THE EFFECT OF THE A GENOME SPECIES (*Triticum monococcum* and *Triticum boeoticum*) ON THE FECUNDITY AND BEHAVIOUR OF *RHOPALOSIPHUM PADI* – BIRD CHERRY-OAT APHID

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Abstract

Triticum monococcum is an A genome diploid species that is closely related to and cross-fertile with *T. uratu* which is now accepted as the donor of the A genome in the hexaploid bread wheat (*T. aestivum*). Ancestral A genome species present good potential sources for further crop improvement through synthetic polyploidisation and introgression into modern wheat cultivars. In this study we examined the antibiotic and antixenotic effect of different A genome diploid species and accessions on the aphid *Rhopalosiphum padi*. In choice tests most of the lines were less attractive to *R.padi* and showed reduction in aphid weight gain and fecundity compared to the hexaploid control. We found through HPLC studies that seedling leaf tissue of the A genome species *T. monococcum* and *T. boeoticum* did not contain the hydroxamic acids found in tetraploid and hexaploid wheats, but did produce two compounds present in the same retention range. Increased

production of both unknown compounds was recorded in the later seedling growth stage, which may have an effect on aphid development, but not as strongly as we have previously seen in the presence of high amount of hydroxamic acids in the B genome species *Aegilops speltoides*.

Keywords: *Triticum monociccum*, *Triticum boeoticum*, *Triticum aestivum*, *Rhopalosiphum padi*, aphid behaviour, aphid fecundity

Összefoglalás

Triticum monococcum az első emberek által termesztett búzafaj, amelynek vad alakja a *Triticum boeoticum*. A *T. monococcum* közeli rokonságban áll a modern hexaploid búza A genom donorjával, a *Triticum urartu*-val. A diploid fajok sok esetben a rezisztencia kutatások tárgyát képezik, mivel kitűnő forrásanyagot biztosítanak a modern búza fejlesztéséhez szintetikus poliploidizáción és introgresszión keresztül.

A fentiek keretében tanulmányoztuk a *Triticum monococcum* és *Triticum boeoticum* fajok néhány képviselőjének antibiotikus és antixenotikus hatását a *Rhopalosiphum padi* levéltetvekre.

Vizsgálataink során megállapítottuk, hogy a diploid fajták kevésbé atraktívak a táplálékválasztási tesztben, az utódszám csökkent és a súlygyarapodás is lassult a hexaploid kontrollon táplálkozó levéltetvekhez képest. HPLC-vel történő vizsgálat során megállapítotuk, hogy a diploid fajták nem tartalmaznak hidroxámsavakat, amiket előzőleg a tetraploid és hexaploid búzafajták levélmintáiban megtaláltunk. A hidroxámsavakkal azonos retenciós intervallumban két ismeretlen vegyület tűnt fel amelyek termelése a növekedési stádium előrehaladtával fokozódott. Vizsgálataink alapján arra következtethetünk, hogy ezek a vegyületek hatással lehetnek a levéltetvek viselkedésére, de mégsem olyan hatékonyak, mint előzőleg a magas hidroxámsav tartalmú B genomú *Aegilops speltoides* esetében voltak.

Introduction

Modern wheat belongs to two species the hexaploid *Triticum aestivum* (2n=42 chromosomes) and the tetraploid *T. turgidum* (2n=28 chromosomes). Polyploid wheat was developed under the influences of ancient human cultivation through amphiploidy between diploid *Triticum* species and *Aegilops* species (Nevo et al,. 2002).

Einkorn wheat incorporates two related A genome species *Triticum monococcum* and *Triticum urartu*. By the early Bronze Age cultivation of tetraploid (AABB) emmer wheat in and around the Fertile Crescent had enabled agricultural societies to thrive (Zohary and Hopf, 1993). The cultivated tetraploid emmer wheat was probably developed from wild emmer wheat *T. turgidum ssp. dicoccoides,* which itself is a result of spontaneous but rare hybridisation between *T. uratu* (AA) and the B genome species *Aegilops speltoides* (Petersen et al., 2006). Although repeated and independent allopolyploidisation events may have occurred this process of speciation inevitably creates a severe evolutionary bottleneck. Modern bread wheat *T. aestivum* (AABBDD) has no wild representatives and certainly arose through a further incident(s) of amphidiploidisation under the influences of human cultivation. Throughout the history of human cultivation of wheat the crop gene pool has therefore always been relatively low in genetic diversity.

CIMMYT in particular has shown the value of resynthesising polyploid wheat in order to introduce greater genetic diversity from representatives of the ancestral diploid species. Most effort has been devoted to the more recent D genome progenitor *Ae. tauschii*. Recent strides in genetic marker technologies offer the potential for faster, targeted introgression of superior, but rare alleles from alien species and have encouraged a renewed interest in alternative compatible

sources in the A and B genomes. For further crop improvement by traditional breeding a wide range of A and B genome wild relatives exist that are cross compatible and could be used for introgression (Nevo et al., 2002).

Aphids, for example the bird cherry-oat aphid, *Rhopalosiphum padi*, are common pests in cereals and are able to cause serious wheat yield losses by direct feeding damage and by transferring plant pathogenic viruses such as barley yellow dwarf virus (BYDV) (Hand, 1989, Thackray et al., 2009). In mild winters in temperate regions the damage could increase under the influences of climate change if aphids are able to continue feeding and reproducing anholocyclically in the wheat crops (Leather, 1993) and therefore increase the risk of secondary spread of virus infection.

The alatae are responsible for the selection of the suitable host plants. The host-selection behaviour is affected by attraction to non-specific visual stimuli, the colour or the form of the host plant (Powell and Hardie, 2001) and plant volatiles, which are detected by antennal olfactory sensillae (Powell et al., 2006).Secondary metabolites can affect aphid behaviour and play an important role in insect resistance. For this reason in our previous work (unpublished data) we concentrated on the hydroxamic acids (HA), which are known as potential aphid resistance factors from the studies of Nicol et al (1992) and Givovich and Niemeyer (1991). However, our results did not support the hypothesis that HAs have antixenotic and antibiotic effects on *R. padi* for the hexaploid and tetraploid varieties tested.

In this study we investigated the antibiotic and antixenotic effects of different A genome diploid varieties on the behaviour and development of *R. padi*. The plants were also subjected to HPLC analysis to confirm the presence or absence of HAs in the leaf tissue.

Methods and material

Aphids

Rhopalosiphum padi was collected from volunteer wheat plants from the field in Thriplow, Herts, UK in August 2006 in September in 2007 and again (refreshing the colony) in 2008. The colony used in this study was established from one aphid using the mildew resistant spring wheat variety Tybalt as the culture plant. The colony was kept in a glasshouse in a temperature range of 12-25°C and light 16:8 L:D.

Plant material

Several accessions of the diploid species *Triticum monococcum* (MDR 002, MDR 037, MDR 043, MDR 044, MDR 049, MDR 050) and *T. boeoticum* (102, 8116, 8150, 8404) were provided by Rothamsted Research (RRes). For the HPLC analysis, the replicated experiment was set up in RRes in a controlled environment room at 20°C+/- 2°C, 16:8 L:D. Plants were grown in vermiculite.

For the fecundity and settling test plants were grown in a glasshouse (16:8 L:D, ≈ 20°C), in compost.

Fecundity test

This test was used to determine the intrinsic rate of population increase by recording how long it takes an aphid from birth to produce the first nymph and how many nymphs were produced over an equivalent time on the test varieties.

Seven alatae were put in a cage with one plant of the test variety for 24 hours to produce pre-conditioned nymphs for the experiment after which the alatae were removed. Nymphs were allowed to develop on those plants for 3-4 days until they reached a reasonable size making them easier to transfer onto the 7 day old test plants. One experimental

3 day old nymph was placed on the middle part of the first leaf in a 2cm diameter clip cage. The developing aphids were monitored at the same time each day. From the first day of nymph production the new nymphs were removed and their numbers recorded daily. The experiment was carried out in a glasshouse at ≈20°C and 16:8 L:D.

From the data the intrinsic rate of population increase (r_m) was calculated using the formula by Wyatt and White (1977). Data were subjected to ANOVA and Student's t test (Microsoft Office Excel).

$$r_m = c (\log_e Md) / d$$

Where c is a constant = 0.74, d = pre-productive period (days) and Md = number of nymphs produced in the reproductive period equal to d

Settling test

Twenty alate *R. padi* were given a choice between a 7 day old seedling of the test plant and a 7 day old control plant, which was the hexaploid variety Solstice. Each cylindrical choice cage (12 cm diameter by 20 cm high) was made of clear acetate sheet and ventilated at the top. Each choice cage was replicated between 6 and 20 times depending upon the variety under test. To keep the humidity high, the experiment was set up on a wet sand tray. Alatae, which had settled on each of the two plants, were counted and recorded after 2, 5 and 24 hours from the beginning of the experiment. The test was conducted in the glasshouse at 20°C, 16:8 L:D. Data were analysed by Student's t test (Microsoft Office Excel).

Weight development and survival studies

Five apterous *R. padi* were put in a clip cage on the first leaf of replicated Solstice, MDR 037, and MDR 049 7-day old seedlings. After

24h, the adults were removed and the nymphs produced were counted. Numbers were reduced to about 10/cage to prevent overcrowding, and the nymphs were left in the clip cages to develop. After 7 days nymphs were recounted and weighed in their batches. Survival rates and average nymph weights were calculated and compared. A mean relative growth rate could not be calculated since birth weights were not taken due to the high mortality incurred during the weighing process in a preliminary trial. Data were analysed by Student's t test (Microsoft Office Excel).

Sample analysis by high pressure liquid chromatography (HPLC)

The leaf tissue of two varieties, MDR 037 and MDR 049, was sampled for the HPLC analysis at the 7, 19 and 28 day growth stage. The middle area was removed from each leaf, weighed into an Eppendorf tube containing three metal beads, which break the tissue during grinding. The Eppendorf tube was frozen in liquid nitrogen and stored on -80 °C until processing. The tissue was ground using a Qiagen tissuelyser for two minutes on 30/s frequency. This equipment enabled 48 samples to be ground at the same time. Before the samples were processed, the plates of the tissuelyser were frozen in liquid nitrogen to ensure that the samples remained frozen during grinding. The buffer, 0.5ml of a methanol 98% and acetic acid 2% mixture, was added to the sample after grinding. It was then sonicated for 10 minutes and centrifuged for 10 minutes at 16000rpm at 4 °C. Supernatant was transferred into a glass vial and analysed by HPLC. The HPLC method was adapted from Baumeler *et al*., (2000). A thermal hypersil C-18 5µ, 250 x 4.6mm column was used, mobile phase (A) HPLC grade water (B) methanol/isopropanol (95/5) + 0.025% acetic acid. The gradient profile of solvent A and B was 0-2 min 10% B; 2-11min 10-50% B; 11-16min 50% B; 16-17min 50 to 10% B. Injection volume 20µl, the flow rate was 1ml/min and the run time 17 minutes.

HA levels were compared and analysed by Student's t test (Microsoft Office Excel).

Results

Settlement choice assay

In the settling test the antixenotic effect of the diploid varieties was tested (Figure 1). Accessions of both *T. monococcum* (MDR 002, MDR 043, MDR 044, MDR 050) and *T. boeoticum* (8404, 8116, 8150) showed reduced attraction for *R. padi* alatae, and for 8404 and MDR 043 the differences were significant (P<0.02). However, the other *T. monococcum* varieties MDR 040 (*P*=0.004) and MDR 037 (*P*=0.0002) had three times more alatae settled on the plants compared to the Solstice control.

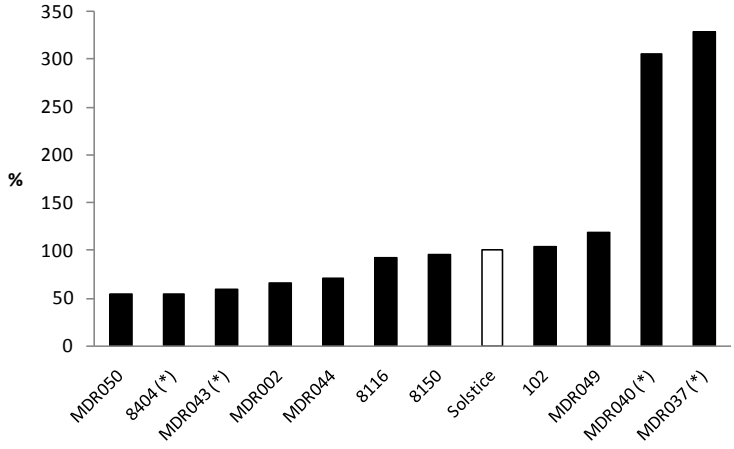

Figure 1. Settlement of *R. padi* on the diploid varieties as a percentage of settlement on Solstice (hexaploid control), which =100%* significantly different to the Solstice control

No choice development assay

The intrinsic rate of population increase (r_m) study showed differences in aphid development rate and fecundity between the varieties (Figure 2). The r_m was reduced on all of the diploid accessions (4-15% lower) compared to the Solstice control. On most of the varieties (MDR 044, MDR 049, 8116, 8150 and 8404) nymph production was significantly reduced (P<0.02) compared to Solstice. On MDR 043, MDR 050, 102, where there were fewer replicates, the differences were not significant (P>0.05).

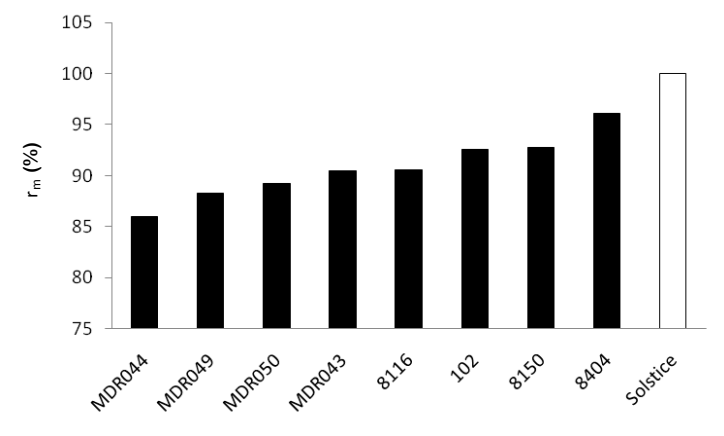

Figure 2. Intrinsic rate of population increase (rm) of *R. padi* on diploid varieties as a percentage of the rm on the hexaploid control (Solstice), which = 100%.

The *R. padi* nymphs on the diploid varieties took 9-10 days to produce the first nymphs. However; nymphs feeding on the hexaploid variety only took 7-9 days from birth. This slower maturation was also reflected in the aphid size. On the diploid varieties aphids were smaller and produced fewer offspring than on the hexaploid variety. The average daily nymph production was 5.9 on Solstice and 4.4 on the diploid varieties.

These results indicate that the diploid varieties may contain attributes that could be important in the resistance breeding against aphids in the future.

Survival and weight development studies

The survival rate of *R. padi* was studied on two *T. monococcum* lines (MDR 037, selected because of the high preference in the settling test and MDR 049 which was selected because it had a reduced r_m value in the fecundity test) and the reference *T. aestivum* (Solstice) cultivar. Differences were noticeable between the species. After 7 days feeding on the hexaploid variety, significantly more nymphs survived (99.4%) than on the two diploid varieties ($P<0.05$) (Figure 3).

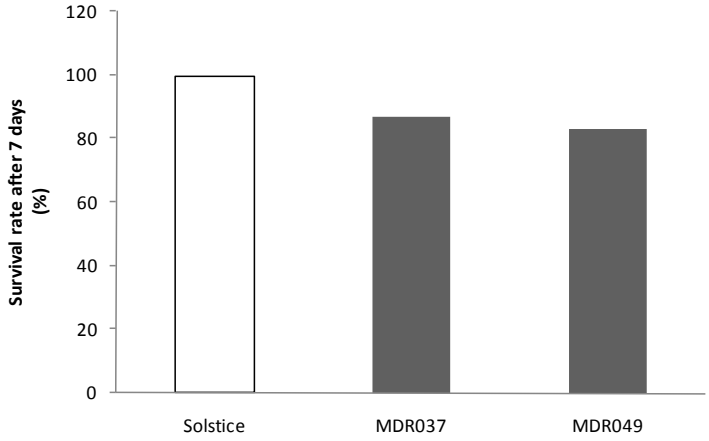

Figure 3. The survival rate of *R. padi* after 7 days on two diploid varieties compared to the hexaploid control (Solstice)

On the hexaploid variety, nymphs gained weight faster than on the diploid varieties (Figure 4). On line MDR 037 nymphs showed significantly lower survival rates, but the weight gain was only slightly reduced compared to aphids feeding on the hexaploid variety. For MDR

049, which was less attractive in the settling test and showed reduced intrinsic rate of development and survival rate, the weight gain was very significantly lower than on the hexaploid control ($P<0.001$).

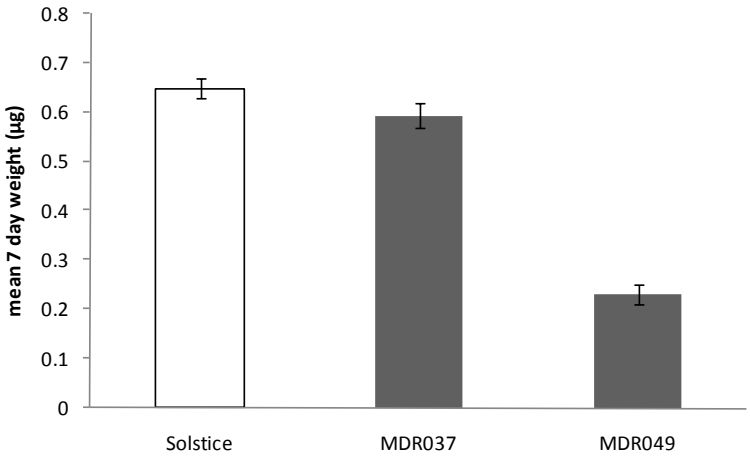

Figure 4. Weight gain of *R. padi* after 7 days on the hexaploid control and two diploid varieties

Result of HPLC analysis

In the previous experiments (Figures 1-4) *R. padi* reproduction and settlement was reduced on the diploid varieties compared to the control. Analysis of the leaf tissue showed that the A genome diploid varieties did not contain known HA related compounds, but two unknown peaks appeared on the HPLC trace at 13.4 (compound I.) and 13.8 minutes (compound II.) which will be investigated further (Figure 5).

