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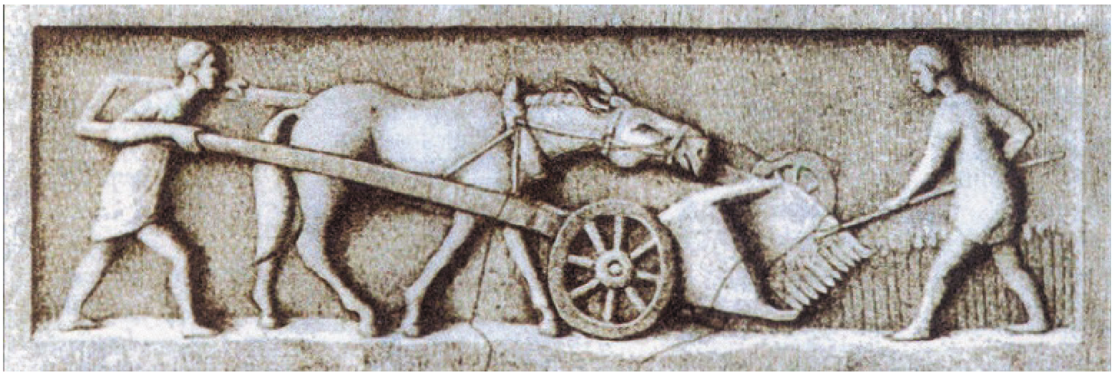

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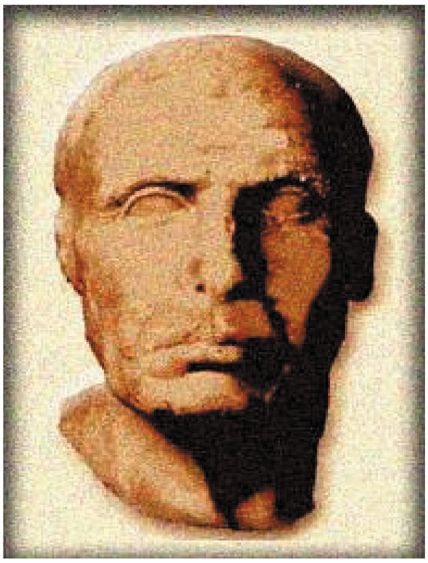
FOREWORD

LECTORIS SALUTEM – GREETINGS TO THE READER



Preparing the preface for this neonate periodical there were two ideas in my mind, how to define the scope and the task of this journal. On one hand it seems to be quite simple, since the title of the journal – Columella – may suggest that. Lucius Junius Moderatus Columella was probably the most popular, and at the same time, the most comprehensive Roman writer dealing with agriculture. His profound knowledge resulted in an exceptionally long active life of his specific book titled "De Re Rustica", which was translated to most languages and used as a basic textbook in agricultural education for almost two millenniums from the reign of Augustus until the end of the nineteenth Century. He was an expert interpreter of various fields of agricultural knowledge. Most of the statements and technical hints regarding land use, soil tillage, crop production, animal breeding, husbandry, forages and food are still valid in our age.

On the other hand it is really hard to describe the mission of the journal. The scope of that is within the field of agricultural and environmental sciences however the task of the journal is rather diverse. In first place, this journal is intended to give floor to the publication and dissemination of scientific results. Also, a major task of that is to provide bridge between scientists of our university and the international scientific community. Furthermore apart from scientific publication, articles in relation with communication, information and review are welcome.

The word "science" has countless definitions. All of them are related somehow to mental activities, however according to the field of that various assessments are available. In general science can be defined as the branch of knowledge involving systematized observation of an experiment with phenomena. Also, on the basis of that it is a formulated knowledge, that pursuit of principles of the subject. One of the most practical definitions was formulated by the late Charles Kellogg who stated, that science is nothing else but the process of understanding the world – we seek, we learn. Actually the two cannot be separated.

I am eager to know about something, so I try to learn about that as much as I can.

