ -N mg L ⁻¹	HWP-C mg L ⁻¹	nm	SUVA L mg ⁻¹ m ⁻¹
7/1	30	5	7.2	2.40	97.94	0.0245	0.025	0.672	1.41	
7/2	24	5	7.6	4.96	55.88	0.0887	0.089	1.743	3.48	
7/3	26	5	8.3	4.34	39.48	0.1099	0.110	1.114	2.05	
7/4	26	5	7.7	4.72	33.84	0.1395	0.139	1.466	1.98	
7/5	23	5	7.6	11.26	61.86	0.1820	0.182	1.836	2.08	
7/6	24	5	7.9	1.80	17.06	0.1055	0.106	2.05	2.65	
7/7	39	4	8.2	55.72	1.00	55.720	421.96	7.844	1.86	
5/1	55	2	8.5	23.50	1.00	23.50	23.500	3.355	2.00	
5/2	54	2	8.4	28.96	1.00	28.96	28.960	4.456	2.02	
5/3	57	2	8.6	26.60	9.20	2.8913	2.891	4.009	2.27	
5/4	66	1	7.4	19.43	1.00	19.43	19.430	2.859	1.58	
5/5	41	3	8.7	90.21	97.27	0.9274	0.927	3.498	1.95	
SzD5%	0.88	0.87	0.54	1.58	3.38	0.0014	3.08	0.43	0.4	

Conclusions

It may be concluded that the C content of hot water percolation (HWP) extracts clearly demonstrates the maturity stage of the composting process, in spite of the rapid extraction process. The results obtained for the two groups of composts demonstrate that the carbon content obtained from the hot water extraction method (HWP) is a good indication of the maturing process, similarly to the absorbance recorded at 254 nm, the SUVA index and the E4/E6 values, which decrease in hot water extracts when maturity is reached (with the exception of SUVA₂₅₄, which increases). The NH₄-N and NO₃-N values recorded for the hot water extracts also clearly

indicate the maturing process. It should be noted, however, that despite these tendencies, the quality of the raw compost materials also influences the values of these parameters. The simple HWP method (Füleky and Czinkota, 1993) can thus be recommended for a rapid, routine determination of compost maturity.

The main advantage of the HWP method is speed, since compost extracts can be prepared in a couple of minutes and measurements can be made rapidly. The quality of the starting material will influence the results, but as more details and data become available, the method has the potential to generate indicative values about the stage of compost maturity cheaply and rapidly.

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Agricultural biotechnology policy review in Africa: A case study of Kenya

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Abstract: Production of genetically modified crops and animals is still a widely debated topic across the globe. There are a lot of players when it comes to the acceptance and adoption of biotechnology in agriculture for the aim of increased food production, quality addition among other goals. One of the key stake holders are policy makers. Many African countries have developed Agricultural policies which address the research, development, production and regulation of genetically engineered crops and animals. Through these policies, A number of new crops have been developed tested and approved, addressing important traits of particular significance for smallholder farmers in Africa. Since most of these policies are still new, there are issues that face the agricultural biotechnology sector in these countries that making it difficult to achieve the potential. The major problems include misinformation and politicization of core issues relating to biotechnology. However, these issues can be addressed easily with implementation of the guidelines delayed in the policy. Kenya developed and adopted such a comprehensive policy in 2006. However, to date, the full implementation and complete adherence to the document guidelines has not been fully achieved. This paper uses several case studies to review the Agricultural Biotechnology policy in Kenya, evaluating what is outlined in the policy adopted slightly more than a decade ago against what has been achieved so far.

Keywords: Agricultural Policy, Agricultural Biotechnology, Genetically engineered crops, Genetically Modified crops, Research and Development

Introduction

Kenya is a country in East Africa with coastline on the Indian Ocean. It encompasses savannah, lakelands, the dramatic Great Rift Valley and mountain highlands. It's also home to wildlife like lions, elephants and rhinos. From Nairobi, the capital, safaris visit the Maasai Mara Reserve, known for its annual wildebeest migrations, and Amboseli National Park, offering views of Tanzania's 5,895m Mt. Kilimanjaro. Agriculture remains the backbone of the Kenyan economy, contributing 25% of GDP. About 80% of Kenya's population work at least part-time in the agricultural sector, including livestock and pastoral activities. Over 75% of agricultural output is from small-scale, rain-fed farming or livestock production. Table 1 and Figure 1 show some basic sociodemographic statistics of Kenya and the climatic zones in the country respectively

Background

Biotechnology is defined as any technological application that uses living organisms, or

derivatives thereof to make or modify new products or improve existing ones. In spite of advances in biotechnology having great potential to improve an economy, it is imperative that it be applied systematically, responsibly and in a way, that responds to a country's priority needs. In this regard, the government of

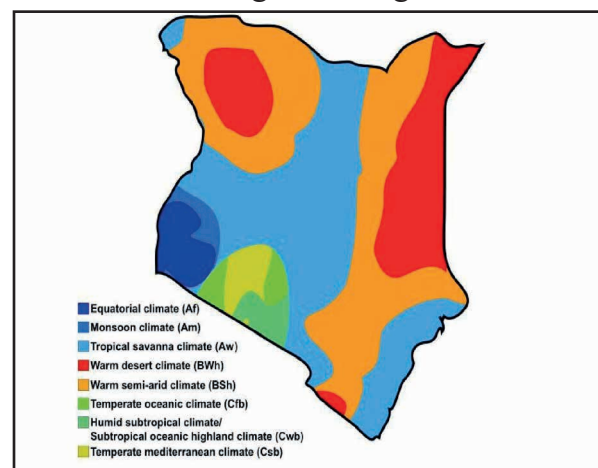


Figure 1. Map of Kenya with Climatic Classification. The map shows the agricultural climate zones as an indicator of the agricultural capacity of the country and the apparent need to adopt biotechnology to attain adequate food production for domestic consumption and possibly export. Source; Wikipedia

Table 1. About Kenya

Population	46,790,758 (2016 <i>est.</i>)
Age structure	0-14 years: 40.87% (male 9,592,017/female 9,532,032) 15-24 years: 18.83% (male 4,398,554/female 4,411,586) 25-54 years: 33.54% (male 7,938,111/female 7,755,128) 55-64 years: 3.84% (male 819,665/female 976,862) 65 years and over: 2.92% (male 590,961/female 775,842) (2016 <i>est.</i>)
Median age	total: 19.5 years male: 19.4 years female: 19.6 years (2016 <i>est.</i>)
Urbanization	urban population: 25.6% of total population (2015) rate of urbanization: 4.34% annual rate of change (2010-15 <i>est.</i>)
Major cities population	NAIROBI (capital) 3.915 million; Mombassa 1.104 million (2015)
Land use and agriculture	Total area 580,367 sq km, land: 569,140 sq km, of which agricultural land covers 48.1%, with arable land taking up 9.8%; permanent crops cover 0.9%; and permanent pasture 37.4%

Kenya developed a comprehensive national policy to guide research, development and commercialization of modern biotechnology products. The policy, which was approved in September 2006, was the result of several years of work involving all major biotechnology stakeholders nationally, internationally working closely with relevant government departments. This paper is a review of the current policy on Agricultural Biotechnology in Kenya.

The Kenya National Biotechnology Development Policy (2006)

The policy covers all biotechnology applications, including tissue culture and micropropagation, biopesticides and biofertilizers, livestock technology, DNA Marker technology, and genetic engineering. It also covers research, development and use of biotechnology in various key fields such as agriculture, environment, human and animal health and industry. The policy takes cognizance of international instruments, such as the Cartagena Protocol on Biosafety. The objectives of this policy include to:

- Prioritize, promote, and coordinate research in basic and applied bio-sciences,
- Promote sustainable industrial development for production of biotechnologically derived products,
- Create enabling administrative and legal frameworks for biotechnological development and commercialization of such related products,

- Develop mechanisms for the provision of sustainable funding for biotechnology research and products' development,
- Support and facilitate capacity building on all aspects of biotechnology including intellectual property access and protection, biosafety and bioethics,
- Support the development and retention of human resources in science, innovation and biotechnology,
- Stimulate collaboration among public, private sectors and international agencies in order to advance biotechnology both locally and internationally.
- Promote public understanding of the potential benefits and address stakeholder concerns on modern biotechnology.