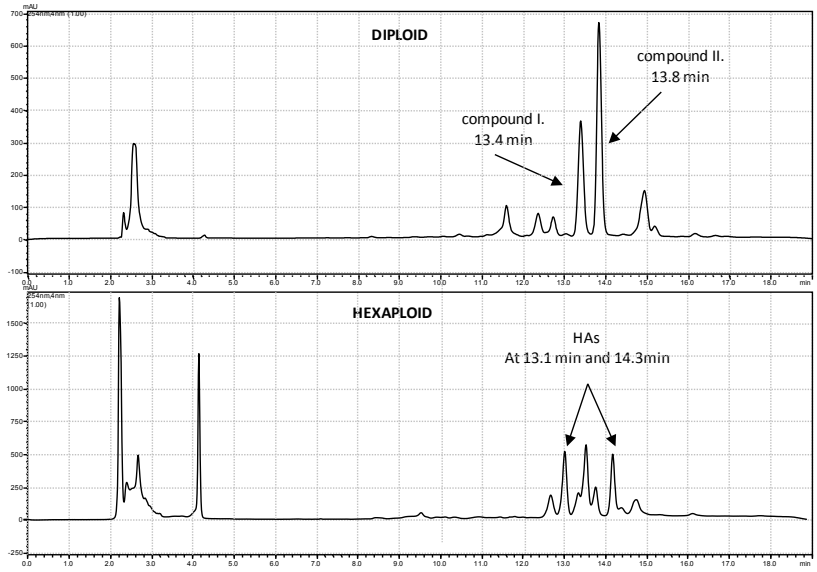

Figure 5. HPLC traces of leaf tissue extracts of an A genome diploid (*T. monococcum* MDR049) and a hexaploid variety (Tybalt). The relative quantities of the peaks are not directly comparable because the sample sizes are not identical.

Leaf samples were taken from two *T. monococcum* varieties MDR 049 and MDR 037 at different growth stages to follow the changes in the levels of the unknown compounds during the early growth of the plants.

Results for compound I. suggest a significant difference between the two lines in the pattern of production. In the case of MDR 037 the compound is absent from the very young leaf, builds to a concentration of 8.0E+05 as the leaf matures and then remains constant in the maturing leaf. Each successive leaf gives similar expression levels. MDR 049 shows a continuing build up of the compound as the leaf ages leading to significantly higher concentrations than for MDR 037 in the later stages of the leaf. Furthermore the 2nd leaf shows significantly higher levels of the compound than the 1st leaf.

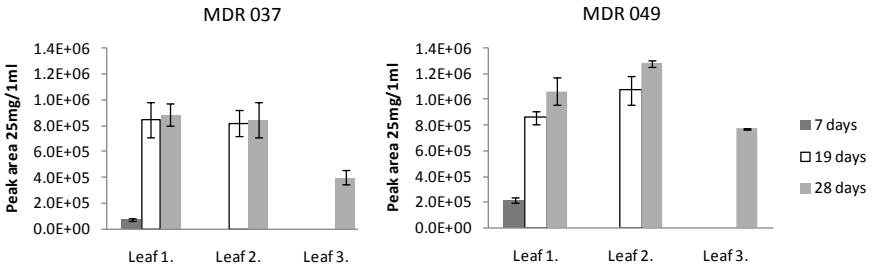

Figure 6. Concentration of compound I. (HPLC retention time 13.4 minutes) in leaf tissue sampled from the first 3 seedling leaves of *T. monococcum* lines after 7, 9 and 28 days. Leaf 2 appeared between days 7 and 19 and leaf 3 between days 19 and 28.

The second unknown peak (HPLC retention time 13.8 minutes), showed a different pattern of production to the other compound. For this compound, the two lines give similar results although MDR 037 may be producing a slightly higher concentration than MDR 049. In each case concentrations increase in subsequent leaves and do not build with time within a leaf suggesting that production of this compound occurs during the early genesis of each leaf.

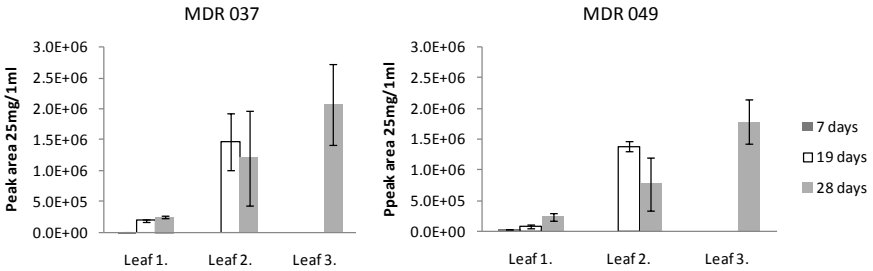

Figure 7. Concentration of compound II. (HPLC retention time 13.8 minutes) in leaf tissue sampled from the first 3 seedling leaves of *T. monococcum* lines after 7, 9 and 28 days. Leaf 2 appeared between days 7 and 19 and leaf 3 between days 19 and 28.

Discussion

In our previous work we have investigated the effect on *Rhopalosiphum padi* of the hydroxamic acids (HAs) in the hexaploid, tetraploid and the B genome diploid species (manuscripts in preparation/ submitted). The importance of this group of secondary metabolites was highlighted in one of the B genome species where a high concentration of HAs, observed in the leaf tissue, had a significant effect on the development of *R. padi*. The defence mechanism in the B genome species could be an option to improve the insect resistance in modern hexaploid wheat.

In the current work, good evidence for reduced fecundity and aphid weight gain has been demonstrated on A genome species, with no corresponding link with increased levels of the known HAs. Indeed, the HPLC analyses failed to find any known HAs. Similar studies by Nomura et al. (2007), found no HAs in *T. boeoticum*.

Of the two novel compounds, the expression pattern of compound II. is similar between the two lines tested and would suggest that this is not responsible for any differences in aphid growth between the lines MDR 037 and MDR 049. Compound I. on the other hand is expressed differently between the lines, with the more aphid resistant line MDR 049 showing higher concentrations in progressively older leaves. Furthermore compound II builds continuously in the leaf. Unfortunately it is not possible to take the conclusion any further than to note the existence of these unidentified compounds and to look to future work to determine if either may have a role as a protective mechanism against aphids.

These results do identify potentially useful A genome accessions that show some degree of resistance to aphid development. The settlement test is based on physical and chemical differences between the

test plant and the control plant (the hexaploid wheat variety Solstice), providing the aphids with a simple choice. Most of the A genome varieties tested were less attractive to *R. padi* alatae. In particular, significantly fewer aphids settled on line 8404 (*T. boeoticum*) and MDR043 (*T. monococcum*) than on the control. MDR040 and MDR037 were highly attractive to *R. padi* and 2.5-3 times more aphids settled on those varieties than on Solstice. For this reason they were not selected for the intrinsic rate of population increase assays.

The intrinsic rate of population increase values for *R. padi* on the A genome varieties were 4-15% lower than on the hexaploid control. On the diploid varieties, the nymph development and maturation was slower; the average daily nymph production on the diploid varieties was 4.4 and on the hexaploid variety 5.2.

The negative effect of some of the diploids on *R. padi* was also demonstrated in the survival and the weight gain studies on lines MDR037 and MDR049. On the diploids, 13-16% fewer aphids survived, which was significantly lower than on the hexaploid control. The weight gain study also provided very interesting results. Weight gain on MDR 037, which was highly attractive in the settlement choice test, was not significantly different from Solstice, and the aphids developed on that diploid variety just as well as on Solstice. On the other hand, aphid settlement on MDR 049 was similar to the control, but the offspring production was significantly reduced in the intrinsic rate of population increase assays. This observation was supported by the weight gain study where aphid development was significantly reduced compared to the hexaploid control.

Although we found a reduction in fecundity and development on some of the diploid lines, it was not as effective as we had seen previously on B genome species *Aegilops speltoides*. The absence of the HAs in *T. monococcum* indicates that the negative effects on the aphid

are caused by a different defence mechanism, which could be combined with the mechanism found in *Ae. speltoides* to provide a pre breeding objective, which could be tested using a *de novo* resynthesis of the allotetraploid (AABB).

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References

Baumeler, A., Hesse, M. and Werner, C. 2000. Benzoxazinoids-cyclic hydroxamic acids, lactams and their corresponding glucosides in the genus Aphelandra (Acanthaceae). *Phytochemistry*. **53.** 213-222

Givovich, A. and Niemeyer, H.M. 1991. Hydroxamic acids affecting barley yellow dwarf virus transmission by the aphid *Rhopalosiphum padi*. *Entomologia Experimentalis et Applicata*. **59.** 1. 79-85

Hand, C.S. 1989. The overwintering of cereal aphids on Gramineae in southern England, *Annals of Applied Biology*. **115.** 17-29

Leather, S.R. 1993. Overwintering in six arable aphid pests: a review with particular relevance to pest management. *Journal of Applied Entomology*. **116.** 217-233

Nevo, E., Korol, A.B., Beiles, A. and Fahima, T. 2002. Evolution of wild emmer and wheat improvement. Springer, Germany. 4-8

Nicol, D., Copaja, S.V., Wratten, S.D. and Niemeyer, H.M. 1992.

A screen of worldwide wheat cultivars for hydroxamic acid levels and aphid antixenosis. *Annals of Applied Biology*. **121**. 11-18

Nomura, T., Ishihara, A., Iwamura, H. and Endo, T.R. 2007. Molecular characterisation of benzoxazinone – deficient mutation in diploid wheat. *Phytochemistry*. **68.** 7. 1008-1016

Petersen, G.,Seberg, O., Yde, M. and Berthelsen, K. 2006. Phylogenetic relationship of *Triticum* and *Aegilops* and evidence for the origin of the A, B and D genomes of common wheat (*Triticum aestivum*). *Molecular Phylogenetics and Evolution*. **39.** 1. 70-82

Powell, G. and Hardie, J. 2001. The chemical ecology of aphid host alternation: How do return migrants find the primary host plant? *Applied Entomology and Zoology.* **36**. 3. 259-267

Powell, G., Tosh, C. R. and Hardie J. 2006. Host plant selection by aphids: behavioral, evolutionary, and applied perspectives. *Annual Review Entomology*. **51.** 309-330

Thackray, D.J., Diggle, A.J. and Jones, R.A.C. 2009. BYDV predictor: a simulation model to predict aphid arrival, epidemics of Barley yellow dwarf virus and yield losses in wheat crops in a Mediterranean – type environment. *Plant Pathology.* **58.** 186-202

Wyatt, J.I. and White, F.P. 1977. Simple estimation of intrinsic rates for aphids and tetranychid mites. *Journal of Applied Ecology*. **14**. 757-766

Zohary D, Hopf M. 1993. Domestication of plants in the Old World: the origin and spread of cultivated plants in West Asia, Europe, and the Nile Valley, 2nd edn. Oxford: Oxford University Press. 1977-1980.

INTENSIVE REARING OF WELS (*SILURUS GLANIS* L.) USING PLANT PROTEIN BASED FEED

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Abstract

In order to replace growingly expensive fish meal, plant based feeds were compared to commercial wels (*Silurus glanis*) feed in a 60 days experiment. Three types of feed were applied: squealer feed (S), squealer feed combined with forage fish (S+F) and commercial wels feed (W). The feed conversion ratio was 0.88±0.21 at the W group, 1.74±0.21 at the S and 1.48±0.23 in the case of S+F group. Specific growth rate was higher at wels feed (2.34%), than in case of the squealer feed (1.77%) or squealer feed with forage fish addition (1.95%). The slaughter loss was the least at the fish fed with forage fish in addition (34.6±2.0%). The relative size of the liver (2.8±0.32% of body weight) and the fat % of the viscera (4.1±1.0% of body weight) were significantly higher at W group than those of the other treatments.

Key Words: wels, intensive rearing, plant protein, growth.

Összefoglalás

Az egyre drágább halliszt kiváltásának érdekében, növényi alapú tápokat hasonlítottunk össze kereskedelmi harcsatáppal egy 60 napos kísérlet során. Háromféle takarmányt alkalmaztunk: harcsatáp (W), malactáp (S), illetve hal-kiegészítés malactáp etetése mellett (S+F). A takarmányértékesítés a harcsatápos kezelés esetében 0.88±0.21 volt, míg a malactápos csoport esetében 1.74±0.21, a hal-kiegészítéses csoportnál 1.48±0.23. A specifikus növekedési ráta a harcsatápos csoport esetében nagyobb (SGR átlag: 2.34%), mint a malactápos (1.77%), ill. a hal-kiegészítéses csoport esetében (1.95%). A törzs vágási veszteségei a hal-kiegészítéses csoport esetén a legkisebbek (34.6±2.0%). A harcsatápos csoport esetében a máj relatív mérete (2.8±0.32%) és a hasűri zsír (4.1±1.0%) szignifikánsan magasabb volt, mint a másik két kezelés esetén.

Kulcsszavak: harcsa, növekedés, növényi fehérje, takarmányhasznosítás

Introduction

Majority of the Hungarian fish production is based on carp-dominant polyculture. The profitability of this segment could be improved by increasing the production of valuable predatory fishes. In traditional pond poly-culture the proportion of carnivorous fishes does not exceed 1-2% of the production, though the market requires the constant and reliable production of these valuable species. This could be provided primarily from intensive rearing facilities using artificial feed. For this purpose in Middle-Europe a native European catfish species, the wels (*Silurus glanis,* Linnaeus*,* 1758) seems to be ideal. There are numerous fortunate properties making wels a good choice for intensive aquacul-

ture. The flesh of wels is boneless, delicious, white coloured, tasty and is of high market value. It is easy to rear it on artificial formulated feed and it utilizes the feed efficiently. Wels tolerates handling stress relatively well and its dissolved oxygen demand is similarly low as that of the carp. Wels production in Hungary is ranging between 1-2 % of the total fish pond production. In 2009 the total wels production was 246 tons, while the capture from natural waters amounted only 166 tons in Hungary (*Pintér, 2010*).

Remarkable part of the running costs (50-90%) of fish production in intensive systems is the feed cost (Müller, 1990). For this reason it is of primary interest to decrease the feed prices by using cheaper feed components.

Nowadays fish in intensive rearing facilities are usually fed with feeds containing fish meal as primary protein source. The catches of species providing the raw material of the fish meal have been stagnating or decreasing globally since the beginning of the 90's (Astles et al., 2009; Caddy & Garibaldi, 2000; Johnsen, 2005). The total marine fish capture in the recent years reached approximately 81-84 million tons per year (FAO, 2009). Due to the overexploitation of seas the sustainability of this practice became uncertain (Tacon & Metian, 2008). Production cost of fish meal increases extremely fast. Since 2004 the price of fish meal has doubled (FAO, 2009), so it is not only ecological but also commercial interest to reduce the amount of the fish meal in commercial fish diets or substitute it with alternative protein sources e. g. plant protein or fermentation products There are many trials aiming fish meal and fish oil replacement (Dias et al., 2009; Panserat et al., 2009; Sánchez-Lozano et al., 2009; Silva et al., 2009) even in the case of different catfish species (Ai & Xie, 2006; Ambardekar et al., 2009; Davies & Gouveia, 2008; Toko et al., 2008; Webster et al., 1997). Channel catfish production in earthen pond is almost exclusively based on plant

protein (Sink et al., 2010) In the present experiment the possibility of rearing wels on plant based feed was examined.

Materials and Methods

This experiment was carried out in the fish laboratory of the University of Pannonia in Keszthely, Hungary. Fish were held in an approx. 4000L recirculation system, which consisted of 9 fish tanks each of 350L (60cm*50cm*130cm) attached to settling, filtering and puffer tanks of 300L. Perlon wool was used as substrate for biofiltering bacteria. Ion exchange marbles were used to reduce the concentration of harmful NH_3/NH_4^+ nitrogen forms. In addition an UV-lamp was built into the system as biocide. The daily water exchange of the whole system was approx. 2.5%. Each fish tanks were supplied with air diffuser. Faeces and uneaten feed were sucked out daily with a rubber pipe. As the wels is a night time predator, the room was dimmed and the temperature was held between 20°C and 25°C (mean±SD: 22.3±1.3°C. Water temperature was measured daily (Figure 1).

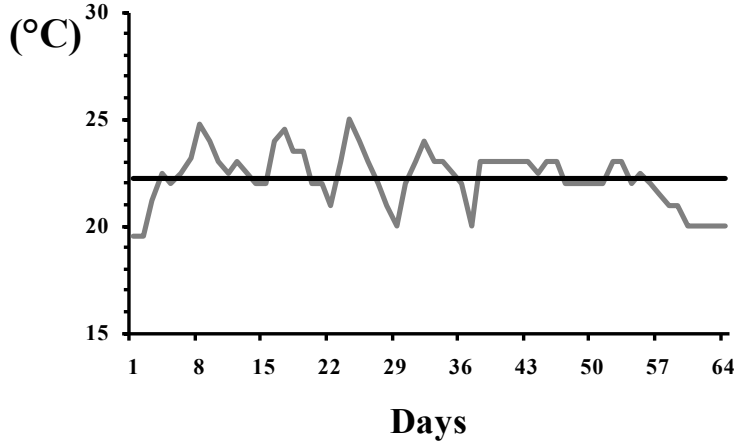

Figure 1. Change of water temperature during the experiment

Three treatments were set up and carried out on three size classes. First group (W group) was fed with commercial wels feed (crude protein content 36%), second group (S group) was fed with squealer feed (crude protein content 20%), while the third group (S+F group) was fed with squealer feed and one day pro week with cut forage fish. The squealer feed contained almost exclusively plant protein (mostly corn, wheat and soybean). The only animal component was 5% milk permeatum. Fish were fed three times a day *ad libitum* by hand feeding. Each time as much granulate were offered as the fish consumed immediately. Tiny cut cyprinids were served as forage fish.

Picture 1. One of the experimental fish (Photo: M. Havas)

Altogether 139 wels (Picture 1) formerly trained to artificial diet were used in the experiment. Starting weights varied between 28.2 and 125.5g according to normal distribution. Since there was great size heterogeneity in the stock, fish was assorted according to individual weight. Three size classes were formed: „small" (S; m_{mean}±SD: 45.9±9.5g), „me-

dium" (M; m_{mean}±SD: 54.1±15g) and „large" (L; m_{mean}±SD: 77.9±14.2g) class. The specimens within one size group were randomly allocated into each treatment. This resulted that there were no significant differences between the initial mean weights of the same size classes of the treatments. 15-16 individuals were held in each fish tank. All three size classes were represented in each of the treatments.

Individual weight was measured weekly – biweekly with tenth gram precision in water. One day before measurements fish weren't fed. To avoid parasitic diseases a short, salty (2.5%) bath was applied during the measures. Body length of fish was measured with 0.5cm precision.

Food conversion ratio (FCR), daily absolute growth (G), specific growth rate (SGR) and condition factor (K) were calculated. By the calculation of food conversion ratio the mass of forage fish was corrected according to its dry matter content (20%).

At the end of the experiment 18 individuals were sacrificed. The weight of the torso (carcass), the liver, and the abdominal fat were measured.

Distribution functions were checked with Kolmogorov-Smirnov test. Comparison of mean values was carried out with one-way analysis of variance (ANOVA) and Tukey's post hoc test. Criteria of significance were determined at 95 % probability ($p<0.05$).