And it is the crucial point where we arrive to a specific problem of any knowledge; - the dissemination of that. The value of the scientific result is in its utility, namely in the broadcasting of that. The scientist, who is loading his results only to the drawer of his writing desk may be clever, diligent, but will render less services to the society. The results have to be delivered to the users to make a benefit.

I do hope that Columella, this new scientific journal may be a medium between scientists and specific experts in the field of agriculture and environment. I would like to encourage scientists to publish their results in this periodical, and I would advise readers to get acquainted with these results.

Csaba Gyuricza
editor-in-chief

EFFECT OF SOIL TILLAGE AND DIGESTATE APPLICATION ON SOME SOIL PROPERTIES

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Abstract

The effect of different methods of tillage on basic physical and chemical properties of soil was tested in a pilot field experiment. The digestate was applied simultaneously and it was tested if this type of dressing did not influence soil in a negative manner. Results obtained in the Variant 2 (i.e. with the minimum tillage) indicated that, as compared with the conventional ploughing, (Variant 1) values of reduced bulk density were increased and those of soil porosity decreased. However, better values of soil humidity were recorded in Variant 2. In Variant 2, a certain trend to increasing values of C_{org}/N_t ratio was observed while in Variant 1 values of soil reaction were decreased. It was concluded that these changes could induce soil degradation processes.

Keywords: ploughing, minimum tillage, soil physical properties, soil chemical properties, digestate

Introduction

There are different opinions concerning not only methods of tillage but also the application of nutrients in the form of mineral fertilisers. One of the most important problems is the formulation of an optimum dose of supplied nutrients (Pokorný and Denešová, 1998). This problem concerns also the application of digestate.

The digestate (i.e. the waste material remaining after the anaerobic digestion of a biodegradable feedstock in biogas stations) represents one of possibilities how to apply nutrients into the soil. When defining the digestate, it is difficult to decide if it is an organic or mineral fertiliser. From the viewpoint of legislation, it is classified into the category of fertilisers of organic type (Lošák, 2010). Because digestate contains predominantly organic substances, it can be considered to be the organic fertilisers.

However, because the technology of biogas production is relatively young and new, there are no data available about effects of digestate matter application as a fertiliser on the soil environment.

If digestate doses are too high and also if it is applied into soils with existing unsuitable conditions, the soil can be excessively compacted and such a compaction can be fairly irreversible.

Materials and methods

The experimental field was situated on loamy Orthic Luvisol in a potato-growing region with the following parameters: altitude 513 m above sea level, long-term sum of precipitation 500 mm (of this, 395 mm in the course of the growing season), and average annual temperature 6.8 °C.

There were two variants of tillage: Variant 1 – conventional ploughing to the depth of 0.22 m, and Variant 2 – minimum tillage with a post-harvest crushing stubble breaking. In both variants, the digestate originating from a local biogas station was applied in the dose of 20 t ha-1 in the autumn of each experimental year. In both experimental variants only the effect of the preceding crop on soil properties was studied. The pH of the digestate was 8.9 and it contained 90.21 % of combustible substances; contents of individual elements (also expressed in % of DM) were as follows: nitrogen 5.47; phosphorus 1.18; potassium 1.53; magnesium 0.71 and calcium 1.1.

When monitoring dynamic changes in physical properties, samples of intact soil were collected by means of Kopecky cylinders. Soil samples were taken in five repetitions from three depths of soil profile (0-0.1 m, 0.1-0.2 m and 0.2-0.3 m). As far as chemical parameters were concerned, recorded were pH values and organic

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carbon to total nitrogen molar ratios (C_{org}/N_t). Soil texture was evaluated on the base of the coefficient of structurality (which expresses the degree of destruction of soil structure) and of the ratio between agronomical valuable (0.25-10 mm) and less valuable (>10 and <0.25 mm) structural elements. Soil samples were taken in three repetitions from two depths (0-0.15 and 0.15-0.30 m). In this paper, results obtained in years 2012 –2013 are presented.