Scope of the Policy

The government of Kenya adopted biotechnology for the purpose of improving the quality of human welfare, maximizing productivity in agriculture and industry and protecting the environment, conserving biodiversity and bioprospecting. The biotechnology policy therefore seeks to address:

- Traditional and modern biotechnology;
- Genetically modified organisms that are human food and animal feeds and pharmaceuticals.

The policy targets to cover all biotechnology applications including tissue culture and

micropropagation, biopesticides and biofertilizers, bioremediation, Livestock technology, DNA Marker technology, and genetic engineering (Karembu et al., 2010). The policy is broad based and covers research, development and use of biotechnology in various fields such as agriculture, environment, human health and industry.

The scope of the policy takes cognizance of local and international agreements and protocols such as the Cartagena Protocol on Biosafety, World Trade Agreements, Application of Sanitary and Phytosanitary Measures, the Agreement on Trade Related Aspects of Intellectual Property Rights, International Convention for the Protection of New Varieties of Plants.

The government recognizes that the domestic regulations governing the importation and use of pharmaceuticals, biologicals, food and feeds, may not be adequate. Therefore, it facilitate the process of aligning the policy to the regulations and policies governing the importation and use of the related products.

The policy outlines six key areas of focus as follows;

1. Agricultural Biotechnology
2. Education
3. Bioresources
4. Environmental Biotechnology
5. Medical Biotechnology
6. Industry and Trade.

Implementation of the Agricultural Biotechnology Policy in Kenya

Regulatory Framework: The National Biosafety Authority of Kenya (NBA), established by the Biosafety Act No.2 of 2009, is under the Ministry of Agriculture, Livestock and Fisheries administratively, but under the Ministry of Education, Science and Technology legally. Kenya Plant Health Inspectorate Service (KEPHIS): under the Ministry of Agriculture, Livestock and Fisheries, oversees the introduction, testing and use of biotechnology plants and seeds. The NBA is the main regulatory agency that oversees agricultural biotechnology in Kenya. It is responsible for regulations and policies,

as well as general supervision and control over the transfer, handling, and use of GE products. Four biotechnology implementing regulations were released following the Biosafety Act 2009:

- Contained Use Regulation, 2011;
- Environmental Release Regulation, 2011;
- Import, Export, and Transit Regulation, 2011;
- Labeling Regulation, 2012; and
- Packaging, Transport, and Identification regulation, 2014

The NBA works together with eight other regulatory agencies that have different roles in regulating Biotechnology products. These regulatory agencies are:

- Department of Public Health, under the Ministry of Health, safeguards consumers' health through food safety and quality control, surveillance, prevention and control of food borne diseases. The Agriculture committee has recommended the establishment of a Food Safety and Control Unit to evaluate food safety of GE foods for human consumption, and to issue import permits for GE foods;
- Kenya Bureau of Standards, (KEBS) under the Ministry of Industrialization and Enterprise Development, develops food standards, quality assurance, and testing;
- National Environment Management Authority (NEMA), under the Ministry of Environment, Water, and Natural Resources, oversees environmental questions and conducts environmental impact assessments. NEMA issues licenses that permit national performance trials (NPTs) on GE crops and plants.
- Pest Control Products Board, (PCPB), under the Ministry of Agriculture, Livestock and Fisheries, regulates pesticide use;
- Kenya Wildlife Service (KWS), under the Ministry of Environment and Natural Resources, handles biodiversity and biotechnology related matters in wildlife and forestry;

- Kenya Industrial Property Institute (KIPI), under the Ministry of Industrialization and Enterprise Development, handles intellectual property issues; and,
- Department of Veterinary Services (DVS).

1. Industry and Trade

The National Biosafety Authority (NBA) is responsible for the approval process of import shipments of GE products. The authoritative legislation, Kenya’s Biosafety Act of 2009, stipulates that the approval process should take 90-150 days. Also, the Kenya Plant Health Inspectorate Service (KEPHIS) requires imported GE plant products to have:

Figure 2 and figure 3 show the process for approving production of Genetically engineered crops developed in Kenya and the regulatory processes of such crops respectively.

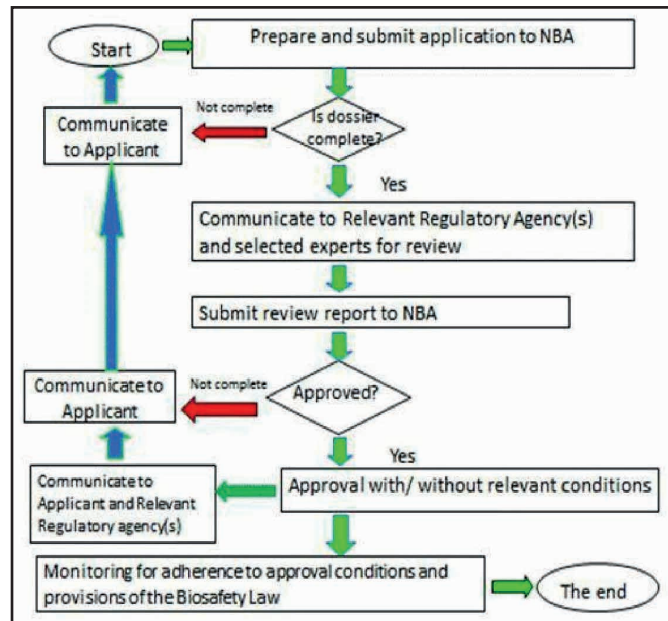


Figure 2. The Approval process for production of Genetically engineered crops developed in Kenya, Source; National Biosafety Association of Kenya. The figure shows a breakdown of the process due to be followed before a scientist, biotechnologist, or any other individuals or companies can be approved to produce genetically engineered crops in Kenya for whichever goal.

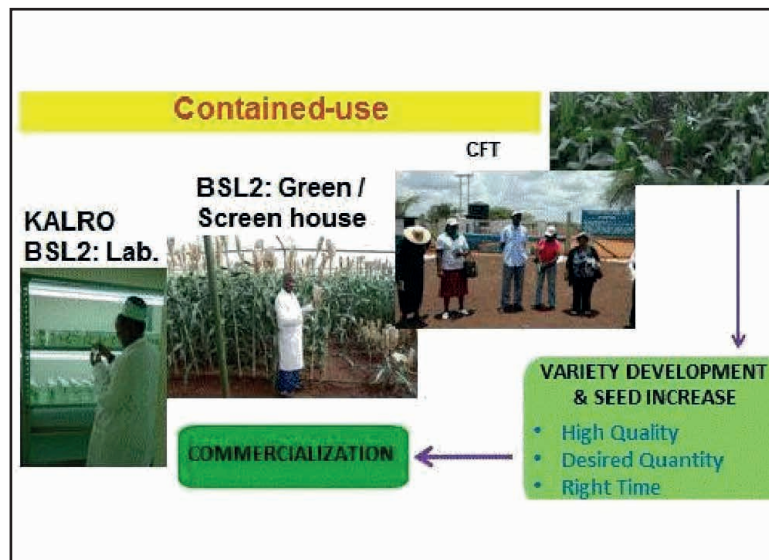


Figure 2. The regulatory processes for genetically engineered crops in Kenya, Source; National Biosafety Association of Kenya. The figure shows the steps involved in the regulatory process for biotechnology crop products in the country.

- A declaration from the country of origin that states the import's GE status, and
- A phytosanitary certificate.

However, progress in agricultural biotechnology suffered a setback after the National Assembly's Agriculture committee recommended that a new food safety law on genetically engineered (GE) products be put in place, before the 2012 import ban is lifted.

The Agriculture committee's move follows an earlier decision by Kenya's National Environment Management Authority (NEMA) to retract the open field trials license for Bt corn. NEMA had previously retracted its license for Bt corn open field trials despite prior approval by NBA, thus, creating confusion in the regulatory framework. NEMA is still reviewing applications for open field trials for Bt corn and Bt cotton, exceeding the 45 days allowed by law.

2. Plant Biotechnology Research, production and commercialization

Participation on the NBA includes representatives from Government Ministries, as well as scientists from civil society and the national universities. Government ministries and key players on the NBA include the Kenya Plant Health Inspectorate Service, Ministry of Agriculture, which oversees the introduction, testing and use of biotechnology plants and seeds; the Ministry of Health and the Kenya Bureau of Standards, which regulate food safety; and the Ministry of Environment and Natural Resources, which oversees environmental questions and conducts environmental impact assessments among others.