Results

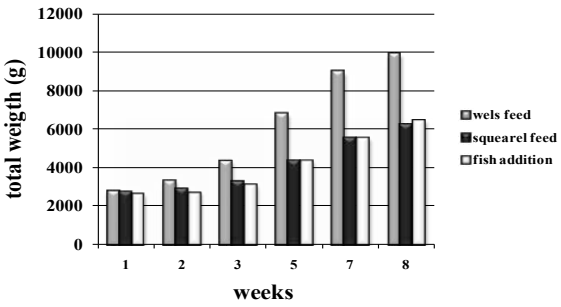

Figure 2. The total weight gain in the different treatments

The treatment groups achieved the following weight gains by the end of the experiment: W group: 7240.2g; S group: 3599.3g; S+F group: 4122.5g. At the end of the experiment the total weight gain in the W group was significantly higher than in groups S or S+F, while these two latter ones didn't differ significantly from each other (Figure 2).

Due to the primary assorting there were no significant differences in the initial mean weight inside any of the size classes. At the end of the experiment the individual mean weight was significantly higher in the case of the W group in all of the three sizes classes. There wasn't any significant difference between the growth rate of S group and S+F group (Figure 3).

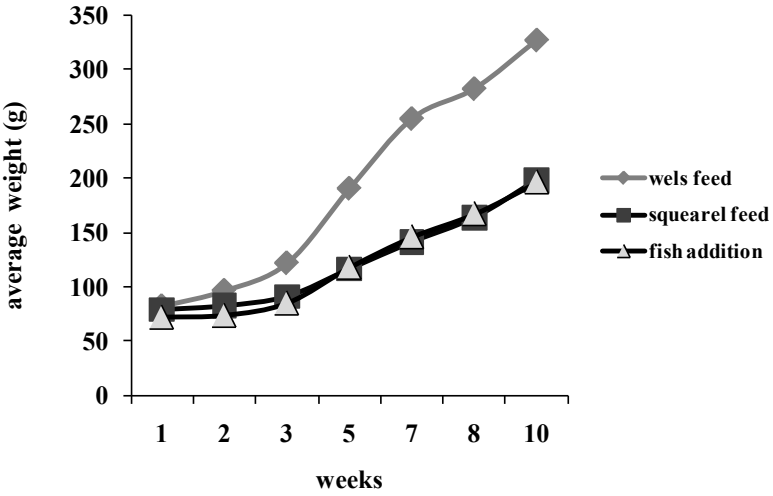

Figure 3. The average individual size of wels in the „L" size class

The condition factor of wels individuals was also better in the W treatment (mean±SD: 0.72±0.02) than in the other two groups (0.62±0.02). The condition of the S+F group significantly increased by the end of the experiment. There weren't any changes of condition in the case of the other groups.

The absolute daily growth of the W group - in the first weekly interval of the experiment - was more than twofold higher (3.60g day^{-1}) than in the other two groups (S group: 1.34 g day^{-1}; S+F group: 1.22 g day^{-1}), though later on this value decreased progressively resulting 2.77g day^{-1} value in the last weekly interval. In groups S and S+F an increase was recorded regarding daily growth resulting 2.56g day^{-1} and 2.73g day^{-1} values respectively in the last two weeks (Figure 4).

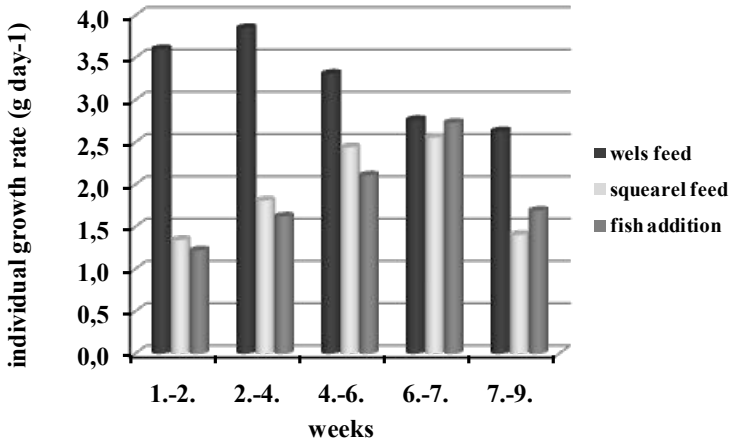

Figure 4. Dial absolute growth rate in the "M" size class

Specific growth rates followed trends similar to individual absolute growth. SGR value varied between 0.89% (S group) and 4.60% (W group). At the beginning of the survey the growth of the individuals fed with wels feed was faster in all of the three size classes (Figure 5). By the end this value decreased to its third. S and S+F groups did not show similar trends regarding changes of SGR values during the experiment.

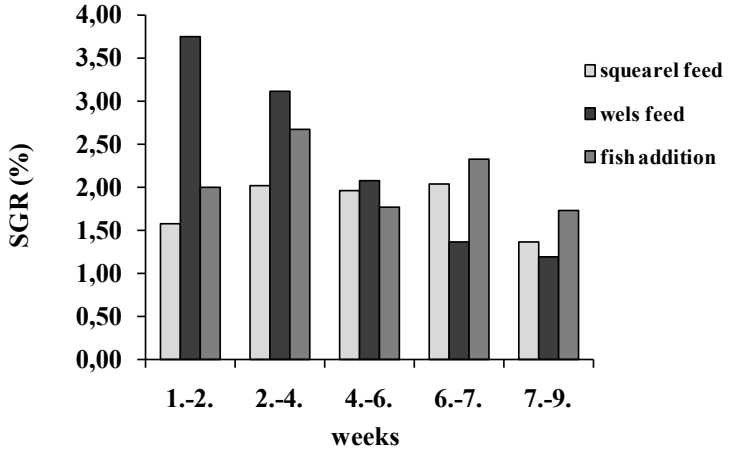

Figure 5. Changes in the specific growth rate during the experiment in the „S" size class

By the calculation of food conversion ratio the quantity of forage fish was considered according to its dry matter content (20%). FCR was the lowest in case of W group, and was highest in case of the S group (Table 1). Food conversion declined in all of the treatments during the experiment.

FCR		*wels feed*			*squealer feed*			*fish addition*		
Nr. of fish tank		1	2	3	4	5	6	7	8	9
weeks	1.-2.	0.64	0.65	0.67	1.54	1.60	1.64	1.21	1.37	1.25
	2.-4.	0.68	0.68	0.67	1.58	1.57	1.54	1.29	1.34	1.27
	4.-6.	0.88	0.91	0.84	1.83	1.67	1.94	1.70	1.53	1.68
	6.-7.	1.17	1.13	1.15	1.56	1.89	2.29	1.47	1.54	1.48
	7.-9.	1.06	1.01	1.12	1.78	1.95	1.77	1.31	1.97	1.82
mean		0.88	0.87	0.89	1.66	1.73	1.83	1.40	1.55	1.50
SD		0.23	0.21	0.24	0.14	0.18	0.30	0.20	0.25	0.25

Table 1. Food conversion ratio values during the experiment

The slaughter values showed that the amount of the fat in the abdomen and the size of the liver were bigger in the case of the W

group. This difference was multiple by the fat content (Table 2). The gut length/total body length ratio was also the biggest in this group.

%	*torso*	*liver*	*fat*	*intestine (length)*
wels feed	64.5±1.8	2.3±0.3	4.1±1.0	71.4±10.4
squearel feed	64.1±2.0	1.8±0.3	1.0±0.8	59.7±6.5
fish addition	65.4±2.0	1.8±0.2	0.5±0.3	60.9±4.2

Table 2. Slaughter values in the proportion of body weight and body length

Discussion

There is practical progress on the field of fish meal partial replacement in feeds of a couple of farmed fishes (Dias et al., 2009; Sealey et al., 2009; Silva et al., 2009). Nowadays feeds used in salmon producing contains only 30% fish meal instead of the former 50% (*FAO Fisheries and Aquaculture Department, 2009*). Up to now it was little done in this direction on wels (*Silurus glanis* L.). In our study we made a tentative experiment what showed that wels can also be reared on plant protein based diet, although the growth rate remained under the growth rate of fish fed by commercial wels feed. This may be due to the differences between both the source and the content of protein in the feeds. There was no mortality neither health problems during the experiment, although the high fat content of the abdomen and the excessive size of the liver can be indicative of health problems may occur if applying the commercial wels feed for longer duration. The increasing speed of daily weight gain indicates that continuing the examination for a longer time would provide better results in the case of the squealer groups. It cannot be excluded that the duration of this examination wasn't long enough for the enzyme system to adapt to the alternative diet. The digestibility of plant source

nutrients - especially for piscivorous fish - is worse than that of the animal nutrients (Cho & Bureau, 2001). It is possible that the duration of this examination wasn't long enough for the enzyme system to adapt to the alternative diet. Panserat et al. (2009) conducted a survey to examine the physiological effects of the total replacement of fish meal and fish oil in the diet of rainbow trout. It was found that diets containing 100% plant protein had no negative effect on the liver functions and metabolism. By today at some industrially farmed species the fish meal has been replaced already in the commercial feeds. The protein in channel catfish feeds applied in the American catfish farms is almost exclusively of plant origin as mostly soybean meal, corn meal and cottonseed (Stickney, 2010).

Further examinations are necessary to develop plant protein based feeds for wels what may result growth rates close to that achieved by using fish meal based feeds. Beside plant protein fermentation product protein should be considered as well. We would like to attain a new type of intensive wels culture in traditional carp ponds.

References

Ai Q., Xie X. 2006. Effects of dietary soybean protein levels on metabolic response of the southern catfish, *Silurus meridionalis*. *Comparative Biochemistry and Physiology* **144** 41-47.

Ambardekar A. A., Reigh R. C., Williams M. B. 2009. Absorption of amino acids from intact dietary proteins and purified amino acid supplements follows different time-courses in channel catfish (*Ictalurus punctatus*). *Aquaculture* **291** 179-187.

Astles K. L., Gibbs P. J., Steffe A. S., Green M. 2009. A qualitative risk-based assessment of impacts on marine habitats and harvested species for a data deficient wild capture fishery. *Biological Conservation* **142** 2759-2773.

Caddy J. F., Garibaldi L. 2000. Apparent changes in the trophic composition of world marine harvests: the perspective from the FAO capture database. *Ocean & Coastal Management* **43** 615-655.

Cho C. Y., Bureau D. P. 2001. A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. *Aquaculture Research* **32** 349-360

Davies S. J., Gouveia A. 2008. Enhancing the nutritional value of pea seed meals (*Pisum sativum*) by thermal treatment or specific isogenic selection with comparison to soybean meal for African catfish, *Clarias gariepinus*. *Aquaculture* **283** 116-122.

Dias J., Conceicao L. E. C., Ribeiro A. R., Borges P., Valente L. M. P., Dinis M. T. 2009. Practical diet with low fish-derived protein is able to sustain growth performance in gilthead seabream (*Sparus aurata*) during the grow-out phase. *Aquacultur*e **293** 255-262.

FAO Fisheries and Aquaculture D. 2009. The State of World Fisheries and Aquaculture-2008. http://www.fao.org/docrep/011/i0250e/i0250e00.htm, 2010. 04. 25.

Johnsen J. P. 2005. The evolution of the „harvest machinery": Why capture capacity has continued to expand in Norwegian fisheries. *Marine Policy* **29** 481-493.

Müller F. 1990. Economical analysis of some superintensive technologies for fish production in Szarvas. *Aquacultura Hungarica* **VI** 235-246.

Panserat S., Hortopan G. A., Plagnes-Juan E., Kolditz C., Lansard M., Skiba-Cassy S., Esquerré D., Geurden I., Médale F., Kaushik S., Corazze G. 2009. Differential gene expression after total replacement of fish meal and fish oil by plant products in rainbow trout (*Oncorhynchus mykiss*) liver. *Aquaculture* **294** 123-131.

Pintér K. 2010. Magyarország halászata 2009-ben. *Halászat* **2010/2.** 43-49.

Sánchez-Lozano N. B., Martínez-Llorens S., Tomás-Vidal A., Cerdá M. J. 2009. Effect of high-level fishmeal replacement by pea and rice concentrate protein on growth, nutrient utilization and fillet quality in gilthead seabream (*Sparus aurata* L.). *Aquaculture* **298** 83-89.

Sealey W.M., Barrows F. T., Smith Ch. E., Overturf K., LaPatra S. E. 2009. Soybean meal level and probiotics in first feeding fry diets alter the ability of rainbow trout *Oncorhynchus mykiss* to utilize high levels of soybean meal during grow-out. *Aquaculture* **293** 195-203.

Silva J. M. G., Espe M., Conceicao L. E. C., Dias J., Valente L. M. P. 2009. Senegalese sole juveniles (*Solea senegalensis* Kaup, 1858) grow equally well on diets devoid of fish meal provided the dietary amino acids are balanced. *Aquaculture* **296** 309-317.

Sink T. D., Lochmann R. T., Kinsey N. R. 2010. Growth and survival of channel catfish, *Ictalurus punctatus*, fry fed diets with 36 or 45% total protein and all plant or animal protein sources. *Journal of the World Aquaculture Society* **41** 124-129.

Stickney R. R. 2010. Cultured Aquatic Species Information Programme, *Ictalurus punctatus* (Rafinesque, 1818). In: FAO Fisheries and Aquaculture Department, http://www.fao.org/fishery/culturedspecies/Ictalurus_punctatus/en 2010. 05. 29.

Tacon A. G. J., Metian M. 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture* **285** 146-158.

Toko I. I., Fiogbe E. D., Kestemont P. 2008. Mineral status of African catfish (*Clarias gariepinus*) fed diets containing graded levels of soybean or cottonseed meals. *Aquaculture* **275** 298-305.

Webster C. D., Tiu L. G., Tidwell J. H., Grizzle J. M. 1997. Growth and body composition of channel catfish (*Ictalurus punctatus*) fed diets containing various percentages of canola meal. *Aquaculture* **150** 103-112.

VALIDATION OF AN AUTOMATED COMPENSATION EVAPOTRANSPIROMETER WITH CADMIUM POLLUTED MAIZE

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Abstract

There are only a few facilities to determine the evapotranspiration of a crop canopy. One of these possibilities is the use of evapotranspirometers. In spite of known shortcomings of the equipment they are widely applied in the everyday practice of crop water loss measurements. In our investigation the traditional compensation evapotranspirometer of Thornthwaite-Matter type was renovated at Keszthely Agrometeorological Research Station in 2011. The volume of the tanks (growing chambers) was 4 m^3 each with surface area of 4 m^2, and the depth of them 1 m. The tanks were layered with soil column characteristic of the surroundings of Keszthely area. The test plant was a short growing season maize hybrid Perlona.

Earlier traditional evapotranspirometers were able to measure the daily sum of lost water. Later on the mechanic construction of the equipment might be automated. The renewed instrument is able to collect the water use of the tank in second's interval. We represent our preliminary results showing diurnal variation of maize

evapotranspiration in two different treatments. One of them contains the impact of cadmium on maize water loss. Detailed discussion on the influence of cadmium pollution on water loss of maize excluded from the study. Performing of polluted crops aimed only an independent treatment in our methodological experiment. Main goal of this investigation was an outline of functioning of converted evapotranspirometers.

Key-words: compensation evapotranspirometer, water loss, cadmium, maize, validation

Összefoglalás

A keszthelyi Agrometeorológiai Kutatóállomáson az 1970-es években telepítettek Thornthwaite-Matter típusú kompenzációs evapotranspirometereket. Ezek mechanikus konstrukcióban a napi párolgásösszeg meghatározására voltak alkalmasak. 2011-ben a műszereket automatizáltuk, mely eredményeként a növények vízfogyasztásának napi változását másodpercenként tudjuk követni. A bemutatásban óraátlagok alapján szemléltetjük a kukorica vízfogyasztásának napi változásait különböző időjárási feltételeknél. Az automatizálás eredményét a kadmium szennyezés evapotranszspirációra gyakorolt hatásának másodpercenként gyűjtött adatokból származtatott óraátlagainak felhasználásával is szemléltetjük. Nem volt célunk a kadmium kukoricára gyakorolt hatásának részletes elemzése, a szennyezett kezelés csak a felújított evapotranspirometer működésének reprezentálására szolgált. A kadmiummal szennyezett növények jelentették a második kezelést.

Introduction

Reference evapotranspiration (ET_o) is one of the most frequently cited processes of evaluating crop water use and irrigation necessity. The definition of ET_o was drawn by *Jensen et al.* (1990) as follows: "the rate at which water, if available, would be removed from the soil and plant surface of a specific crop, arbitrarily called a reference crop." Although any crop could be a reference crop, the 0.2 m tall clipped grass or 0.5m tall alfalfa is widely current.

Grismer (2002) rated 50 methods to evaluate reference evapotranspiration. He concluded that the assumptions, data demands, mainly meteorological ones, and results scatter on high extent in different methods. The FAO standard counting the ET_o is the well known Penman Monteith (*Allen et al. 1998*) equation:

$$ET_0 = \frac{0.408\,\Delta(R_n - G) + \gamma \dfrac{900}{T+273} u_2 (e_s - e_a)}{\Delta + \gamma\ \Delta(1 + 0.34u_2)} \qquad (1)$$

where ET_o is the hypothetical reference crop evapotranspiration rate in mm d^{-1}, T is mean air temperature in °C, and u_2 is wind speed in m s^{-1} at 2 m above the ground. RH or dew point and air temperature, needed to get saturation deficit (e_s-e_a), are also measured at 2 m above the soil surface. The Rn is the net solar radiation, the G is the soil heat flux (W m^{-2}). The psichrometric constans is γ (0.5 g m^{-3} K^{-1}), the Δ denotes the slope of saturated vapour pressure-temperature relation (Pa $°C^{-1}$).

This worldwide applied assumption lies on well discussed physical and biological (physiological) basis.

There is other way in evapotranspiration evaluation, measuring the daily loss of a water body. The proper equipment to achieve this

goal is the class "A" pan. Although the accuracy of class "A" pans in reference evapotranspiration measurement is controversial, its simplicity, practicality, wide availability and low cost make them widely applicable in irrigation management (*Medeiros et al.* 2001). On the other hand the FAO do not recommend any more the use of "A" pans in evaluation of reference evapotranspiration. At a push in estimation of ET_o FAO recommends a minimum 10-day or longer time period for irrigation purposes.

Cadmium is a toxic heavy metal that accumulates in all branches of the food chain. In plants it can be easily taken up. We chose the maize as test plant, because the sensitivity of maize to cadmium was extremely high (*Wojcik and Tukendorf* 1999). Earlier investigations showed depression of crop evapotranspiration due to Cd pollution (*Kirkham 2006*). Impact of Cd on other maize properties was summed among others by *Bi et al. (2009*).

The purpose of the study was the review of functioning of a renewed mechanic evapotranspirometer. We used two different water treatments in validation of the equipment. Of the two water treatments one was polluted with cadmium. Detailed investigation on the impact of cadmium on maize evapotranspiration excluded from our observation. The polluted crops represented an independent water treatment only. Reconstruction aimed the accommodation to the most up-to-date data acquisition system of the research area (*Berke* 2007).