Results concerning physical properties of soil and characterising their changes within the whole study period are presented in Tabs 1 and 2.

As one can see, in Variant 1 values of reduced bulk density of soil were always lower than in Variant 2. On the other hand, however, the content of water in soil was always higher in Variant 2. In this variant, values of soil aeration were better but only in the upper soil layer.

Table 1. Physical properties of soil – Budisov, 2012

Variants	Soil depth (m)	Bulk density ($g.cm^{-3}$)	Total porosity (%)	Momentary content of water	Momentary content of air	Maximum capillary capacity	Minimum air capacity
				(% vol.)		(% vol.)	
1	0-0.1	1.39	48.44	14.00	34.44	34.93	13.51
	0.1-0.2	1.38	48.88	16.88	32.00	37.11	11.77
	0.2-0.3	1.38	48.95	15.56	33.39	34.64	14.31
	Mean	1.38	48.76	15.48	33.28	35.56	13.20
2	0-0.1	1.41	49.65	15.54	34.10	34.07	15.58
	0.1-0.2	1.57	43.76	18.36	25.40	30.90	12.87
	0.2-0.3	1.53	45.27	15.94	29.33	31.48	13.79
	Mean	1.51	46.23	16.62	29.61	32.15	14.08

Table 2. Physical properties of soil – Budisov, 2013

Variants	Soil depth (m)	Bulk density ($g.cm^{-3}$)	Total porosity (%)	Momentary content of water	Momentary content of air	Maximum capillary capacity	Minimum air capacity
				(% vol.)		(% vol.)	
1	0-0.1	1.38	47.76	19.00	28.76	38.65	9.11
	0.1-0.2	1.41	46.89	18.14	28.76	34.83	12.06
	0.2-0.3	1.33	49.77	17.54	32.23	35.38	14.39
	Mean	1.37	48.14	18.23	29.92	36.29	11.85
2	0-0.1	1.38	48.02	22.70	25.32	35.24	12.78
	0.1-0.2	1.48	44.02	24.01	20.00	33.77	10.24
	0.2-0.3	1.46	44.99	21.11	23.88	34.88	10.10
	Mean	1.44	45.68	22.61	23.07	34.63	11.04

Results and discussion

When studying effects of tillage on physical properties of soil, individual samplings were performed always at the beginning and to the end of the growing season.

In the second experimental year, values of reduced bulk density of soil were generally lower (above all in Variant 2). Different methods of soil management and of application of any kinds of organic fertilisers influence and change physical properties of soil.

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When using a new form of fertiliser (i.e. the so-called digestate), it is necessary to define not only its doses but also the method of its application.

The term digestate is used as a name of the waste material remaining after the anaerobic digestion of a biodegradable feedstock in biogas stations. There are different kinds of digestate and that can be classified from different points of view, e.g. with regard to biodegraded raw materials, according to the method of their use or on the base of the content of dry matter (Marada et al., 2008). In contrast to well examined and exactly known kinds of biomass (e.g. well fermented sludge and compost), properties of digestate are not known in detail and also the methods of its use in agriculture still remain to be unexplored (Ferri et al., 2010; Tampone et al., 2010). In Table 3 and 4 is statistical evaluation of Bulk density and Content of water. The results are statistically significant between variants, years and depths Values of the coefficient of structurality are presented in Tab. 5. These values indicated that in Variant 1 there was a tendency to improving soil texture. In 2013, parameters of soil texture were generally better than in 2012.

It was possible that the application of the fore crop straw into the soil performed in 2012 contributed to this improvement of soil structure.