In terms of commercialization of bio-engineered food products in studies done in 2003, 2006 and 2007 by the International Maize and Wheat

Improvement Centre (CIMMYT), KALRO and Kansas State University, Kenyan consumers were found to accept agricultural biotechnology and genetic modification of foods at rates well below 50 percent. Processors and retailers showed a higher level of acceptance, especially with regard to genetically modified foods. This showed the need for public education, awareness creation and sensitization on the stringent measures put in before the production and commercialization of products to ensure the safety of the consumers. Table 2 shows the Awareness on Agricultural Biotechnology in 2007

A new approach to the comprehensive agriculture policy that includes capacity and confidence building, policy stability in form and application from year-to-year and production and trade enhancing characteristics is still needed in Kenya before the full benefits of agriculture biotechnology can be realized. Poor policies mean farmers minimize their investment in agriculture, because of their inability to predict/expect profits from efforts (Adato - Meinzen-Dick 2002).

Top government leaders, cereal millers, traders, and agricultural research scientists widely acknowledge that modern biotechnology is an important tool for improving agricultural production in Kenya, and have continued to publicly support agricultural biotechnology. Agricultural biotechnology awareness campaigns initiated by institutions like BioAware, ISAAA, Open Forum on Agricultural Biotechnology, African Biotechnology Stakeholders Forum and Africa Harvest Biotech Foundation International avail credible information and demystify misconceptions related to agricultural biotechnology. Kenya has advanced in agricultural biotechnology governance, as evidenced by the Biosafety Act of 2009, establishment of NBA,

Table 2. Awareness on Agricultural Biotechnology in 2007

Type	Area or Industry	Number of respondents	Awareness (Percentage)	
			Biotechnology	GM crops
Urban consumers	Nairobi	612	46	38
Rural consumers	Western Kenya	121	16	13
	Eastern Kenya	400	63	31
Gatekeepers	Milling companies	32	67	87
	Supermarkets	40	83	79

Source: The International Maize and Wheat Improvement Center (CIMMYT)

regulations and policies. To maximize on these gains, Kenya needs encouragement to:

- Reverse the GE foods import ban;
- Commercialize Bt cotton;
- Continue public awareness on modern biotechnology and biosafety; and
- Continue capacity building on biotechnology to manage and strengthen research, development and trade

Case Studies for the implementation of the Policy

To further clarify the state of affairs in regard to research activities and the need for action, the following are some case studies of recent agricultural research and development work in Kenya.

Case Study 1: Cotton

Cotton production in Kenya has declined over the years due to yields being affected by bollworm, necessitating the search for varieties that will be resistant to bollworm. Work on Bt. Cotton began with Bt. cotton seeds with a gene of resistance against the bollworm being imported for trials from South Africa in late May 2004 (Kameri-Mbote 2003). This was after the plant regulatory authority, the Kenya Plant Health Inspectorate Service (KEPHIS) granted KARI a permit to introduce the seeds. The trials were done at KARI Fiber research station in Mwea Tabere whose biosafety facilities have been inspected and approved by KEPHIS on behalf of the National Biosafety Committee. However, upon the success of the project, the seeds were not commercialized. If these seeds were handed to the farmers, this would have a very significant impact on the Kenyan Cotton industry, and the country's economy by proxy.

Case study 2: Maize

The main thrust of agricultural research on maize in Kenya has traditionally focused on breeding for both higher yields and drought tolerance (Smale - Jay 2003). Not much attention has been given

to breeding for pest and disease tolerance and consequently, small-scale farmers have been affected substantially as they plant improved maize varieties under very poor pest and disease management conditions. They end up not benefiting from the yield potential of such varieties.

Stem borers pose one of the most serious threats to the production of maize in Kenya, with losses estimated to be about 15 % of the harvest. These problems have continued to intensify as most subsistence farmers are poor and cannot afford to buy pesticides to curb the menace posed by the borers.

The Insect Resistant Maize for Africa (IRMA)

The Insect Resistant Maize for Africa project started in 1999 by KALRO working together with the International Maize and Wheat Improvement Centre (CIMMYT) with funding from the Syngenta Foundation for Sustainable Agriculture. The overall objective of the project was to increase maize production and enhance food security through the development and deployment of insect resistant maize that is adapted to various agro-ecological zones in Kenya (IRMA).

In furtherance of the objectives of the project, maize leaves with Bt. toxins were imported into Kenya from Mexico and these underwent trials at various KALRO research stations (Kameri-Mbote 2003). The project was continued in a green house and controlled environments until seeds which were approved as fit, safe and stable for human consumption were obtained at the end of the project.

By the end of the project in 2014, the project had succeeded in developing maize varieties that can better resist attack by the three major insect pests in Kenya – stem borers, maize weevils, and the larger grain borer (LGB).

Nine maize varieties (both open pollinated and hybrid) with remarkable resistance to stem borers were released. They can control three of the four main stem borers (IRMA).

IRMA project achievements

It identified new germplasm sources of resistance to stem borer and post-harvest insect pests among landraces, open pollinated varieties (OPVs) and CIMMYT lines (CMLs), and developed new insect resistant germplasm. Kenya released 13 stem borer-resistant (SBR) conventional maize varieties (three OPVs and 10 hybrids) and four storage pest-resistant (SPR) hybrids. Kenya has also nominated several stem borer and four postharvest-resistant hybrids for national performance trials (IRMA).

Three insect-resistant varieties were commercialized in Kenya by Monsanto, Wakala Seeds and the Kenya Agricultural Research Institute Seed Unit. These are the KH 414-1 SBR and 414-4 SBR hybrids, and the OPV Pamuka (IRMA). However, the uptake of these commercialized varieties was low since the Kenyan policy was not particularly clear on the matter at the time of release, therefore making it difficult to advertise or market the varieties.

Key Policy Recommendations*I. Prioritization and Coordination of Research and Development*

The policy recommends establishment of a National Biotechnology Enterprises Programme that will consist of a National Commission on Biotechnology, a National Biotechnology Education Centre and a National Biosafety Authority. Although the NBA has already been established, the establishment of the NCB and NBEC is necessary.

II. Public Education and Awareness Creation

1. Creation of public awareness on biotechnology issues and investment opportunities;
2. Access to information held by public authorities;
3. Public participation in decision making process;
4. Access to judicial and administrative provisions.

III. Public Protection and Support

1. Protecting Intellectual Property Rights (IPR) is a critical aspect of biotechnology innovation, and ensuring effective public and private sector participation in research and product development.
2. The Government recognizes the existing policies and legislation on protection of traditional knowledge and resources.

IV. Infrastructure, Facilities and Equipment

The National Biotechnology Enterprises Programme to put in place mechanisms to create linkages and networks among public research institutes and universities for optimum access and utilization of available resources while supporting initiatives for the establishment of biotechnology parks at R & D institutions as incubators to stimulate the growth of small and medium size businesses with potential to mature into high technology companies.

V. Financial and Business Support

The key recommendations here are;

1. Create incentives to encourage partnerships between public research institutes and the private sector
2. Waiver of taxes on research materials and equipment to encourage further research
3. Encourage specialized technological financing agencies to provide loans to firms or consortia and research institutions.
4. Direct public budgetary allocation to biotechnology research and development.

Conclusion

The policy provides a road map for agricultural biotechnology and should effectively guide the country into a pre-eminent position of a knowledge-based economy for overall sustainable economic growth, poverty alleviation and wealth creation.

The policy is well structured and covers the most key issues dealing with biotechnology in the country. However, its slow implementation and unclear distinction of the parts played by different

agencies defined within it may cause conflicts and thus slow down the progress of the country towards having an efficient biotechnology framework. It is crucial for the Government to come up with an evaluation strategy to evaluate the effectiveness and efficiency of

the policy so as to find out rising issues as the implementation roles out further. Moreover, the evident interference on the implementation of the policy by the political class should be closely monitored and strictly regulated.

