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# Columella

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## Foreword

### Scientific illiteracy

Chemistry is the basic science that enables us to understand biological phenomena. Life, including environment, infra and supra individual live organisms from microbes to giant mammals is driven by chemical processes. Therefore life sciences are dedicated to the knowledge of principles to make our life better and easier, also to learn the means and the ways of influencing natural processes in favour of enhancing environmental quality, to produce food and feed, to create and use advanced materials as well as to save our lives by improved medical and pharmaceutical patterns. Consequently chemistry should be reasonably a part of public literacy at least on a rudimentary level. Regrettably the case is much different. Scientific illiteracy, and the growth and spreading of pseudoscience and false philosophies degrade the knowledge of the society.

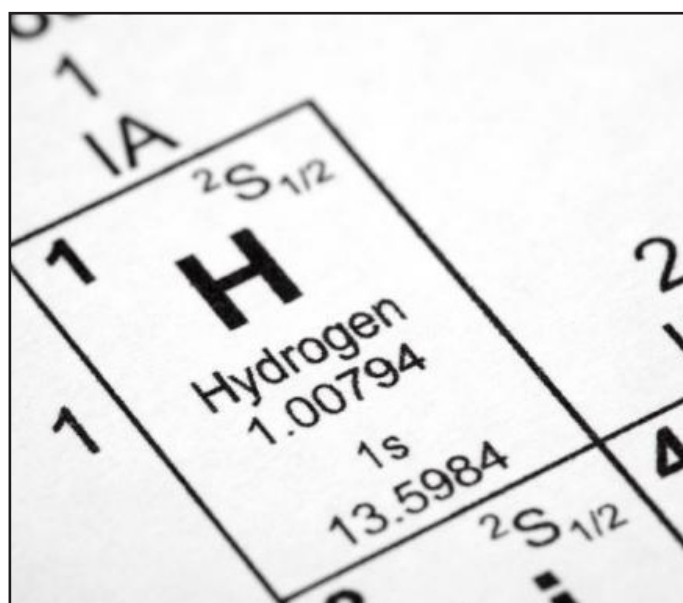


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Recently I had a conversation with a high rank government official who had rather strong belief and an irrevocable opinion blaming nitrogen to be a poisonous chemical substance. For my greatest surprise I was the first person to inform her that nitrogen is an essential macro element for any live organism, and the fact, that some 78 per cent of the atmosphere is made of this material. After this meeting I had a feeling that there must be some problems with our education system.

Hundred years ago uneducated cleansing women had sufficient knowledge on basic household chemicals, their effect and possible interactions. The use of acids and alkaline substances was an everyday practice with no major problems in general. According to press information eight toilettes were blown up by mixing non appropriate doses of bleaches and acids, and two swimming pools had to be closed temporarily for developing chlorine gas in one single year only in our country.

However the case is more serious than meeting people with less information on chemical processes. Today there are worldwide movements of rather aggressive nature to propagate pseudoscientific theses and to influence – sometimes violently – the society to accept their superstitious ideas. Quite frequently they reach the level of politics and turn to be active participants in the processes of decision making.

The story of dihydrogen monoxide is a good example for highlighting the system of manipulating the public with arguments of no scientific value as well as making attempts to influence politics. First in 1997 activists circulated protesting charts that were undersigned by twenty eight thousand citizens in favour of influencing the government to ban dihydrogen monoxide – the invisible killer! All facts written were true concerning this molecule. These are as follows: “Dihydrogen monoxide is colourless, odourless, tasteless, and kills uncounted thousands of people every year. Most of these deaths are caused by accidental inhalation, but the dangers of dihydrogen monoxide do not end there. Prolonged exposure to its solid form causes severe tissue damage. Symptoms of dihydrogen monoxide ingestion can include excessive sweating and urination, and possibly a bloated feeling, nausea, vomiting and body electrolyte imbalance. For those who have become dependent, dihydrogen monoxide withdrawal means certain death. Dihydrogen monoxide is also known as hydric acid, and is the major component of acid rain. It contributes to the *Greenhouse Effect*. It may cause severe burns. It contributes to the erosion of our natural landscape. It accelerates corrosion and rusting of many metals. It may cause electrical failures and decreased effectiveness of automobile brakes. It has been found in excised tumours of terminal cancer patients”. Thanks to God, governments did not respond in this case and did not ban the use of water. But they do regularly in many other – almost similar – cases.

What can we do? There is quite a lot to do. Lack of knowledge can only be treated by education, dissemination of information, convincing the public and in general by teaching the young generation. A scientific paper has a double task in this process. We have to encourage scientists to publish their research results, and on the other hand any journal has a responsibility in providing a bridge between authors and readers.

*Márton Jolánkai*





## Laboratory studies on the effects of a neonicotinoid-containing seed treatment product on non-target soil animals

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**Abstract:** Cruiser 350 FS (Syngenta) is a widely applied thiamethoxam-containing seed treatment product. Despite of this fact, little is known about its side effects on non-target organisms other than bees. In this study, the effects of Cruiser 350 FS mixed in soil in different concentrations (according to OECD standards) were investigated on the mortality and reproduction of a Collembola species (*Folsomia candida*). On the basis of springtail mortality data, an  $LC_{50}$  of 223.6 mg/kg and a NOEC of 24.5 mg/kg were determined for *Folsomia candida*. The following toxicological limits were obtained on the basis of the reproduction data from the springtail test:  $EC_{50}$ : 61.73 mg/kg, NOEC: 12.27 mg/kg. Moreover, mortality tests were performed in microplates on two nematode species of different feeding and reproductive strategies. After 24 hours of exposure, treatment had no effect on mortality up to a concentration of 35 g of thiamethoxam/l in the r-strategist bacterivorous *Panagrellus redivivus*; while the species-specific  $LC_{50}$  was determined to be 0.19 g/l for *Xiphinema vuittenezi*. Our results proved the K-strategist plant-feeding *X. vuittenezi* to be more sensitive than the r-strategist bacterivore *P. redivivus*. Our results highlight the difference in the sensitivity of nematodes of different feeding and functional groups, suggesting the importance of a more sophisticated study approach.

**Keywords:** side effect, thiamethoxam, Collembola, Nematoda

### Introduction

The main environmental problem related to plant protection products is that they may pose a risk to non-target organisms, thereby decrease the diversity of species and functions.

The use of insecticide neurotoxins that are similar to plant-based nicotine and belong to the class of neonicotinoids has become widespread in the past two and a half decades. The most problematic issue is their sublethal effect on non-target organisms (Blacquièrre et al. 2012). These agents have received attention because of their effect on bees (Apidae), the most important pollinators. An association was found between the use of neonicotinoids and Colony Collapse Disorder (CCD), a syndrome that occurs in Western honeybees (*Apis mellifera* L.) (Pisa et al. 2015), and probably at bumblebees (*Bombus* spp.), which is the second most studied group (Laycock et al. 2012). Neonicotinoids are effective against insect pests both in the case of direct contact and ingestion. Their mode of action is blocking acetylcholine receptors on the

post-synaptic side, thus disrupting stimulation, leading to the death of the insect (Honda et al. 2006). These agents can interfere with the very precise coordination between the neural and the hormonal systems of an insect, disrupting the series of behavioural and physiological events that lead to egg laying (Desneux et al. 2007). Neonicotinoids are effective at very low concentrations, provided that the duration of exposure is sufficiently long (Tennekes 2010). Findings of Whitehorn et al. (2012) show that imidacloprid reduced bumblebee colony growth and the rate of egg laying by queens under laboratory conditions. For this reason, after a moratorium period of two years in 2013-2015, the EU introduced a ban on the use of neonicotinoid pesticides. Despite of this fact, the side effects of these compounds in terrestrial systems are still rather poorly known.

Thiamethoxam-containing seed treatment products reach the soil, these may affect soil biota that live there and play a central role in soil decomposition and nutrient turnover

processes. Despite this, hardly any literature data are available concerning the effects of these agents on soil fauna (Bonmatin et al. 2015). Springtails are important regulators of decomposition processes in soil through the selective consumption of soil microbes (Fountain and Hopkins 2005; Seres et al. 2007; 2009). El-Naggar et Zidan (2013) studied the effects of thiamethoxam on soil fauna under field conditions. Soil samples were obtained from two depths following seed treatment with a 70% Cruiser WG (2 g/kg seeds) and spraying with 25% Actara WG (20 g/100 l). The number of individuals belonging to the following soil animal groups was determined weekly for five weeks: Collembola, Psocoptera, Oribatida, Actinedida, Gamasida. When thiamethoxam-containing plant protection products were used, a clear increase was noted in the number of individuals of soil animals studied, mainly that of springtails. This phenomenon was probably due to death of predatory mites. For springtails, one single study was found to investigate the effects of Cruiser 350 FS, a thiamethoxam-containing insecticide under laboratory conditions (Alves et al. 2014). In acute test, it was found that the product was lethal to the springtail *Folsomia candida* only at the highest concentration tested (1,000 mg/kg). In the chronic test, thiamethoxam had no effect on reproduction of springtails at the concentrations tested (0.06 mg/kg, 0.12 mg/kg, 0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg). The following toxicological limits were derived from the study: a NOEC of 500 mg/kg in the acute test and a NOEC of 1 mg/kg thiamethoxam in the chronic test for reproduction.

Nematodes are a part of soil microfauna and are important participants in soil processes due to their high numbers, diverse feeding types and life history strategies. As for the effects of neonicotinoid insecticides to free-living nematodes, scientific literature is restricted to the sensitivity of entomopathogenic nematodes (EPN) to these compounds (Koppenhöfer et al. 2003; 2015).

Usually, bacterivorous r-strategist nematodes that are easy to breed in the laboratory are

used in toxicological studies. The species most commonly used as test animals are *Caenorhabditis elegans* (Maupas, 1900) and *Panagrellus redivivus* (Linné, 1767). However, several results show that there may be huge differences in the sensitivity to xenobiotics of nematodes with different life history and feeding strategies (Bongers and Bongers 1998; Nagy 2009). Therefore, apart from performing tests on *Panagrellus redivivus*, we aimed to involve a further test species of considerably different feeding type and life strategy as compared to the usually applied bacterial feeder test species.

Springtails and free-living nematodes applied in ecotoxicology were used to explore the effects of the seed treatment product on soil animals. The questions to be answered during the research were:

- (i) At what concentrations and at what rate does the tested insecticide cause mortality in the springtail species (*Folsomia candida*)?
- (ii) Does thiamethoxam have an effect on the reproduction of the tested springtail species?
- (iii) What is the mortality rate caused by the insecticide in two nematode species with different feeding type and life strategy (*Panagrellus redivivus*, *Xiphinema vuittenezi*) in the acute mortality test at different concentrations?

## Materials and methods

### *Collembolan reproduction test*

The test was performed on the basis of OECD 232 protocol (OECD, 2009). The parthenogenetic species, *Folsomia candida* (Willem 1902) was used as test animal. Synchronised cultures were established first because same aged individuals (9–12 days) are required for the test. Test agent was Cruiser 350FS, a thiamethoxam-containing seed treatment product. Seven concentrations were set in the test (3.5 g/l, 1.75 g/l, 0.875 g/l, 0.437 g/l, 0.219 g/l, 0.109 g/l, and 0.055 g/l) on the basis of results from a previous range finding test (unpublished data). Four replicates were applied for each concentration and 8 in the negative control without pesticide. These

concentrations were equal to 786 mg/kg, 393 mg/kg, 196 mg/kg, 98 mg/kg, 49 mg/kg, 24 mg/kg, and 12 mg/kg, respectively, as calculated to dry soil weight. Water holding capacity of soil was determined before the test and soils were moistened to 60% thereof. Standard soil was mixed at proportions according to the protocol as follows: 5% sphagnum peat, 20% kaolin clay, 74% sand and 1% calcium carbonate. An amount of 24.5 g of OECD soil was placed into plastic test vessels. They were moistened with 5.5 ml of distilled water and a water solution of the insecticide at a given concentration. Ten randomly selected 10-12 days old specimens of *Folsomia candida* were introduced into each of the test vessels with an aspirator. The animals were provided baker's yeast at the start of the test and once weekly thereafter. Duration of the test was 28 days, during which test vessels were kept in an incubator (TS 606CZ/4-Var) at  $20 \pm 1^\circ\text{C}$ . Test validity criteria in the control groups were: adult mortality should not exceed 20%, the number of juveniles should reach 100 and the coefficient of variation calculated for the number of offspring should not exceed 30%. At the end of the test, the number of adults and juveniles was counted. Animals were washed from soil in the vessels and were counted after transferring them into a Petri dish and adding blue ink for better visibility. The endpoints of the test were mortality, i.e. the number of dead individuals, and reproduction, i.e. the number of offspring.

#### *Acute mortality test on nematodes*

The test was performed involving two free-living soil nematode species. One of these is the r-strategist bacterivorous *Panagrellus redivivus*. The other species is the K-strategist plant-feeding nematode, *Xiphinema vuittenezi* (Luc, Lima, Weischer and Flegg, 1964). The test is not standardised but *P. redivivus* has been commonly used since decades as its testing is simple, quick and does not require special equipment (Samoiloff 1987). Endpoint of the tests was mortality, with an exposure time of 24 hours. We also considered the nematode tests valid if mortality in the control treatment did

not exceed 20%. Thiamethoxam concentrations used in the first two range-finding tests for both species were as follows: 350 g/l, 35 g/l, 3.5 g/l, 0.35 g/l and 0.035 g/l. Eight replicates were applied for each concentration and 16 in negative control without pesticide. Tests were performed in disposable microtiter plates with 8x12 wells (Bioster). An amount of 370  $\mu\text{l}$  test solution, or in the control distilled water were pipetted into each well. Subsequently, five *P. redivivus* and three *X. vuittenezi* specimens were introduced with a micropipette to the holes of the microtiter plates. Adult female individuals were randomly selected from synchronised cultures in the case of *P. redivivus*. *X. vuittenezi* females were extracted from samples collected from grapevine soil in a garden in Isaszeg, Hungary (GPS coordinates: 47.52945, 19.39419). It is difficult to maintain cultures of this species under laboratory conditions. For soil extractions we used a modified version of Cobb's decanting and sieving method (Brown and Boag 1988), which is based on the positive hydrotaxic response of the animals. Nematodes were stored in a refrigerator at  $4^\circ\text{C}$  until the initiation of testing. The third test was set on the basis of the previous ones. Based on the results of the range finding tests, the definitive test was carried out only in *X. vuittenezi* in 6 replicates. Apart from concentrations and numbers of replicates, the definitive test was identical to the *Xiphinema* range finding test. The used concentrations were as follows: 3.5 g/l, 1.75 g/l, 0.875 g/l, 0.437 g/l, 0.219 g/l, 0.109 g/l, and 0.055 g/l of thiamethoxam. Subsequently, plates were placed into a temperature-controlled incubator (TS606-CZ/4-WAR) until the end of the test at  $20 \pm 1^\circ\text{C}$ . Test plates were checked under a microscope (Olympus SZH 10). Immobility and no response to physical stimulations are signs of mortality in nematodes. Moreover, in case of *X. vuittenezi*, open C-shaped body posture is a typical form of dead animals. In contrary *P. redivivus* individuals become straight after death, similarly to other small bacterivorous species.

#### *Statistical analysis*

The results were analysed using R software

package (R Core Team 2013). After confirming that the conditions of the utility, one-way ANOVA and a post-hoc Dunnett's test were used. ToxRat statistical software was used in order to determine NOEC, LOEC, LC<sub>50</sub> and EC<sub>50</sub> levels.