Materials and Methods

Study on the measurement of maize evapotranspiration was carried out at Keszthely Agrometeorological Research Station in the growing season of 2011. The soil was Ramann's brown forest soil with a mean bulk density of 1.46 Mg m^{-3} in the top 1 m of the profile and an

available water capacity of 150 mm m^{-1}. Nutrients (180, 80 and 120 kg ha^{-1} N, P and K, respectively) were applied in spring, immediately prior to sowing. The usual agronomic measures (plant protection, weed control) recommended for the location by the staff of the University of Agricultural Sciences, Keszthely, were applied. The test plant was the short- season maize hybrid Sperlona.

Two treatments were applied at "ad libitum" watering in Thornthwaite type compensation evapotranspirometers. Half of the crops were polluted with cadmium. Characteristics of the instrument see also below. The actual evapotranspiration was calculated as a residual term of the water balance after *Antal* (1968).

The Cd concentration used for pollution was 10^{-5} M [$Cd(NO_3)_2 \times 4H_2O$]. A motorised sprayer (SP 415) was used to apply the pollutant in the field at weekly intervals.

Differences in seasonal mean evapotranspirations were compared using the LSD test at the P=0.05 significance level (STATA 5.0 program package). In diurnal variation of evapotranspiration and water uptake the influence of cadmium was analysed using paired t- test.

Results and Discussions

Conversion of traditional mechanic evapotranspirometers at Keszthely

The aim of installation of Agrometeorological Research Station at Keszthely was the foundation of an evaporation measurement system in Hungary including crop evapotranspiration. The beginning of observations started in the early 1970'-es. To gauge daily water loss of crops twenty four Thornthwaite-Matter type compensation evapotranspirometers were settled into two measuring units (two cellars). The instruments were self-made ones as in general worldwide, because ready installations were not available.

The evapotranspirometers contain two separate units (*Fig. 1*); the growing chamber or tank in the field (1) and the measuring cellar (2) below the soil surface at the edge of the field. A tube connects the two units of the evapotranspirometer.

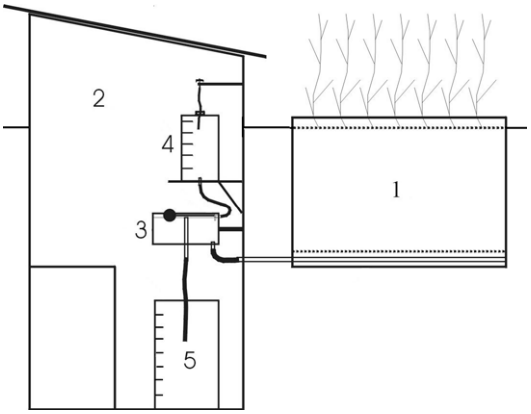

Figure 1. Schematic representation of the Thornthwaite-Matter type compensation evapotranspirometer

The tanks were settled in the middle of a field, where the irrigation facilities were also at our disposal. The irrigation and size of field guaranteed the existence of an irrigated canopy just surrounding of our growing chambers. The field layout, evapotranspirometers and crop fetch covered a total area of about 0.7 ha. In the tanks a constant water table level was kept at a depth of 0.90 m. The growing chambers were metal containers with a volume of 4 m^3 (2×2 m in area, 1 m in depth), filled with a monolith from the surrounding areas, layered as in the natural state. Each evapotranspirometer unit consisted of three measuring pots mounted into the cellar (see Fig. 1). The water level controlling pot (3) was placed in the middle. Above and below this central unit the compensation (4) and leakage (5) pots were placed, respectively. A mechanic float as water level controller was fixed in the controlling pot.

The basic principle of evapotranspirometers functioning is the communicating vessels. If additional liquid is added to one vessel (controlling pot), the liquid will again find a new equal level in all the connected vessels (tank in the field) (en.wikipedia.org/wiki/Communicating_vessels). In our everyday work the components of the water balance were registered each day of the season, expressing evapotranspiration as the residual term. The inputs were the daily rainfall amount (P), the additional watering added by compensation pot (C) and in case of drought the irrigation water (I). When the precipitation exceeded the water holding capacity of the soil column of tanks, the leakage water (L) was an output in addition to evapotranspiration (ET_o):

$$P+C+I = ET_o+L \qquad (2)$$

The traditional evapotranspirometers were renewed in 2011. The control system was changed from mechanical to electronic. Two pots, the compensation and leakage ones were omitted. The compensation pot was replaced by an electronic switch connected to the water supplier tap (*Fig. 2*).

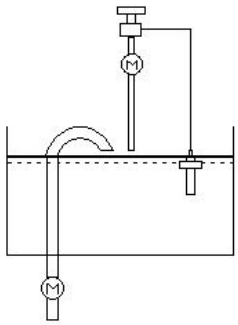

Figure 2. The reconstructed compensation system of the evapotranspirometer with float ring on the right and siphon (inverted U shape tube). M denotes flow meters

The electronic switch was controlled by a reed switch and a magnet built into a float ring. Finally the water level was adjusted by the float ring. At declining water level (in case of crop water use) the float ring's magnetic field opens the electric tap. When the water level reaches the adjusted level the float ring closes the water supply. When the amount of precipitation is higher than the water holding capacity of the soil column, the surplus water flows into the controlling vessel and the additional water is siphoned off. The picture of the renewed system is presented on the Photo 1.

Photo 1. One unit of water supplier with the data logger of the evapotranspirometer (Photo: G. Soós)

A meteorological data logger of HYGACQ V1.3 type was connected to each cellar. The frequency of data sampling is 1 second. The logger calculates hourly sums and memorizes them. The collected hour-

ly data can be loaded by PC using the WHYGACQ computer program. The program has a graphical surface providing a quick view about temporal variation of water supply.

Weather of the studied season

Seasonal air temperature was 1.2°C higher in 2011 than the climatic norm (*Fig. 3*). Extremely low precipitation sum amounting to about half the average figure (54%) was observed. The season of 2011 was the driest from the beginning of meteorological observations (1871) in Keszthely. In the course of monthly meteorological data there was only one exceptional months, the month July, when both climate means (air temperature and precipitation sum) were in good correspondence with the long-term average. All in one, the other periods were warm and dry in 2011.

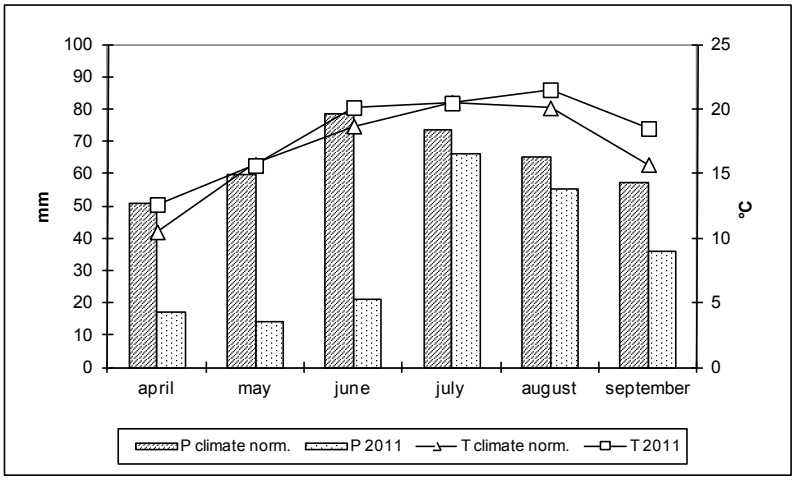

Figure 3. The weather of the season of 2011 at Keszthely. The climate norms were calculated for 1971-2000.

Actual evapotranspiration of maize

At non limited watering the size of actual evapotranspiration follows the changes in transpiration surface (leaf-area -index) and radia-

tion intensity. In the early stage and in the end of the season less water loss is observed than in the middle of the vegetation period. Arid season of 2011 resulted in extremely high total water losses in maize. Yearly evapotranspiration sums in control and polluted maize totaled in 463.9 mm and 428.9 mm, respectively (*Fig. 4*). The seasonal evapotranspiration sums differed significantly at the 5% probability level. The greatest value of daily water loss was measured during flowering in 9th July 2011 (7.1 and 6.7 mm in control and polluted crops). Cadmium pollution did not modify the date at which peak daily evapotranspiration loss was observed.

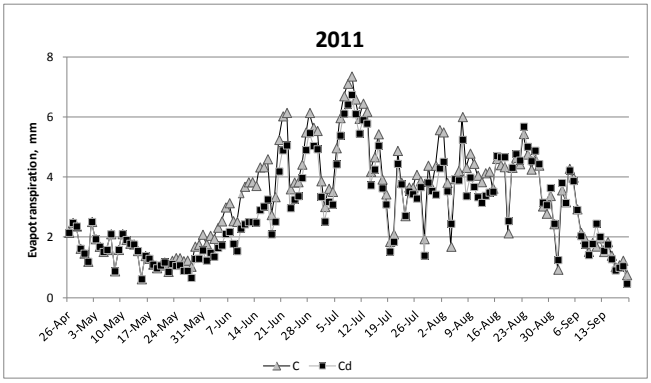

Figure 4. Seasonal variation of actual evapotranspiration in maize at Keszthely

The sum of yearly evapotranspiration of Cd polluted maize significantly lowered with 13% during 2011. Decline was the most pronounced in the second half of June and on some extremely hot days. The monthly mean temperature of June exceeded the climate norm of Keszthely with 1.5°C. In the beginning and in the end of the vegetation period the impact of Cd was more moderate on maize water loss.

Impact of atmospheric cadmium pollution on the daily dynamic of system's water uptake

The renewed evapotranspirometer permitted the hourly imitation of growing chamber's water compensation, by presenting the member C in Eq. 1. This is the amount of additional water offered to soil and crops grown in the growing chamber. We demonstrated hourly distribution of compensation water for different radiation regimes; for overcast and completely clear days separately. On cloudy days, both radiation and water replenishment were low and consolidated (*Fig. 5*). Radiation intensity was expressed by total incoming radiation (global one, W/m^2). The impact of Cd was also moderate. Decreased water use of cadmium polluted maize was in accordance to earlier investigations (*Fodor* 2003).

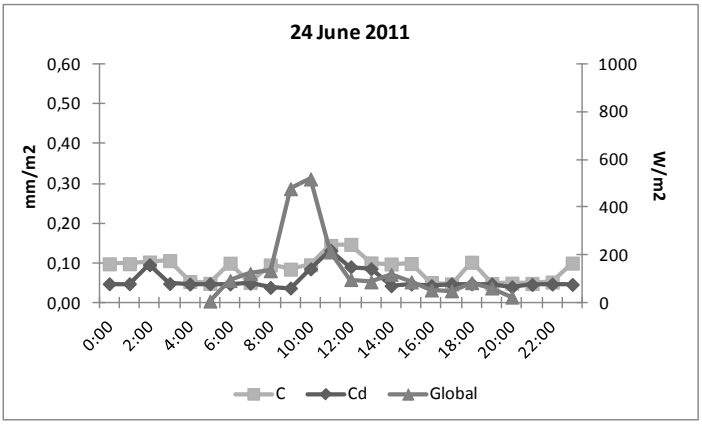

Figure 5. Daily variation in water uptake during overcast day

At the beginning and in the end of the season (low solar angles and small leaf size) the daily water compensation (*Fig. 6)* is only a few mm (1-2 mm/m^2). The time of highest irradiation using DST (daylight saving time) is at 13:00 in Hungary (about 10 minutes earlier at Keszthely). In our case it means Central European Time +1:00, or UTC+2:00. The

time of largest system's water demand did not coincide with the highest solar angle; the peak water uptake was late about two to three hours. It means that the water demand follows better the air temperature curves then radiation changes.

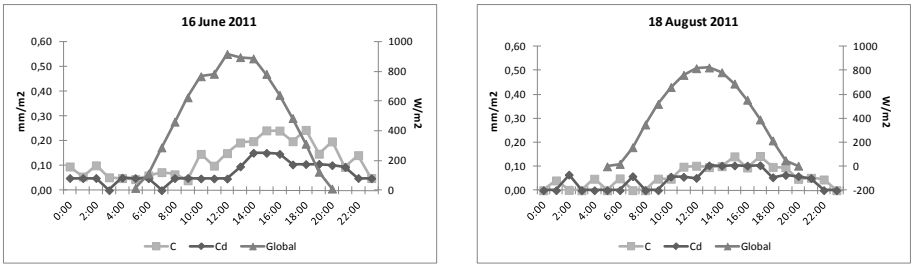

Figure 6. Hourly water consumptions during the time of canopy closure (16 June) and wax ripe (18 August)

The top water demand was observed at the time of tasseling (*Fig. 7*). Daily compensation water amounts totaled 5.99 and 4.45 mm/m^2 in control and polluted treatment, respectively. These observations are in accordance to earlier studies for maize (*Steduto and Hsiao 1998*), where peak rates of water losses were reached during the most active stage of growth, at tasseling independently on water supply. The impact of cadmium on clear days was significant ($P > 0.0000$) and high. In our clear sky sample day decline in water use of polluted treatment reached the 30%. In spite of undisturbed radiation (cloudiness) and "ad libitum" watering, the water consumption was more variable than the radiation curve calling the attention to other influencing environmental factors (air moisture, wind etc.). This small "noises" appeared parallel in the two treatments suggesting impact of non living environment more than influence of biological origin.

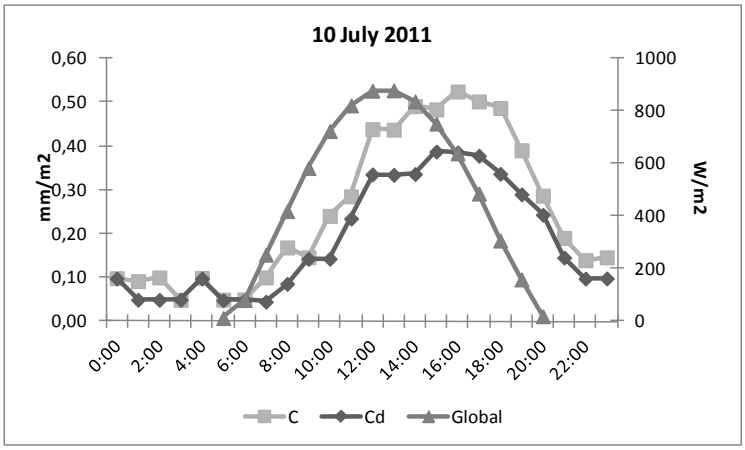

Figure 7. Peak water uptake of maize in tasseling on completely clear sky conditions

Hourly actual evapotranspiration of maize was also calculated (*Fig.8*). Our sample day was in tasseling, at the same time as discussing amounts of compensation water. The impact of cadmium on maize evapotranspiration was significant (P > 0.0000).

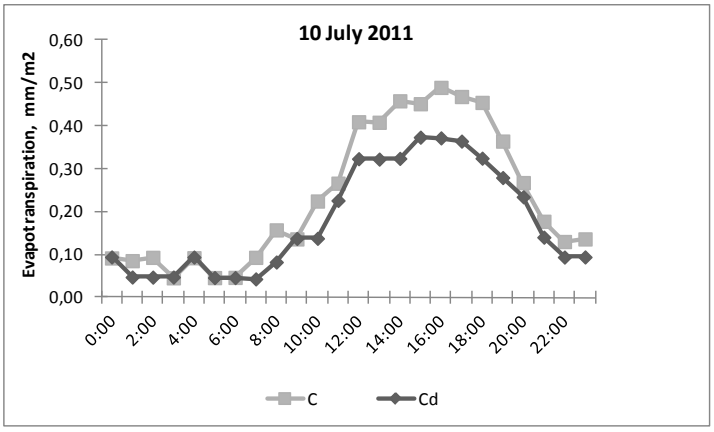

Figure 8. Diurnal variation of maize evapotranspiration during tasseling

Water capacity of the tank's soil, mainly in the upper layers, might be low due to drought; the potential evapotranspiration was extremely high (7.7 mm). This was the reason why the amounts of compensation water and the counted evapotranspiration were close to each other. Almost the whole amount of additional water was turned into evapotranspiration, their rations were 93% (5.6 mm) and 96% (4.3 mm) in control and polluted crops, respectively. Only the remaining part of water enhanced the soil water capacity in daytime hours. Peak maize water losses were measured at 16:00 DST, at the time of warmest air temperatures.

Conclusion

Reconstruction of mechanic evapotranspirometer fulfilled our expectations. We could follow the dynamic of crop water uptake and reference evapotranspiration. In our sample the cadmium pollution declined with 13% the yearly sum of maize evapotranspiration. Our results were in accordance to earlier investigation of *Greger and Johansson* (1992) for beans. The impact of cadmium was the most pronounced in clear sky conditions.

With one exception, our observation covered an overview of diurnal variation of addition water, the compensation water. Discussion of hourly variation in maize evapotranspiration needs deeper consideration later.

Acknowledgement

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References

Allen, R.G., Pereira, L.S., Raes, D., and Smith, M. 1998. Crop evapotranspiration, guidelines for computing crop water requirements. FAO Irrig. and Drain. Paper 56, Food and Agric. Orgn. of the United Nations, Rome, Italy. 300 pp.

Antal, E. 1968. Irrigation planning by using meteorological data. PhD Thesis, Budapest (In Hungarian)

Berke, J. 2007. Measuring of Spectral Fractal Dimension. J. of New Mathematics and Natural Computation, Print ISSN: 1793-0057, Online ISSN: 1793-7027, 3/3: 409-418, DOI: 10.1142/S1793005707000872.

Bi, X., Feng, X. Yang, Y., Li, X. Shin, G. P. Y., Li, F., Qiu, G., Li, G., Liu, T. and Fu, Z. 2009. Allocation and source attribution of lead and cadmium in maize (*Zea mays* L.) impacted by smelting emissions. *Environ. Pollution.* **157**. 834-839.

Fodor, F. 2003. Lead and cadmium stress in crops. *Bot. Közlemények.* 90. **1-2.**107-120. (In Hungarian)

Greger, M., Johansson M. 1992. Cadmium effects on leaf transpiration of sugar beet (Beta vulgaris) *Physiologia Plantarum.* 86. **3**. 465-473.

Grismer, M.E., Orang, M., Snyder, R., Matyac, R., 2002. Pan evaporation to reference evapotranspiration conversion methods. *J. Irrig. Drain. Eng*. **128**. 180–184.

Jensen, M.E., R.D. Burman, and R.G. Allen (eds.) 1990. Evaporation and irrigation water requirements. ASCE Manuals and Reports on Eng. Practices No. 70., Am.Soc. of Civil Eng., NY, 360 pp.

Kirkham, M.B. 2006. Cadmium in plants on polluted soils: Effects of soil factors, hyperaccumulation, and amendments. *Geoderma.* **137**. 19–32.

Medeiros, G. A., Arruda, F. B., Sakai, E., Fujivara, M. 2001. The influence of crop canopy of evapotranspiration and crop coefficient of beans (Phaseolus vulgaris L.). *Agric. Water Manag*. **49**. 211-224.