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Climate change research review – 10th anniversary of the VAHAVA report

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Abstract: Climate change processes highly affect agriculture. The impacts of climate change may influence almost all fields of agricultural activities; production efficiency, quantitative and qualitative deterioration of crop yields produced for alimentary purposes, and to determine postharvest manifestation of agricultural products inducing hazard in the field of food safety, transport, storage and distribution. There are many factors like soil-climatic conditions, amount and distribution of precipitation, anomalies and extremities of temperature as well as various manifestation of air movement from stand still to storms that may influence agriculture. Climate change may have indirect impacts as well like pollution, which has been considered solely as the presence of unfavourable alien matter in the environment, but in reality pollution is far more than that. Agri-environmental pollution is less anthropogenic since many pollution or degradation processes may begin with no direct relationship to human activities. Soil degradations or irreversible damage to natural ecosystems by climatic factors (drought, flood, water logging and salinity) are to be seen as the most frequent consequences. Biological pollution, like weed infestation, epidemics and gradations, pollen allergy, the poisonous effect of mycotoxins on farm animals and humans, new pests and diseases, the emission of greenhouse gases and biological factors which cause quality deterioration represent an increasing pressure on agri-environment. This paper is intended to give an overview of some research activities and their results in relation with climatic aspects of agri-environment in Hungary on the occasion of the 10th anniversary of the edition of the VAHAVA report, a major national climate change research project. The majority of its postulates are still valid. Novel follow up research results are presented in the review.

Keywords: climate change, agri-environment, VAHAVA project

Introduction

Agriculture in general and crop production in particular are highly affected by climate change impacts. Results of recent climate change researches have highlighted, that climate change impacts may influence almost all fields of agricultural activities; production efficiency, quantitative and qualitative deterioration of crop yields produced for alimentary purposes, and determine postharvest manifestation of agricultural products inducing hazard in the field of food safety, transport, storage and distribution (Czelnai, 2003; Láng et al., 2007; Jolánkai, 2008). Agriculture has a special place in human activities since it is closely linked to nature and wildlife. For many centuries pollution was considered simply as the presence of unfavourable alien matter in the environment altering its qualitative and quantitative characteristics. Actually pollution is far more than that.

A basic characteristic of agri-environmental pollution is that, unlike industrial or urban pollution, it is largely independent of mankind, since a wide range of pollution or degradation processes may begin with no direct relationship to human activities (Láng et al., 2004; Láng et al., 2009; Jolánkai, 2010; Jolánkai et al 2012). Examples of this are soil erosion, or irreversible damage within natural ecosystems by climatic factors (drought, flood, water logging, salinity etc.). Biological pollution, like weed infestation in agricultural fields, epidemics and gradations from abandoned or set-aside areas, which may also be a source of human ailments like pollen allergy, the poisonous effect of mycotoxins on farm animals and humans, new pests and diseases, the emission of greenhouse gases by soils and ruminants, and biological factors which cause quality deterioration represent an increasing pressure on agri-environment.

Table 1. Main climatic characteristics of Hungary,
Source: KLIMAKKT, 2008

Annual precipitation	580 mm
Annual mean temperature	11 °C
Altitude	78-1014 m
Heat amount in vegetation period	1280-1465 °C
Dry matter production	8.3-17.6 t/ha/year
Photosynthetic active radiation	1518-1612 MJ/m ²
Annual snow coverage	41 days/year

The problems of any of the above are manifold:

- High variability of yield performance in accordance with weather extremities.
- Economic losses in agricultural and food production.
- Quantitative and qualitative deterioration of food and feed products.
- Lack of sustainable long term vertical and horizontal technology structures.
- Limited chances for forecast and prevention, as well as for technological implementation.
- Environmental hazards affecting agro-ecology as a whole.

In case of Hungary, two facts can be observed in the Carpathian basin (Harnos and Csete, 2008; Várallyay, 2011). In first place, the ascending levels of temperature rise, with a magnitude of 1° C during the past century. The other is the decreasing trend-line of annual precipitation according to what during one century 83 mm rainfall has disappeared. Hungary is a country in the centre of Europe in the Carpathian basin with a most peculiar geographic location regarding the possible impacts of any sort of climatic changes

due to the physical environment and the high ratio of agri-ecosystems of the country (Fig 2; WWF, 2010). The climate of the region has always been highly variable (Table 1; UN, 2011, Fig 3).

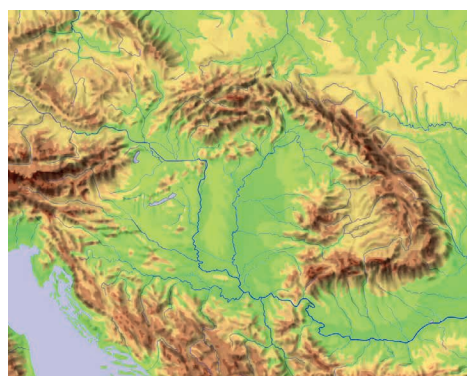
Expert teams of various fields of agriculture have been working within the framework of three major projects between 2003 and 2010: the national VAHAVA and the KLIMAKKT projects, and the European ADAM project. From 2012 a new national climate research network has been established; AGRÁRKLÍMA and its succession the AGRÁRKLÍMA-2. The main task of these research projects was to study climate change impacts and possible responses in the respective fields. The working hypotheses of the projects were based on international surveys as follows:

- The warming of the climate will be stronger in the Carpathian Basin;
- We may expect the decreasing of annual average precipitation;
- The number and intensity of extreme weather events will be increasing.
- Adaptation strategies need to be supported by decision making based on reliable nationwide scientific research results.

The hypotheses of the VAHAVA project have been approved by recent results with one exception concerning the precipitation scenarios (EEA 2017).

Climate change research results

The VAHAVA report was edited in 2007 and 2010 with auxiliary follow up research results.



Over 80 % of the territory of Hungary is covered by agro-ecosystems

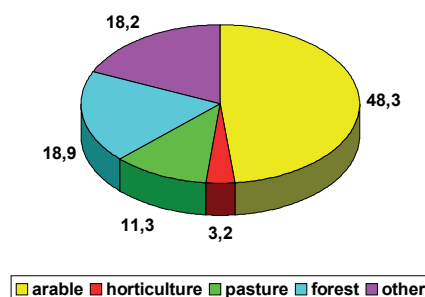


Figure 2. Geographic location and land use distribution of Hungary,
Source Jolánkai, 2008

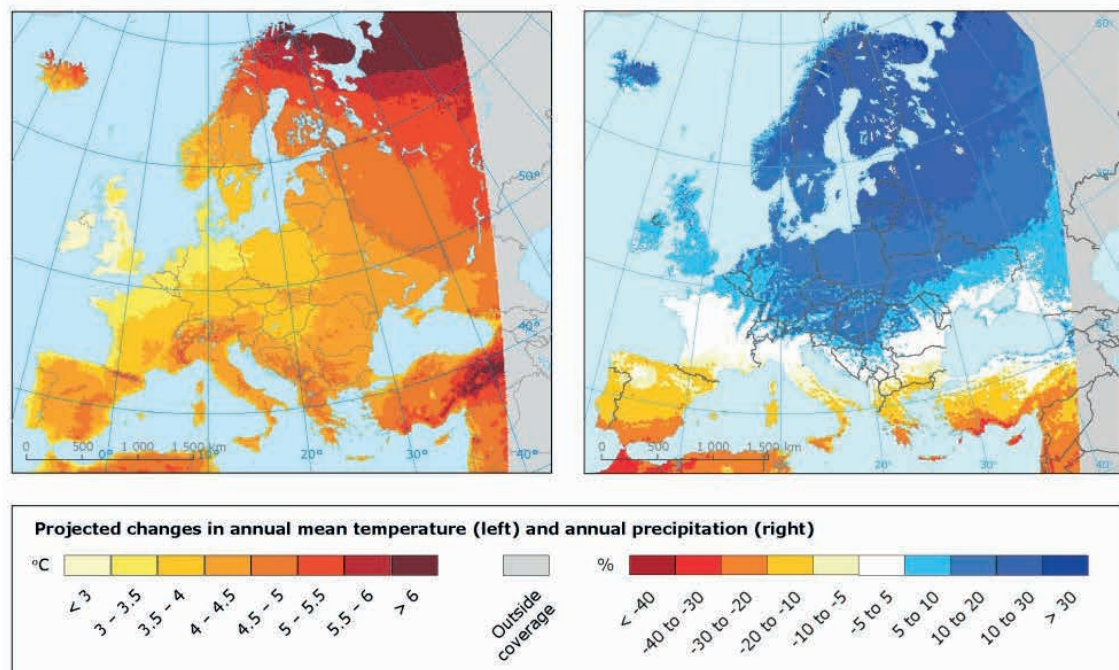


Figure 1. Changes in temperature and precipitation by RCP model scenarios. Source: EEA, 2017

The ADAM report and the KLIMAKKT report were both published in 2008. The result of expert teams' work was summarized in these volumes. The following passages present a digest of the respective reports' agri-environmental postulates (Láng et al., 2007; ADAM, 2008; KLIMAKKT, 2008; Harnos and Csete, 2008; VAHAVA, 2010; Jolánkai et al 2013; Bidló et al 2014; Mátyás 2016).