Table 3. Statistical evaluation of Bulk density

Source	SS	df	MS	F	p	sign.
Intercept	244.0294	1	244.0294	28585.36	0.000000	*
Year	0.0413	1	0.0413	4.84	0.029824	*
Variante	0.2658	1	0.2658	31.13	0.000000	***
Season	0.0473	1	0.0473	5.54	0.020247	*
Depth	0.1001	2	0.0500	5.86	0.003773	**
Error	0.9732	114	0.0085			

*** P = 0.001; **P = 0.01; *P = 0.05

Table 4. Statistical evaluation of Water content

Source	SS	Degr. of	MS	F	p	sign
Intercept	39884.69	1	39884.69	6250.222	0.000000	***
Year	572.43	1	572.43	89.703	0.000000	***
Variante	228.28	1	228.28	35.773	0.000000	***
Season	3534.61	1	3534.61	553.898	0.000000	***
Depth	76.25	2	38.12	5.974	0.003408	**
Error	727.47	114	6.38			

*** P = 0.001; **P = 0.01; *P = 0.05

Table 5. Coefficient of structurality (Budisov 2012, 2013)

Variants	Soil depth (m)	2012		2013	
		spring	autumn	spring	autumn
1	0-0.15	1.87	1.97	3.42	3.05
	0.15-0.30	1.90	2.49	2.62	3.87
	Mean	1.88	2.21	2.98	3.42
2	0-0.15	2.01	1.87	1.08	2.08
	0.15-0.30	1.82	1.88	1.14	2.96
	Mean	1.91	1.88	1.11	2.46

Table 6. Statistical evaluation of Structure

Source	SS	Degr. of	MS	F	p
Intercept	81.10104	1	81.10104	234.5237	0.000000
Year	1.21034	1	1.21034	3.5000	0.088191
Variante	2.51817	1	2.51817	7.2819	0.020712
Season	1.16006	1	1.16006	3.3546	0.094209
Depth	0.11358	1	0.11358	0.3284	0.578106
Error	3.80393	11	0.34581		

Differences between variants weren't statistically significant (Tab.6). The C_{org}/N_t ratios calculated for both variants of tillage are presented in Tab. 7. On the one hand, a well-balanced carbon-to-nitrogen ratio is important for the decomposition of organic matter contained in soil while on the other it is also closely associated with the reduced bulk density. As it is well-known that the rate of organic matter decomposition is dependent on the C_{org}/N_t ratio, organic compounds with the C_{org}/N_t below 10:1 are decomposed very quickly (and are very easily available for soil microorganisms) while those with the C_{org}/N_t ratio above 50:1 are decomposed very slowly (Váňa, 1994). Results obtained within the framework of our experiments indicated that the rate of organic matter decomposition was nearly the same in both variants of tillage. In 2013, a slightly increased C_{org}/N_t ratio was recorded in deeper soil layers in Variant 2. Properties of the digestate are dependent on the quality and parameters of materials used for anaerobic digestion. Contents of other elements are usually different and depend on the properties of the fermented feedstock (Dostál, Richter, 2008). It was found, that values of soil reaction in year 2013 were decreased in Variant 1. This process is associated with the decomposition of organic matter to mineral that are for plants more available (Pokorný et al., 1998). It is therefore probable that the digestate caused an acidification of soil in ploughed Variant 1.

Table 7. C_{org}/N_t ratio in variants with different methods of tillage and digestate application (Budisov 2012, 2013)

Variants	Soil depth (m)	2012		2013	
		spring	autumn	spring	autumn
1	0-0.15	10	10	10	10
	0.15-0.30	13	11	9	9
	Mean	12	11	10	10
2	0-0.15	12	10	12	12
	0.15-0.30	13	13	16	13
	Mean	13	12	14	13

Conclusions

Results of a two-year monitoring of physical and also some chemical properties of soil in different variants of tillage and application of digestate indicate that basic physical properties of soil and its texture were influenced above all by the method of tillage.

In Variant with minimum tillage, the C_{org}/N_t, ratio was increased in the second experimental year. This means that in this variant the content of C organic substances was higher than in Variant with ploughing.

Acknowledgement

These results were obtained within the framework of a (partial) institutional support of the long-term conception of the research organisation and also thanks to the support provided by the Ministry of Agriculture of the Czech Republic within the framework of the research project NAZV QJ1210263.