**Results**

*Collembolan reproduction test*

The test satisfied validity criteria as follows: mean mortality of adults in the control group was 1.25%; mean number of offspring in the control group was 376±65.38 individuals, variation coefficient: 17.39. Based on ANOVA results, treatment had a strongly significant effect on both mortality (F=77.39, p<0.001) and reproduction (37.69, p<0.001). Dunnett's test revealed a significant difference in the mortality of springtails as compared with control at 393 mg/kg (0.875 g/l) concentration and at higher concentrations (Figure 1). On the basis of mortality data, LC<sub>50</sub> was defined as 223.6 mg/

kg (0.996 g/l) while mortality NOEC level was found to be 24.5 mg/kg (0.109 g/l). Reproduction differed from the control group at 49.1 mg/kg concentration level and at higher concentrations (Figure 2). The following toxicological limits were determined on the basis of reproduction data from springtail test: EC<sub>50</sub>: 61.73 mg/kg, (0.275 g/l), NOEC: 12.27 mg/kg (0.055 g/l).

*Acute mortality test in nematodes*

In the case of the bacterivorous species, *P. redivivus*, the highest concentration resulted in 100% mortality, while lower concentrations lead to significant differences in two cases: at 0.35 g/l and at 3.5 g/l (Table 1). However, mortality did not differ from the control group when the 35 g/l concentration was used. In test with *Xiphinema* species, the two highest concentrations resulted in 100% mortality and high mortality was observed at the next two concentration levels as well (3.5 and 0.35 g/l) (Table 1). A significant difference was found in each case compared to the control, except

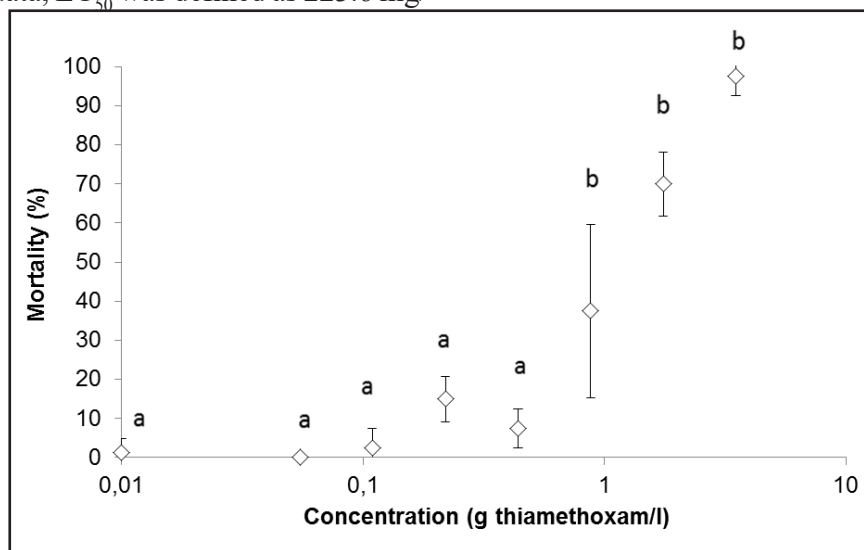


Figure 1. Mean mortality of adult *Folsomia candida* individuals (± SD) on a logarithmic scale. Different letters indicate significantly different results (p<0.001) according to Dunnett's test results.

Table 1. Mortality of *Panagrelus redivivus* and *Xiphinema vuittenezi* individuals (%) in range-finding test (mean of 8 replicates ± SD).

	Concentration (g/l)					
	Control	0.035	0.35	3.5	35	350
<i>P. redivivus</i>	0 ± 0	2.5 ± 6.2	17.5 ± 19.9	22.5 ± 14.7	0 ± 0	100 ± 0
<i>X. vuittenezi</i>	12.5 ± 15.2	4.2 ± 10.4	83.3 ± 22.2	91.7 ± 20.8	100 ± 0	100 ± 0

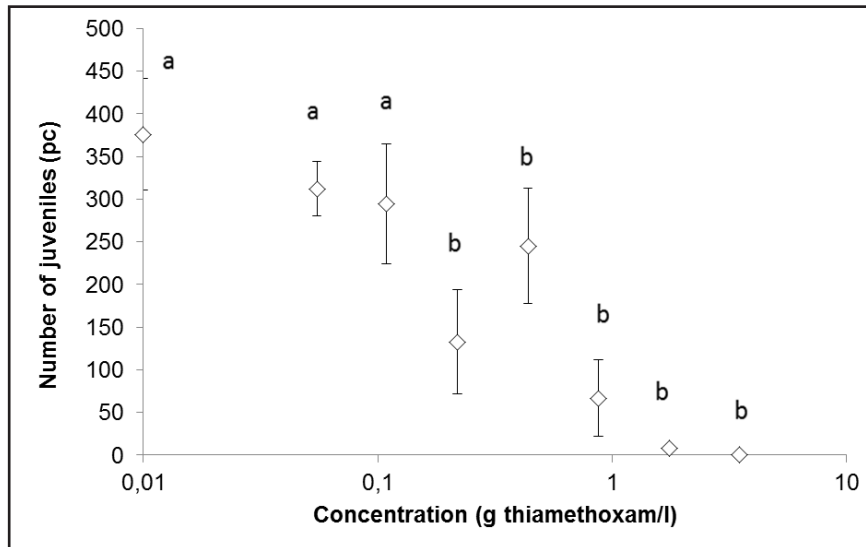


Figure 2. Mean number of *Folsomia candida* offsprings during each treatment ( $\pm$  SD) on a logarithmic scale. Different letters indicate significantly different results ( $p < 0.001$ ) according to Dunnett's test results.

the lowest concentration. The second test with *X. vuittenezi* yielded more specific results regarding sensitivity of the species (Figure 3). A mortality of 100% was experienced at the three highest concentration levels. Dunnett's test showed a significant difference ( $F=2.81$ ,  $p < 0.05$ ) in comparison with control group already at the lowest concentration (0.055 g/l). The next concentration level (0.109 g/l) did not differ statistically from the control group, but higher concentrations resulted a strong significant difference in each case ( $p < 0.001$ ). The species-specific  $LC_{50}$  was determined to be 0.19 g/l.

### Discussion

The results from the OECD reproduction test with springtails show that the applied pesticide caused a complete mortality only at a very high concentration (786 mg/kg), which is not realistic under field conditions of normal agricultural practices (Bonmatin et al. 2015). Mortality rate differed significantly from that observed in the control group at concentrations of 196 mg/kg and above where a mortality of 37.5% was experienced. A significant effect on reproduction was found at a lower concentration, i.e. 49.1 mg/kg. When the above toxicity indices

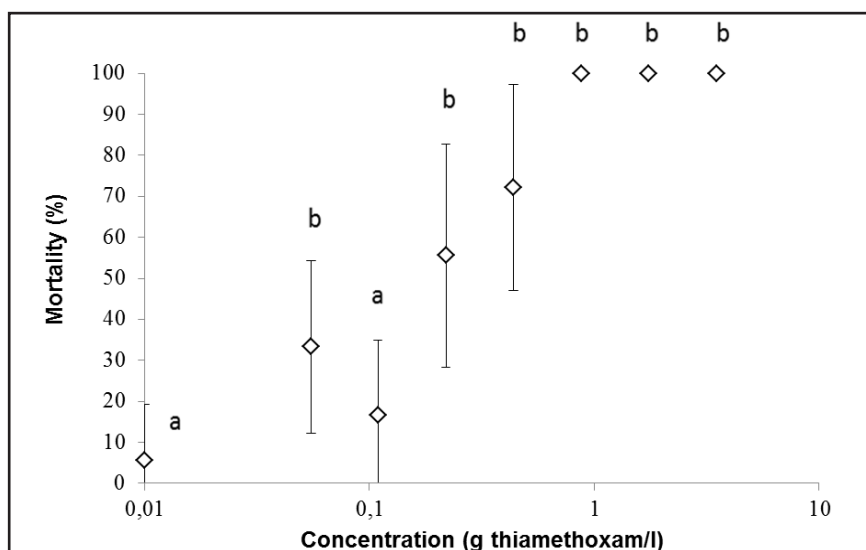


Figure 3. Mean mortality of adult *Xiphinema vuittenezi* females ( $\pm$  SD) on a logarithmic scale. Different letters indicate significantly different results ( $p < 0.001$ ) according to Dunnett's test results.

are compared with the results by Alves et al. (2014), it is to be noted that lower mortality NOEC and LC<sub>50</sub> values were obtained, thus springtails were found to be more sensitive to thiamethoxam in the present experiment. This may be due to the fact that Alves et al. (2014) examined mortality during a two-week subacute test, while in our study a four-week chronic test was used. Regarding inhibition of reproduction, Alves et al. (2014) determined a NOEC of 1 mg/kg. In the present test a higher NOEC of 12.27 mg/kg was calculated. The difference in results is explained by the test concentrations chosen. These concentrations are well above that are used and potentially present in the soil under realistic circumstances. PEC of the thiamethoxam-containing Cruiser F350 is 0.201 mg/kg based on literature data (Alves et al. 2013). Field concentrations used in practice most probably do not have a negative side effect on springtails, but discharge of this agent into the soil in high concentrations (for example, in the case of an accident or inadequate application) poses a risk to this group of soil animals. Our results explain the phenomenon seen in studies by El-Naggar et al. (2013). The number of springtails increased at the expense of other soil-dwelling microarthropods because these animals are not sensitive to thiamethoxam and their number increased after the death of their predators.

The tests performed with the two free-living nematode species showed that, following 24 hours of exposure, treatment had no mortality effect up to a concentration of 35 g of thiamethoxam/l in the r-strategist bacterivorous *Panagrellus redivivus*; however, a 100% mortality rate was observed at a concentration of 350 g/l. Hardly any studies that specifically examine the side effects of neonicotinoids on free-living nematodes were found in literature. Only the effectiveness of entomopathogenic nematode (EPN) species has been tested, with the focus on

their ability to control insect pest as influenced by the insecticide treatment. According to Kopenhöffer et al. (2015), neonicotinoid treatments usually showed a synergistic interaction when applied in combination with several EPN species (e.g. *Steinernema glaseri*, *Heterorhabditis bacteriophora*). The combined effects of thiamethoxam and entomopathogenic nematodes of the order Rhabditida against pest insects were investigated both under field and laboratory conditions. During the tests performed by Kopenhöffer et al. (2003), thiamethoxam did not have a negative effect on the reproduction of entomopathogenic nematodes. This supports the results of our study concerning *P. redivivus*, which also belongs to the order Rhabditida, i.e. nematodes of this group were not sensitive to thiamethoxam. As for the mortality of *Xiphinema vuittenezi*, the difference versus the control group was significant even at the lowest concentration after the exposure period of 24 hours. Mortality rate was 100% when the test concentration of 0.875 g/l was used. According to our results obtained so far, the sensitivity of the K-strategist plant-feeding *X. vuittenezi* considerably exceeds that of *P. redivivus*. This finding makes it worthwhile to carry out further tests on the sensitivity of different plant-feeding nematodes for the environmentally realistic concentration levels of neonicotinoid compounds. Furthermore, our results highlight the difference in the sensitivity of nematodes belonging to different feeding and life history groups. This underlines the importance of a more sophisticated study approach than the generally applied use of few r-strategist bacterial feeder species only.

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## Correlation between fertilization and baking quality of winter wheat cultivars

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**Abstract:** In a long-term experiment, a complex study of the baking quality parameters of four modern winter wheat genotypes (GK Csillag, Mv Csárdás, Mv Toldi, GK Békés) was carried out in fertilizer treatments with increasing dosages (control, N<sub>60</sub>+PK, N<sub>120</sub>+PK) in two different years (2013 and 2014) on chernozem soil in Eastern Hungary. In the control and the N<sub>120</sub>+PK treatments, the protein content ranged within the boundaries of 8.88-11.46% (in 2013) and 6.73-11.19% (in 2014) and 11.03-13.30% (in 2013) and 10.53-14.29% (in 2014), respectively. The wet gluten content values were 24.88-37.18% (in 2013) and 18.03-23.53% (in 2014) in the control and 35.30-43.16% and 33.28-39.10% in the N<sub>120</sub>+PK treatment. Using Pearson's correlation analysis, a tight correlation was found between fertilization and the protein content ( $r = 0.571^{**}$ - $0.739^{**}$ ) and between fertilization and the wet gluten content ( $r = 0.587^{**}$ - $0.859^{**}$ ). A medium correlation was observed between fertilization and the farinographic value ( $r = 0.275$ - $0.484^{**}$ ) and between fertilization and gluten elasticity ( $r = 0.322^{**}$ - $0.466^{**}$ ). Fertilization did not have an impact on the falling number ( $r = -0.014$ - $0.226$ ). Strong correlation was found between the protein and the wet gluten contents ( $r = 0.817^{**}$ - $0.950^{**}$ ).

**Keywords:** winter wheat, varieties, fertilization, baking quality parameters, yield

### Introduction

Foods made from wheat have been the bases of human nutrition for several thousands of years. Along with the social changes, the human diet has also changed significantly as a result of which the consumption of cereals was reduced in the developed countries. In line with this, the demand has increased for wheat from which high-quality bread and bakery products can be made with the lowest possible amount of additives. Yield and bread-making quality influenced by genotype, growth conditions and fertilization regime (Johansson et al. 2004)

The ecological conditions, the genetical bases of the grown winter wheat varieties and the agrotechnical elements are important satisfactory for the production of wheat with a good baking quality (Pepó et al. 2005, Babulicová. 2014). The baking quality of winter wheat is basically defined by the genetically determined characteristics of the variety, which can be modified by the ecological conditions (primarily by weather) and the agrotechnical elements (primarily by fertilization) to a lower or higher extent (Pollhamerné 1973, Borghi et al 1997, Shewry et al. 2000, Zhao et al. 2005, Drezner et al. 2007, Har Gil et al. 2011). From among the agrotechnical elements, the nutrient supply

(mainly that of nitrogen) has direct and indirect impacts on the baking quality of winter wheat (Goos et al. 1982, Peterson et al. 1992, Vida et al. 1996, Pechanek et al. 1997, Ragasits 1998, Pepó et al. 2005, Zecevic et al. 2010). Responses of wheat to nitrogen (+PK) application have been well recognized for many varieties under different ecological conditions (Johansson et al. 2004, Erekul et al. 2012). A more favourable nutrient supply, the increasing nitrogen fertilizer dosages – in addition to a harmonized NPK and mezo- and microelement fertilization – increased the protein and wet gluten contents of wheat, but the gluten elasticity, the falling number and the farinographic value were less affected (Jolánkai 1993, Johansson 2002, Pedersen and Jorgensen 2007, Wang et al. 2009, Erekul et al. 2012). The research results proved that NPK fertilization increased the farinographic value of winter wheat varieties, but the increment varied with the year (Barič et al. 2004, Mikulikova et al. 2009, Balla et al. 2011), and the year-dependent stability of the quality was much lower under deficient or moderate NPK fertilization (Holford et al. 1992). International and Hungarian experimental results proved that the baking quality parameters modified by NPK fertilization depended on genotype (Ragasits 1992, Jolánkai et al. 1998, Pepó 1999, Lloveras et al. 2001.),

so variety-specific fertilization needed in the practical management to improve the quality of wheat. *Vaughan et al. (1990)*, *Pepó et al. (2005)* and *Erekul et al. (2012)* found that there were of varying correlation among the different baking quality parameters, which were strongly dependent upon the year.

The aim of our research was to analyze the baking quality of modern wheat genotypes under different levels of NPK fertilization and in different years and to determine the correlations between the agronomical parameters, the yields and the quality of wheat varieties in a long-term experiment in Eastern Hungary (Hajdúság).

### Materials and methods

The long-term experiment was set up on calcareous chernozem soil at the Látókép Experimental Farm of the University of Debrecen 15 km from Debrecen (NL 47° 33', EL 21° 27') in 1983 Eastern Hungary. The humus content of the experimental soil was 2.7-2.8%, the pH value was near neutral ( $\text{pH}_{\text{KCl}} = 6.46$ ). The width of the humus layer was 0.8-1.0 m. The original AL-soluble  $\text{P}_2\text{O}_5$  and  $\text{K}_2\text{O}$  contents of the top 0-0.25 m soil layer were 135  $\text{mg kg}^{-1}$  and 240  $\text{mg kg}^{-1}$ , which were significantly modified in the different fertilization treatments during the past 30 years of the experiment. The experimental chernozem soil has excellent water management characteristics.