The VAHAVA results' postulates (Láng et al 2007, Láng et al 2009, VAHAVA 2010)

Climate change impacts in crop production can be prevented or reduced by the following measures. Water preserving soil tillage that may contribute to storage of higher amounts of annual precipitation. Increment of irrigation. Novel crop production technologies, breeding and use of drought tolerant crop varieties. Establishment of appropriate cropping structures and crop rotations.

Water supply of crop production involves three major sources; annual precipitation in rainfed cropping depending on the amount and distribution as well as the preservation and storage of that; irrigated cropping where rainfall is considered as additional or modifying means of water supply only; and flood irrigation systems that are mainly independent from precipitation

impacts. In favour of preventing harmful climate change impacts the two latter cropping systems should be given priority in the future.

Climate change impacts may have an influence on the trends of temperature as well as on the vegetation period of various field crops. Ascending levels of temperature induce alterations in the physiological requirements of heat amount. This may result in a change of duration of crop variety vegetation periods, and also, there is a chance for alterations in yielding ability, winter hardiness, phenological phases etc.

Warming and drying may have an effect on plant nutrition. In general there is scientific evidence that high levels of mineral fertilization may counteract the harmful effects of drought. In particular there are several crop species that may respond with yield declines in case of permanent drought. Abundant nutrient supply may result in higher concentrations that may be less beneficial to crop performance. Optimal soil conditions are required for better crop plant development.

Abiotic stress resistance of wheat varieties is a major issue in Hungary. The major task of plant breeding is to provide high yielding wheat varieties of marketable quality that are

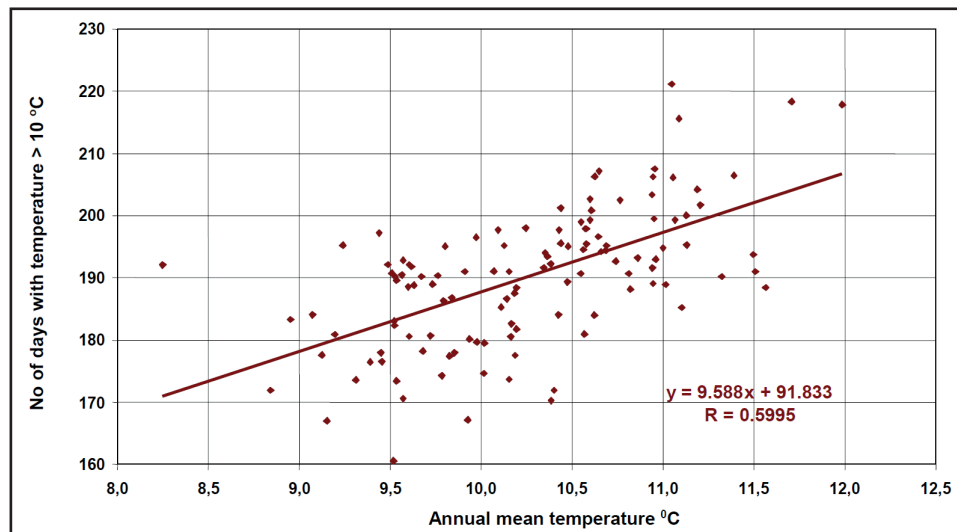


Figure 3. Correlation between the annual mean temperature and the duration of warm periods (>10°C), Source VAHAVA, 2010

less susceptible to climate change impacts. Any variety has to meet a threefold demand: grain quality, quantity and yield stability.

Seed production is a field where climate change impacts may have both positive and negative effects. Arid conditions and weather extremities may risk the results of seed production and processing. On the other hand climate change may contribute to favourable conditions that may be essential for producing seed of new species and varieties.

Agricultural mechanization is also facing new challenges induced by climate change. Such are: Technology improvements (water preserving tillage technologies); combined or reduced number of field operations (to prevent or lessen unfavourable soil conditions); more quick, flexible and efficient machinery; security equipment (installation of special machinery for emergency uses only); propagation of tram line production systems; use of adapted machinery.

Agricultural mechanization may have a major role in mitigation processes, like CO₂ emission control and carbon sequestration. Specific tillage technologies, mulching and appropriate stubble operations may contribute to a better soil water budget.

Plant protection is highly affected by climate change. There is an invasion of new plant

diseases, insect pests and weed species. To counteract the harmful effects improved methods of prevention, defence and remediation are needed. The major fields of that are as follows: comprehensive and efficient forecasting systems, extension services, integrated pest management, application of high tech implements, site specific precision methods. Genetic resistance and/or tolerance of crop plants has to be improved by breeding. Means of biological control has to be studied and applied.

A most specific field of agriculture is the grassland and pasture management. In Hungary over 1.1 million hectares of grasslands are exposed to climate change impacts, but on the other hand provide new adaptation chances for agriculture and for the country as a whole.

Specific climate change research statements

In relation with the climate change research results of indirect processes statements have been formulated during the work of the VAHAVA project and its follow up research activities.

Water scarcity and drought represent physiological water stress causing irreversible changes in live structures (Láng et al, 2007; Láng et al 2009). The genetic type of soils provides less information in comparison with water management categories. From among water management categories infiltration rate and water retention are the most

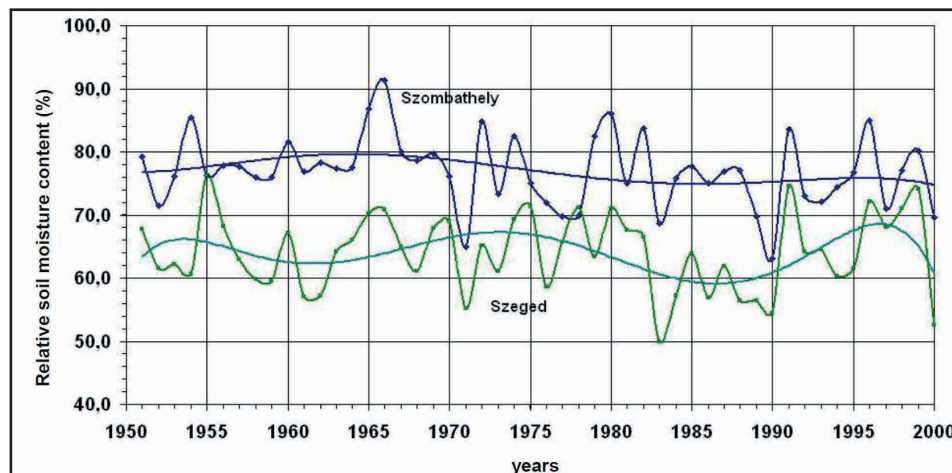


Figure 4. Annual changes in soil moisture content at two locations, Source VAHAVA, 2007

influential properties, however their impact may be modified by permeability, hydraulic conductivity and field capacity properties of the given soil (Fig 4). The depth of groundwater is a most influential factor in drought assessment of an agricultural region however its relation with drought indices is highly affected by the water management properties of the very soil. Various drought indices are applied to identify these processes regarding aridity and water scarcity conditions of an agro-ecological site (Pálfai, 2010, Jolánkai et al 2016).

Excesswater in ecosystems according to the amount and/or uneven distribution of atmospheric precipitation may result in floods, water logging and various sorts of physiological stresses (Jolánkai, 2008; UN, 2011). The consequences of temporary, long term and permanent presence of excess water in an agri-environmental ecosystem are physical and chemical deterioration of soils as well as direct and indirect degradation processes like water and wind erosion (Várallyay, 2009; Várallyay, 2011).