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APPLICATION OF MEASURES OF GOOD AGRICULTURAL PRACTICE TO CONTROL DIFFUSE N POLLUTION ORIGINATED FROM LIVESTOCK MANURE

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Abstract

The polluting effects of organic farm wastes can occur in a number of different ways and therefore require a broad range of approaches to control. For example, all watercourses (notably lakes, ponds, rivers, streams and field ditches) adjacent to the production, storage or application of organic wastes are potentially at risk of point source pollution. This risk is different from the diffuse pollution that occurs when the microbial breakdown of manure applied to the soil occurs out of phase with the N uptake of a growing crop and leads to nitrate leaching. Bosnia and Herzegovina is divided into basic river basins as follows: Una, Vrbas, Bosna and Drina flow to the Sava River, which drains into the Danube, while the Neretva, Trebišnjica and Cetina flow to the Adriatic Sea. The biggest emission of organic pollution, then nitrogen and phosphorous is from the Bosna River basin (around 20%). This is followed by Una (15%), Sava (13%), Drina (12.5%), and Vrbas (around 10%). About 30% of above mentioned pollutants goes to the catchment of Adriatic Sea. The spatial distribution of emission is in accordance with the size of the river basin, and the size of the river basin is in accordance with other parameters (population, agricultural activities, cattle-breeding). Exceptions are the river basins of Sava and Ukrina with a bigger concentration of population and more intensive agricultural activities.

Keywords: manure, nitrogen, pollution, best agricultural practices, organic farming

Introduction

In the EU Countries various policy instruments and practical measures are currently used and/or are in preparation for implementation in the countries in order to promote the control of water pollution by agriculture (e.g. to implement national policy objectives, prepare for joining EU or comply with international conventions). These include: regulatory, economic and advisory/informative instruments and measures.

The current policy objectives and strategies of Bosnia and Herzegovina concerning the control of water pollution and other ecosystems caused by agriculture do not exist (red zone). This includes the point sources of pollution and diffuse sources of pollutions. When we talk about "water pollution by agriculture" we mean the presence of harmful substances in water which is caused by agricultural activity. This includes substances that are derived from: i) agrochemical inputs, such as mineral fertilizers and pesticides, that are used deliberately by farmers to improve crop and animal production, ii) farm wastes, such as silage effluent and animal manure, that are produced during usual agricultural activities, iii) natural processes, such as soil erosion, that are enhanced by usual agricultural activities.

Sustainable management of manure and organic matter in general could lead to the conclusion that the value of Soil Condition Index (SCI) could indicate negative, stable or improved organic matter trend condition in soil (Čustović et al., 2006). The implementation of pollution control measures at the farm level will only be successful and sustainable if the farmers can determine that it is in their economic interest to undertake such measures. While livestock manures were traditionally viewed as a valuable soil improver and important source of nutrients, changes in agricultural practices over the last 60 years have caused that manures are now regarded as a serious waste product. This is particularly the case where there is an imbalance between land suitable for livestock manure application and the rapid growth and intensification of confined livestock. Water is the primary medium for transporting diffuse pollution (Ćerić et al., 2003). Average number of rainy days in Bosnia and

Herzegovina is about 130 or 35%. Distribution of precipitation, characteristics of soil, phase of growth and development of crops and evapotranspiration are the main factors influencing the leaching of nitrogen (Mesić et al., 2007).

Materials and methods

Statistical data on livestock were analyzed and production of manure and quantity of nitrogen were calculated based on average values for all animal categories. Analysis was made for 2002 and 2011 according to the entities (Federation of BiH (FBiH) and Republika Srpska (RS)), and the aggregate values were interpreted for the entire Bosnia and Herzegovina (hereinafter BiH).The analyzed data were collected from statistical sources at the entity, cantonal and municipality levels. The values of total nitrogen in kg t^{-1} are the average values obtained by the Kjeldahl analysis.