In the long-term experiment, the nutrient requirements and fertilizer response of winter wheat varieties with different genotypes are studied at six fertilization levels. In addition to the control, the fertilizer treatments were the basic treatment of  $\text{N} = 30 \text{ kg ha}^{-1} + \text{P}_2\text{O}_5 = 22.5 \text{ kg ha}^{-1} + \text{K}_2\text{O} = 26.5 \text{ kg ha}^{-1}$  and its twofold, threefold, fourfold and fivefold dosages. The quality tests were performed on the samples of the control, the  $\text{N}_{60} + \text{PK}$  and the  $\text{N}_{120} + \text{PK}$  treatments in four repetitions. The long-term experiment was set up in a split-split-plot design, the size of the plots is 10  $\text{m}^2$ , the number of repetitions is 4. During the 30 years of the experiment, 100% of the phosphorus and potassium fertilizers and 50% of the nitrogen fertilizers were applied in

the autumn. The remaining 50% of the nitrogen was applied in early spring. The forecrop was sweet corn in all years.

A standard, modern agrotechnique (tillage, sowing, plant protection, harvest) was applied in the experiment. In the experiment four modern, new Hungarian winter wheat genotypes were tested: GK Csillag, Mv Csárdás, Mv Toldi, GK Békés.

The baking quality of the wheat samples collected at harvest was determined by the accredited laboratory of the University of Debrecen Centre for Agricultural Sciences Central Laboratory. The quality tests were performed according to the relevant standards: wet gluten content (HS ISO 5531:1993), gluten elasticity (HS ISO 6369-5:1987), farinographic value (HS ISO 5530-3:1995), Hagberg's falling number (HS ISO 3093:1995), wheat protein content (ICC 159:1995).

The experimental data were evaluated using the programs SPSS for Windows 13.0 and Microsoft Office 2013 Excel by two-way analysis of variance and Pearson's correlation analysis.

### Results and discussion

The year had a significant effect on both the quantity and the quality of yield in the different winter wheat varieties. There were great differences between the experimental years in the weather parameters (*Table 1*). The dry autumn period of the season of 2012/2013 hindered the germination and early development of the stands. The winter period, the cold weather in early spring (in March) and the extreme amount of precipitation (136.3 mm) also had a harmful effect on the vegetative development and the tillering of plants. Although the total amount of precipitation in the season of 2012/2013 was higher than the 30-year average (466.6 mm), its distribution had a negative effect on the amount of yield. The lower than average precipitation and the above-average temperature in the period of ripening (June-July) were partly favourable for protein building, however, they had a negative impact on the composition of proteins. The year of 2013/2014 was very special,

Table 2. Monthly average precipitation and temperature in the vegetation period of winter wheat (Debrecen, 2013-2014)

	Oct.	Nov.	Dec.	Jan.	Feb.	March	Apr.	May	June	July	Total (mm)/ Average (°C)
<b>Rainfall (mm)</b>											
2012/2013	22.4	16.6	65.8	38.7	52.4	136.3	48.0	68.7	30.8	15.6	495.8
2013/2014	39.1	51.5	0	39.2	26.0	11.3	39.6	69.4	7.9	128.0	412.0
30 year average	30.8	45.2	43.5	37.0	30.2	33.5	42.4	58.8	79.5	65.7	466.6
<b>Temperature (°C)</b>											
2012/2013	11.1	7.2	-1.2	-1.0	2.3	2.9	12.0	16.6	19.6	21.2	9.07
2013/2014	11.8	7.6	0.5	2.0	7.8	8.9	12.3	15.4	19.0	21.2	10.65
30 year average	10.3	4.5	-0.2	-2.6	0.2	5.0	10.7	15.8	18.7	20.3	8.27

mainly regarding the monthly mean temperatures (Table 1). In this year, the temperatures of the winter months were considerably higher than the multi-year average (in December 0.5 °C as compared to the multi-year average of -0.2 °C, while the corresponding values were 2.0 °C and -2.6 °C in January and 7.8 °C and 0.2 °C in February), which enabled an undisturbed, continuous vegetative development. As a result of that the stands developed a huge vegetative mass and the wheat phenological phases occurred 2-3 weeks earlier than usual.

The large vegetative mass caused an early and significant lodging under increasing fertilizer dosages, which also had a detrimental effect on the levels of infection by leaf diseases. Due to the accelerated plant development, the dry and warm weather in June had a weaker impact on the baking quality parameters.

The plant height and lodging values and the yields of the tested winter wheat varieties gave a good indication of the year effect and the fertilizer response (Table 2, including the values of all fertilizer treatments). In 2013, the plant height (41.9-64.7 cm in the control) was unfavourable due to the negative weather effects, especially in the control and the low-dosage fertilizer treatments (N<sub>30</sub>+PK), it increased to an average value with increasing fertilizer dosages (80.4-94.3 cm in the N<sub>120</sub>+PK treatment). Due

to the average vegetative development, lodging did not occur in the stands (except for GK Békés N<sub>120</sub>+PK = 17% and N<sub>150</sub>+PK = 39% lodging, which are considered minimal). The weaker vegetative development could be detected also in the yields, especially in the control treatment where the yields of the tested varieties ranged between 1316 and 1686 kg ha<sup>-1</sup>. In 2013, the yields of the varieties in the N<sub>120</sub>+PK treatment varied from 5200 to 6281 kg ha<sup>-1</sup>, which can be considered average under the given ecological and agrotechnical conditions. The highest yield was measured in GK Békés in 2013. As opposed to that, the weather conditions of 2014 resulted in a large vegetative biomass. Due to the excellent natural nutrient-providing capacity of the chernozem soil, the plant height was also high in the control treatment (78.1-94.2 cm), which was further increased as a result of the fertilizer treatments (ranging from 96.3 to 119.6 cm in the N<sub>120</sub>+PK treatment). Due to the large vegetative mass, there was an early lodging in the stands in 2014 (in April) and the degree of lodging continuously increased until the harvest. Lodging at harvest varied between 0 and 100% in the N<sub>120</sub>+PK treatment, depending upon the genotype. Resistance to lodging was favourable in Mv Toldi (0% lodging) and partly in Mv Csárdás (41%). The favourable natural nutrient supply of the soil was proven by the control yields (3847-5431 kg ha<sup>-1</sup>, the highest

Table 2. Effect of fertilization and cropyear on the yield, plant height and lodging of winter wheat varieties (Debrecen, 2013-2014)

Variety (V)	Fert. (F)	Yield (kg ha <sup>-1</sup> )		Lodging (%)		Plant height (cm)	
		2013	2014	2013	2014	2013	2014
GK Csillag	Ø	1651	5431	0	0	41.9	86.1
	N <sub>30</sub> +PK	3107	7818	0	0	58.6	89.8
	N <sub>60</sub> +PK	4639	8350	0	0	69.7	92.4
	N <sub>90</sub> +PK	5981	7926	0	56	77.6	94.6
	N <sub>120</sub> +PK	6207	7519	0	100	80.4	96.3
	N <sub>150</sub> +PK	6562	6871	0	100	84.2	99.1
Mv Csárdás	Ø	1316	3847	0	0	64.7	94.2
	N <sub>30</sub> +PK	2451	7025	0	0	74.2	104.6
	N <sub>60</sub> +PK	3834	6772	0	0	82.6	116.1
	N <sub>90</sub> +PK	4832	6418	0	0	91.5	116.9
	N <sub>120</sub> +PK	5200	6136	0	41	94.3	117.6
	N <sub>150</sub> +PK	5819	5419	0	68	96.1	119.7
Mv Toldi	Ø	1547	4372	0	0	60.7	88.7
	N <sub>30</sub> +PK	3300	7563	0	0	71.2	100.6
	N <sub>60</sub> +PK	4172	8520	0	0	79.6	112.7
	N <sub>90</sub> +PK	5367	8286	0	0	88.3	117.9
	N <sub>120</sub> +PK	5616	8019	0	0	90.2	119.6
	N <sub>150</sub> +PK	6183	7780	0	0	92.6	120.8
GK Békés	Ø	1686	5172	0	0	53.8	78.1
	N <sub>30</sub> +PK	3272	7915	0	0	64.6	98.0
	N <sub>60</sub> +PK	4419	7131	0	12	75.9	112.1
	N <sub>90</sub> +PK	5464	6942	0	25	82.7	115.2
	N <sub>120</sub> +PK	6281	6574	17	81	90.1	116.6
	N <sub>150</sub> +PK	5807	6386	39	100	92.6	117.3
LSD <sub>5%</sub> fert. (F)		350	420	6	8	6.8	7.1
LSD <sub>5%</sub> var. (V)		274	319	4	6	4.9	6.4
LSD <sub>5%</sub> (F x V)		681	706	11	14	9.6	11.6

value measured in GK Csillag). In the N<sub>60</sub>+PK treatment, the yields of the tested wheat varieties varied between 6772 and 8520 kg ha<sup>-1</sup>, which was reduced by lodging in the N<sub>120</sub>+PK treatment (6136-8019 kg ha<sup>-1</sup>).

The baking quality examinations (Table 3) proved that the flour protein content and the wet gluten content values significantly increased as a result of the fertilizer treatments (increasing NPK dosages) in both years. In the control, the flour protein content ranged from 8.88 to 11.46% (in 2013) and from 6.73 to 11.19% (in 2014) depending upon the variety. The wet gluten content varied between 24.88 and 37.18% (in 2013) and between 18.03 and 23.53% (in 2014) in the control treatment. Both the protein and the gluten contents increased significantly as a result of the fertilizer treatments in both years (Table 3). In the N<sub>120</sub>+PK treatment, the

protein content ranged from 11.03 to 13.30% and from 10.53 to 14.29%, while the wet gluten content varied between 35.30 and 43.16% and between 33.28 and 39.10%. The protein content of Mv Toldi was favourable in both years and the gluten content of GK Békés could also be described as good.

Fertilization and the year had only a moderate effect on gluten elasticity (Table 3). The gluten elasticity values ranged within the optimum interval (2-6 mm) in both years (with a few exceptions). The fertilizer dosages minimally augmented the gluten elasticity, however, the changes were not significant. Due to the favourable dry weather at harvest, the Hagberg's falling number values were favourable (Table 3). The falling numbers of the wheat varieties varied between 374 and 430 s in 2013 and between 325 and 421 s in 2014. The fertilizer

Table 3. Effect of fertilization and cropyear on the baking quality parameters of winter wheat varieties (Debrecen, 2013-2014)

Variety (V)	Fert. (F)	Wet gluten (%)		Gluten elasticity (mm)		Falling number (s)		Farinograph value		Protein content (%)	
		2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
GK Csillag	Ø	24.88	18.03	3.3	1.7	381	350	58.43	53.48	8.88	6,73
	N <sub>60</sub> +PK	28.51	30.71	3.4	3.5	398	356	58.58	66.18	9.16	10,61
	N <sub>120</sub> +PK	35.30	33.28	5.3	4.5	387	354	63.60	66.87	11.03	10,53
Mv Csárdás	Ø	37.18	19.35	5.6	2.9	374	372	56.83	72.58	11.46	6,91
	N <sub>60</sub> +PK	38.13	31.78	6.3	3.8	425	410	57.23	56.37	12.14	10,66
	N <sub>120</sub> +PK	43.16	34.61	7.3	4.5	400	404	58.30	63.67	13.07	12,04
Mv Toldi	Ø	36.16	20.22	3.4	4.0	417	421	56.70	51.10	11.41	7,22
	N <sub>60</sub> +PK	36.38	31.50	3.9	3.0	413	393	65.05	78.28	11.92	12,00
	N <sub>120</sub> +PK	40.65	33.76	4.6	2.1	430	397	61.00	84.30	13.10	14,29
GK Békés	Ø	33.69	23.53	3.9	1.8	423	325	64.50	62.08	11.02	11,19
	N <sub>60</sub> +PK	37.92	35.81	5.6	4.1	386	359	63.53	70.95	11.94	12,18
	N <sub>120</sub> +PK	42.53	39.10	6.3	5.6	374	387	62.43	73.05	13.30	12,37
LSD <sub>5%</sub> fert. (F)		1,67	5,89	1,3	1,4	36	38	3,75	6,91	0,57	1,04
LSD <sub>5%</sub> var. (V)		1,43	2,19	0,9	1,2	31	27	3,20	5,82	0,43	0,89
LSD <sub>5%</sub> (F x V)		2,47	3,73	1,5	2,0	53	47	5,55	10,09	0,74	1,54

treatments did not have a significant effect on the falling number. The most complex baking quality parameter of wheat is the farinographic value. The experimental results proved (Table 3) that the farinographic value was influenced both by the year and the fertilization treatment. Depending upon the fertilizer treatment and the genotype, the farinographic value ranged from 56.70 to 64.50 in 2013 and from 51.10 to 84.30 in 2014. The impact of fertilization was moderate in 2013. The farinographic values were between 56.70 and 64.50 in the control and between 58.30 and 63.60 in the N<sub>120</sub>+PK treatment. The highest value was obtained in the control treatment in the variety GK Békés. In 2014, the farinographic value increased due to the fertilizer treatments, however, the differences were not always significant or consequent (Mv Csárdás gave the highest value in the control treatment). A significant improvement could be observed as a result of fertilization in Mv Toldi which had excellent stem strength in 2014 (control: 51.10, N<sub>60</sub>+PK: 78.28, N<sub>120</sub>+PK: 84.30).

The effect of fertilization on the baking quality of winter wheat varieties of new genotypes was studied in a long-term experiment in two different

years (2013 and 2014) on chernozem soil. For determining the cause and effect relationships behind the changes in quality, the plant height and lodging values and the yields of wheat varieties were used. The two years were greatly different as regards weather which was proven by the vegetative development of the stands (plant height) and by the lodging values. In 2013, the development of the varieties was very moderate in the control treatment, lodging did not occur even under increasing fertilizer dosages. Yields in 2013 in the control and the N<sub>120</sub>+PK treatment were 1316-1686 kg ha<sup>-1</sup> and 5200-6281 kg ha<sup>-1</sup>, respectively. In 2014, the stands developed a large vegetative biomass, which resulted in an early and significant lodging (except for Mv Toldi). The excellent nutrient-providing capacity of the chernozem soil was shown by the yields of the control treatment (3847-5431 kg ha<sup>-1</sup>) in 2014. The yields of the varieties significantly increased due to fertilization in the N<sub>60</sub>+PK treatment (6772-8520 kg ha<sup>-1</sup>), but then they were reduced in the N<sub>120</sub>+PK treatment (6136-8019 kg ha<sup>-1</sup>).

From among the baking quality parameters, the flour protein content and the wet gluten content were significantly influenced by the variety and

Table 4. Study of interrelation among the fertilization and baking quality parameters of winter wheat using by Pearson correlation (Debrecen, 2013-2014)

Cropyear	Parameters	Wet gluten	Gluten elasticity	Falling number	Protein content	Farinograph value
2013	Fertilization	0.587**	0.522**	-0.014	0.571**	0.275
	Wet gluten	1	0.7139**	0.101	0.950**	0.083
	Gluten elasticity	0.713**	1	-0.196	0.637**	-0.019
	Falling number	0.101	-0.196	1	0.144	0.045
	Protein content	0.950**	0.637**	0.144	1	0.121
	Farinograph value	0.083	-0.019	0.045	0.121	1
2014	Fertilization	0.859**	0.466**	0.226	0.739**	0.484**
	Wet gluten	1	0.546**	0.172	0.817**	0.475**
	Gluten elasticity	0.546**	1	0.162	0.157	-0.021
	Falling number	0.172	0.162	1	0.076	0.045
	Protein content	0.817**	0.157	0.076	1	0.574**
	Farinograph value	0.475**	-0.021	0.045	0.574**	1
2013-2014	Fertilization	0.643**	0.440**	0.111	0.636**	0.363**
	Wet gluten	1	0.694**	0.292**	0.841**	0.134
	Gluten elasticity	0.694**	1	0.163	0.389**	-0.177
	Falling number	0.292**	0.163	1	0.175	0.090
	Protein content	0.841**	0.389**	0.175	1	0.353**
	Farinograph value	0.134	-0.177	-0.090	0.353**	1

the fertilization similarly to the results of *Pepó* et al. (2005), *Zecevic* et al. (2010) and *Erekul* et al. (2012). In accordance with the research by *Johansson* (2002), *Petersen* and *Jorgensen* (2007) and *Pechanek* et al. (1997), it was found that fertilization had only a moderate effect on gluten spreading, the falling number and the farinographic value and the effects were not significant. Baking quality parameters (protein, wet gluten etc.) and grain yields of winter wheat cultivars were strongly affected by growing season (*Mikulikova* et al. 2009, *Balla* et al. 2011).