Soil degradation is usually a complex process in which several features can be recognized that contribute to unfavourable changes in soil processes and soil properties; loss or decrease in soil fertility and productive capacity; limitations in normal soil functions; and/or serious environmental deterioration (WWF, 2010; UN, 2011). Soil degradation may be the result of natural factors and/or human activities. Climate change may lead to increasing

temperature with heterogeneous spatial and temporal distribution. These changes are reflected in changes of vegetation and land use patterns with considerable impacts on soil formation processes, the moisture regime and soil degradation processes (Lal et al., 1994; Lawlor, 2002; Várallyay, 2011).

Land use and soil tillage manifest in a basic agronomic technological process which has a direct influence on the efficiency of crop production. The methods and tools used for soil tillage induce various agro-ecological and soil degradation problems (Birkás et al., 2006). Soil compaction, water and wind erosion and alterations in physical and textural characteristics can be considered as special manifestations of pollution processes. Soil properties depend greatly on land use practices. Alleviation and remediation are complex tasks involved in soil tillage (Birkás et al., 2008). The basic attribute of modern food technologies during processing, storage and transport is of *food safety*. The foods must be protected from microbiological, physical chemical pollution and oxidative deterioration until they are consumed. Some constituents of food are exposed to environmental contaminants. Certain environmental conditions, during improper processing, storage and transport, could facilitate the formation (fermentation) of a wide range of mycotoxins (Láng et al., 2004, Eser et al 2017).

Pests and diseases. Weeds increase the yield loss in the dry periods caused by the global

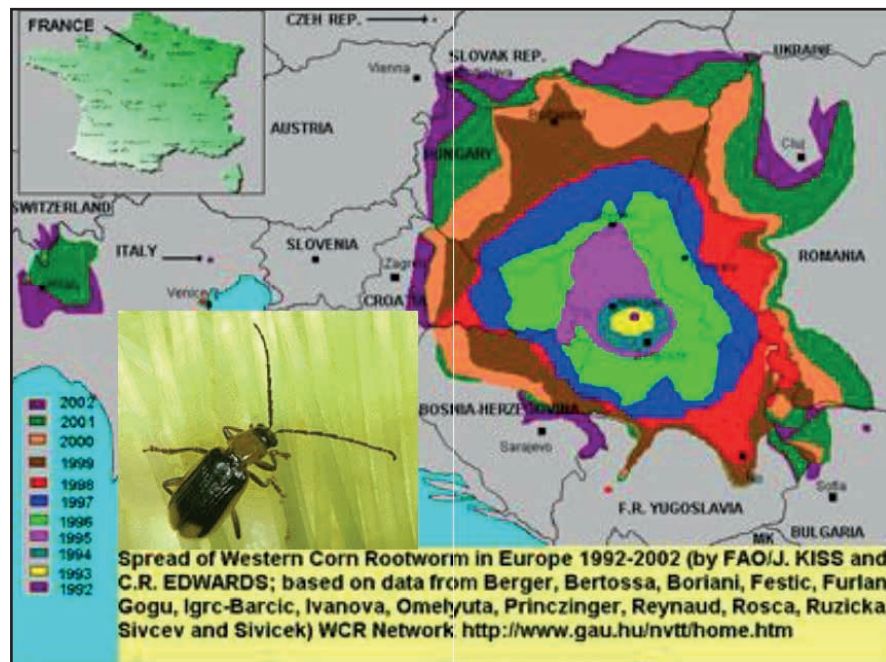


Figure 5. Spread of *Diabrotica virgifera* in Europe, Source Kiss and Edwards, 2002

climate change (Solymosi, 2005). Eight of the ten most important weeds in the Carpathian basin have C_4 -type photosynthesis. These species (*Amaranthus* spp., *Sorghum halepense*, *Echinochloa crus-galli*, *Panicum* spp., *Cynodon dactylon*, *Portulaca oleracea*, etc.) are capable of effective photosynthesis and water utilisation even in high temperature and dryness, when the stomata are closed (Czimer, 2004). The spread of western corn rootworm (*Diabrotica virgifera virgifera*) is much influenced by climatic conditions. This insect was brought to Europe during the Yugoslav war in 1992 to the Belgrade airport from where huge tracts of Eastern Europe were then occupied (Fig 5). The very unusual year of 1999 when spring drought was followed by moist summer allowed a 150 km distance migration (Kiss and Edwards, 2002; Jolánkai et al. 2015).

Alterations in climate are likely to have considerable impacts on most or all *ecosystems*. The distribution patterns of many species and communities are determined to a large part by climatic parameters; however, the responses to changes in these parameters are rarely simple (Tuba et al., 2004). Ecotones are transition areas between adjacent but different environments: habitats, ecosystems, landscapes, biomes or

eco-climatic regions. Climate change due to excessive environmental pollution is expected to exacerbate desertification. Reduced precipitation and increased evapotranspiration will change the spatial features of ecotones (e.g. the coalescence of patches, on the one hand, and increased fragmentation on the other). Furthermore, the overexploitation of the vegetation, which is typical in semi-arid drylands (WWF, 2010; UN, 2011) combined with climate change, will further increase habitat loss and hence the loss of biodiversity, ecosystem services and the potential for adaptation. The overall contribution of agriculture to CO_2 emissions is very low. However, grasslands and especially low input grassland, is believed to act as a sink for carbon dioxide and nitrous oxide (Tilman, 1999), but this cannot be considered as generalised figure. The vegetation of the Carpathian basin suffers from precipitation anomalies, rather than temperature changes (Czimer, 2004). Finally, since there is no chance to give any summary or plausible forecast regarding the direct and indirect problems emerging from climate change phenomena, there is only one hint we may accept: „avoiding the unmanageable, managing the unavoidable” as it has been stated by John Schellnhuber, a spiritual father of European climate change research (ADAM, 2008).

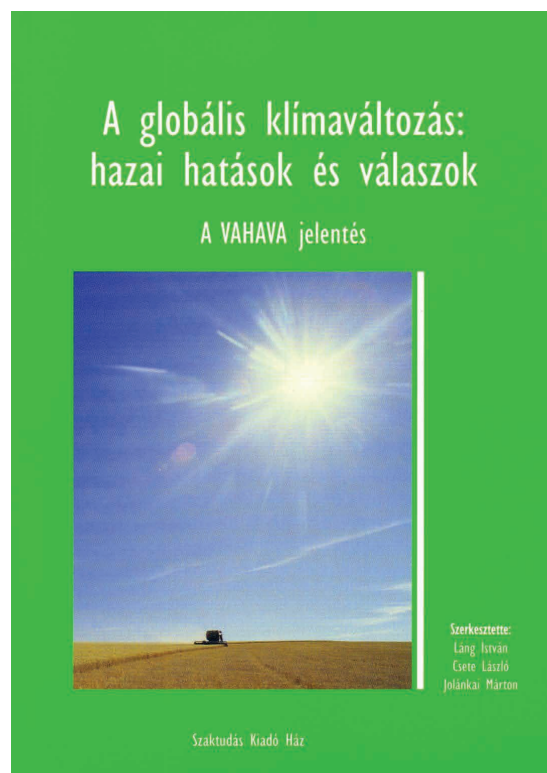


Figure 6. The VAHAVA Report (first Hungarian edition), Source Szaktudás Kiadó Ház 2007

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The profitability of millet cultivation on heavy soils

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Abstract: Between years 2013 and 2015, field treatments with millet variety Biserka was carried out in locality Milhostov, where experimental place of Agroecology Research Institute is situated. Two soil tillage technologies (conventional and reduce tillage) and two fertilization variants (control and soil conditioner PRP SOL) were examined. The economic effectiveness of individual variant of millet growing was evaluated. In experimental years 2013 – 2015 the highest costs (more than 560 € ha⁻¹) were determined for conventional tillage at variant with soil conditioner PRP SOL. The lowest costs, on level 330 € ha⁻¹, was on control variant under reduce tillage. In year 2015 the weather was very severe, which was the cause of the lowest millet yields and so the lowest gains were achieved, too. The highest profitability was determined for control variants under reduce tillage. Lower gains from variants with application of PRP SOL soil conditioner will be compensate in next years, when impact of this conditioner on soil environment will be more significant and will be effected of consecutive crops.