Results and discussion

State of soil and production of manure in Bosnia and Herzegovina

By delimitation from 1985 BiH is divided into four agricultural regions: mountain region (57%); hilly (27%); flat (11%); and, Mediterranean region (5%). The huge heterogeneity of soil, relief, climate, geology, hydrology and hydrographic network in BiH, uncontrolled cultivation of sloped terrains, plowing of sloped grassland are just some of the very complex issues related to soil and water use and management.

BiH covers 51,129 km^2. In total area of BiH there are 2,533,000 hectares of agricultural land. Arable land takes up 1,593,000 ha or 62.9% of total agricultural area and non-cultivable plots take up 940,000 ha or 37.1%.

Table 1: Number of livestock in BiH in 2002 and 2011 (thousand)

Categories of livestock	BiH year		FBiH year		RS year	
	2011	2002	2011	2002	2011	**2002**
Cattle	455	459	213	223	236	235
Sheep	1,021	821	520	375	499	445
Pigs	577	168	87	81	483	88
Horses	19	22	649	13	12	9
Goats	65	50	38	40	27	10
Poultry	18,703	7,142	8,789	4,514	9,653	2,628

Table 2: Estimated Manure Production and Nitrogen in BiH in 2011

Type of livestock	Number of animals (thousand)	Fresh manure per animal in t/year	Total manure production in t/year	Quantity of N in kg t^{-1} of manure	Total N production from manure in t/year
Cattle	455	8.7	3,954,000	4.5	17,793
Sheep	1 021	0.53	540,360	10.0	5,404
Pigs	577	1.18	681,480	5.0	3,407
Horses	19	4.8	91,200	5.5	502
Goats	65	0.7	45,500	10.0	455
Poultry	18 703	0.006	112,218	15.0	1,683
Total			5,424,758		29,244

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From a total of 1,009,000 hectares of ploughed fields and gardens, the sowed area in 2011 totaled 527,000 ha or 52.2%, about 4,000 ha was under nurseries, while 478,000 ha or 47.4% were fallow and uncultivated area (Agency for statistics, 2012).

Organic fertilizers – manure is a serious source of water pollution as well as pollution of environment in general, but on the other hand it is a considerable source of biogenic elements required by plants. Tables 1 and 2 show data on livestock numbers and quantity of produced manure and nitrogen.

In the past decade there has been an increase in number of sheep by 24%, pigs by 343% and poultry by 261%. Produced quantities of manure and nitrogen from it represent significant sources of diffuse pollution whose ultimate destination are the watercourses. In addition to manure, a lot of mineral fertilizers such as urea, 27% KAN and complex NPK fertilizers, usually 7:14:21 and 15:15:15 are used in BiH. However, the quantities of mineral fertilizers used in some crops are still very low, untimely applied and with inadequate NPK ratio.

According to some information, mineral fertilizers are applied on arable land, primarily ploughland, in the amount of 30 kg of P, 30 kg of K and about 50 kg of N per hectare, which is only 1/3 of the required dose. The reason is of an economic nature and has to do with poor position of the agrarian sector in society. Manure has been increasingly used in organic farming and production of vegetables in greenhouses and outdoors.

Emission of pollutants by catchments

BiH is divided into basic river basins as follows: Una, Vrbas, Bosna and Drina flow to the Sava River, which flows into the Danube, while the Neretva, Trebišnjica and Cetina flow to the Adriatic Sea. The biggest emission of organic pollution, then nitrogen and phosphorous comes from the Bosna River basin (around 20%). This is followed by Una (15%), Sava (13%), Drina (12.5%), and Vrbas (around 10%). About 30% of above mentioned pollutants goes to the catchment of Adriatic Sea. All watercourses should reach "Good Ecological Status" by 2015 with the exception of four water bodies on the Bosna River. All bodies under pressure were classified as "Exposed to risk in the first approximation". Because of this it is necessary to develop a strategy of sustainable soil and water management and make agriculture a generator instead of a victim of development. In such conditions, the issues of managing animal manure and other animal and human waste in rural areas will be not easy to solve.