The effect of the year and the fertilization on the baking quality parameters and the correlations between the quality parameters were determined by Pearson's correlation analysis (Table 4). The correlations between fertilization and the flour protein content ( $r = 0.571^{**}$ ,  $r = 0.739^{**}$ ) and between fertilization and wet gluten content ( $r = 0.587^{**}$ ,  $r = 0.859^{**}$ ) were medium in 2013 and tight in 2014. There was a medium

correlation between fertilization and gluten elasticity ( $r = 0.522^{**}$  in 2013 and  $r = 0.466^{**}$  in 2014) and between fertilization and the farinographic value ( $r = 0.275$  in 2013,  $r = 0.484^{**}$  in 2014). In our study, no correlation was found between fertilization and the falling number ( $r = -0.014$ ,  $r = 0.226$ ). According to scientific results of *Erekul* and *Köhn* (2006) and *Erekul* et al. (2012) there was no impact of fertilization on the falling number of wheat varieties in the studied years. As an average of the experimental years, fertilization had the greatest impact on the wet gluten content ( $r = 0.643^{**}$ ) and on the flour protein content ( $r = 0.635^{**}$ ). A moderate correlation was found between fertilization and gluten elasticity ( $r = 0.440^{**}$ ) and the farinographic value ( $r = 0.363^{**}$ ). These results justified the conclusions of *Mikulikova* et al. (2009) and *Pepó* et al. (2005). The results verified a very tight correlation between the protein content and the wet gluten content ( $r = 0.950^{**}$  in 2013,  $r = 0.817^{**}$  in 2014,

$r = 0.841^{**}$  as an average of the two years). A medium-strong correlation was found between the gluten content and gluten elasticity ( $r = 0.713^{**}$ ,  $r = 0.546^{**}$ ,  $r = 0.694^{**}$  as an average of 2013 and 2014).

### Conclusion

The yields and baking-quality parameters of winter wheat were affected by ecological factors (weather in the vegetation period), genotypes and nitrogen (+PK) fertilization on chernozem soil. Our scientific data in long-term experiment proved the results of *Johansson et al.* (2004) and *Erekul et al.* (2012): In favourable crop year (mild winter period) chernozem soil could provide excellent natural nutrient sources for the macroelement uptake of winter wheat genotypes (in control 3800-5400 kg ha<sup>-1</sup>) comparing with stress crop year (in control 1300-1700 kg ha<sup>-1</sup>). The yield increases of N(+PK) fertilization were much higher when the water supply was limited during vegetative period (in 2013 the yield surpluses

varied between 3900-4600 kg ha<sup>-1</sup> and in 2014 1400-3600 kg ha<sup>-1</sup>, respectively). According to the results of *Jolánkai* (1993), *Shewry et al.* (2000), *Drezner et al.* (2007) we proved that the most important determining factor in winter wheat baking quality was the genotype and its traits (tolerance to diseases and lodging etc.). We found strong correlations between fertilization and wet gluten content, medium correlation fertilization and gluten elasticity and farinograph value, and there was no correlation between fertilization and falling number. *Waugham et al.* (1990) and *Erekul et al.* (2012) found similar correlation indexes among the wheat baking quality parameters and genetic traits and weather conditions.

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## Effect of coloured shade nets on some nutritional characteristics of a kapia type pepper grown in plastic tunnel

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**Abstract:** Sweet pepper is the most important vegetable crop of the Hungarian greenhouse industry. Production of red coloured cultivars, having very high nutritive value, is getting popular recently. Shading of plastic tunnels is a common practice in Hungary, but research about its effects on the nutritional characteristics of sweet pepper has just started. The objective of this study was to investigate the effect of different shading methods (shading paint, white, green, yellow and red coloured shade nets) on some nutritional characteristics of a red coloured kapia type pepper, under Hungarian climatic conditions cultivated in unheated walk-in plastic tunnels. Shade net colour slightly affected radiation and hence temperature conditions of the tunnels; especially ratio of supra-optimal temperature values ( $32\text{ }^{\circ}\text{C} <$ ) was changed. Dry matter content was not influenced by shading method, in contrary to sampling time which had a significant effect on this parameter. Vitamin C and total polyphenol contents were affected by both of these factors and also by their interaction; shading effect on these phytonutrients changed with harvest times. However, total carotenoid content was affected just by this latter factor. Heat stress, higher ratio of supra-optimal temperature values, increased total polyphenol concentration and decreased total carotenoid content. In overall, white shade net resulted sweet peppers with the best nutritive quality, but harvest time had a more pronounced effect on content of nutritive constituents than shade net colour.

**Keywords:** *Capsicum annuum*, carotenoids, vitamin C, polyphenols, harvest time

### Introduction

Sweet pepper is by far the most important crop of the Hungarian greenhouse vegetable industry, accounting for about 40% of both total production area and total product (FruitVeB, 2015). Besides the commonly cultivated sweet wax-yellow type, production of red-coloured kapia type peppers is getting popular in Hungary recently. Sweet peppers have one of the highest nutritional values among vegetables (Rubatzky and Yamaguchi 1997). Peppers are not only one of the richest plant sources of vitamin C, but also contain pro-vitamin A carotenes, tocopherols, niacin, riboflavin and thiamine in considerable amounts (Bosland and Votava 2012). Red pepper pods contain twice as much vitamin C and 6 to 90 times more carotenoids than similar green fruits, with capsanthins and capsorubin being the main colour components (Frary and Frary 2012). An even higher, up to seventeen-fold increase in

vitamin C concentration during maturation was observed for chilli pepper hybrids (Nagy et al. 2015). Flavonoid and phenol compounds also contribute to the strong antioxidative property of this vegetable. Antioxidant capacity of pepper pods usually increases with maturity (Frary and Frary 2012). Environmental factors, such as radiation and temperature can greatly affect accumulation of antioxidants, such as carotenoids, vitamin C and polyphenols (Pék et al. 2011).

Low-cost, 2-meter-high walk-in plastic tunnels are still commonly used for vegetable forcing in Hungary, accounting for one third of total greenhouse area (Fodor 2014). Due to high temperatures during late spring, summer and early autumn, overheating could be a serious problem in these tunnels. To reduce the heat load generally ventilation, shading paint, external shade nets and/or mist irrigation are applied (López-Marín et al. 2012; Zhu et al. 2012).

Under high radiation conditions, moderate (20% to 30%) shading results in high bell pepper yield of good quality due to decreased incidence of sunburn and improved water use efficiency (Zhu et al. 2012; Díaz-Pérez 2013; Kitta et al. 2014). Photosensitive shade netting was developed around the beginning of the new century (Fallik et al. 2009). Compared to the traditional method (black shade net), application of red, pearl (white) and yellow nets resulted better yield and fruit quality, reduced infestation by pests and improved post-harvest quality (Stamps 2009; Goren et al. 2011; Shahak 2014). Yellow shade net was found to increase pepper yield and quality compared to traditional black nets in Israel in a field experiment (Fallik et al. 2009). Ambrózy et al. (2016) also reported significantly higher pepper yield under yellow net compared to white net and unshaded control.

By modifying the spectra and microclimate, coloured shade nets can also affect the concentration of phytonutrients in pepper pods. Coloured shade nets have increased nutritional value of pepper fruits according to several studies. In Israel Kong et al. (2013) reported higher vitamin C contents and increased antioxidant capacity under white net compared to the traditional black one in net house sweet pepper cultivation. In South Africa Selahle et al. (2015) also found that white net was more effective in increasing vitamin C and total polyphenol concentrations, and total antioxidant activity than yellow and red nets. Meanwhile  $\beta$ -carotene and lycopene contents were the highest under the control black net. In an experiment conducted in Hungary, the highest total carotenoid content was also achieved by white shade net (Ambrózy et al. 2016). In contrary of these favourable results Nagy et al. (2016) found that red, white and green nets all decreased vitamin C content of a yellow coloured chilli hybrid compared to the unshaded control.

The above mentioned results were all derived from field experiments. However, Milenkovic et al. (2012) and Ilic et al. (2015) observed that the effect of photosensitive shade net treatments on phytonutrient contents of pepper and tomato

is different if they are combined with plastic sheet cover of tunnels. This phenomenon was also experienced in Hungarian chilli pepper experiments (Nagy unpublished data 2014; Nagy et al. 2016). For example pepper vitamin C content was significantly the highest in the red shade net treatment in plastic tunnels, while in the parallel field experiment this colour resulted significantly lower values than black, white and blue nets (Milenkovic et al. 2012).

The current study aimed to investigate the effect of different coloured shade nets on some nutritional characteristics of a red coloured kapia type pepper cultivated in unheated walk-in plastic tunnels under Hungarian climatic conditions.

## Material and Methods

### Methods of the cultivation

The experiment was carried out in 2012 in a private farm close to Lajosmizse (NL 47°00', EL 19°64'). Kapia type, red coloured 'Kárpia F1' pepper hybrid was cultivated in six unheated walk-in plastic tunnels. Main parameters of the tunnels were the following: 5 m width, 2.3 m height and 40 m length. Tunnels were clad by Extra S-24 type (Solarker Ltd., Kecskemét, Hungary) light stable polyethylene film, having 180  $\mu$ m thickness and 8.5 m width.

Forty-day old seedlings were transplanted on the 24<sup>th</sup> of April in double rows at a distance of 0.3 m and 0.7 m between the rows. Distance between neighbouring plants in the rows was 0.25 m. As a result of this planting design, density reached 8 plants per m<sup>2</sup>. Plants were trained in a one-stem system.

Pepper plants were fertirrigated daily through a drip irrigation system. Tunnel ends and side vents were open over 22 °C day-time and 15 °C night-time tunnel air temperature. Overhead spray irrigation was applied when temperature exceeded 30 °C. Hard, full-grown, red coloured fruits were harvested in 7-10-day intervals.

### Treatments

Treatments consisted of different methods of shading; one tunnel corresponded to one

treatment. Four tunnels were covered with different coloured shade nets (white, green, yellow and red) of 34 g m<sup>-2</sup> weight (Első Magyar Kenderfonó PLC, Szeged, Hungary) at the top of the polyethylene cladding for the whole growing season. One tunnel was shaded by Shadefix paint (Royal Brinkman B.V., 's-Gravenzande, Holland) applied at two occasions during the season with a dose of 100 kg ha<sup>-1</sup>. The sixth tunnel remained unshaded and represented control.

### Measurements

Shading effect of treatments in the spectral range between 325 and 1075nm was measured by a portable spectroradiometer (FieldSpec® HandHeld 2, ASD Inc., Boulder, Colorado, USA) using white reference panel. Four measurements per treatment were recorded outside and inside each tunnel on the 2<sup>nd</sup> of August. Air temperature in the tunnels was determined by thermometers (Conrad Electronic SE, Hirschau, Germany) at every 30 minutes from 27<sup>th</sup> April to 27<sup>th</sup> September. For each tunnel three data loggers were situated in the centre of the tunnels at the height of plant apex. Data loggers were protected from direct sunlight and irrigation, and their situation was adjusted to plant height every second week.

Fruits used for chemical analysis were sampled twice in the season, on 19<sup>th</sup> July and on 25<sup>th</sup> September. One sample was composed of five fully coloured fruits, and four samples were taken

from each treatment. Dry matter was measured after freeze drying of the homogenized plant part materials. Ascorbic acid content was analysed using high performance liquid chromatography (Dong and Pace 1996). Total carotenoids were separated and cleaned on Al<sub>2</sub>O<sub>3</sub> column, and finally quantified spectrophotometrically at 470 nm (MÉTÉ 1977). The analyses of total polyphenols was performed according to the Folin-Denis method, spectrophotometrically at 760 nm, using catechin as standard (AOAC 1990).

### Statistical analysis

Nutritional results were expressed as the mean of the four replications ± standard errors. Air temperature values were evaluated by one-way analysis of variance (ANOVA). Nutritive characteristics were analysed with two-way ANOVA with the factors of treatment and sampling time. Mean separations were performed using Fisher's protected least significant difference test at P ≤ 0.05. Correlation analysis was applied to reveal relations among different parameters using Microsoft Excel 2007 software (Microsoft Inc., Redmond, Washington, USA).

## Results and Discussion

### Radiation and temperature conditions

Considerable differences among the shading effect of the treatments were revealed by the experiment. Compared to the white reference values measured

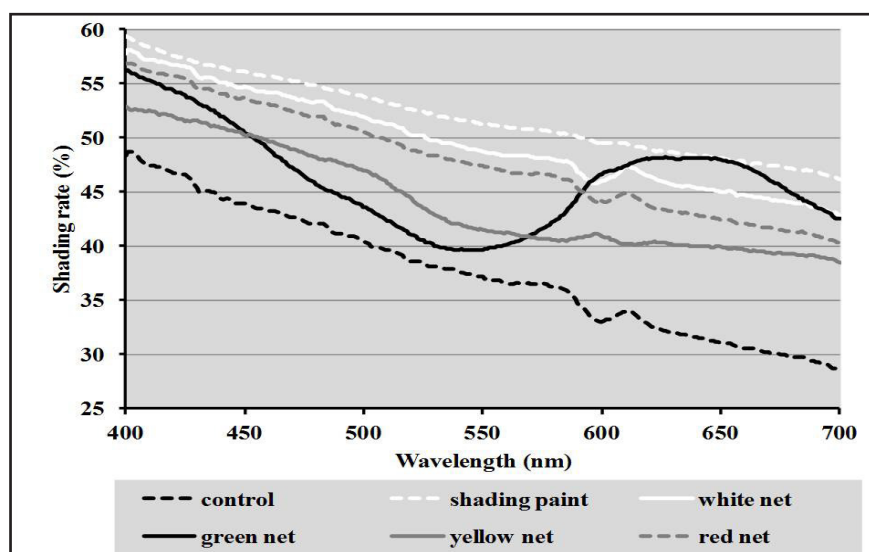


Figure 1. Shading rate of the applied treatments in the PAR range

outside of the tunnel, the cladding material itself showed a 31% cumulative shading ratio for the reflectance spectra between 325 and 1075 nm; while this value was detected 46% for the shading paint, 44% for the white net, 39% for the green net and yellow net, and 42% for the red net treatments. In the photosynthetically active radiation (PAR) range shading effect decreased continuously in relation to increasing wavelength. The order of the treatments was constant in the whole PAR range, except for the green net (Figure 1.). Green shading net resulted in slightly higher shading ratio (47%) in the 600 to 700 nm (red) spectral range than the other three net treatments (40% to 45%).

Except for the yellow shade net, average air temperature of tunnels was not affected by the treatments (Table 1), presumably due to proper ventilation and regular misting. Significant correlation was not found between shading ratio and average air temperature. Significantly the highest average air temperature was recorded for the yellow treatment, both for the whole growing period and for the 4-week period prior to the second sampling. These latter results are in accordance with the findings of Selahle et al. (2015). Unfortunately, based on the gathered data we could not find the reason for significantly higher air temperature in the yellow shade net treatment. One possible explanation is that

this shading material captured emitted infrared radiation in the tunnel more than the other ones. Prior to the second sampling average air temperatures were measured 6-7 °C lower compared to the period prior to the first sampling, when air temperatures were considerably higher than the optimal 21-23 °C range of pepper cultivation (Wien 1997). In accordance with the higher average temperature, ratio of supra-optimal temperature values was also the highest under the yellow net, especially prior to the second harvest (Table 1). During the period prior to the first sampling, ratio of high temperature values was the lowest in the white and in the green net treatments. Hence, plants of these tunnels were exposed to less heat stress.