Introduction

The millet (*Panicum miliaceum* L.) is one of the oldest cultivated cereals in the world. At present, it is a basic food for over 400 million people. Millet contains polyphenols with antioxidant impact (Léder 2010). This cereal does not gluten and hence it is suitable for food production for people with celiac disease (Janovská 2014). Millet is also used for malt production, which is further utilized to brew celiac disease (Zarnkow et al. 2010; De Meo et al. 2011). Under climate change, with frequent and longer periods of drought, the millet cultivation is very interesting by reason of its high dryness (Agdag et al. 2001; Seghatoleslami 2008). PRP SOL soil conditioner is usually applied for amelioration of unfavourable properties of heavy clay soils and for formation of soil environment with positive impact on soil fertility, soil structure, soil water management and improvement of nutrients transport, too.

The objective of this manuscript was comparison the effect of soil conditioner in relation to millet yield and profitability of its cultivation under different tillage.

Material and methods

In year 2013, the field stationary treatment with millet, variety Biserka, was carried out. Studied crop was included in right crop rotation as follows: sweet sorghum (*Sorghum bicolor* L.

Moench.) – buckwheat (*Fagopyrum esculentum Moench.*) – millet (*Panicum miliaceum* L.) – amaranth (*Amaranthus* sp. L.).

Gleyic Fluvisol in Milhostov is characterized as heavy, clay-loamy soil with average content of clay particles 53.08 % in topsoil. Gleyic Fluvisol was formed on heavy alluvial sediments during the long-time contact with groundwater and surface water. The topsoil has lump aggregate structure with high binding ability and it has a weak perviousness in its whole profile. In the depth 0.7–0.8 m of soil profile, a layer of dark grey clay is found. The level of underground water is high. Agronomical properties of Gleyic Fluvisol are significantly influenced by the high content of clay particles. The basic properties of topsoil of this soil type are as followed: average particle density was 2 607 kg m⁻³, average bulk density 1 451 kg m⁻³, average total porosity 44.35 % (Kotorová, Kováč 2017). The average values of chemical properties of the topsoil (depth from 0.0 to 0.3 m) are as follows: available phosphorus content 50 mg kg⁻¹ (Mehlich III), available potassium content 240 mg kg⁻¹ (Mehlich III), available magnesium content 460 mg kg⁻¹ (Mehlich III), exchangeable calcium content 5 200 mg kg⁻¹ (Mehlich III), soil reaction (1 M KCl) 6.3, humus content 3.2 %, the type of humus is from humate-fulvic to fulvic-humate with humic acids and fulvic acids ratio from 0.8 to 1.2 (Šoltysová 2013).

Two tillage technologies, namely conventional tillage (CT) and reduce tillage (RT), were examined. At conventional tillage, after harvest of forecrop, were made agrotechnics arrangements as follows: stubble ploughing, later mean ploughing, pre-sowing soil prepare by skive-cultivator and sowing by sowing machine Great Plains. At reduce tillage, after harvest of forecrop, was made stubble ploughing by skive plough-harrow and before sowing the soil was prepared by skive-cultivator.

Soil conditioner PRP SOL was examined in millet stand. Two variants were monitored and it: 1. control variant – without mineral fertilizers nor conditioners, 2. PRP SOL variant – dose 200 kg ha⁻¹ of PRP SOL conditioner was applied in pre-sowing soil prepare.

The norms in reference to Kavka et al. (2006) and Abrham et al. (2007) were used to cost evaluation of set of machines and working procedures. It was recalculated in conditions of heavy soils of the East Slovak Lowland. The total production was calculated on base of real production for regional processor according to approved contract price. Economy effectiveness of production technologies was evaluated in accordance with methodology Poláčková et al. (2010).

The calculation of economy effectiveness:

- production [€ ha⁻¹] = yield [t ha⁻¹] × realization price [€ t⁻¹]
- profit/loss [€ ha⁻¹] = production [€ ha⁻¹] - costs [€ ha⁻¹]
- profit/loss [€ t⁻¹] = realization price [€ t⁻¹] - costs [€ t⁻¹]
- profitability of costs per 1 hectare [%] = [profit/loss : costs] × 100
- income threshold for null profitability [t ha⁻¹] = costs [€ ha⁻¹] : realization price [€ t⁻¹]

Results and discussion

The millet is not of such commercial importance as wheat and barley, but interest in its cultivation is increasing, mainly for its using in alimentary production. It is also in connection with healthy

eating and gluten-free diets. The quality of millet proteins is higher than winter wheat proteins (Kalinová and Moudrý 2006). The millet is unpretentious crop, but its cultivation requires attention at stand foundation (Agdag et al. 2006; Káš and Janovská 2011) as well as at its fertilization (Turgut et al. 2006).

The weather course has significantly effect on growth and development of cultivated plants. Experimental area is characterized as warm and very dry lowland continental climate region T 03 (Linkeš et al. 1996). The sum of the precipitation and average air temperature were compared to long-time normal (LTN) from years 1961–1990 (Mikulová et al., 2008). The long-term mean yearly precipitation shows 550 mm, during vegetation season 348 mm, the mean annual temperature is 8.9 °C, during vegetation season 16.0 °C. Course of meteorological factors were evaluated according to Kožnarová and Klabzuba (2002). Weather conditions at the site in experimental years are shown in Table 1.

Table 1. Evaluation of weather conditions

Year	Average air temperature			Sum of precipitation		
	°C	variation [°C]	eva.	mm	% LTN	eva.
LTN	8.9	0.0	N	550	100.0	N
2013	10.3	+1.4	VW	530	96.4	N
2014	11.1	+2.2	EW	613	111.5	H
2015	11.0	+2.1	EW	447	81.3	D

Where: eva. – evaluation, LTN – long-term normal, N – normal, VW – very warm, EW – extremely warm, H – humid, D – dry

The year 2013 from the point of view of the average air temperature indicated that this year was very warm. Average air temperature in years 2014 and 2015 was higher than long-term normal by 2.2 and 2.1 °C and both experimental years were extremely warm. From point of view of the sum precipitation, year 2013 was normal (96.4 % of LTN), year 2014 was humid (111.5 % of LTN) and year 2015 was dry with sum of precipitation only 81.3 % of LTN. Economic effectiveness of trial variants for 2013 year is shown in Table 2.

Material costs were higher for variant with PRP SOL application, what is related to the costs

Table 2. Inputs and economics of millet cultivation in year 2013

Parameter	Unit	Control		PRP	
		CT	RT	CT	RT
Material cots	[€ ha ⁻¹]	81.10	102.85	225.10	246.85
Costs of mechanized works	[€ ha ⁻¹]	208.23	133.74	214.56	140.07
<i>Variable costs common</i>	<i>[€ ha⁻¹]</i>	<i>289.33</i>	<i>236.59</i>	<i>439.66</i>	<i>386.92</i>
Fixed costs	[€ ha ⁻¹]	120.52	93.79	124.72	97.99
Total costs	[€ ha⁻¹]	409.85	330.38	564.38	484.91
Yield	[t ha ⁻¹]	1.93	2.18	2.10	2.48
Exercise price	[€ t ⁻¹]	400.00	400.00	400.00	400.00
Total production	[€ ha ⁻¹]	772.00	872.00	840.00	992.00
Result of farming per hectare	[€ ha⁻¹]	362.15	541.62	275.62	507.09
Profitability per hectare	[%]	88.36	163.94	48.83	104.57
Income threshold for zero profitability	[t ha ⁻¹]	1.02	0.83	1.41	1.21

Where: CT – conventional tillage, RT – reduce tillage of the purchase soil conditioner. For variant with PRP SOL higher costs for mechanized works, in connection of its application, were determined, too. At conventional soil tillage, for variant with PRP Sol conditioner the total costs reached almost 565 € ha⁻¹. For variant with PRP SOL under reduce tillage, the total costs were lower by nearly 80 € ha⁻¹. The lowest total costs were at reduce tillage and it 330.38 € ha⁻¹ on control variant. The millet yields, after PRP SOL application, under conventional soil tillage increased only about 0.25 t ha⁻¹ and under reduce tillage it was about 0.38 t ha⁻¹.

The exercise price of millet in year 2013 was on level 400 € t⁻¹. from point of view of the highest total production was achieved for PRP Sol variant under reduce soil tillage and it was 992 € ha⁻¹. On this variant the profit amounted to 507.09 € ha⁻¹. Despite lower millet yield from the hectare, more profit was obtained from control variant under

reduce soil tillage (541.62 € ha⁻¹). Profitability per hectare for this variant was 163.94 % and control variant under reduce tillage would be profitable at yield higher than 0.83 t ha⁻¹.