STATA 5.0 (1996) Stata Corporation LP Texas, USA, www.stata.com

Steduto, P. and T. C. Hsiao 1998. Maize canopies under two soil water regimes: II. Seasonal trends of evapotranspiration, carbon dioxide assimilation and canopy conductance, and as related to leaf area index. *Agric. and Forest Meteor.* 89. **3-4**. 185-200.

Wojcik, M. and A. Tukendorf 1999. Cd tolerance of maize, rye and wheat seedlings. *Acta Phys. Plant.* **21**. 99-107.

en.wikipedia.org/wiki/Communicating_vessels

EXAMINATION OF EYE IRRITANCY BY USING TWO ALTERNATIVE METHODS

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Abstract

Agrochemicals must undergo numerous toxicological tests before registration. To get knowledge about eye irritation, nowadays only the *in vivo* Draize-test is accepted, which is one of the most criticized methods because of the injuries of the test animals. Therefore, several *in vitro* tests have been developed to replace *in vivo* eye irritation testing. Two of these alternative methods, the MTT-Assay and the HET-CAM test (Hen's Egg Test – Chorioallantois Membrane) were used in our comparative screening, with a set of agrochemicals to establish parallel data on *in vitro* and *in vivo* (Draize) results. The examined products were:

Systhane 12 E (miclobuthanyl, 125 g/l), Clinic 480 SL (glyphosate isopropylamin salt 360 g/l), Targa Super 5 EC (quizalofop-P-ethyl

5%), Trend™ (isodecyl acetate), Silwet L-77 (polyalkylenoxid 84%, iso-propylene 16%) and Substral (66 g/l nitrogen, 30 g/l P_2O_5 water-soluble phosphate, 67 g/l water-soluble K_2O).

In these experiments, the same results were obtained in the alternative tests and in the Draize test which shows that the methods applied may be possible the replace the *in vivo* test as a test system in the future.

Keywords: chorioallantoic membrane, *in vitro*, rabbit, Draize-test, HET-CAM test, MTT-Assay

Összefoglalás

A mezőgazdasági vegyi anyagok forgalomba hozatalát megelőző, előírt toxikológiai vizsgálatokban a szemirritáció meghatározására jelenleg az élő nyulakon elvégzett vizsgálatok eredményeit fogadják el. Mivel a módszer a kísérleti állatokra nézve erős fájdalommal járhat, az erősödő állatvédő mozgalmak hatására több olyan *in vitro* módszert is kidolgoztak, amely nem csak csökkentheti az ecélra felhasznált emlős állatok számát, hanem esetleg teljes mértékben ki is válthatja ezek alkalmazását. A lehetséges alternatív módszerek közé tartoznak a tyúktojás chorioallantois membránját (CAM) használó tesztek és az *in vitro* citotoxicitási tesztek.

A kísérletekben 6 mezőgazdasági vegyi anyagot használtunk fel, a kísérleti anyagok károsító hatásait az alternatív tesztrendszerrel (HET-CAM, MTT-Assay) és *in vivo* (Draize-teszt) módszerrel vizsgáltuk. Az alternatív tesztekből származó eredményeket összevetettük az élő állaton végzett teszt eredményeivel. Az irritációs kategóriákba történt besorolás után az *in vitro* és az *in vivo* módszerek eredményei jelen esetben teljes egyezést mutattak, ami alapján feltételezhető, hogy a későbbiekben az alternatív tesztek tesztrendszerként alkalmasak lehetnek

a szemirritáció mértékének becslésére, így a kísérletekben felhasznált állatok számának csökkentésére.

Introduction

Recently, all chemicals have to be tested before putting on the market. In this process toxicological examinations play an important role, because they can show several features of the ingredients that preclude the possibility of their distribution in the European Union. During drug and pesticide research and development different toxicological test are used for evaluation of their safety. These tests are necessary to determine the potential risk of chemicals for humans, pets and livestock animals, and to investigate their possible deleterious effects on the living organisms. The people can get into direct contact with the chemicals during the manufacture or using, and the animals can be contaminated with these agents accidentally during the authorised application or due to the irregular storage. The contact of these poisonous materials with the eyes can even lead to irreversible changes or blindness (Bordás, 1971). To detect the ocular irritation, only the Draize eye irritation test (OECD 405, 2002) is accepted now that is one of the most criticized methods because of the pain induced to the test animals. Several *in vitro* methods have been used to investigate the toxicity of potential eye irritants with a view to replacing *in vivo* eye irritation testing. To detect severe irritancy or corrosivity, the Isolated Chicken Eye Test is acceptable (OECD no. 438, 2009)

The HET-CAM (Hen's egg test – chorioallantoic membrane) test, using the chorioallantois membrane (CAM) of the chick embryo, is an alternative test to replace the Draize rabbit eye test (Walum et al., 1992). The CAM of the chick embryo has been used extensively for many years in various fields of biological research including virol-

ogy, bacteriology and toxicology, as it is a complete tissue including arteries, capillaries and veins, and is technically easy to study. In this test chemicals are placed in direct contact with the chorioallantoic membrane of the hen's egg. The occurrence of vascular injury or coagulation in response to a compound is the basis for employing this technique as an indication of the likelihood that a chemical would damage mucous membranes (especially the eye) *in vivo* (Leighton et al., 1985).

The MTT-Assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a simple method to determine the viability/number of cells in culture, through the formation of a coloured product to which the cell membrane is impermeable. Determination of the ability of cells to reduce MTT to the formazan product after exposure to test compounds enables the relative toxicity of test chemials to be assessed.

In our studies the HET-CAM test (Luepke and Kemper, 1986) and the MTT were used as *in vitro* methods. The results from the *in vitro* tests were compared with results from *in vivo* Draize rabbit eye test.

Materials and methods

Test materials

The test materials were: Systhane 12 E (miclobuthanyl 125 g/l), Clinic 480 SL (glyphosate isopropylamin salt 360 g/l), Targa Super 5 EC (quizalofop-P-ethyl 5%), Trend™ (isodecyl acetate), Silwet L-77 (polyalkylenoxid 84%, iso-propylene 16%) and Substral (66 g/l nitrogen, 30 g/l P_2O_5 water-soluble phosphate, 67 g/l water-soluble K_2O).

All of the chemicals were applied in their original form in the HET-CAM test and in the Draize rabbit eye irritation test. In the MTT assay, the test materials were tested in different dilutions.

Methods

HET-CAM Test

Shaver Rusticbrow chicken eggs were used in the study, incubated in a Ragus incubator. The temperature was 37 ^{o}C, the relative humidity was 60-70%. The eggs were rotated for 8 days to prevent the attachment of the embryo to one side of the egg. The eggs were prepared for assaying on day 10. The section of shell above the air chamber was removed with scissors. The membrane was moistened carefully with 0.9% NaCl solution.

Two eggs were used as control, treated with 0.9% NaCl, 2 eggs as standards, treated with 1% Sodium dodecyl sulphate and 0.1 M NaOH. The test materials were applied on 6 eggs, on 4 separated replicates.

The membrane was removed carefully with tapered forceps. 0.1 ml of test pesticide was added to the chorioallantoic membrane and the effect was observed over a period of up to 300 seconds. Hemorrhage, vascular lysis or coagulation can be seen on the chorioallantoic membrane. Each reaction occurred were recorded in seconds. A computer software was used to evaluate data (Invittox Protocol no. 47). The computer software uses the following algorithm:

$$RI = \frac{301\text{-}secH}{300} \times 5 + \frac{301\text{-}secL}{300} \times 7 + \frac{301\text{-}secC}{300} \times 9$$

Where H = haemorrhage, L = vascular lysis, C = coagulation, RI = irritation index, and sec = start second.

The irritation categories can be seen in Table 1.

Irritation index	Irritation category
0-0.9	no irritation
1-4.9	weak irritation
5-8.9	moderate irritation
9-21	severe irritation

Table 1. Classification of HET-CAM test

MTT-Assay

Maintained fibroblast-like cells from the kidney of the African green monkey (Vero-Hektor cells, ECACC No.: 03092503) were used for the MTT assay, according to Invittox protocol No.: 17. The cells were distributed into the wells of a microtiter plate 24 hours before the test. Test samples were diluted with cell culture medium, diluted 10, 20, 40, 80, 160, 320, 640 and 1280 times and added to the cells. After 24 hours dissolved MTT (Sigma M 5655) was added, which in turn was converted to water insoluble purple formazan by mitochondrial dehydrogenases. At the end of the incubation period of two hours the formazan was dissolved in methanol and its absorbance was measured at a wavelength of 570 nm. Reduction of the value (OD570- OD630) compared to the same value of non-treated cells indicates cell destruction due to the test chemical. For comparison among the test chemicals LCC_{50} (50% lethal concentration for cells) was assessed. For the assessment approximately linear correlation was supposed between concentration and effect in the vicinity of LCC_{50}. The irritation categories were detected based on effects of the dilution used (Table 2).

LCC_{50} (mL/L)	**Irritation category**
> 10.000	no irritation
5.000 – 1.250	weak irritation
1.25 – 0.078	moderate irritation
< 0.078	severe irritation

Table 2. Irritation categories from the MTT-Assay

Draize Rabbit Eye Test

The test was performed on 2 or 3 New-Zealand rabbits, depending on the symptoms observed. Using of separated control group is not necessary as the untreated right eye serves as control. Rabbits were kept in individual cages of a climatic animal room. The temperature was 22-25 ^{o}C and the relative humidity was 50-70%. The animals were fed with laboratory rabbit diet, and tap water was served *ad libitum* (OECD Guidelines for Testing of Chemicals, Number 405, 2002). The animal study was performed by the permission of the Animal Testing Work Committee.

A volume of 0.1 ml agrocemical was instilled into the conjunctival sac of each rabbit. Ocular irritation on the treated eye was evaluated at 1 hour, then 1, 2, 3 and 7 days post installation (Draize et al., 1944). Individual scores were recorded for each animal. The time interval with the highest mean score (Maximum Mean Total Score – MMTS) for all rabbits was used to classify the test substance by the system of Kay and Calandra (1962), (Table 3).

MAXIMUM MEAN SCORE		PERSISTENCE OF SCORE	DESCRIPTION RATING (AND CLASS)
0.0 to 0.5	Group mean total score at 24 hours = 0 Group mean total score at 24 hours > 0		Non-irritant (1) Practically non-irritant (2)
0.5 to 2.5	Group mean total score at 24 hours = 0 Group mean total score at 24 hours > 0		Practically non-irritant (2) Minimal irritant (3)
2.5 to 15	Group mean total score at 48 hours = 0 Group mean total score at 48 hours > 0		Minimal irritant (3) Mild irritant (4)
15 to 25	Group mean total score at 72 hours = 0 Group mean total score at 72 hours > 0		Mild irritant (4) Moderate irritant (5)
		More than half of the individual total scores at 7 days 10 or less	Moderate irritant (5)
25 to 50	Group mean total score at 7 days 20 or less	More than half of the individual total scores at 7 days > 10 but no individual total score at 7 days > 30	Moderate irritant (5)
		More than half of the individual total scores at 7 days > 10 and any individual score at 7 days > 30	Severe irritant (6)
	Group mean total score at 7 days > 20		Severe irritant (6)
		More than half of the individual total scores at 7 days 30 or less	Severe irritant (6)
50 to 80	Group mean total score at 7 days 40 or less	More than half of the individual total scores at 7 days > 30 but no individual total scores at 7 days > 60	Severe irritant (6)

Table 3.

Classification of eye irritation scores from Draize eye irritation test

		More than half of the individual total scores at 7 days > 30 and individual total score at 7 days > 60	Very severe irritant (7)
	Group mean total score at 7 days > 40		Very severe irritant (7)
		More than half of the individual total scores at 7 days 60 or less	Very severe irritant (7)
80 to 100	Group mean total score at 7 days 80 or less	More than half of the individual total scores at 7 days > 60 but no individual total score at 7 days > 100	Very severe irritant (7)
		More than half of the individual total scores at 7 days > 60 and individual total score at 7 days > 100	Extremely severe irritant (8)
	Group mean total score at 7 days > 80		Extremely severe irritant (8)
100 to 110	Group mean total score at 7 days 80 or less Group mean total score at 7 days > 80		Very severe irritant (7) Extremely severe irritant (8)

Table 3.

Classification of eye irritation scores from Draize eye irritation test (Continued)

Results

Results of the HET-CAM Test

The numerical data can be seen in Table 4. After the treatment with Systhane 12 E lysis was observed between 5 and 12 sec, and haemorrhage between 14 and 28 sec. By these results the irritation index is 11.50 and the fungicide is severely irritative.

On the chorioallantois membranes treated with Clinic 480 SL vascular lysis started between 2 and 7 sec, and there was slight haemor-

rhage from 7 to 19 sec. Based on irritation index of 11.74, it is a severe irritative pesticide.

Vascular lysis was noted between 4 and 6 sec after treatment with Targa Super 5 EC, and haemorrhage was found from 8 to 14 sec. On the basis of the 11.70 irritation index, Targa Super 5 EC is severely irritative.

During the observation period there was vascular lysis from 3 to 10 sec after treatment with Trend™, followed by haemorrhage started between 9 and 21 sec. The irritation index of Trend™ is 11.66, thus it is a severely irritative agent.

On the chorioallantois membranes treated with Silwet L-77 vascular lysis started between 2 and 7 sec, and there was mild haemorrhage from 7 to 16 sec. On the base of the 11.75 irritation index, this pesticide is severely irritative.

During the observation period mild haemorrhage was observed from 8 to 19 sec after the treatment with Substral. Based on the irritation index of 4.80, Substral is a weakly irritative pesticide.

Pesticide	**Irritation index**
Systhane 12 E	11.50
Clinic 480 SL	11.74
Targa Super 5 EC	11.70
Trend™	11.66
Silwet L-77	11.75
Substral	4.80
NaOH 0.1 M + SDS 1%	11.53

Table 4. Irritation indices from HET-CAM test

The obtained irritation indices can be evaluated using the classification categories of Table 1.

Results from the MTT-assay

The numerical data can be seen in Table 5.

The destruction of the cells treated with Systhane 12 E was over 50% in all the dilutions applied up to 1280 times dilution. The pesticide showed high citotoxicity.

Clinic 480 SL also showed high citotoxicity. More than 50% of the cells were destroyed by the treatment with the highest dilution.

More than 50% of the cells treated with 1280 times diluted Targa Super 5 EC died after the treatment, which shows the herbicide to be highly citotoxic.

The destruction of the cells treated with Trend™ was over 50% in all the dilutions applied up to 1280 times dilution. The pesticide showed high citotoxicity.

More than 50% of the cells treated with 1280 times diluted Silwet L-77 died after the treatment, which shows the pesticide to be higly citotoxic.

Substral, applied 10, 20 and 40 times diluted, destroyed more than 50% of the cells. The test material diluted 80 times caused no destruction of the cells which shows the chemical to be less citotoxic.

Test material/ Dilution	Systhane 12 E	Clinic 480 SL	Targa Super 5 EC	Trend™	Silwet L-77	Substral
10x	98%	98%	86%	98%	96%	98%
20x	98%	96%	97%	98%	97%	92%
40x	98%	97%	96%	98%	98%	60%
80x	98%	97%	96%	98%	98%	0%
160x	98%	96%	96%	98%	98%	0%
320x	98%	95%	97%	98%	98%	0%
640x	96%	97%	96%	97%	96%	0%
1280x	87%	97%	97%	97%	97%	0%

Table 5. Destruction of cells in different dilutions

The results show that the test materials with higher irritation potential were toxic to the cells in higher dilution as well.

Results from the Draize rabbit eye irritation test

The obtained numerical data are summarised in Table 6. After instillation of Systhane 12 E, positive conjunctival responses with severe redness and, chemosis, and strong discharge were noted until the end of the observation period. The iris lesion was seen within 1 hour. On the cornea moderate opacity was noted from the first day which was not reversible during the 7 days observation period. On the basis of the irritation index (78.33), Systhane 12 E is very severely irritative to the rabbit eye.

Severe redness and chemosis, and strong discharge were recorded during 7 days after treatment with Clinic 480 SL. Iris lesion was seen from day 1 or 2 and it was not reversible during the observation period. On the cornea slight opacity was noted from 1 hour in all animals until the end of the observation period. Clinic 480 SL is very severely irritative to the rabbit eye on the basis of the irritation index (56.67).

After instillation of Targa Super 5 EC, positive conjuctival responses with moderate redness and chemosis, and strong discharge were found after the first hour. Iris lesion was seen at 24 hours after the treatment and the inflammation was present until the end of the observation period. Slight opacity was seen on the cornea from 1 hour that was not reversible but stronger until day 7. On the base of the irritation index (69.00), Targa Super 5 EC was very severe irritative to the rabbit eye. Based on the symptoms observed on the first and second animal, the third rabbit was not treated due to animal welfare reasons.

Moderate redness and chemosis, and strong discharge were noted at the first observation post instillation of Trend™ and the symptoms became stronger on day 2 and 3. The iris was changed after 1 day and

did not recover until the end of the observation period. Moderate opacity was noted on the cornea from the first hour until the end of the observation period. Based on the irritation index (64.00), Trend™ has very severe irritative potential to the rabbit eye. Based on the symptoms observed on the first and second animal, the third rabbit was not treated due to animal welfare reasons.

Positive conjuctival responses with moderate redness and chemosis, and strong discharge were observed during the observation post instillation of Silwet L-77. Iris lesion was seen after 1 hour. Slight opacity was noted on the cornea from the first hour and it became stronger during the observation period. On the basis of its irritation index (70.70), Silwet-L-77 was very severely irritative to the rabbit eye.

Moderate redness and slight chemosis were noted in the first hour post instillation of Substral. After 24 hours the treated eyes returned to normal. The iris remained normal. On the cornea no opacity was noted during the observation period. On the basis of these results, Substral was minimally irritative to the rabbit eye.

Pesticide	**Irritation index**	**Category**
Systhane 12 E	78.33	Very severe
Clinic 480 EC	56.67	Very severe
Targa Super 5 EC	69.00	Very severe
Trend™	64.00	Very severe
Silwet L-77	70.70	Very severe
Substral	3.30	Minimal

Table 6. Irritation indices from Draize rabbit eye test with corresponding irritation categories

Discussion

The HET-CAM test and the MTT-Assay, as *in vitro* methods have several advantages including their rapidity, simplicity, sensitivity, ease of performance and their relative cheapness. One of the disadvantages of the HET-CAM test is the subjective nature of the evaluation of the results. In addition, the evaluation can not be done in case of opaque and colour chemicals, because these materials cover up the membrane and make the emulsion colourful. To test powder or granulate formulations by these methods is more complicated.

In case of chemicals tested in present study, all the results from the *in vitro* MTT-Assay and HET-CAM test were equal with the *in vivo* data from Draize rabbit eye test. Based on these data, both of these methods may be appropriate to predict the prospective irritation caused by liquid agrochemicals. The results in comparison can be seen in Table 7.