#### Dry matter content

According to the results of the two-way ANOVA, shading did not significantly affect dry matter content of the kapia type sweet pepper fruits ( $P = 0.451$ ) (Figure 2). This result is in agreement with the findings of Goren et al. (2011) and Kong et al. (2013), but is in contrast with the data of Milenkovic et al. (2012) and Selahle et al. (2015). On the other hand, performing correlation analysis on prior to sampling average air temperature and dry matter content data separately for the two sampling times, significant positive correlations were found if results of the yellow shade net treatment were disregarded.

Table 1. Effect of shading method on temperature conditions of walk-in plastic tunnels

Treatment	Average air temperature (°C)			Ratio of supraoptimal (32°C<) temperature values		
	Whole period	Prior to 1 <sup>st</sup> sampling	Prior to 2 <sup>nd</sup> sampling	Whole period	Prior to 1 <sup>st</sup> sampling	Prior to 2 <sup>nd</sup> sampling
	22.04. – 02.10.	24.06. – 19.07.	28.08. – 25.09.	22.04. – 02.10.	24.06. – 19.07.	28.08. – 25.09.
Control	22.8 b*	25.9 ab	18.5 b	15.8% b	29.0% ab	2.3% b
Shading paint	22.7 b	26.6 a	18.9 b	15.3% b	30.9% a	4.1% b
White net	22.6 b	25.6 b	18.9 b	14.3% b	25.2% c	3.8% b
Green net	22.4 b	25.4 b	19.2 b	15.1% b	24.5% c	8.3% b
Yellow net	23.6 a	26.5 a	20.9 a	21.0% a	31.0% a	17.8% a
Red net	22.6 b	25.6 b	18.9 b	15.1% b	26.3% bc	5.2% b
Significance						
P-value	0.001	0.018	0.002	0.049	0.009	0.036

\*mean separation within columns according to Fisher's protected least significant difference test at  $P \leq 0.05$

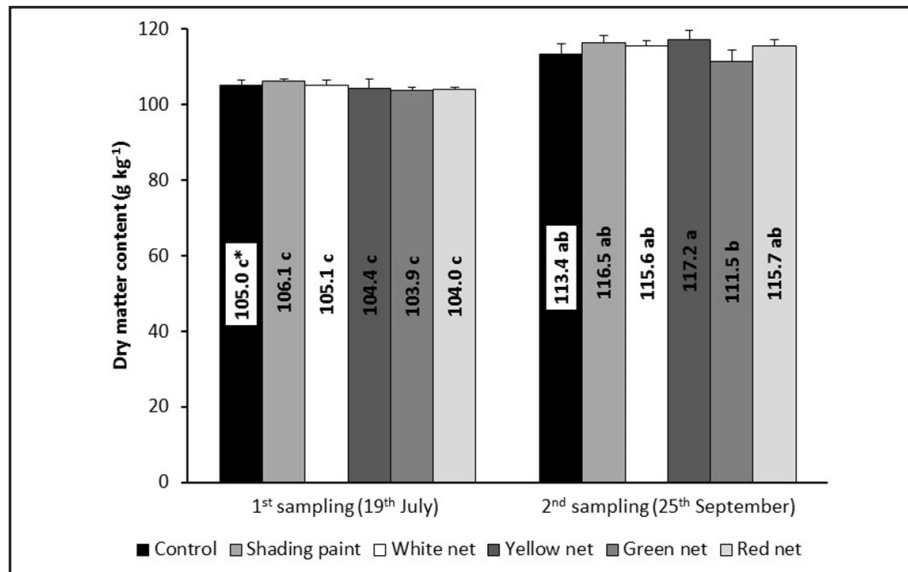


Figure 2. Effect of shading method and sampling time on dry matter content of kapia type red sweet pepper fruits \* Mean separation was performed using Fisher's protected least significant difference test at  $P \leq 0.05$ .

Hence despite of the ANOVA results, it can be stated that in this experiment colour of shading net affected dry matter content of pepper pods in some extent. Meanwhile, fruits sampled at the end of September had significantly higher dry matter content than the ones harvested in July ( $P = 2.28 \times 10^{-11}$ ). It is presumed that less irrigation and misting in September, may have resulted in higher dry matter accumulation in the pepper pods. The treatment x sampling time interaction did not have a significant effect on the dry matter content ( $P = 0.728$ ).

#### Vitamin C content

As sampling time had a significant effect on dry matter content, beside the fresh weight (FW) base, the investigated nutritional characteristics were also expressed on dry weight (DW) base, for ensuring better comparability (Table 2). Measured vitamin C values varied quite considerably, ranging between 1.29 and 2.40 g kg<sup>-1</sup> FW, which is considered around the average value for red coloured sweet pepper cultivars (Table 2). Vitamin C contents were affected by both shading and sampling time and also by their interaction. Kong et al. (2013) and Nagy et al. (2016) also found that shading effect on ascorbic acid content of sweet pepper changed with harvest times. On average of the two sampling times white and yellow

shade nets resulted significantly the highest DW based vitamin C content, while the red net treatment and the control had the lowest values (Table 2.). This order of the treatments is in good agreement with the findings of field experiments of Kong et al. (2013), Tinyane et al. (2013) and Selahle et al. (2015), but partly contradicts to results of Milenkovic et al (2012). Latter authors found significantly the highest ascorbic acid concentration under red shade net in plastic tunnels, while the same red net produced significantly the lowest vitamin C value under field conditions. Mashabela et al. (2015) assumed that more favourable red/far red (R/FR) photon ratio under the white net could improve the ascorbic acid content and the antioxidant scavenging activity in sweet pepper fruits. Among the coloured shade nets white has the best light-diffusing capability in the PAR range, resulting in deeper light penetration into the canopy (Kong et al. 2013). This effect can increase the lower vitamin C content of pepper pods situating in more shaded parts of the plants (Milenkovic et al. 2012). High vitamin C content under the yellow shade net could be the consequence of higher air temperature in this treatment (Lee and Kader 2000).

Difference among the treatments was proved considerably lower at the first sampling than for the second one. On average of the six shading

Table 1. Effect of shading method and sampling time on some phytonutrient contents (mean  $\pm$  SE) of kapia type red sweet pepper fruits

	Vitamin C		Total carotenoids		Total polyphenols	
	g kg <sup>-1</sup> FW	g kg <sup>-1</sup> DW	mg kg <sup>-1</sup> FW	mg kg <sup>-1</sup> DW	g kg <sup>-1</sup> FW	g kg <sup>-1</sup> DW
1 <sup>st</sup> sampling (19.07.)						
Control	1.76 $\pm$ 0.04 d*	16.8 $\pm$ 0.2 cd	75 $\pm$ 11 c	712 $\pm$ 104 c	2.54 $\pm$ 0.04 a*	24.21 $\pm$ 0.18 a
Shading paint	2.04 $\pm$ 0.09 bc	19.3 $\pm$ 0.9 ab	80 $\pm$ 3 c	754 $\pm$ 34 c	2.49 $\pm$ 0.03 ab	23.37 $\pm$ 0.10 ab
White net	1.70 $\pm$ 0.03 d	16.1 $\pm$ 0.2 d	103 $\pm$ 14 c	974 $\pm$ 120 c	2.48 $\pm$ 0.03 abc	23.59 $\pm$ 0.17 ab
Green net	1.77 $\pm$ 0.05 d	17.0 $\pm$ 0.3 bcd	81 $\pm$ 2 c	778 $\pm$ 39 c	2.36 $\pm$ 0.07 bcd	22.58 $\pm$ 0.54 bc
Yellow net	1.74 $\pm$ 0.06 d	16.8 $\pm$ 0.6 cd	86 $\pm$ 5 c	833 $\pm$ 52 c	2.31 $\pm$ 0.06 cd	22.26 $\pm$ 0.52 bcd
Red net	1.80 $\pm$ 0.05 cd	17.3 $\pm$ 0.5 bcd	91 $\pm$ 3 c	874 $\pm$ 26 c	2.37 $\pm$ 0.10 abcd	22.83 $\pm$ 0.94 abc
2 <sup>nd</sup> sampling (25.09.)						
Control	1.34 $\pm$ 0.10 e	11.9 $\pm$ 1.0 e	181 $\pm$ 19 ab	1586 $\pm$ 136 ab	2.33 $\pm$ 0.07 bcd	20.53 $\pm$ 0.51 e
Shading paint	1.83 $\pm$ 0.08 cd	15.7 $\pm$ 0.6 d	204 $\pm$ 8 ab	1756 $\pm$ 77 ab	2.43 $\pm$ 0.09 abc	20.88 $\pm$ 0.82 de
White net	2.40 $\pm$ 0.16 a	20.8 $\pm$ 1.4 a	221 $\pm$ 24 a	1915 $\pm$ 206 a	2.53 $\pm$ 0.06 ab	21.86 $\pm$ 0.43 cde
Green net	2.18 $\pm$ 0.15 ab	18.6 $\pm$ 1.4 abc	174 $\pm$ 27 b	1479 $\pm$ 204 b	2.48 $\pm$ 0.06 abc	21.11 $\pm$ 0.18 de
Yellow net	1.74 $\pm$ 0.03 d	15.6 $\pm$ 0.3 d	169 $\pm$ 17 b	1521 $\pm$ 156 b	1.92 $\pm$ 0.08 e	17.17 $\pm$ 0.28 f
Red net	1.29 $\pm$ 0.08 e	11.2 $\pm$ 0.7 e	197 $\pm$ 12 ab	1710 $\pm$ 125 ab	2.20 $\pm$ 0.04 d	19.05 $\pm$ 0.41 f
Significance (P-value)						
Treatment	2.85*10 <sup>-7</sup>	2.99*10 <sup>-6</sup>	0.14	0.12	3.32*10 <sup>-6</sup>	3.37*10 <sup>-6</sup>
Sampling time	0.88	1.3*10 <sup>-3</sup>	9.05*10 <sup>-15</sup>	4.47*10 <sup>-14</sup>	4.13*10 <sup>-3</sup>	8.25*10 <sup>-13</sup>
Treatment x time	3.94*10 <sup>-8</sup>	1.77*10 <sup>-7</sup>	0.72	0.74	3.31*10 <sup>-3</sup>	5.97*10 <sup>-3</sup>

\*mean separation within columns according to Fisher's protected least significant difference test at  $P \leq 0.05$

treatments DW based vitamin C content was significantly higher for the July sampling than for the samples gathered in September, under cooler conditions. This result is in accordance with the general assumption that heat stress usually results in higher vitamin C accumulation (Lee and Kader 2000). Another factor could be the higher irradiation during summer compared to that in September, as higher solar radiation favours the biosynthesis of ascorbic acid (Tinyane et al. 2013). In previous pepper studies positive correlation between total soluble solids and vitamin C content was found (Milenkovic et al. 2012). However, in this present experiment dry matter and vitamin C content correlation could not be proved. Neither significant correlation between shading ratio and vitamin C content was revealed, in contrary to the findings of Tinyane et al. (2013).

#### Total carotenoid content

According to the results of two-way ANOVA, significant effect of shading treatment on total carotenoid content was not demonstrated. However, according to Fisher's post-hoc test, white shade net resulted in significantly higher carotenoid content compared to the green and the yellow ones at the second sampling time (Table 2). This result is in accordance with the results of Selahle et al. (2015) who found significantly higher  $\beta$ -carotene and lycopene content in red peppers under white shade net compared to red and yellow nets. Also, in the experiment of Ambrózy et al. (2016) the highest total carotenoid content was measured under the white shade net treatment. Light level and quality can affect carotenoid accumulation in red pepper pods (Kim et al. 1978; Russo and Howard 2002). For tomatoes it was found that



fruit-localized phytochromes, sensitive to R/FR ratio, mediate lycopene accumulation in the berries (Alba et al. 2000). Sampling time had a highly significant effect on the carotenoid content. On the average of the six treatments carotenoid level in the fruits of the July sampling was only half of that of the September sampling. This result is in good agreement with the findings of Ambrózy et al. (2016). This phenomenon can be explained by the temperature conditions, as it is well known that supraoptimal temperatures can inhibit carotenoid synthesis in fruits of tomato, an another Solanaceous vegetable (Helyes et al. 2007). In this study, a significant negative correlation was found between the ratio of supraoptimal temperature prior to harvest and total carotenoid content ( $r = -0.90$ ,  $N = 12$ ,  $P < 0.001$ ). Meanwhile correlation was not revealed between shading ratio and carotenoid results.

#### Total polyphenol content

Similarly to the vitamin C results, total polyphenol content was significantly affected by both investigated factors and also by their interaction (Table 2). In the average of the two sampling times the red and the green shading nets resulted significantly lower total polyphenol content than the other four treatments. Tinyane et al. (2013) and Selahle et al. (2015) also found inferior polyphenol content under red shade net in tomatoes and red peppers, respectively. Mashabela et al. (2015) supposed that reduced R/FR ratio could be responsible for lower total polyphenol content. As for the sampling time, total polyphenol content was significantly higher in July than in September, especially when it was expressed on DW basis (Table 2). Polyphenol content increasing effect of heat stress is a well-known phenomenon (Bita and Gerats, 2013). Accordingly, in the present experiment a significant positive correlation between ratio of supra-optimal temperature values prior the samplings and DW based total polyphenol content was found ( $r = +0.59$ ,  $N = 12$ ,  $P < 0.05$ ).

#### **Conclusion**

Effects of shade net colour and sampling time on nutritional characteristics of kapia type sweet pepper cultivated in plastic tunnels were investigated. Net colour influenced light level, light quality and tunnel temperature, while sampling time had a much bigger effect on temperature conditions. Shade net colour significantly affected vitamin C and total-polyphenol contents of pepper fruits, presumably due to modification of light quality. Considering vitamin C, total carotenoid and total polyphenol contents, white shade net improved nutritional value of pepper the most in average of the two sampling times compared to the control treatment. This favourable effect is probably due to higher ratio of diffused light and more favourable R/FR ratio under this net. Hence, if the main goal is producing pepper with better nutritional quality, this colour of shade net is advised to use. It is intriguing that this simple growing practice can significantly improve nutritional quality of sweet pepper produced in plastic tunnels, especially in Hungary where per capita pepper consumption is very high. Our results were in good accordance with previous field and net house experiments but not with a previous research conducted in shade net covered plastic tunnels. Hence, more detailed research is necessary for investigating the combined effect of shade net and plastic sheet covers of tunnels. During these future researches special attention should be paid to the R/FR photon ratios to understand physiological mechanisms. Harvest time influenced nutritive characteristics at a much greater extent than shade net colour, due to significant differences in ratio of supra-optimal temperature values. Heat stress increased total polyphenol concentration and decreased total carotenoid content of pepper pods.

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## Effect of leaf litter mulching on the pests of tomato

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**Abstract:** Agricultural techniques essentially change the weed flora, pests and diseases of cultivated plants. Our aim was to investigate the effect of leaf litter mulch on the pests of tomato, especially on the weed flora and on three important plant protection problems: late blight (*Phytophthora infestans*), cotton bollworm (*Helicoverpa armigera*) and root-knot nematodes (*Meloidogyne* spp.). Besides mulching, the experiment consisted of irrigation, mycorrhiza-inoculation and artificial *Meloidogyne*-infestation. We recorded the natural *P. infestans* infection, *Helicoverpa armigera* damage and the species composition and cover of weeds as well. We examined whether leaf litter mulching had any effect on pests, and on the generative and vegetative production of tomato. Our results were that only mulching had a significant positive effect on almost all the measured generative and vegetative production parameters of tomato, while irrigation, mycorrhiza application and artificial nematode infestation had no significant effects on these parameters. Negative correlation was found between *P. infestans* infection and *H. armigera*-damage on tomato fruits. Mulching also reduced significantly the number of *Meloidogyne*-induced galls on the roots of tomato. Application of leaf litter mulch did not affect soil organic matter and soil pH within the growing season. In the beginning of the growing season mulching suppressed weed cover. Later, there was no significant difference between weed cover in the treatments. It can be concluded that leaf litter mulch had a more pronounced influence on the quantity of yield than on the damage and presence of pests and weeds.