The best practices of managing diffuse pollution can be grouped into structural, such as the construction of appropriate basins for the collection and storage of manure, change of method and timing of soil tillage, application of organic and mineral fertilizers, sowing structure (Šarić et al., 2004); and non-structural ones which pertain to the prevention of pollution through education, institutional measures etc. (Ćerić et al., 2003).

The application of measures of good agricultural practice aimed at preventing the formation of sources of diffuse pollution is necessary if we are to protect the water, soil and nature. In our circumstances this can be achieved by introducing the measures of good agricultural practice through integrated production and organic farming. At the state level of BiH, the area under organic farming, both certified and in the process of conversions, is 681 ha. This area is cultivated by 92 organic farmers (GIZ, 2012). Organic farmers try to be self-sufficient when it comes to crop nutrition and thus aim to ensure that as many nutrients as possible are recycled on the farm. Consequently, manure and slurry from housed livestock are carefully managed during storage and application to minimize nutrient losses.

Crop rotation is a fundamental measure suitable to maintain and improve soil fertility, stabilize humification and mineralization processes, increase water and nutrient efficiency, microbial activity of soil and nitrogen intake. In organic farming a principle has been established "plough shallow, hoe deep". The principle is to turn soils as little as possible. This implies that the most suitable way of tilling the soil is through various soil-protecting methods, using cultivators and various attachables, rotating harrows, etc. Generally, on lighter soils and in wet conditions, farmyard fertilizer is applied deep, while on heavier soils and in drier conditions only shallow. It is advisable to fertilize with organic fertilizers more often, i.e. in intervals of 2-3 years and with smaller doses (Šarapatka et al., 2009). If the vegetation is seen as a barrier to surface and underground flows of nutrients, the role of vegetation cover in many strategies to control diffuse pollution sources becomes very important. Growing of cover crops of oat and rye in alternation with corn and soybean leads to the reduction of nitrate losses for more than 70% (Longsdon et al., 2002, cit. Oljača and Dolijanović, 2013).

Conclusions

Diffuse pollution from agriculture, especially from organic manures, poses a considerable problem in Bosnia and Herzegovina as it has never been addressed institutionally, and the increase in livestock can only contribute to an increase of diffuse pollution of water from agriculture.

The application of measures of good agricultural practice through organic farming can be the directions of agricultural development that will contribute to the reduction of such pollution, especially with regard to the fact that this type of production has been certified and that the protection of environment is one of the postulates on which it operates, and the possibilities for its expansion in the territory of BiH are there.

Diffuse pollution is a wide spread problem which affects the quality of surface and ground water in both rural and urban areas. Agricultural activities that cause diffuse pollution are mainly related to livestock farming. Livestock farming causes soil compaction, excessive grazing and overproduction of manure. The situation is further aggravated by the application of mineral fertilizers, protective and other chemicals, while the size of the load will depend on the type and amount of applied matters, type of soil and scale and intensity of precipitation. Diffuse pollution can be discharged into (or transported over) the land surface, atmosphere and rivers, lakes and ground waters.

Acknowledgements

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EFFECTS OF FERTILIZATION ON SOME QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF WINTER WHEAT IN GREAT CUMANIA

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Abstract

Due to the drastically increasing food prices during the last few years in Europe and also in Hungary, good and medium quality winter wheat varieties produced at low costs with high yield potential came to the front. Although food overproduction is characteristic to one part, while shortage of food to the other part of the world, the newest tendencies show an increasing role of the production and consumption of high quality food that can be produced only from high quality raw materials. When a new variety is certified, the qualitative parameters are more and more important beyond the quantitative ones. Several programmes were started to breed and produce winter wheat varieties with excellent qualitative parameters including the determination of the adequate, variety- and habitat specific agro-techniques and plant nutrition. In Karcag Research Institute plant breeding and long-term fertilization experiments have been carried out separately from one another for several decades. In the vegetation period of 2012/2013 a new series of experiments was started that includes the determination of the fertilizer doses optimal for our high quality winter wheat varieties with high yield potential. The determination of the adequate and optimal fertilizer doses are essential not only from economic point of view and lower environmental loads, but also for the most effective utilization of the genetic potential of our winter wheat varieties without the degradation of the qualitative parameters. After the assessment of the data we established that extreme high doses of fertilizers are needed for the spectacular improvement of the parameters we examined. As the financial possibilities of farmers are limited, the fertilizer cost is a determining factor: they have to consider if the yield or the quality of a given variety can be increased at an affordable fertilizer input. Our goal is to determine the often empiric hence not so accurate fertilizer doses more precisely providing the producers a proper production technology and plant nutrition recipe adequate to the varieties bred by us. Due to the complexity of this topic high number of further examinations is required.