Pesticide	**Category from HET-CAM test**	**LCC_{50} (mL/L) from cytotoxicity test**	**Category from Draize rabbit eye test**
Systhane 12 E	Severe	<0.078	Very severe irritative
Clinic 480 EC	Severe	< 0.078	Very severe irritative
Targa Super 5 EC	Severe	< 0.078	Very severe irritative
Trend™	Severe	< 0.078	Very severe irritative
Silwet L-77	Severe	< 0.078	Very severe irritative
Substral	Weak	1.25–2.50	Minimal irritative

Table 7. Irritation categories by HET-CAM test and MTT-Assay in comparison to irritation categories by Draize rabbit eye test

In several previous experiments a good correlation was found between the results from *in vitro* HET-CAM test and the results from *in vivo* Draize eye irritation test (Luepke and Wallat, 1987; Budai and Várnagy, 2000; Budai et al., 1995, 1997, 1998, 2002). In 1992 the German authorities accepted the use of HET-CAM data for the classification of R41 chemicals in the notification of new industrial chemicals (Balls et al., 1999).

In this study a good (100%) correlation was seen between the data from *in vitro* and *in vivo* methods. The HET-CAM test or the MTT-Assay could not replace the currently used Draize eye irritation toxicological test, but it could diminish investigations in mammals as well as limit or eliminate pain and damage in animal experiments. In this form the HET-CAM test and the MTT-Assay can be useful for prescreen methods as part of an *in vitro* system.

References

Balls. M., Berg, N., Bruner, L.H., Curren R., deSilva, O., Earl, L.K., Esdaile, D.J., Fentem, J.H., Liemsch, M., Ohno, Y., Prinsen, M.K., Spielmann, H. and Worth, A.P. 1999. Eye irritation testing: the way forward. The report and recommendations of ECVAM workshop 34. *ATLA* **27**. 53-77.

Bordás S. 1971: A növényvédő szer mérgezés elsősegélynyújtása. Mezőgazdasági és Élelmezésügyi Minisztérium. Budapest, 1971. **21**.

Budai P., Fáncsi T., Várnagy L. and Fejes S. 2002. Effects of irritative pesticides and a technical component on tissue structure of CAM. *Georgikon for Agric.*, **13**. 87-96.

Budai P., Kiss Zs.I., Somlyay I.M. and Várnagy L. 1998. ln vitro irritancy test on hen's eggs with some pesticide components. *Med. Fac. Landbouww. Univ. Gent*, **63/2a**. 315 - 319.

Budai P., Somlyay I., Várnagy L. and Varga T. 1995. Comparison

of in vitro (HE'I'-CAM) and in vivo (Draize) irritation tests using different pesticides. *Med. Fac. Landbouww. Univ. Gent*, **60/2b**. 593 - 597.

Budai P. and Várnagy L. 2000. In vitro ocular irritation toxicity study of some pesticides. *Acta Vet. Hung.*, **48**. 221-228.

Budai P., Várnagy L., Somlyay I. and Varga T. 1997. Ocular irritation study of some pesticides in HE'I'-CAM test. *Med.. Fac. Landbouww. Univ. Gent*, **62/2a**. 265 - 268.

Draize, J.H., Woodard, G. and Calvery, H.O. 1944. Methods for the study of irritation and toxicity of substance applied to the skin and mucous membranes. *J. Pharmac., exp. Ther*. **82**. 377 p.

Invittox Protocol Number **17**. MTT ASSAY 1990.

Invittox Protocol Number **47**. HET-CAM test 1990.

Kay, J.H. and Calandra, J.C. 1962. Interpretation of eye irritation tests. *J. Soc. Cos. Chem*.; **13**. 281-289.

Leighton, J., Nassauer, J. and Tchao, R. 1985. The chick embryo in toxicology: an alternative to the rabbit eye. *Fd. Chem. Toxic.* **23**. No. 2, 293-298.

Luepke NP.and Kemper FH. 1986. The HET-CAM test: an alternative to the Draize eye test. *Fd. Chem. Toxic.* **24**, No. 6/7, 495-496 p.

Luepke, N.P. and Wallat, S. 1987. HET-CAM reproducibility studies. *Alternative Methods in Toxicology* **5**. 353-363.

OECD 2002. OECD Guidelines for the Testing of Chemicals No.**405**: Acute Eye Irritation/Corrosion.14pp. Paris, France: Organisation for Economic Cooperation and Development. OECD Guideline for Testing of Chemicals no. **438**. Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants. Adopted 08th September 2009.

Walum, E., Balls, M. et al. 1992. ECITTS: An integrated approach to the application of *in vitro* test systems to the hazard assessment of chemicals. *ATLA*. **20**. 416.

SOIL POROSITY INVESTIGATIONS IN TRUFFLE ORCHARDS

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Abstract

Those who are engaged in the production and research of the truffle as a special ecological trait agree that beside of the climatic attributes , the soil circumstances play important role in the sucessful production. Thank to the extensive investigations large amount of the information is available about this fungus, but there are still questions remaining. One of these questions is the importance of the air in the soil in truffle production.

The amount of soil air is one of the most important thing of the soil life, which is in centre of interest. The aim of the investigation was to find out what kind of effect has the air permeability of soil pores to the production of truffle.

The investigation of aggregate composition has shown the structure of the genetical soil layers, which is in close connection with the air permeability of the soils.

We have investigated basic soil parameters to learn the physical and chemical properties of soil which determine the life conditions of truffle.

Water retention capacity and –indirectly– the differential porosity of the investigated soils were characterized by pF-measurements.

The in situ measurement of air permeability of the soils is a rather new technology in our country, and one of our aims was to get acquainted with the method itself. The great advantage of the in situ measurements is that the soils can be investigated in their original structure without disturbance and in this way we can get more exact data about the examined parameter. At the same time the disadvantage is that the measurement cannot be repeated (at the same place). A well-planned measurement protocol can minimize the possibility of the potential mistakes.

Our investigations revealed that advanced truffle growing technology should focus not only on nutrient and water supply but on soil air permeability in order to satisfy special needs of these unique fungi.

Key-words: soil porosity; truffle; truffle orchard; air in the soil in truffle production

Összefoglalás

A szarvasgomba termesztésével és kutatásával foglalkozó szakemberek egyetértenek abban, hogy a klimatikus jellemzők mellett a talajtani viszonyok is meghatározó szerepet játszanak a termés eredményessége szempontjából. A kiterjedt kutatásoknak köszönhetően mára a legtöbb ismeret a rendelkezésünkre áll, de sok kérdésre még nincs kielégítő és egybehangzó válasz. Ezen kérdések egyike a talajlevegő jelentősége a szarvasgomba termesztésben.

A szarvasgomba tenyésztestek, és termőtestek légzésintenzitását nagymértékben befolyásolja a talaj levegőgazdálkodása. A légcsere minőségét, és mennyiségét közvetlenül a talajok szerkezete, fizikai félesége, közvetve pedig a mésztartalom, és a talajok víztartalma határozza meg. Az összefüggések megismerése céljából kielemeztük az említett tényezőket, és megkerestük a köztük fennálló összefüggéseket, melyek befolyásolják a talajok légáteresztő képességét.

Az aggregátum összetétel vizsgálat megmutatta az egyes genetikai szintek szerkezetét, ami szoros összefüggésben áll a talajok légáteresztő képességével.

A talajtani alapvizsgálatok során megismertük a vizsgált talajok fizikai és kémiai tulajdonságait, melyek meghatározzák a szarvasgomba életfeltételeit.

A pF mérések a talajok víztartó-képességének, illetve közvetve a talajok differenciált porozitásának megismerését tették lehetővé.

Légáteresztő-képesség helyszíni mérése egy viszonylag új technológia hazánkban, melynek megismerése egyik fő célja vizsgálatunknak. A helyszíni mérések legnagyobb előnye, hogy a talajokat eredeti szerkezetükben vizsgálhatjuk, azok bolygatása nélkül, így sokkal pontosabb képet kaphatunk a vizsgált paraméterről. Hátránya viszont, hogy egyazon mérés ismételt elvégzésére nincs lehetőség. A jól megtervezett metodika, és annak betartása azonban jelentősen szűkíti a hibalehetőségek körét, és számát.

Vizsgálataink bizonyították, hogy a jövőben a szarvasgomba termesztés élvonalában azok sorakoznak majd, akik a hangsúlyt nemcsak a tápanyag, és vízellátásra helyezik, hanem ezt a talaj levegőellátásának vizsgálatával kiegészítve igyekeznek megismerni a földalatti gombák egyedülállóan speciális igényeit.

Introduction

The culture of truffle consumption seems to revive in Hungary. The evidence of is the growing number of special food on the market made with truffle. The possibility of the growing mycorrhiza mushrooms is examined for a long time, in fact, the growing of so called „early mycorrhiza mushrooms" is solved in French, in Italy, and Spain (Chevalier et al., 2005).

Beside of the climatic features, pedological relations are determined for the successful growing. Thanks to the wide-spread research, most of the knowledge about the growing is available, but there are some questions which are not answered yet. One of these questions is the importance of soil air in the truffle growing.

Truffles are also performing gas exchange with their environment, which is determined by the „air management" of the soil. The quality and quantity of the gas exchange depends on the structure, the physical feature, and indirectly on the amount of lime, and water in the soil (Stefanovits, 1999). We've examined the factors above, and the relationships between them that can influence air permeability od soils.

The aim of our research was the examination of the air permeability of the soil in truffle plantations, and to test an in situ field method. The experimental field was a truffle orchard. Soil investigations were made after planting and before harvesting, which can serve a good basis for further comparisons and well characterize relationship between soil air supplying and truffle growth.

We hypothesized that there is a relationship between the yield of the truffle and the air permeability of the soil. By finding relationship between basic soil examination and the field air permeability investigations we can get fast and precise picture about the soil porosity. With

help of this – and with some other investigations – we can draw conclusions on the suitability of the soil for truffle production solely from the soil-air management indices. With a fast and simple measurement we can determine the necessity of the volume of soil improvements and with the help of this we can establish better conditions for the truffle.

Materials and methods

The truffle orchard in Keszthely is situated in the botanical garden of University of Pannonia, Georgikon Faculty. Regarding the hydrological circumstances the field is periodically under influence of water. The causes of this are that the physical type of the upper 35-55 cm layer is clayey loam, which makes the moving of the gravitation water slower; and at 200 cm depth there is a layer, which is impermeable to water. The truffle orchard has on Ramann-type brown forest soil (Haplic Cambisol).

The Szentgál truffle orchard is situated near to the village on a former arable land. In the Szentgál valley there are clayey brown forest soils (Dövényi, 2010). These soils belong to the physical type of loam, and they have favorable water budget. There were two sampling points at the field. The first was at a lower point of the valley and it can be classified as a slope alluvium. The second point was at the upper part of the sample area. The soil of this sampling point is a typically clayey brown forest soil (Haplic Luvisols).

From the genetic layers we collected disturbed samples for the laboratory examinations. The basic tests were made according to the protocol of the Hungarian soil examinations (Buzás, 1993) in the laboratory of University of Pannonia, Georgikon Faculty.

In situ measurements of the air permeability of the soils were also performed which were used for further evaluations. For these

measurements, PL300 permeameter from Eijkelkamp Agrisearch were used. This equipment can be used either for field measurements or laboratory measurements. There are two accessories to the equipment: a tensiometer, and a TDR probe with the help of these we can measure the water content of the soil, and the capillary suction force of the soil pores.

We collected undisturbed soil samples from both fields in 100 cm^3 sample rings for the measurements of water capacity and differential porosity from the soil layers where we measured the soil air permeability.

The pF value shows the water retention capacity of the soil against a given pressure. We can fit a function to the measured values in every pressure and with the help of this fitted curve, we can read the water retention value. From the form of the curve and the designated points we can conclude to the ratio of different soil pores (differential porosity) (Várallyay, 1973).

The water retention capacity of the soils were measured at the adequate pressure values: pF: 0.0, 0.4, 1.3, 2.2, 2.5, 4.2, 6.2 with the porous ceramic plate pF measuring equipment type Soil moisture Equipment Corporation LAB 23. After the measurement, the soils were dried at 105°C till permanent weight, and their weight was measured. From the dry weight and from the weight values in different pressures, the water content for each pressure can be calculated.

Results

The results of the basic soil examinations are shown in the Table 1. Comparing our data to the literature (Barna, 1998) it can be stated, that the pH, the lime content, and the humus content of the examined soils are not quite optimal for truffle growing.

Profile/level	Depth (cm)	K_A	Density (g/ cm^3)	pH distilled water	pH KCl	$CaCO_3$ (%)	Humus (%)
Szentgál 1/A	0-30	45	2.33	6.63	5.58	Ø	2.49
Szentgál 1/B1	30-55	51	2.28	6.72	5.44	Ø	0.79
Szentgál 1/B2	55-80	52	2.38	7.17	6.35	0.34	0.68
Szentgál 2/A1	0-30	42	2.44	6.83	5.97	Ø	2.39
Szentgál 2/A2	30-60	38	2.57	6.82	5.86	Ø	1.46
Szentgál 2/B1	60-85	40	2.5	6.66	5.24	Ø	0.67
Keszthely /1	0-20	35	2.5	7.63	7.15	1.35	2.14
Keszthely /2	20-40	32	2.57	7.84	7.2	1.05	1.49
Keszthely /3	40-55	38	2.5	8.1	7.35	9.27	0.71
Keszthely /4	55-75	45	2.56	8.16	7.54	20.23	0.41

Favorable pH value: 7.0 – 8.3

Optimal $CaCO_3$: 5 - 50 %

Proper organic material %: 1.5 – 8.0 %

Table 1. The most important data of the soils of sample areas (Barna, 1998)

On the Figures 1 and 2 the relationships between air permeability and pF curve are shown. The pF curve is described from the measuring points with the help of a soil expert program (Fodor-Rajkai, 2005). The in situ measured values of the air permeability are shown with the soil moisture content in the pF curve of the same soil sample. The difference of the volumetric soil moisture content at pF 2.5 and pF 0 shows the amount of the gravitation pores in the original structure soil. The braces on the figure (namely the difference between the pF0 and the soil moisture content at the measuring time) shows the pore volume (%), which were filled with air at the moment of the measurement. These pores are responsible for the air supply of the soil organisms and of the soil itself.

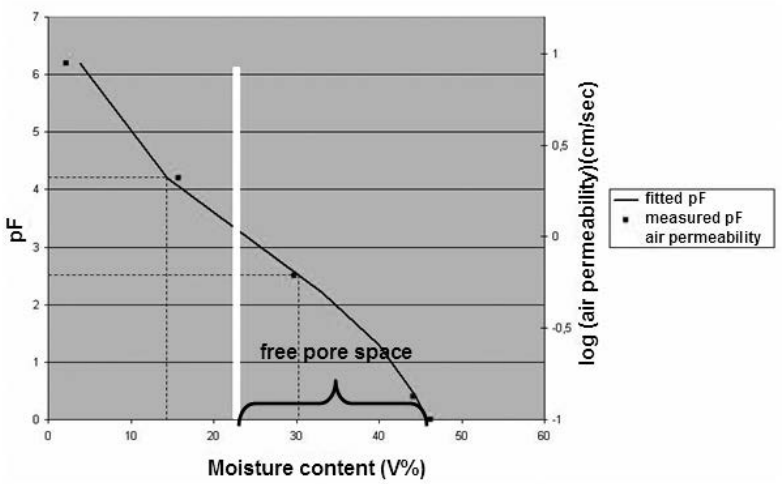

Fig. 1. The connection between soil air permeability and pF curve in large free pore space, measured at actual soil moisture content.

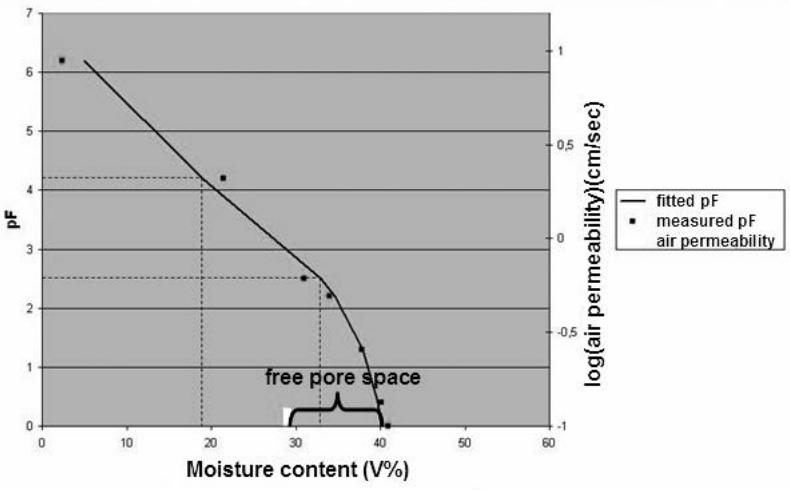

Fig. 2. The relationship between soil air permeability and pF curve in small free pore space, measured at actual soil moisture.

The evaluation of the results of air permeability examinations. Air permeability was made with the statistical program SPSS for Windows 13.1 (Ketskeméty-Izsó, 1996). The connection of air permeability and certain soil features were evaluated with lineal regression method. The received results give information about the connection and it strength between the examined parameters. The strength of relationship between the parameters can be seen well on the figures and on the R^2 value (determination coefficient).

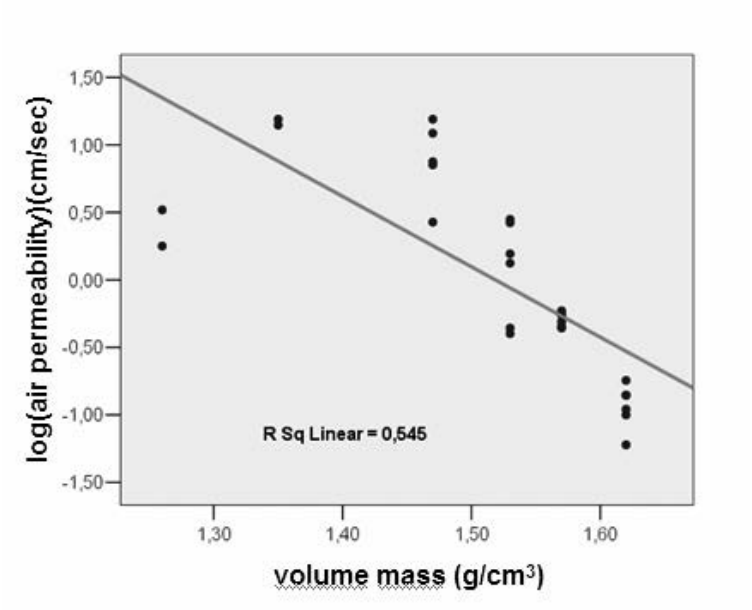

Fig. 3. Relationship between the air permeability and bulk density

The total porosity of the soil can be calculated on the basis of the bulk density, so it can be supposed the relationship between the air permeability and the bulk density. On the Figure 3 can be seen, that in case of the examined soil samples with the growing of the bulk density the air permeability decreasing, the relationship is medium tight.