**Keywords:** *Meloidogyne* sp., leaf litter mulching, *Phytophthora infestans*, tomato, weed

### Introduction

Mulching is multifunctional: it is able to regulate the nutrient level, porosity, temperature and water management of the soil (Balázs 1989). Moreover, mulching hampers weed emergence therefore contributes to decreasing the frequency of weeding, irrigation and nutrient supply (Makkai 2008).

The role of organic mulching in weed control is much more important in the beginning of the growing season than later. Peat, chopped wheat straw and woodchips successfully suppressed weed germination. Grass clippings were also effective, but only before starting to decompose. When decomposition started, it had not any effect on weeds (Jodaugienė et al. 2006).

Straw-mulching and mechanical weed control resulted in a different composition of weed flora than the tillage-based method. Furthermore,

mulching was more effective to control weeds. The straw mulching could suppress most of annual weeds, but it was also effective especially in the case of the sturdier, perennial species (Zalai et al. 2015).

In the case of newspaper-mulching, a 7.6 cm thick layer of chopped newspaper suppressed 90 % of weeds and conserved soil moisture. However, thicker newspaper-mulching resulted in a reduced soil temperature (Monks et al. 1997).

Examining the effect of polyethylene mulch on *Phytophthora infestans* on tomato, it suppressed the disease more effectively than fungicides (Shtienberg et al. 2010). Oat straw mulch negatively correlated with *Phytophthora cinnamomi* in the case of avocado (You and Sivasithamparam 1995). Researchers found that different kinds of animal manure and fresh green manure reduced the viability of oospores and the incidence of

*Phytophthora capsici* (Núñez-Zofío et al. 2011). In case of potato, arbuscular mycorrhiza induced systemic resistance, decreased the leaf infection by *P. infestans* (Gallou et al. 2011).

The use of newspaper mulching decreased the number of nematodes (*Pratylenchus penetrans*), and thus reduced nematode damage in apple (Forge et al. 2008).

When mulched with plastic, especially with black plastic, tomato plants produced significantly higher number of fruits and the weight of fresh fruit per plant was also significantly higher. In addition, black plastic decreased root-knot nematode (*Meloidogyne javanica*) infestation (Ogwulumba and Ugwuoke 2011).

Our aim was to investigate the effect of leaf litter mulching on the yield and on the pests of tomato, especially on weed cover and on the presence and damage of *P. infestans*, *Helicoverpa armigera* and *Meloidogyne* species. Besides mulching, the experiment consisted of irrigation, mycorrhiza-inoculation and artificial *Meloidogyne*-infestation.

### Materials and methods

#### Biological materials

The experiment took place on the trial field of Szent István University in Gödöllő. The tomato seeds („Dány” gene bank sample) were provided

by Plant Diversity Center, (Növényi Diverzitás Központ, NöDiK), with the help of Research Institute of Organic Agriculture (Ökológiai Mezőgazdasági Kutatóintézet, ÖMKi).

The „Dány” gene bank sample is a determinate type of tomato. Its fruit is bright red coloured, round-shaped with 75- 85 g average fruit weight. The disease-resistance of the variety is medium (Cseperkálóné Mirek et al. 2014).

Artificial infestation was used to introduce root-knot nematodes (*Meloidogyne* spp.). The inoculum was collected from polytunnel raised peppers, in Jászfényszaru. It contained spontaneously infested root pieces and soil.

The mycorrhiza inoculation was obtained by using SYMBIVIT® that contains the following species: *Glomus claroideum*, *G. etunicatum*, *G. geosporum*, *G. intraradices*, *G. microaggregatum* and *G. mosseae* (Albrechtova et al. 2011). Since these species were all renamed after issuing the permission for marketing of SYMBIVIT®, their recent scientific names are: *Claroideoglomus claroideum*, *Claroideoglomus etunicatum*, *Funneliformis geosporum*, *Rhizophagus intraradices* (Schüßler and Walker 2010), *Rhizoglossum microaggregatum* (Sieverding et al. 2014) and *Funneliformis mosseae* (Schüßler and Walker 2010). Leaf litter was used as a mulching material, and it was provided by Zöld Híd Régió

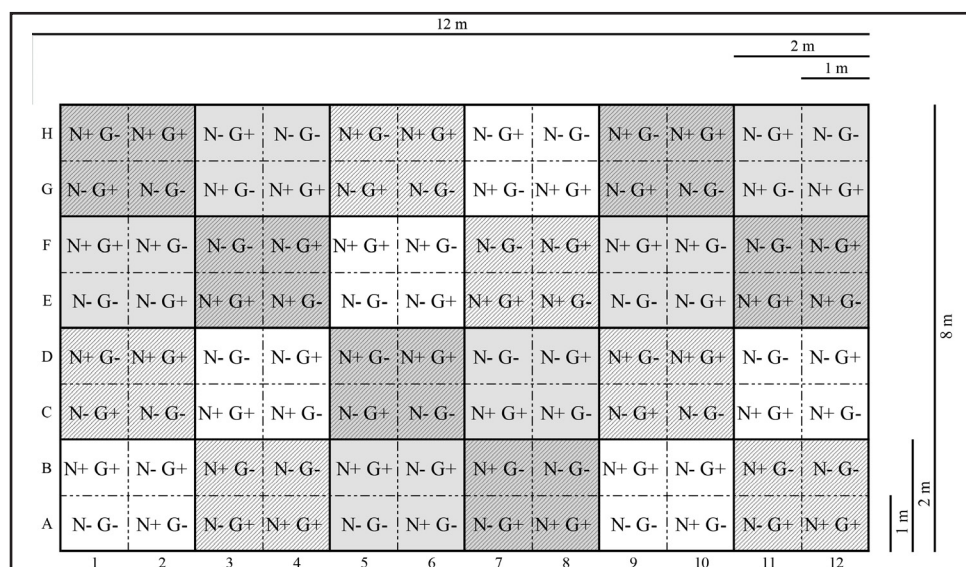


Figure 1. Arrangement of treatments (N: Root-knot nematode, G: *Glomus*, Greycolored: Irrigation, Lined: Mulching).

Ltd. It consisted mostly of the leaves of maples (*Acer* spp.). The leaf litter was collected in Gödöllő in October 2015, and was stored in open field circumstances as a pile (without any handling or processing) approximately 60 m far from the experimental plots until application as mulch in June 2016. Thickness of mulch was 15 cm at the beginning of the experiment.

#### Interaction experiment

On 18 March, a frame was made from pinewood to separate the various treatments. Altogether, there were 24 pieces of 2 x 2 m plots on a 96 m<sup>2</sup> total area. After the pinewood-frame was in place, leaf litter was spread on every second plots. Tomato seeds were sown on 14 April into potting compost. After 2 weeks, the seedlings were planted into pots. On 2 June, before planting, we weeded the area. After weeding, leaf litter mulch and a dripping irrigation system was installed, and all the irrigated plots were given dripper stakes. So there were four treatments and six replications of the 24 plots:

1. mulched and irrigated (M+I+)
2. mulched and non-irrigated (M+I-)
3. unmulched and irrigated (M-I+)
4. control unmulched and non-irrigated (M-I-)

In every plot, 4 plants were planted (1 plant/m<sup>2</sup>), and every single plant received a different treatment within each plot:

1. control (N-G-) (after the terms “Nematode” and “*Glomus*”)
2. only *Meloidogyne*-infested (N+G-)
3. only mycorrhiza-inoculated (N-G+)
4. both *Meloidogyne*-infested and mycorrhiza-inoculated (N+G+).

In case of mycorrhiza-inoculation, 25 g SYMBIVIT® product was used, as described on the instruction of the producer. To reach the planned *Meloidogyne* infestation, 20 g infested soil with pieces of galled roots were placed under the roots of tomato plants during planting. (For the *Meloidogyne* infestation not J2 nematodes (Hooper et al. 2005) were used, because our opinion was that although the number of nematodes are known in that way, but the juveniles can get easily injured during the extraction process. Therefore they may lose their virulence. On the other hand, we can achieve a more realistic inoculation with infested soil and galled root pieces.)

Altogether, 16 treatment combinations with 6 replications were used in a combined block

Table 1. Values of scales elaborated by Zeck (1971), Garabedian and Van Gundy (1984), Mukhtar et al. (2013) (modification of Taylor and Sasser 1978) for estimation of root-knot nematode (*Meloidogyne* spp.) damage on roots.

Scale-values	Zeck (1971)	Garabedian and Van Gundy (1984)	Mukhtar et al. (2013) (mod. Taylor and Sasser 1978)
0	no galls	no infection	0 gall
1	very few small galls	1-20% infection (trace)	1- 2 galls
2	numerous small galls	21-40% infection (slight)	3- 10 galls
3	numerous small galls, some of which are grown together	41-60% infection (moderate)	11- 30 galls
4	numerous small and some big galls	61-80% infection (severe)	30- 70 galls
5	25% of roots severely galled	81-100% infection (very severe)	71- 100 galls
6	50% of roots severely galled		> 100 galls
7	75% of roots severely galled		
8	no healthy roots but plant is still green		
9	roots rotting and plant dying		
10	plant and roots dead		

setting (Figure 1). As there was a rainy period at the beginning of summer, irrigation started only on 20 June. Daily weather dataset was the basis to calculate the optimal irrigation quantities (Helyes and Varga 1994). Irrigated blocks were supplied with water three times a week. In case of rain, irrigation quantity was corrected with the quantity of the rainfall. During the whole growing season, 153 mm water was used for the irrigation treatment, while 213 mm rain fell.

From 13 July, the formation of the generative parts were observed and noted. In case of every observation, quantity of the formed bunches, flower buds, flowers and later, fruits were recorded. On 8 of August, the height of the plants and the widest diameter of their canopies were measured.

During the growing season, weeding was done after every weed survey (26 May, 27 June, 18 July, 5 August and 28 August). The plots were weeded and hoed, and the demand of time for weeding and the species composition of weed flora was recorded for every plot. The early symptoms of tomato late blight (*Phytophthora infestans*) were noticed on 2 August. In order to reduce the spreading of the

disease, Trifender (*Trichoderma asperellum*) and Boni Protect (*Aureobasidium pullulans*) were sprayed. Besides *P. infestans* infection, the tomato fruits were damaged by the larvae of the cotton bollworm (*Helicoverpa armigera*). Infected and damaged fruits were removed and measured. The experiment was terminated on 30 August. The tomato yield was measured. Soil samples were collected from the root zone. After washing the roots, *Meloidogyne* damage was estimated with the help of the scales elaborated by Zeck (1971), Garabedian and Van Gundy (1984), Mukhtar et al. (2013) (modification of Taylor and Sasser 1978) (Table 1). Finally, fresh shoots and roots were measured. After 2 weeks of air-drying, dry weight of shoots and roots were measured as well.

#### Laboratory examinations

In order to identify the *Meloidogyne* species in the inoculum from Jászfényszaru, a preparation method by Hartman and Sasser (1985) was used. 10 individuals of female root-knot nematodes were examined for species determination. A revised version (Szakálas et al. 2015) of Baermann funnel (Baermann 1917) was used to extract active nematodes from

Table 2. Generative production parameters of tomato plants receiving the following treatments: unmulched and mulched (M), non-irrigated and irrigated (I), non-infested and artificially nematode-infested (N), non-inoculated and artificially mycorrhiza-inoculated (G). (p-value: Welch test)

treatment +/-	Mulching (M)		Irrigation (I)		Root-knot nematode (N)		Glomus (G)	
	-	+	-	+	-	+	-	+
number of repetitions	48	48	48	48	48	48	48	48
Maximum number of bunches/plant								
mean ± CI 95%	18.5 ± 1.4	27.8 ± 1.9	22.4 ± 2.2	23.8 ± 2.1	22.5 ± 2.3	23.7 ± 2	22.9 ± 2.2	23.4 ± 2.1
p-value	< 0.001		0.373		0.45		0.767	
Maximum number of buds/plant								
mean ± CI 95%	22.5 ± 2	31.5 ± 2.7	27.5 ± 2.5	26.5 ± 2.8	26.6 ± 2.7	27.4 ± 2.6	27.06 ± 2.3	26.9 ± 3
p-value	< 0.001		0.612		0.658		0.94	
Maximum number of flowers/plant								
mean ± CI 95%	25.7 ± 2.6	39.4 ± 3.4	29 ± 3.7	36 ± 3.3	31.3 ± 3.8	33.8 ± 3.3	32.1 ± 3.5	33 ± 3.7
p-value	< 0.001		0.006		0.341		0.719	
Total number of fruits/plant								
mean ± CI 95%	16.4 ± 2	31.9 ± 3.8	25.4 ± 4.1	23 ± 3.4	24.3 ± 3.9	24.1 ± 3.7	24.1 ± 4.1	24.2 ± 3.4
p-value	< 0.001		0.368		0.945		0.982	



Table 3. Vegetative production parameters of tomato plants receiving the following treatments: unmulched and mulched (M), non-irrigated and irrigated (I), non-infested and artificially nematode-infested (N), non-inoculated and artificially mycorrhiza-inoculated (G). (p-value: Welch test)

treatment +/-	Mulching (M)		Irrigation (I)		Root-knot nematode (N)		Glomus (G)	
	-	+	-	+	-	+	-	+
no. of repetitions	48	48	48	48	48	48	48	48
Height of plant (cm)								
mean ± CI 95%	48 ± 1.6	55.9 ± 2	51.8 ± 1.9	52 ± 2.3	52.3 ± 1.8	51.6 ± 2.4	51.4 ± 2.3	52.4 ± 1.9
p-value	< 0.001		0.913		0.661		0.510	
Widest diameter of canopy (cm)								
mean ± CI 95%	74.7 ± 4.8	98.7 ± 5	86.9 ± 5.9	86.4 ± 6	84.1 ± 5.9	89.2 ± 5.9	87.2 ± 6.2	86.2 ± 5.7
p-value	< 0.001		0.904		0.234		0.828	
Fresh shoot weight (g)								
mean ± CI 95%	180.9 ± 16.6	321.6 ± 32.2	249.8 ± 32.8	252.8 ± 32.3	253.8 ± 32	248.7 ± 33	264.1 ± 36.2	238.4 ± 27.9
p-value	< 0.001		0.899		0.828		0.273	
Fresh root weight (g)								
mean ± CI 95%	30.4 ± 2.8	58.3 ± 6.1	46.7 ± 6.8	42 ± 5.4	42.8 ± 6.6	45.9 ± 5.8	45.9 ± 6.8	42.8 ± 5.6
p-value	< 0.001		0.304		0.501		0.501	

soil samples, including predatory nematodes. Soil samples were used for measuring soil organic matter (Walkley 1947) and pH with distilled water and with potassium chloride (Buzás 1988). Samples were processed per plots.

Statistical methods

Welch test, correlation analysis and One-way ANOVA (Tukey’s pairwise comparisons) were

used for data analysis in “PAST” statistical software (Hammer et al. 2001).