In year 2014 the costs in comparison to year 2013 significantly altered, but exercise price decreased to 350 € ha⁻¹. At conventional tillage the millet yields were significantly higher than 3 t ha⁻¹ and from this reason for conventional tillage variants not only the total production, but also higher profit in comparison to reduce tillage variants were achieved (Table 3.). The control variants without fertilization were more profitable than PRP SOL variants. The highest profit was obtained from control variant under conventional tillage and it was 377.32 € ha⁻¹, the profitability per hectare was more than 166 %.

In year 2015 the costs of millet cultivation were not radically changed. The millet yields in this year were significantly lower in comparison

Table 3. Inputs and economics of millet cultivation in year 2014

Parameter	Unit	Control		PRP	
		CT	RT	CT	RT
Material cots	[€ ha ⁻¹]	80.93	103.43	240.93	263.43
Costs of mechanized works	[€ ha ⁻¹]	206.23	132.44	212.51	138.72
<i>Variable costs common</i>	<i>[€ ha⁻¹]</i>	<i>287.16</i>	<i>235.87</i>	<i>453.44</i>	<i>402.15</i>
Fixed costs	[€ ha ⁻¹]	120.52	93.79	124.72	97.99
Total costs	[€ ha⁻¹]	407.68	329.66	578.16	500.14
Yield	[t ha ⁻¹]	3.1	2.2	3.2	2.19
Exercise price	[€ t ⁻¹]	350.00	350.00	350.00	350.00
Total production	[€ ha ⁻¹]	1085.00	770.00	1120.00	766.50
Result of farming per hectare	[€ ha⁻¹]	677.32	440.34	541.84	266.36
Profitability per hectare	[%]	166.14	133.57	93.72	53.26
Income threshold for zero profitability	[t ha ⁻¹]	1.16	0.94	1.65	1.43

Where: CT – conventional tillage, RT – reduce tillage

Table 4. Inputs and economics of millet cultivation in year 2015

Parameter	Unit	Control		PRP	
		CT	RT	CT	RT
Material costs	[€ ha ⁻¹]	85.55	109.85	249.55	273.85
Costs of mechanized works	[€ ha ⁻¹]	195.71	125.61	201.72	131.63
<i>Variable costs common</i>	<i>[€ ha⁻¹]</i>	<i>281.25</i>	<i>235.46</i>	<i>451.27</i>	<i>405.48</i>
Fixed costs	[€ ha ⁻¹]	120.52	93.79	124.72	97.99
Total costs	[€ ha⁻¹]	401.77	329.25	575.99	503.47
Yield	[t ha ⁻¹]	1.65	1.58	1.93	1.88
Exercise price	[€ t ⁻¹]	380.00	380.00	380.00	380.00
Total production	[€ ha ⁻¹]	627.00	600.40	733.40	714.40
Result of farming per hectare	[€ ha⁻¹]	225.23	271.15	157.41	210.93
Profitability per hectare	[%]	56.06	82.36	27.33	41.90
Income threshold for zero profitability	[t ha ⁻¹]	1.06	0.87	1.52	1.32

Where: CT – conventional tillage, RT – reduce tillage with yields in 2014, mainly for control variant. In year 2015, the exercise price increased on 380 € ha⁻¹, but total production from 1 hectare was lower than in year 2014 (table 4.). For experimental variants, viz. control and PRP SOL variants, the profit was obtained, but more profit was determined for control variant. These results confirm statistically significant effect of meteorological factors on millet yield and also effect of exercise price on profitability of millet cultivation. The lowest profit, namely only 157.41 € ha⁻¹, and the profitability per hectare only 27.33 % were reached from variant with PRP Sol application under conventional tillage.

The result of farming per hectare between 2013 and 2015 years are shown on fig. 1. From fig. 1 it becomes clear, the lowest profits were achieved in year 2015, because from point of view of weather conditions it was dry and extremely warm year. Similarly, the lowest profits were determined for

variant with PRP SOL conditioner application under conventional tillage of heavy soil.

For economy of plant production is very important evaluation of plant cultivation profitability. From development of this economics parameter of millet cultivation on fig. 2 resulted, that the highest profitability per 1 ton of millet grain was achieved from variant without PRP SOL application under reduce soil tillage technology.

Conclusions

The highest production of millet was achieved in year 2013 at reduce tillage with PRP SOL application and that was 992 € ha⁻¹ with a profit of 507.09 € ha⁻¹. The control variant without fertilization under reduce tillage was more profitable (541.62 € ha⁻¹) and profitability per 1 hectare was 163.94 %. In year 2014 from conventional tillage variants was higher production and also higher profit in comparison

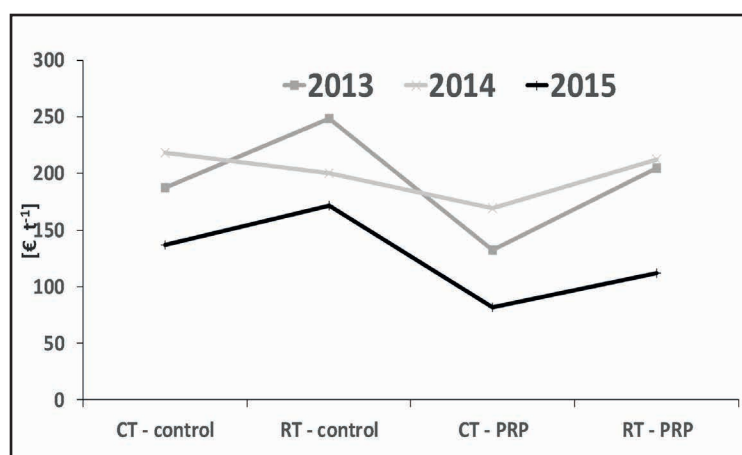


Figure 1. Profitability of millet cultivation per 1 ton

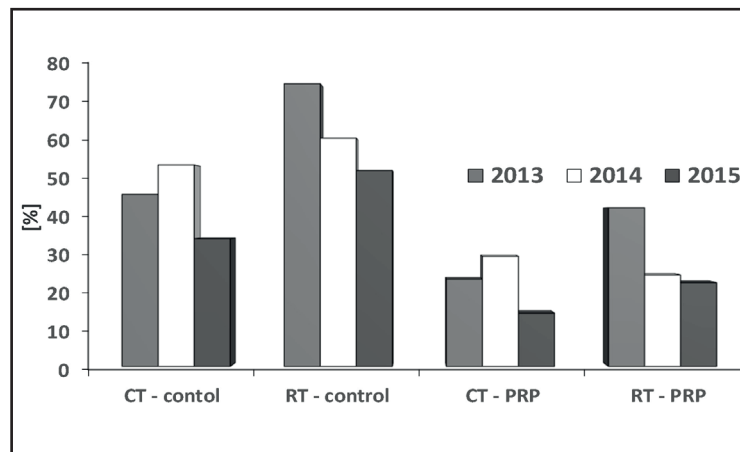


Figure 2. Result of farming per 1 ton of millet cultivation

to reduce variants. From comparison control and PRP SOL variants result more profitable were control variants without fertilization. The highest profit (677.32 € ha⁻¹) was determined for control variant under conventional tillage.

In year 2015 the total production from all variants was lower than in year 2014. All control variants given higher profit. The lowest profit (157.41 € ha⁻¹) was found for PRP sol variant under conventional tillage.

The highest profitability was reached under reduce tillage of soil at no-fertilized control variant. In all more profitable were no-fertilized control variants in compared with variants PRP SOL.

Soil conditioner PRP SOL, from point of view of farmers, increase total costs on millet cultivation in 1st year of growing, but PRP SOL activity in soil profile is distributed during two to the three follows years. Favourable effect of PRP SOL on soil properties was confirmed in our experiments. In our experiments the need to combine application of soil conditioner PRP SOL with mineral fertilizers, mainly nitrogen, were also validated.

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Portrait of Columella, in Jean de Tournes, Insignium aliquot virorum icones.
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Lucius Junius Moderatus Columella

(AD 4 – 70) is the most important writer on agriculture of the Roman empire. His *De Re Rustica* in twelve volumes has been completely preserved and forms an important source on agriculture. This book was translated to many languages and used as a basic work in agricultural education until the end of the 19th Century.