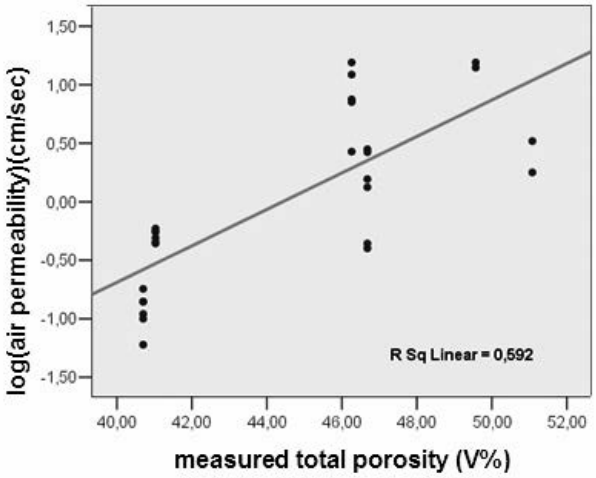

Fig. 4. Relationship between the air permeability and the total porosity

The examination of the correlation between the measured total porosity and the air permeability gave the expected result. On Figure 4 it can be seen, that with the increasing of the pore volume, the air permeability of the soil also increases. . Between the two factors there is a medium tight relationship.

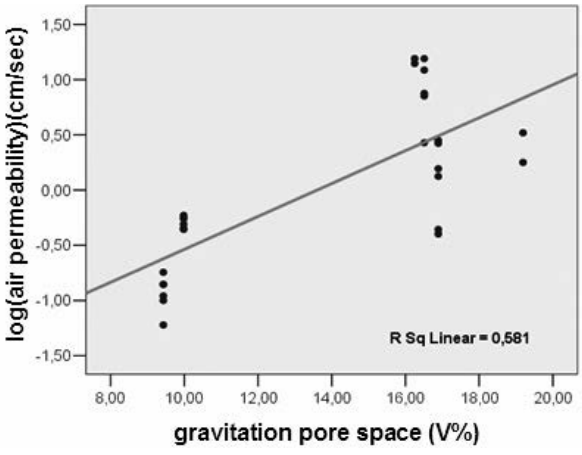

Fig. 5. Relationship between the gravitation pore space and air permeability

Air permeability occurs through the gravitation pore space in the soils. To comparing the pF curves with air permeability data can be seen, that where the proportion of the gravitation pore space was higher, the air permeability of the soil increased (Figure 1, 2). On the Figure. 5 can be seen, that the air permeability of the soil was higher where the volumetric amount of the gravitation pores were higher.

Conclusions

Because of the gravitation pore space, the measured total porosity, the bulk density and the air permeability of the soils are in significant linear correlation with each other, there is a possibility to work out a method, which enables fast diagnosis of aeration and compaction of the soils.

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References

BUZÁS I. (ed., 1993): The physical, water management and mineralogical investigation of soil (in Hungarian). Soil- and agrochemical investigation methodology handbook 1. INDA 4231 Press, Budapest. 357 p.

CHEVALIER, G. et al. (ed., 2005): The European black truffle (in Hungarian). EMSZE Press, Budapest. 266 p.

DÖVÉNYI Z. (ed., 2010): Survey of land of Hungary. Hungarian Academy of Sciences, Geographical Research Institute, Budapest. 1023 p.

FODOR N., RAJKAI K., (2005): A computer program for calculating the characteristics of the soil's physical and water management from other soil parameters (in Hungarian).

(TALAJTANonc1.0). Agrochemistry and Soil Science, 54.

KETSKEMÉTY L.-IZSÓ L. (1996): Basic SPSS for Windows (in Hungarian).

SPSS Partner Bt. Budapest 118. p.

SZEGLET P., DONGÓ A., SZABÓ I. (2002): Situation of the mushroom breeding at the Georgikon Faculty of PU, Keszthely (in French). Mycelium no. 6. 2002 p. 8-9.

SZEGLET P., MÉSZÉROS GY. (2009): The use of in-situ mikorrhized forest saplings in the territory of HM VERGA near Szentgál. The beginning experiences (in Hungarian). Forestry Papers, vol. CXLIV. p.: 382-383.

STEFANOVITS P. (ed., 1999): Soil Science (in Hungarian). Agronomy Press, Budapest. 470 p.

VÁRALLYAY, GY. (1973): The potential of the soil and a new device of the determinating in low tension range (in Hungarian). Agrochemistry and Soil Science, 22. p.1-22.

SOLAR TRACKING SIMULATOR DEVELOPMENT FOR SOLAR COLLECTOR TESTS

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Abstract

Our department has been commissioned to carry out stress and energy tests of vacuum tube solar collectors recently developed in Hungary. In order to be able to carry out the energy tests, we have developed and installed an outdoor solar tracking simulator equipment set, accompanied by a matching computer assisted measuring system. We also developed a test system based on the standard MSZ EN 12975-2:2006 (E). With the solar simulator built, we tested vacuum collectors and issued evaluation reports on the results. Our tests were carried out between October 2006 and November 2007. The necessary investment was implemented with the support of the West Pannon Regional Development Council.

Keywords: solar energy, vacuum tube solar collectors, solar tracking simulator, computer assisted measuring system, energy tests.

Összefoglalás

Tanszékünk új fejlesztésű, hazai vákuumcsöves napkollektorok terhelési és energetikai ellenőrző vizsgálatainak elvégzésére kapott megbízást. Az energetikai vizsgálatok elvégzéséhez kifejlesztésre került egy külsőtéri napkövető vizsgáló berendezés (szolár szimulátor) és a hozzá tartozó számítógépes mérőrendszer (4. és 5. ábra). Az MSZ EN 12975-2:2006 (E) szabvány alapján vizsgálati módszert dolgoztunk ki. A megépített szolár szimulátorral három különböző konstrukciójú vákuumcsöves kollektort teszteltünk, az eredményekről záró szakvéleményt adtunk. A mért paramétereket és mérési pontokat a 4. ábra mutatja. A szükséges beruházások a Nyugat-dunántúli Regionális Fejlesztési Ügynökség támogatásával valósultak meg.

A kialakított hőtechnikai mérőrendszer napkollektor-egységek terhelés vizsgálatára, a kollektorhatásfok és a termelt hőenergia mennyiség meghatározására, valamint összehasonlító mérésekre alkalmas. A szimulátoron egyszerre két kollektor-egység vizsgálható, melyeket a napkövető szervo rendszer képes folyamatosan a nap felé fordítani, azaz síkjukat állandóan a nap sugaraira merőleges irányban tartani. A vizsgálatok ideje alatt az adatgyűjtő számítógép regisztrálja a kollektorok által termelt hőmennyiséget, a napsugárzás intenzitását és az egyéb időjárási adatokat. Az elvégzett vizsgálatok két fő csoportra oszthatók: a termikus és mechanikai terhelhetőség vizsgálatára és a hőteljesítmény-vizsgálatra. A terhelési vizsgálatok során elvégeztük a kollektor-egységek belső nyomás-tesztjét, a magas hőmérsékleti ellenállás tesztjét, a külső-belső hősokk tesztet, az esővíz-áteresztés, fagyállóság és leüríthetőség vizsgálatát, valamint a sugárzás-tűrés tesztet. A hőteljesítmény-vizsgálatban meghatároztuk a kollektorok kimenő teljesítményét, valamint felvettük az egyes típusok kollektor-hatásfok-görbéjét.

A vizsgáló berendezés nemcsak a sík- és vákuumcsöves kollektorok tesztelésére alkalmas, hanem optimális lehet a koncentrátoros kollektorok vizsgálatára is. A szolár szimulátor alapvetően alkalmas kollektorok hőtechnikai tesztelésére. A hosszabb idejű biztonságos működéshez és a mérőrendszer mérési pontosságának megfelelő értéken tartásához a jelenlegi fejlesztési szinten még felügyelet és esetenként kézi beavatkozás is szükséges lehet. A berendezés teljes automatizálási szintjének eléréséhez és a mérési hibák minimálisra csökkentéséhez korrekciók, további fejlesztések szükségesek.

1. Introduction

One of the most important parameters of solar collectors is the heat produced by a unit of area in a given period of time and the thermal efficiency available in the given weather conditions. The performance of solar collectors is characterised by an efficiency curve, which shows what fraction of the solar energy input can be utilised as heat energy (Farkas, 2003). The efficiency curve is defined with the help of a series of test which can be carried out in open air, on a sun tracking simulator or on a simulator equipped with an artificial light source placed indoors. According to the Hungarian standard MSZ EN 12975-2:2006 (E) the simulator we developed is suitable to carry out qualifying tests to measure heat energy as well as thermal exposure stress tests. Besides, our system makes it possible to carry out comparative analyses of different types of collectors under identical radiation conditions.

2. Materials and methods

2.1. The structure of the solar simulator

Our experimental system is a solar domestic water heating system using fluid for heat transfer, equipped with a circulation pump. It has a 150-litre insulated storage tank and it transfers the collected heat to the water with a built in heat exchanger. The system comes complete with built-in elements necessary for proper operation: circulation pump, expansion chamber, safety valve, temperature gauges and pressure meter) as well as the automatic electronic controller, and some of the instruments needed to carry out the tests (Fig. 1).

The solar collector unit to be tested is attached to the system with temporary binds, and a flexible pipe. There are two ways to place and fix the solar collector: it can be placed at a suitable angle on the sloping roof surface of the building facing to south, or it can be mounted on a frame similar to seen in Fig. 2. This allows two collector units to be fixed, which then can be tested in parallel as their orientation and angular position is the same. Besides it is possible to operate a solar collector unit with known conditions. For control measurements a vacuum flat collector was used.

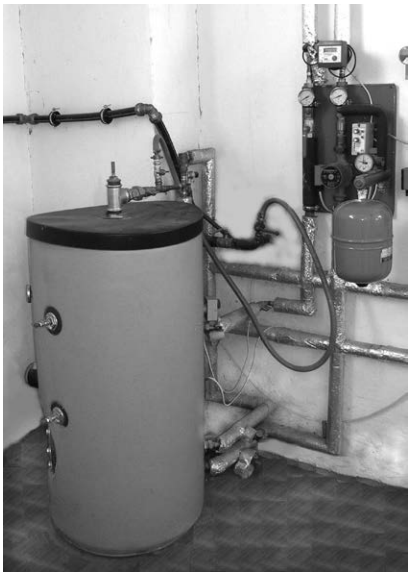


Figure 1. Components of the test equipment installed indoors at the Laboratory of University of Pannonia, Georgikon Faculty, Department of Agricultural Mechanization

Figure 2. Mounting frame of the simulator (University of Pannonia, Georgikon Faculty, Department of Agricultural Mechanization)

The vacuum necessary to operate the vacuum tube solar collectors is provided by a vacuum pump fixed on a mounting frame. The vacuum present in the system is displayed by a 10 mbar scale division vacuum meter. The continuous wind stream above the collector tubes necessary for heat performance tests is provided by an axial ventilator. The scaffolding has wheels to make the simulator unit mobile so when the tests are over, it can be pulled into a closed workshop. The support also has a fork attached to which can be turned round a vertical axis to allow the frame holding the collector to be tilted along a horizontal axis. The frame makes it possible to exactly orientate the collector tested and to set and fix the tilt angle.

The frame allows fixing of different size and type of collectors reaching up to the length of 1.7 m and the width of 1.2 m. The frame is suitable for on-by-one fixing the glass tubes of the vacuum tube collectors as well. The collector tubes fit into nests at the foot of the plinth board, while at their necks at the top they are fixed with thin plastic straps.

The vacuum tubes are attached to a common distributor or collector pipe with union nuts, so they can be changed simply and so after some short fitting it is possible to test a further evacuated tubular collector type.

The sun-tracking servo system is an electromechanical unit which is capable of making a tool turn in the direction of the sun that is keep a designated plan moving at a right angle (90 °) to the rays of the sun.

The necessary control loop consists of

- A sensor, which transforms any changes of the sun position or the momentary position of the moved object into electronic signals;
- A processing unit, which compares the signal characterising the different positions and according to the results of the comparison provides the elements generating the movement with electric energy;

Actuators which do the mechanical work with the help of the electronic energy, which in this case is a newel/screw shaft/screw spindle mechanism operated by a step motor. The rotation around the horizontal and the vertical axes is carried out by such a unit. (Fig. 3).

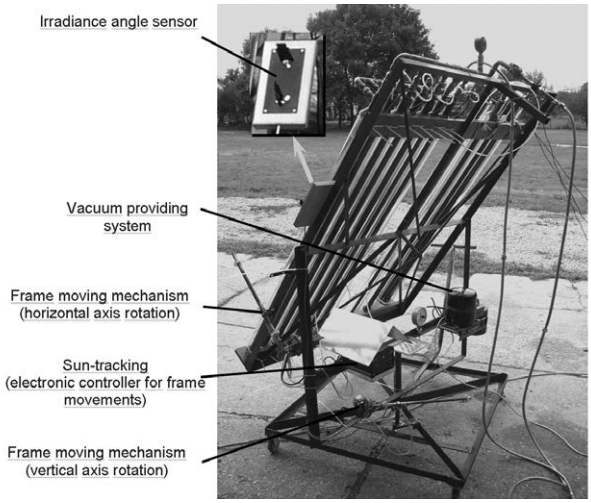
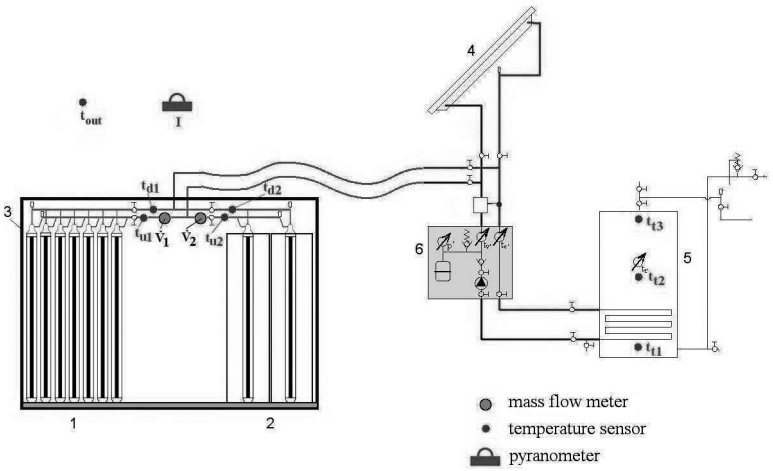


Figure 3. The mechanism moving the frame and the system providing the vacuum (University of Pannonia, Georgikon Faculty, Department of Agricultural Mechanization)

2.2. The development of the measuring-controlling and data acquisition system

The thermal-technology system we developed is suitable to carry out for stress tests of solar collector units, to measure collector efficiency, to measure and define thermal energy produced as well as to carry out comparative tests. The thermal energy produced by the collectors to be tested can be measured by temperature sensors installed inlet and outlet stream in the heat transfer fluid and volume flow rate signal device. During the tests it is essential that the environmental temperatures, global radiation intensity and storage tank temperatures should be measured (Fig. 4).



1.-2. – tested collectors; 3. – solar simulator; 4. - control-collector; 5. – hot water tank; 6. - solar device unit

Figure 4. Measuring points of the monitoring, controlling and data logging system

The output signals of the sensors are received by a ‘Basic Stamp 2’ micro computer and a software we developed transmits it to the data

logger PC which collects the measured data in an Excel table and calculates the energetic characteristics of the collector for a certain point of time (Nagy, 2007). Global radiation can be measured with a Kipp & Zonen CM 21 type pyranometer. During the tests it is advisable to measure other weather parameters as well, e.g. wind speed, direction of wind, relative moisture content, air pressure, which can be registered with the help of a mobile meteorological station equipped with a digital data logger. The simulator equipped with the measuring devices can be seen in Fig. 5.

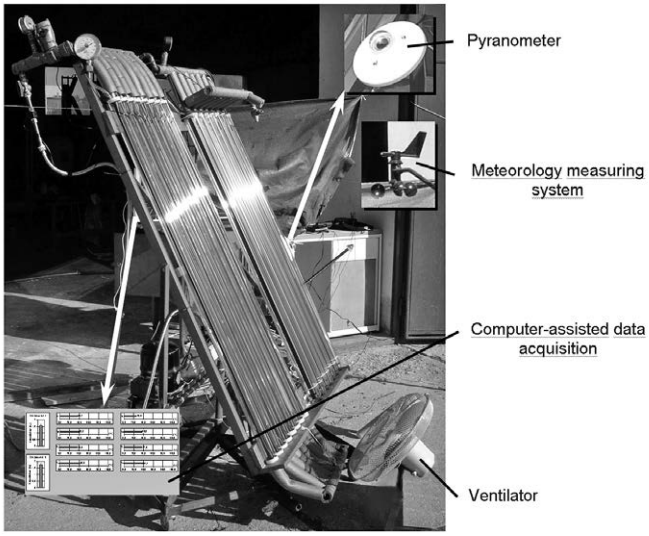


Figure 5. The simulator equipped with the test system (University of Pannonia, Georgikon Faculty, Department of Agricultural Mechanization)

2.3. Measurements taken with the test equipment

In our tests we examined and qualified vacuum tube collectors of three different types according to Chapter 5 of the standard MSZ EN 12975-2:2006 (E)**.**

Tests were divided into two main groups: those of thermal and mechanic stress (exposure) and those of heat performance (Drück,

1997, Fischer, 1998). During the stress (exposure) tests we carried the collector units interior pressure tests, interior and exterior thermal shock tests, rain penetration tests, freeze resistance tests as well as drain-down tests and radiation exposure tests. In the thermal performance test we defined the output of the collectors and drew the efficiency-curves of the respective collectors (MSZ EN 12975-2:2006). The collectors were fixed on the solar simulator. The automatic sun tracking system ensured that the sun rays always fell onto the surfaces at a right angle (90 °) on. The hot or cold water circulation necessary for the respective tests was ensured by the water loop of our equipment. To test thermal performance we used the gauge system of our test equipment (Cseke, 2008).

3. Results

The test carried out allowed not only the qualification of the collectors but they provided a lot of information about the suitable operation of the testing equipment as well. First we managed to put the mounting frame of the simulator to a test, which only went to show that the light profile steel structure was not able to steadily hold the mass of the collector tubes filled up with water. So it was necessary to fix the frame with further bracing. Besides this when collectors with different masses were being tested, it was necessary to apply extra weights to counterbalance the differences.

A precondition of collector performance testing is that there is a cloudless sky and even radiation. Under such conditions the automatic sun-tracking was able to follow the sun with an error of about 1% for both the sun height and the azimuth value, which was acceptable for our tests. However, under cloudy conditions, (at decreased solar radiation) in some cases the device was not working reliably, which necessitated manual intervention. As during the test periods, the sky was always

bright, this did not cause any trouble in carrying out the tests. In long-term tests, however, (e.g. duration of several weeks) it would have been a great advantage if it had not been necessary to provide constant supervision of the system.