**Results and discussion**

Only mulching had significant effect on all the measured parameters of tomato. It increased the maximum number of bunches, buds and flowers, and the total number of fruits. In addition, the maximum number of flowers was influenced by irrigation as well (Table 2). Mulched plants

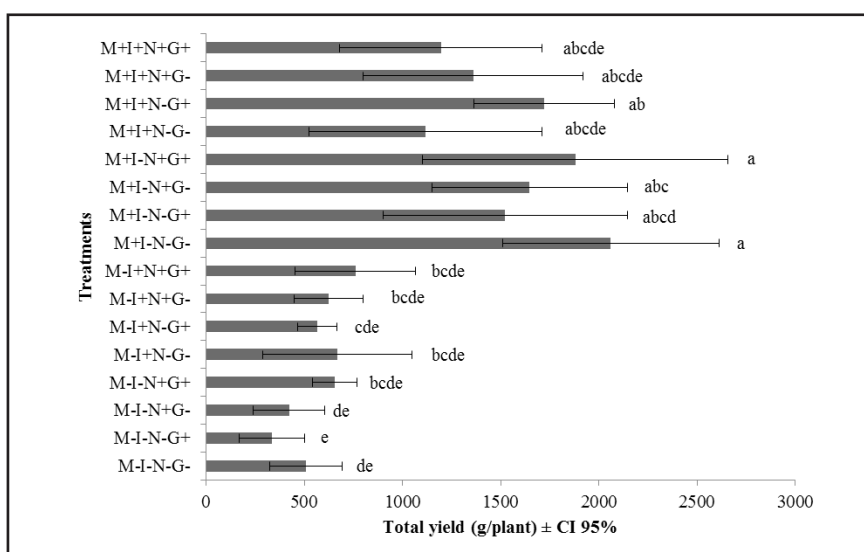


Figure 2. Total yield of tomato for each treatment combinations (M: Mulching, I: Irrigation, N: Root-knot nematode, G: Glomus). (One-way ANOVA, Tukey’s pairwise comparisons; the same letters indicate the lack of significant difference at p<0.05 level)

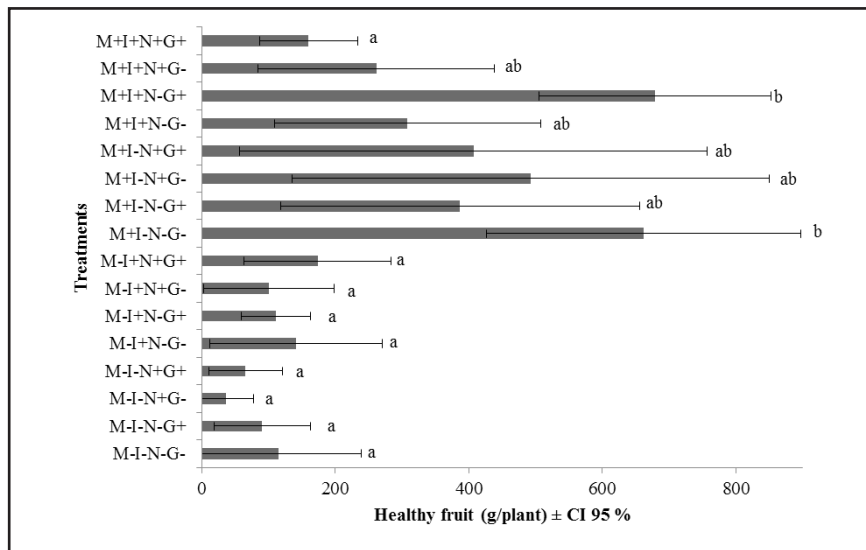


Figure 3. Healthy yield of tomato for each treatment combinations (M: Mulching, I: Irrigation, N: Root-knot nematode, G: *Glomus*). (One-way ANOVA, Tukey’s pairwise comparisons; the same letters indicate the lack of significant difference at  $p < 0.05$  level)

grew higher and had wider canopies, even when compared to the irrigated unmulched treatment. The weight of fresh shoots and roots of plants were significantly higher in mulched plots (Table 3). It is in a line with the findings of Soltész (1997), who published that mulching increased nutrient uptake, and therefore the growth of plants as well.

The presence of mulch resulted in higher yields (Figure 2). The most healthy fruits were measured in two mulched, but non-infested treatment combinations (M+I-N-G- and M+I+N-G+) (Figure 3). Despite the fact that mulching

increased the quantity of healthy fruits, it did not affect the percentage of damaged (infected by tomato late blight and/or damaged by cotton bollworm) fruits. This is in contrast with earlier data published by Shtienberg et al. (2010), who experienced a *Phytophthora*-reducing effect of mulching. In our results, the presence of leaf litter mulch influenced the quantity of yield more than the damage and presence of pests. While treatments had no effect on late blight symptoms or cotton bollworm damage, a negative correlation was found between the proportion of damaged fruits by cotton bollworm

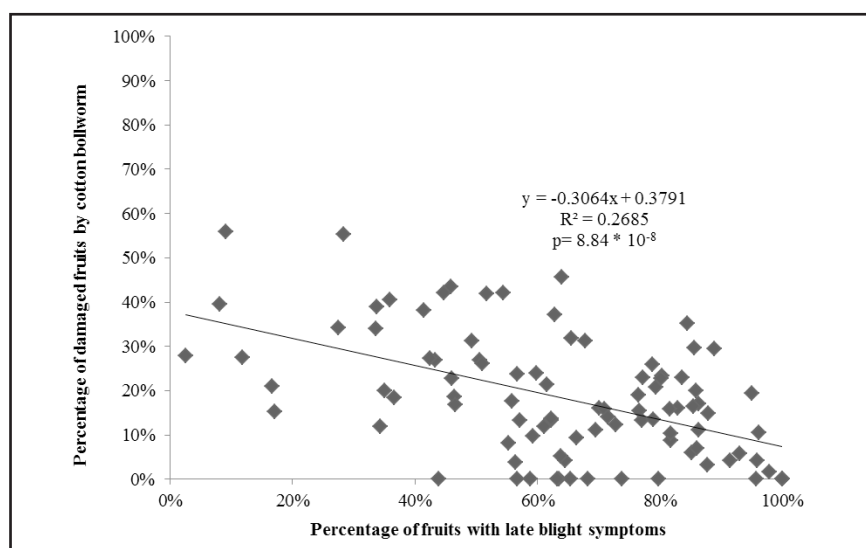


Figure 4. Correlation between percentage of tomato fruits damaged by cotton bollworm and percentage of fruits with late blight symptoms. (Each dot represents a tomato plant.)

Table 4. Values of soil organic matter and pH (with distilled water and potassium chloride), depending on mulching and irrigation. (p-value: Welch test)

treatment +/-	Mulching (M)		Irrigation (I)	
	-	+	-	+
number of repetitions	12	12	12	12
organic matter content (%)				
mean ± CI 95%	2 ± 0.4	1.9 ± 0.6	2 ± 0.6	1.8 ± 0.4
p-value	0.819		0.626	
pH with distilled water				
mean ± CI 95%	7.8 ± 0.1	7.8 ± 0.1	7.8 ± 0.1	7.9 ± 0.1
p-value	1.000		0.078	
pH with potassium chloride (KCl)				
mean ± CI 95%	6.8 ± 0.1	6.8 ± 0.1	6.8 ± 0.01	6.8 ± 0.1
p-value	0.242		0.404	

Table 5. Estimation of root-knot nematode (*Meloidogyne incognita*) damage on the roots of unmulched and mulched (M), non-irrigated and irrigated (I), non-infested and artificially nematode-infested (N), non-inoculated and artificially mycorrhiza-inoculated (G) tomato plants with scales elaborated by Zeck (1971), Garabedian and Van Gundy (1984), Mukhtar et al. (2013) (modification of Taylor and Sasser 1978). (p-value: Welch test)

treatment +/-	Mulching (M)		Irrigation (I)		Root-knot nematode (N)		Glomus (G)	
	-	+	-	+	-	+	-	+
nof repetitions	48	48	48	48	48	48	48	48
Zeck (0-10)								
mean ± CI 95%	1.77 ± 0.56	1.10 ± 0.38	1.44 ± 0.47	1.44 ± 0.5	0.44 ± 0.27	2.44 ± 0.49	1.63 ± 0.53	1.25 ± 0.43
p-value	0.056		1.000		< 0.001		0.285	
Garabedian and Van Gundy (0-5)								
mean ± CI 95%	1.25 ± 0.49	0.60 ± 0.22	0.94 ± 0.41	0.92 ± 0.37	0.25 ± 0.16	1.60 ± 0.46	1.19 ± 0.47	0.67 ± 0.27
p-value	0.022		0.941		< 0.001		0.064	
Mukhtar et al. (0-6)								
mean ± CI 95%	2.06 ± 0.7	0.91 ± 0.33	1.52 ± 0.58	1.46 ± 0.57	0.35 ± 0.23	2.63 ± 0.63	1.73 ± 0.64	1.25 ± 0.5
p-value	0.005		0.881		< 0.001		0.248	

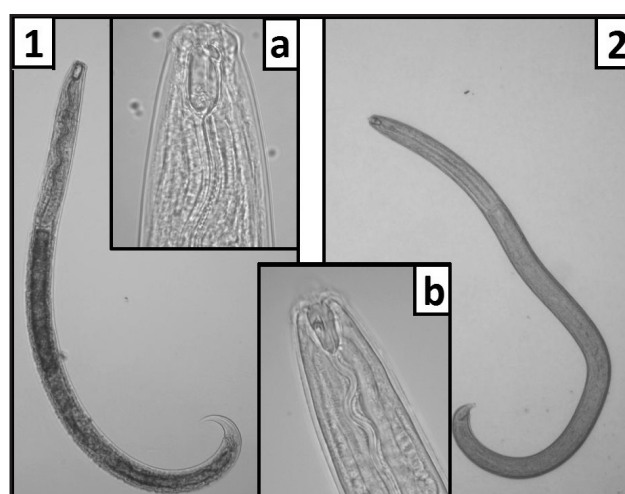


Figure 5. Predatory nematodes (1a: *Clarkus* sp., 2b: *Mylonchulus* sp.) found in the soil samples of tomato plots.

Table 6. Species of weed flora, their lifestyles and the percentage of weed covering for mulched (M+), unmulched (M-), irrigated (I+) and non-irrigated (I-) plots.

Species	Life forms	M- I-	M- I+	M+ I-	M+ I+
<i>Stellaria media</i>	T1	80	60	23	75
<i>Elymus repens</i>	G1	2	3	25	25
<i>Chenopodium album</i>	T4	11	20	5	6
<i>Convolvulus arvensis</i>	G3	8	6.5	10	20
<i>Setaria viridis</i>	T4	0.5	0.2	17	10
<i>Setaria pumila</i>	T4	1	0.5	15	5
<i>Echinochloa crus-galli</i>	T4	2	2	10	8
<i>Glechoma hederacea</i>	H2	4	2	10	1
<i>Solanum tuberosum</i>	G2	3	1	5	10
<i>Taraxacum officinale</i>	H3	3	8	10	3
<i>Portulaca oleracea</i>	T4	1	0.8	6	4
<i>Amaranthus retroflexus</i>	T4	0.1	0.4	1	5
<i>Galinsoga parviflora</i>	T4	1.3	0.5	5	5
<i>Digitaria sanguinalis</i>	T4	4	2.5	4	4
<i>Acer spp.</i>	Ph	0.2	3	1.5	2
<i>Capsella bursa-pastoris</i>	T1	2	0	0	0
<i>Conyza canadensis</i>	G1	0	0	0	2
<i>Polygonum aviculare</i>	T4	1.5	0.3	2	0.2
<i>Solanum nigrum</i>	T4	0	0	0.5	2
<i>Humulus lupulus</i>	H3	0	0.2	0	1.5
<i>Oxalis corniculata</i>	H2	1	0.1	0	0
<i>Amaranthus chlorostachys</i>	T4	0	0	0.5	0
<i>Ambrosia artemisiifolia</i>	T4	0	0.5	0	0
<i>Daucus carota</i>	T4	0.5	0.2	0	0
<i>Lamium purpureum</i>	T1	0.5	0.1	0	0
<i>Anagallis arvensis</i>	T4	0.2	0.4	0.2	0.4
<i>Ailanthus altissima</i>	Ph	0	0	0	0.3
<i>Artemisia vulgaris</i>	H5	0	0.2	0	0
<i>Chelidonium majus</i>	H5	0.1	0	0.2	0
<i>Stenactis annua</i>	T4	0.2	0.1	0	0
<i>Ballota nigra</i>	H5	0	0	0	0.1
<i>Lolium perenne</i>	H1	0	0	0	0.1
<i>Medicago lupulina</i>	T4	0.1	0.1	0	0
<i>Raphanus raphanistrum</i>	T3	0	0.1	0	0
<i>Solanum nigrum</i>	T4	0	0.1	0	0

and proportion of fruits with the symptoms of late blight (Figure 4), probably because female moths preferred uninfected fruits for egg-laying.

According to the literature, irrigation increased (Karajeh and Mohawesh 2016) and mycorrhiza-inoculation decreased (Diedhiou et al. 2003) the number of *Meloidogyne*-induced galls on

the roots. We did not experience these effects in our trial, probably due to the weather conditions of the year of the experiment. Results of next years with the same experimental design may answer this question. In our experiment, leaf litter mulching decreased the damage of root-knot nematodes, similarly to the results of Forge et

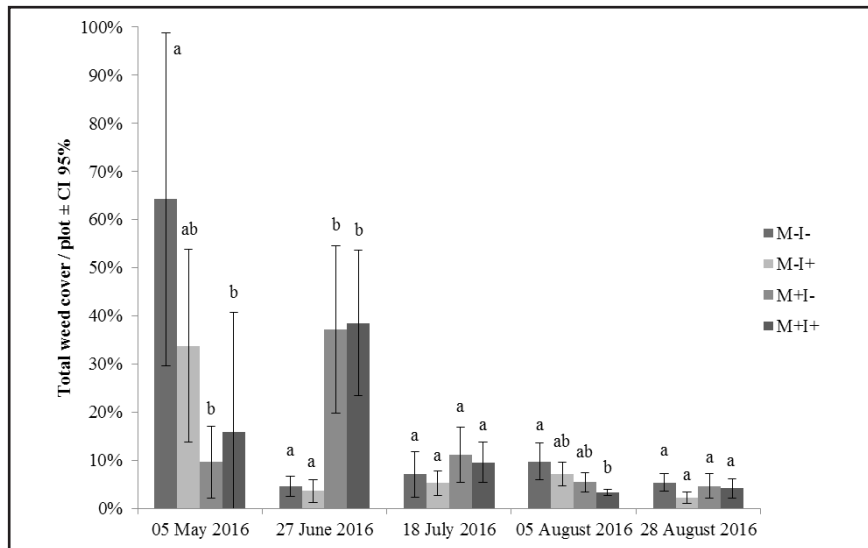


Figure 6. Change of total weed cover in tomato plots receiving the following treatments: mulched and unmulched (M+ and M-), irrigated and non-irrigated (I+ and I-). (One-way ANOVA, Tukey's pairwise comparisons; the same letters within a group of columns of the same date indicate the lack of significant difference at  $p < 0.05$  level)

al. (2008), who used newspaper mulch against *Pratylenchus penetrans*, or Ogwulumba and Ugwuoke (2011), who used plastic mulch against *Meloidogyne javanica*. Although these three experiments used totally different mulch materials, the results were highly similar. This may suggest that nematode-suppression of mulch is mainly not due to chemical changes in the soil, but rather to physical or biological changes.

Neither mulching, nor irrigation influenced soil organic matter content and pH (measured with distilled water and with potassium chloride) (Table 4). Considering the fact that mulch was spread in the spring, therefore it had probably not enough time to influence these soil parameters yet. So these parameters cannot explain the reduced *Meloidogyne*-symptoms and increased yield in the mulched plots compared to the unmulched control. The experiment is going to be continued exactly at the same place with the same experimental design next years, so as many parameters as possible, like tannin content and nutrient content of mulch and soil should be measured periodically in the future.

During the species identification of root-knot nematodes, only *Meloidogyne incognita* individuals were observed. This result met our expectations as inoculum was collected from a polytunnel. Mulching reduced the damage of

root-knot nematodes (Table 5). As *M. incognita* is a thermophilic nematode species (Andrássy and Farkas 1988), we assume that the circumstances (like temperature) in the mulched soil were not preferable to this species. We plan to test the native *M. hapla* species next year in a similar experiment. Another hypothesis for the explanation of the reduced *M. incognita* infestation in the mulched plots is the increased presence of antagonist organisms of root-knot nematodes. However, neither the density of other nematodes in the soil, nor the number of predatory nematodes was influenced by treatments. Two predatory nematode genera were found: *Clarkus* and *Mylonchulus* (Figure 5). Distribution of predatory nematodes was random which is in line with earlier data published by Renčo and Kováčik (2012). So we cannot explain reduced *Meloidogyne* damage on the roots of the mulched plants with the presence and density of potential antagonist nematodes in our experiment.

In the first two weed surveys, at the beginning of the growing season, mulched and unmulched plots showed significant difference in weed cover by different reasons. At the first survey, unmulched plots were highly covered by chickweed (*Stellaria media*). At the second survey, the mulched plots were weedier mainly

caused by the high presence of field bindweed (*Convolvulus arvensis*) which can be poorly controlled in a thick mulch layer. Later, there was no difference between the weed cover of the treatments, probably because of the decomposition of the leaf litter and because of the germination and reproduction biology of the dominant weed species (Table 6, Figure 6).

### Conclusions

Our results suggest that the effect of leaf litter mulch should be subjected to further studies. Application of leaf litter mulch had a significant positive effect on tomato yield and on the generative and vegetative parameters of the plant. Mulching reduced *Meloidogyne*-infestation, but did not influence *Phytophthora infestans* infection and *Helicoverpa armigera* damage. It should be tested in the future whether the leaf litter mulch provides matrix for other tomato

pests, or beneficial organisms, like antagonists of root-knot nematodes. Suppression of weeds was experienced only at the beginning of the growing season, so repeated application of leaf litter mulch should be tested next years, extended with survey of composition – especially nutrient content and allelopathic substance content – of different leaf litter types.

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## Study of the degradation patterns of thermophilic fungi from special digested wastewater sludge samples

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**Abstract:** Digested wastewater sludges have high content of thermophilic fungi because of the anaerobe biodegradation. This study shows the ecophysiological properties (cellulose, hemi-cellulose, lignin and fatty-acid ester degradations) of thermophilic fungi which were isolated from digested wastewater sludge samples. The samples came from three Hungarian wastewater treatment systems. In this study four types of mycological agars were applied. This study shows digestion properties and growing temperature range of eight thermophilic fungal species. This study demonstrated wide ranges of digestion properties. Most of the isolated species are appropriate for degradations of several biopolymers.

**Keywords:** fungal species, wastewater treatment, ecophysiological properties

### Introduction

The biodiversity and the ecosystems are basic for the human and nature. An important part of the ecosystems are the fungi (psychrophilic, mesophilic and thermophilic species). About 100.000 fungal species have been described worldwide (Valencia et al., 2013). Fungi have been used in pharmaceutical and biotechnological industries to produce important materials (Lange et al., 2012). Thermophilic fungi also have been used in biogas production. Wastes from food industry and other agricultural systems have high methane potential and high content of thermophilic fungi (Palatsi et al., 2009). In biogas production the lipid content is hydrolysed by extracellular enzymes of fungi. Fatty acids are degraded through  $\beta$ -oxidation process (Palatsi et al., 2009).

The average growing temperature range of the mesophilic and thermophilic fungi are between 10°C and 60°C. While, the optimal growing temperature of extreme thermophilic fungi is up to 90°C (Sterflinger et al., 2012). Most of the thermophilic fungal species can use carbon and nitrogen contents of materials to their processes (Souza et al., 2013). The best nitrogen sources are potassium-nitrate, sodium-nitrate and asparagine (Wagner et al., 2013), while the ammonium-nitrate and sodium-nitrite are poor

sources to thermophilic fungi (Chmielewski et al., 2012). The high rate of unsaturated fatty acids is the reason of the resistant cell membrane in thermophilic fungal species (Palatsi et al., 2009; Watanabe et al., 2012). Therefore, the transport processes could work at higher temperature through the cell membrane (Dobolyi et al., 2008; Souza et al., 2013). In the study of Wagner et al. (2013) nine complex organic substrates from three biochemical classes (protein-, lipid-, and cellulose-rich) were investigated in batch experiments and compared with controls in order to evaluate their potential use as substrates for biogas production and in order to have a comparable set of data, which independent of digestion system, reactor type, and design. The complex substrates were applied with a constant final carbon concentration to facilitate comparability (Wagner et al., 2013).

The study of Watanabe et al. (2012) described the production system of biofuels by cellulose degradation capacity of fungi. The cellulose degradation is one of the most important parts of the biofuel production. The work reported the most 25 powerful cellulose-degrading fungi. The study of Souza et al. (2013) presented the enzymatical background of cellulose degradation. The enzymatic degradation of cellulose involves a complex set of enzymes as cellulases: three most relevant classes are endoglucanases,

exoglucanases and  $\beta$ -glucosidases (Souza *et al.*, 2013). Endo and exoglucanases hydrolyze internal  $\beta$ -1,4 linkages of cellulosic polymers to celooligosaccharides and cellobiose and that will be hydrolyzed by  $\beta$ -glucosidases to glucose (Watanabe *et al.*, 2012; Souza *et al.*, 2013). The process needs high cellulose concentration.

Several thermophilic fungi have been determined in a digested wastewater sludge. The fungal contamination depended on the origin of sludge: coming from communal or industrial wastewater (Sterflinger *et al.*, 2011). The complex, cooperative and dynamic processes of digestion involved the microbiological communities and a rapid community structure succession. Some species of the microbiological communities could degrade many special materials (e.g. cellulose, hemicellulose, lipids, fatty-acids). Studying the changes of the microbe communities was important to follow the material transporting processes (Zhao *et al.*, 2013). The possible transport processes should determines the species composition of the microbiome.

### Materials and methods

Three types of digested wastewater sludge samples were used in our research. These samples came from three different hungarian wastewater treatment systems. Three types of agars were used to determine the thermophilic fungal species from the samples.

#### *Digasted wastewater sludge samples*

Three types of wastewater sludge samples were used in this research which came from three different hungarian wastewater treatment systems. These samples came from the wastewater treatment systems of Bánhalma, Hungary (Sample 1<sup>st</sup>), Pálhalma, Hungary (Sample 2<sup>nd</sup>) and Kecskemét, Hungary (Sample 3<sup>rd</sup>).

#### *The mycological agars*

Four types of mycological agars were used in the experiment. The agars were the Potatoe-glucose agar, Malate-extract agar, Martin-type agar and the Mycrocristalle cellulose agar. These agars had some unique content which were

fit to growing fungus. The Potatoe-glucose agar content potatoe-extract and glucose in high concentrations. In the Malate-extract agar were malate-extract and élesztő-extract. In the Martin-type agar were sacharose and  $K_2HPO_4$ . In the Mycrocristalle cellulose agar were  $KNO_3$  and élesztő-extract and peptone in high concentrations.

The thermophilic fungus species were determined from the wastewater sludge samples quantitatively. All of the agars were used to all of the samples in the experiment. So the optimal growing temperature and the optimal degradation properties could be determined because on each agars could grown only the fungus species which could use the agar contents (e.g. cellulose, peptone).

All of the sample with all of the fungus species were temperatured in a heated box with temperature settings (the determined temperature was 65°C, 68°C and 71°C).

### Results and discussions

The ammount and the quality of thermophilic fungus were measured in the wastewater sludge samples. High numbers of thermophilic fungus species were determined in the wastewater sludge samples. We found eight species in the samples but these species were not in all of the samples because the species were growed only on the agars which contents the best materials to them.

#### *Ammount of thermophilic fungus in samples*

First of all the ammount of thremophilic fungus were measured in the wastewater sludge samples (Figure 1.).

Sample 1<sup>st</sup> (Bánhalma, Hungary) –  $3,7 \times 10^4$  CFU/g fungus ammount

Sample 2<sup>nd</sup> (Pálhalma, Hungary) –  $4,8 \times 10^3$  CFU/g fungus ammount

Sample 3<sup>rd</sup> (Kecskemét, Hungary) –  $7,7 \times 10^4$  CFU/g fungus ammount

#### *Determiations of isolated fungus species*

Eight thermophilic fungus species were determined in the wastewater sludge samples.

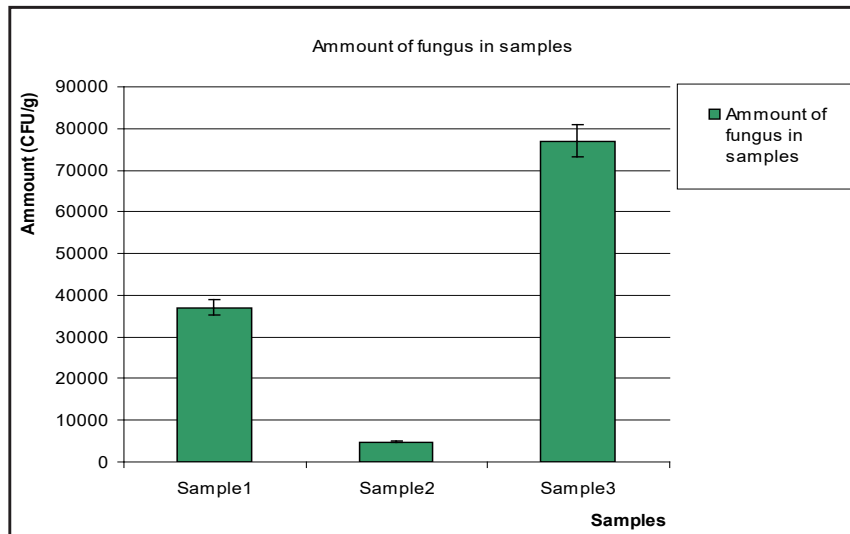


Figure 1. The amount of thermophilic fungus in samples

The following species were found: *Malbranchea cinnamomea*, *Thermomyces lanuginosus*, *Scytalidium thermophilum*, *Myceliophthora thermophila*, *Paecilomyces sp.*, *Thermoascus aurantiacus*, *Myriococcum thermophilum* and *Rhizomucor pusillus*. An international standard were used to determined which isolated fungus were which fungus species.

The degraded wastewater sludge is a unique matter because it has some organic matters and high water content. The thermophilic fungus could growing if the environment contains high water amount and several organic matters (e.g. cellulose, lignin, hemi-celluloses). That was the reason of the research of organic matter degradation properties.

*Degradation properties of isolated fungus*

The following table (Table 1.) shows the degradation properties of each isolated thermophilic fungus species.

In comparison of fungus species cellulose degraded by three species, xilan, mannan and fatty-acid ester by seven species, lignin and ceratine by only one fungi, and phospathe by four species.

In an other comparison (in the point of isolated species): the most important organic matter cellulose could degraded by *Thermomyces lanuginosus*, *Scytalidium thermophilum*, and *Paecilomyces sp.* Three matters from seven organic matters could degraded by seven fungus. The mannan, xilan were not degraded

Table 1. The degradation properties of isolated fungus species

Isolated species	Degradation properties (+ or -)						
	cellulose	xilan	mannan	lignin	fatty-acid ester	ceratine	phosphate
<i>Malbranchea cinnamomea</i>	-	+	+	-	+	-	-
<i>Thermomyces lanuginosus</i>	+	+	+	-	+	+	+
<i>Scytalidium thermophilum</i>	+	+	+	-	+	-	+
<i>Myceliophthora thermophila</i>	-	+	+	-	+	-	+
<i>Paecilomyces sp.</i>	+	+	+	+	+	-	+
<i>Thermoascus aurantiacus</i>	-	+	+	-	-	-	-
<i>Myriococcum thermophilum</i>	-	+	+	-	+	-	-
<i>Rhizomucor pusillus</i>	-	-	-	-	+	-	-

by only one fungi *Rhizomucor pusillus*. This fungi could degrade only the fatty-acid ester. Most of the fungus could degrade five or six matters from seven organic matters. This could be a reason of fungus methabolic processes.

The thermophilic fungus could use the same matters than the other fungus but they use these on higher temperature. This is the reason we measured the optimal growing (degrading) temperature. There were no significant difference between the optimal growing temperatures of isolated fungus. The grewed between 65°C and 71°C. Most of the fungus were grewed at 65-68°C. Only *Rhizomucor pusillus* grewed at 71°C. The optimal growing temperaure means the optimal size of fungus in three days old life.

### Conclusions

The digested wastewater sludge contains several fungus species (and wilde range of thermophilic fungus species). The sludge contains several

organic matters which could use by thermophilic fungus to their methabolic processes. The eight isolated thermophilic fungus species were in all samples and this is the reason of the wild range of degrading. The cellulose and the other matters are useful to fungus growing. The thermophilic fungus could use these matters but they must degrade to use these in better chemical formats. We recommend to research the other important matters (e.g. organic matters in more difficult chemical formats) degradation because we would like to determine the whole degradation spectrum of thermophilic fungus.

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## OBITUARY

### **István Láng (1931-2016) agrochemist, soil scientist and environmentalist has passed away**



István Láng (source: mta.hu)

“Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs.” István Láng was a scientist working hard on man-and-biosphere issues. His whole life was dedicated to scientific research in favour of building an environmentally sound coexistence on our planet.

István Láng was born on the 26<sup>th</sup> December 1931 in Mohács, Hungary. By profession he studied chemistry and agriculture and graduated as an agrochemist and pedologist. He started to work at the Institute of Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences in 1955. During the next eight years he was engaged in various research programmes. Also, this was the time when he began experiments in sandy soil research, and founded the Nyírlugos long term trial which contributed to novel scientific results in the field of acidification of sandy soils.

In 1963 he was appointed by the Academy for certain tasks of science administration. He started a life-long service during which period he was governing the research network of the Academy first as a deputy, later as the general secretary. He was a responsible and a wise leader of the Academy for almost a quarter of a century. His contribution to the development of the scientific network is remarkable. During his leadership numerous national and international research institutions have been founded and many nationwide scientific programmes were managed.

He was founder and later president of the National Scientific Research Fund (OTKA). He was a member of the National Council of Environment Protection. He was also an active expert in various international scientific missions with the UN. His participation in the work of the Brundtland Committee was a most remarkable task for him as well as for the scientific community.

Milestones of his scientific career: he defended his CSc degree in 1961 and the DSc degree in 1973, after which he was elected to be the corresponding member of the Hungarian Academy of Sciences. Since 1985 he was full member of the Academy. From 1993 he was acting as a university professor. Also, his international activities were appreciated. He was member of several foreign

academies - the Polish Academy of Sciences, the Academy of Agricultural Sciences of Russia, the Academy of Sciences of Mongolia, the Academy of Agricultural Sciences of Romania, and last but not least the European Academy of Sciences and Art.

His personality was remarkable as well. Apart from being a talented and highly educated person he had special characteristics and attributes. His work and lifestyle both were driven by accuracy and a restless diligent manner. He was a wise man with good skills in leadership. All along his life, even during the periods of carrying a bureaucratic burden, he remained an open-minded scientist participating in field work with a strong bond to nature and the environment. Also, he was fond of outdoor activities such as hiking and bird watching.

István Láng has passed away. An exceptional scientist left us. However his mental heritage will contribute to the success of environmental research on a long term. Professor Láng, rest in peace!

*Márton Jolánkai*

### **Source of the graphics**

*Front cover:*

Gallo-Roman harvesting machine, called Vallus. Source: U. Troitzsch - W. Weber (1987): Die Technik : Von den Anfängen bis zur Gegenwart

*Rear cover:*

Portrait of Columella, in Jean de Tournes, Insignium aliquot virorum icones. Lugduni: Apud Ioan. Tornaesium 1559. Centre d'Études Supérieures de la Renaissance - Tours





### **POSTA Katalin, editor-in-chief**

DSc /agric/, dean of the the Faculty of Agricultural and Environmental Sciences of the Szent István University, Gödöllő, Hungary, member of the Soil Science, Water Management and Crop Production Committee of the Hungarian Academy of Sciences. Professional fields: soil microbiology, arbuscular mycorrhizal fungi, plant protecton by soil microorganisms.



### **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 19<sup>th</sup> Century.