The driving mechanism of the sun-tracking system worked properly, the screw shaft mechanism has a slowing down so it is capable of generating even a small angle move the mounting frame, which is very advantageous from the point of view of exact/sensitive sun-tracking. However, it is not an ideal solution for the rotation around a vertical shaft as it is capable of covering a maximum of 120-140° rotation range, which would cause problems in the case of a whole-daylong test without supervision. The accuracy of the sensors of the measuring system is basically satisfactory. In case there is a satisfactory temperature increase of the fluid, then the DT measurement error is still acceptable. Should the temperature increase remain low, (e.g. because of low radiation), it would cause such a big error in the measurement of DT that it would make heat capacity measurements impossible.

To establish the collector efficiency under static conditions, it is an important requirement that the temperature of the water entering the collector should be kept constant. The accepted deviation is ±1K. In our testing equipment, we solved this problem by making the heat transfer fluid rotate in the heat exchange loop of the necessary length till it reached the constant temperature of the water in the tank. This simple cooling was – in most cases – enough to maintain constant temperature. In some cases, however, the fluctuation was bigger than acceptable thus the periods with no constant temperature may not be included on the evaluation, which would justify a control circle being built into the system.

4. Discussion

It was experienced in the tests carried out so far that with the help of the equipment it was possible to significantly lengthen the testing periods in comparison with the fixed test benches. It is a further advantage that it is possible to adjust the angle difference between the collector and the direction of the solar radiation, so it was possible to quickly determine the correlation between the collector performance and the incidence/irradiation angle. The testing equipment is not only suitable to test flat and vacuum tube collectors but it might prove ideal to test concentrating collectors as well.

Basically the test equipment is suitable for thermal tests of collectors. Under present conditions, in order to achieve a longer term operation and to keep the accuracy of the system on an acceptable level, however, constant supervision and several instances of manual interventions are necessary. To reach the fully automated level and to further decrease the error, the following development and corrections are necessary.

- It is important to achieve that the automatic sun-tracking operates accurately independently of the radiation conditions. The problem is possible to solve with the installation of a light-intensity sensor and minor adjustment of the power circuit of the equipment.
- The transformation of the rotation mechanism of the simulator around the vertical axis, so that it can follow the full orbit of the sun. It is possible to solve with the installation of a worm drive.
- It is necessary to develop the control of the water temperature entering the collector. It necessitates the installation of an engine-operated mixing valve, which will mix the well warmed water coming from the outlet with a properly accurate regulation and the cold water coming from the tank and lets the mixture to the inlet side.

- To achieve a more accurate measurement of DT it is necessary to create a measuring circuit more accurate than the present one.
- Instead of the three different data logger units, (Basic Stamp, pyranometer, meteorological station) and the accompanying software, we would need a single data acquisition unit and one single software to go with it. It can be done with a more sophisticated Basic Stamp unit which is capable of receiving the data of the pyranometer and the meteorological station as well.
- In order to minimise heat losses, better insulation material quality should be used in the vicinity of the inlet stubs. The present polyfoam pipe insulation should be replaced with moulded polystyrene insulation elements.

Acknowledgement

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References

Cseke, B., László, A., Szabó, B. (2008). Qualifying tests of vacuum tube solar collectors developed in Hungary, XXXII. MTA-AMB K+F Tanácskozás, Gödöllő.

Drück, H., Peter, M., Hahne, E. (1997). Leistungsprüfung von Solaranlagen zur Brauchwassererwärmung nach den zukünftigen CEN-Normen des TC 312, Tagungsband zum siebten Symposium Thermische Solarenergie, Otti-Technologie-Kolleg, Regensburg, 387-391.

Fischer, S., Hahne, E. (1998). Thermische Prüfung von Sonnencollectoren nach CEN-Norm. *Sonnenenergie und Wärmetechnik* **5.**

Farkas, I. et al. (2003) Solar energy in agriculture, ‘Mezőgazda’ publishers, 53-99.

MSZ EN 12975-2:2006 (E), Thermal solar systems and components. Solar collectors. Part 2: Test methods. English version.

Nagy, P., Lönhárd, M., Cseke, B. (2007). Development of micro-regulated data collecting systems at the Department of Agricultural Mechanisation of PE GMK. IV. ‘Erdei Ferenc’ Scientific conference, Kecskemét.

CHECKLIST OF FRESHWATER TRICLADS (PLATYHELMINTHES: TRICLADIDA) OF HUNGARY

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Abstract

This paper lists the free living freshwater triclad (flatworms: turbellarians) species reported from the territory of Hungary, along with the main faunistical characters referred to Hungary. Seventeen (17) identified species were reported up to 2011 year: fifteen (15) native freshwater triclads and two (2) non-native freshwater triclads.

Keywords: turbellarians, freshwater planarians, Tricladida, Hungary, checklist

Összefoglalás

Jelen publikáció felsorolja a Magyarország területéről közölt édesvízi planáriák (laposférgek: örvényférgek) szabadban előforduló fajait Magyarországra vonatkozó főbb faunisztikai jellemzőivel. Tizen-

hét (17) azonosított faj került elő eddig: tizenöt (15) őshonos és kettő (2) nem őshonos édesvízi planária.

Kulcsszavak: örvényférgek, édesvízi planáriák, Tricladida, Magyarország, fajlista

Introduction

This work lists free living freshwater triclad (Platyhelminthes: "Turbellaria": Tricladida) species within the present borders of Hungary, Middle Europe. (Those species were not mentioned in this paper which were registered in the historical "Great Hungary", but the localities are out of the present political borders since the Treaty of Trianon, 1920.) The first data on triclad from Hungary was published in the report by Margó (1879) from Budapest and its region. The checklist of Parádi (1899) was referred to the Great Hungary. The following checklist of G. Dahm and Gourbault (1978) was referred to the whole Carpathian Basin, including Hungary.

Materials and Methods

The Checklist of Freshwater Triclads in Hungary contains taxonomy, species names, and some basic information referred to Hungary:

Current ***Genus species*** (Auctor, year) [= outdated *Genus species* Auctor, year; other outdated *Genus species* (Auctor, year)] <current Hungarian name (= former Hungarian name), body length, habitat, occurrence, main character>: (citation of the first PUBLISHER from Hungary)

Taxonomy and the nomenclature follow Kenk (1974) and Tyler et al. (2006–2011). The origin of the Hungarian names were based on the works of Méhelÿ (1925), Gelei (1930), Dudich (1942), Lukács

(1955), Hartwich (1977), Andrássy (1984), Reichholf (1998), Bährmann (2000), Kovács and Fülep (2011).

Results

Seventeen (17) identified species were reported in Hungary up to 2011 year: fifteen (15) native freshwater triclads and two (2) non-native freshwater triclads.

Phylum: **Platyhelminthes** Minot, 1876 [= Plathelminthes Schneider, 1873] <laposférgek>

Suborder: **Tricladida** Lang, 1884 <hármasbelűek>

Infraorder: **Continenticola** Carranza, Littlewood, Clough, Ruiz-Trillo, Baguna, Riutort, 1998

Superfamilia: **Planarioidea** Stimpson, 1857

Familia: **Dendrocoelidae** Hallez, 1892 <fodros planáriák>

Genus: **Dendrocoelum** Müller, 1774 [= Dendrocoelum Örsted, 1844; Dendrocoelides de Beauchamp, 1919]

1. ***Dendrocoelum album*** (Steinmann, 1910) [= *Polycladodes alba* Steinmann, 1910] <10–14 mm, region of Balaton lake: Vászoly>: (Gelei, 1931)

2. ***Dendrocoelum hankoi*** (Gelei, 1927) [= *Dendrocoelides hankoi* Gelei, 1927; *Paradendrocoelum hankoi* (Gelei, 1927)] <6–10 mm, region of Balaton lake: Vászoly, Kádárta, Kővágóörs>: (Gelei, 1927)

3. ***Dendrocoelum lacteum*** (Müller, 1774) [= *Fasciola lactea* Müller, 1774; *Dendrocoelum lacteum* Örsted, 1844; *Eudendrocoelum lacteum* (Müller, 1773)] <tejfehér planária (= tejfehér örvényféreg), 10–30 mm, lentic, productive waters, common>: (Margó, 1879)

4. ***Dendrocoelum pannonicum*** (Méhely, 1927) [= *Dendrocoelides pannonicus* Méhely, 1927; *Dendrocoelum mrazekii pannonicum*

(Méhely, 1927); *Dendrocoelides mrazeki pannonica* (Méhely, 1927)] <12 mm, Mecsek mountains: waters of Mánfai-kőlyuk Cave, endemic>: (Méhelÿ, 1927)

5. ***Dendrocoelum romanodanubiale*** (Codreanu, 1949) [= *Palaeodendrocoelum romanodanubialis Codreanu*, 1949] <dunai planária, 5–9 mm, lentic, Ponto-Caspian, non-native>: (Fülep and Nosek, 2010)

Familia: **Planariidae** Stimpson, 1857 <valódi planáriák>

Genus: **Crenobia** Kenk, 1930

6. ***Crenobia alpina*** (Dana, 1766) [= *Hirudo alpina* Dana, 1766; *Fasciola alpina* Dana, 1766; *Planaria alpina* Dana, 1766)] <szarvasplanária (= alpesi planária, alpesi örvényféreg, kétszemű szarvasféreg), 10–16 mm, lotic, mountainous springs, glacial relict>: (Méhelÿ, 1918)

Genus: **Phagocata** Leidy, 1847 [= Fonticola Komárek, 1926]

7. ***Phagocata albissima*** (Vejdovský, 1883) [= *Planaria albissima* Vejdovský, 1883; *Fonticola albissima* (Vejdovský, 1883)] <8–12 mm, lotic, Bükk mountains: Vörös-kő-völgy, Bánya-lápa>: (Lukács, 1958)

8. ***Phagocata vitta*** (Dugès, 1830) [= *Planaria vitta* Dugès, 1830; *Fonticola vitta* (Dugès, 1830)] <önmentő planária, 8–15 mm, lotic, Bükk mountains: Létrás-tető, Fekete-sár, Mátra mountains: Kékes>: (Fülep, 2006)

Genus: **Planaria** Müller, 1776

9. ***Planaria torva*** (Müller, 1773) [= *Fasciola torva* Müller, 1773] <mocsári planária, 10–15 mm, lentic, lowland waters, common>: (Margó, 1879)

Genus: **Polycelis** Ehrenberg, 1831

10. ***Polycelis felina*** (Dalyell, 1814) [= *Planaria felina* Dalyell,

1814; *Planaria cornuta* Johnson, 1822; *Polycelis cornuta* Johnston, 1822] <sokszemű szarvasplanária (= forrás örvényféreg, sokszemű planária, seregszemű szarvas planária, sokszemű szarvasféreg, seregszemű szarvasféreg), 8–15 mm, lotic, mountains, common>: (Hankó and Dudich, 1924)

11. ***Polycelis nigra*** (Müller, 1773) [= *Fasciola nigra* Müller, 1773] <fekete planária (= sokszemű planária), 8–12 mm, lentic, common>: (Margó, 1879)

(Exact distinguishing from *P. tenuis* needs genital examination.)

12. ***Polycelis tenuis*** Ijima, 1884 <seregszemű planária, 8–12 mm, lentic, common>: (Gelei, 1928)

(Exact distinguishing from *P. nigra* needs genital examination.)

13. ***Polycelis tothi*** Méhely, 1927 <15 mm, Mecsek mountains: waters of Mánfai-kőlyuk Cave, endemic>: (Méhelÿ, 1927)

Superfamilia: **Geoplanoidea** Stimpson, 1857

Familia: **Dugesiidae** Ball, 1974

Genus: **Dugesia** Girard, 1851 [= Euplanaria Hesse, 1897; Geopaludicola Komarek, 1919]

14. ***Dugesia gonocephala*** (Dugès, 1830) [= *Planaria gonocephala* Dugès, 1830*; Euplanaria gonocephala* (Dugès, 1830)] <füles planária (= nyílfejű örvényféreg), 10–25 mm, lotic, hills and mountains, common>: (Méhelÿ, 1918)

15. ***Dugesia lugubris*** (Schmidt, 1861) [= *Planaria lugubris* Schmidt, 1861] <gyászplanária (= gyászos planária, gyászörvényféreg), 11–25 mm, lentic, lowland waters, common>: (Margó, 1879)

(Exact distinguishing from *D. polychroa* needs genital examination.)

16. ***Dugesia polychroa*** (Schmidt, 1861) [= *Planaria polychroa* Schmidt, 1861] <11–20 mm, lentic, lowland waters, common>: (Gelei, 1928)

(Exact distinguishing from *D. lugubris* needs genital examination.)

17. ***Dugesia tigrina*** (Girard, 1850) [= *Planaria maculata* Leidy 1847; *Euplanaria tigrina* (Girard, 1850)] <foltos planária, 11–25 mm, lentic, North American, non-native>: (Kender, 1939)

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References

Andrássy, I. 1984. Phylum of flatworms – Platyhelminthes. *In* Móczár, L. (ed) Key to Hungarian animals 1. (in Hungarian) Tankönyvkiadó, Budapest, 36–39.

Bährmann, R. (ed) 2000. A key to the invertebrates – Illustrated keys for field practices of zoology. (in Hungarian) Mezőgazda Kiadó, Budapest, 384.

Dudich, E. 1942. 7. phylum: Platyhelminthes. – Flatworms. *In* Dudich, E. (ed) The world of nature. The animal and its life. Part two. (in Hungarian) Királyi Magyar Természettudományi Társulat, Budapest, 57–68.

Fülep, T. 2006. New data to the distribution of Turbellaria species (Platyhelminthes: Turbellaria) in the Bükk Mountains, North-Eastern Hungary (Bükk Plateau, Csondró Valley). (in Hungarian) *Acta Biologica Debrecina Supplementum Oecologica Hungarica.* **14**. 123–133.

Fülep, T. and Nosek, J. 2010. Contribution to the macroinvertebrate fauna of the Hungarian Danube VI. Triclads (Platyhelminthes: Tricladida). *Folia Historico-naturalia Musei Matraensis.* **34**. 05–09.

G. Dahm, A. and Gourbault, N. 1978. Tricladida et Temnocephalida (Turbellaria). *In* Illies, J. (ed) Limnofauna Europaea. Eine Zusammenstellung aller die europäischen Binnengewässer bewohnenden mehrzellingen Tierarten mit Angaben über ihre Verbreitung und Ökologie Gustav Fischer Verlag, Stuttgart, New York, Swets & Zeitlinger B. V., Amsterdam, 16–20.

Gelei, J. 1927. Eine neue Blindtriclade aus Ungarn. *Zoologischer Anzeiger* **72**. 35–46.

Gelei, J. 1928. Turbellarii Hungarici 1. Tricladen aus der Umgebung von Szeged. (Angaben zur Variabilität der Turbellarien). *Acta biologica. A Magyar Királyi Ferencz József Tudományegyetem Tudományos Közleményei – Természettudományi szakosztály Biologiai értekezései*, **1.1**. 1–17.

Gelei, J. 1930. 1. class: Turbellarians (Turbellaria). (in Hungarian) *In* Brehm, A. (ed) Brehm – The world of animals **18**. (in Hungarian) Digital edition: Arcanum Adatbázis Kft. 2000. http://mek.niif.hu/03400/03408/html/index.html

Gelei, J. 1931. Neue Artmerkmale von *Polycladodes alba* (Steinm.). *Zoologischer Anzeiger* **93**. 284–287.

Hankó, B. and Dudich, E. 1924. Über das Vorkommen von *Polycelis cornuta* (Johns.) in Ungarn. – Verhandlungen der Internationale Vereinigung für theoretische und angewandte Limnologie, Innsbruck **2**. 324–331.

Hartwich, H.-J. 1977. Phylum of flatworms – Plathelminthes. *In* Urania Animals. Inferior animals. (in Hungarian) Gondolat Kiadó, Budapest, 121–167.

Kender, J. 1939. Study on limnobiology of the lake of Saint Lukács Thermal Bath. (in Hungarian) *Palaestra Calasanctiana. PhD Thesis of Piarist,* **25**. 24.

Kenk, R. 1974. Index of the Genera and Species of the Freshwater Triclads (Turbellaria) of the World. *Smithsonian Contributions to Zoology* **183**. 90.
http://www.sil.si.edu/smithsoniancontributions/Zoology/pdf_hi/SCTZ-0183.pdf

Kovács, K. and Fülep, T. 2011. The occurrence of invasive triclad species (Platyhelminthes: Tricladida) in Northwest Hungary. (in Hungarian) *Acta Biol. Debrecina Suppl. Oecologica Hungarica* **26**. 153–160.

Lukács, D. 1955. Zoological and environmental relations of the warm waters of Eger. (in Hungarian) *Heves megyei Füzetek* **5**: 19–27.

Lukács, D. 1958. Data to the ecology of *Fonticola albissima* Vejd. (Probursaria [Tricladida paludicola]). (in Hungarian) *Acta Academiae Paedagogicae Agriensis* **4**. 493–497.

Margó, T. 1879. Budapest and its regions in the respect of zoology. *In* Gerlóczy, Gy. and Dulácska, G. (ed) Natural historical, medical and public educational description of Budapest and its surroundings. (in Hungarian) Magyar Királyi Egyetem, Budapest, 295–432.

Méhelÿ, L. 1918. Distribution of triclads in the High Tatra and in the Kőszeg Mountains. (in Hungarian) *Mathematikai és Természettudományi Közlemények* **34/2**. 109–131.

Méhelÿ, L. 1925. Triclads (Platyhelminthes: Tricladida) in the Hungarian middle mountain ranges of Bükk mountains, Bakony mountains and Mecsek mountains. (in Hungarian) *Matematikai és természettudományi értesítő* **41**: 178–184.

Méhelÿ, L. 1927. Neue Würmer und Krebse aus Ungarn. Budapest, 1–11., 12–19.

Parádi, C. 1899. Subphylum. Plathelminthes. Classis. Turbellaria. *In* Fauna Regni Hungariae. Animalium Hungariae hucusque cognitorum enumeratio systematica. Királyi Magyar Természettudományi Társulat, Budapest, 1918, 29–30.

Reichholf, J. 1998. Nature guide. The world of waters. European inland waters, streams and marshes. (in Hungarian) Magyar Könyvklub, Budapest, 224.

Tyler, S., Schilling, S., Hooge, M. and Bush, L.F. (comp) 2006–2011. Turbellarian taxonomic database. Version 1.7 http://turbellaria.umaine.edu (08.02.2012.)

ERRATUM

1. Menyhárt, L. and A. Anda (2011) Global radiation and albedo in the radiation system of Lake Balaton. In: Georgikon for Agriculture Vol.14. No 1: 1-20.

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