Keywords: winter wheat, fertilization, yield, Falling Number, Zeleny Sedimentation Value

Introduction

The region of Great Cumania is of great importance from the point of view of high quality winter wheat production in Hungary. The main goal of our investigations is to determine how to produce winter wheat varieties bred for the extreme agroecological conditions of the region providing such quantitative and qualitative parameters that make them attractive to the farmers. We used conventional agro-techniques but variety-specific fertilizer doses in our experiment.

One of the most cardinal elements of wheat production is the seeds, as not only their quantity but the quality is very important, therefore seeds must be produced at the highest level as possible (Jolánkai et al., 2006). The main goal of seed production is to gain high quality seeds demanded by the market economically. This goal must be in harmony with the purpose of the actual plant breeding research (Klupács and Tarnawa, 2007).

According to Peterson at al. (1998) environmental factors have significant effect on the quality of winter wheat, but the wheats with different genotypes have different reaction to the environmental effects. The conditions of wheat growing are considerably determined by the soil and climatic features. The effects of these features are cannot or hardly be influenced, even they basically determine the aim of the production, the species and variety of the plant to be produced, the applicable agrotechnical operations and the economy of the activity (Jolánkai et al., 2004).

At the recent level of agriculture plant nutrition (mainly provided in the form of artificial

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fertilizers) is of great importance for winter wheat (Ragasits et al., 2000). Wheat is a nutrient-demanding plant with good nutrient reaction. The biggest problem of wheat nutrition (mainly with nitrogen) is the fact that the optimal interval of nutrient supply is narrower compared to other plants, therefore over- or underdosage can happen very easily.

The nutrient demand of wheat is considered similar by wheat growing experts, 25-30 kg N, 10-15 kg P_2O_5 és 15-20 kg K_2O substances are needed for 1 t of grain yield and the accompanying straw and roots as an average (Pepó, 2009).

Not only the detailed examination of species specific-, but variety specific fertilizer reaction is also important, as the N-demand and nutrient reaction of wheat varieties with different genotypes are significantly different (Pepó, 2001).

It is well known from the national and international literature that different doses and rates of nutrients are needed for the optimal plant nutrition at different habitats, hence the importance of differentiated, habitat-specific nutrition is emphasized by several experts (Debreczeniné and Ragasits, 1996).

Materials and methods

The indicator crop was Hunor, a winter wheat variety bred in Karcag Research Institute. It was registered in 1996, but it is still very popular among wheat growers due to its morphologic and agrotechnical features.

It is a waxy variety with medium maturity and short-medium height. It has excellent stem stability, winter- and drought tolerance. It has good fertilizer reaction and yield capacity with Harvest-index of 45-46%. Its Farinograph value is A2 - B1. It has medium sensitivity for most of the well-known diseases of winter wheat.

A small-plot field trial with 4 replications was set on a meadow chernozem soil in the territory of Karcag Research Institute using the data base of the National Long-term Fertilization Experiments and the data of production year of 2012/2013 (Table 1.).

More than 15 quantitative and qualitative parameters were examined during the investigation, among them yield, plant height, thousand grain weight (TGW), hectoliter test weight, Zeleny-index and the falling number are analyzed in this paper.

Table 1. The investigated treatments of the experiment

Treatments (kg ha^{-1})	N_0	N_{50}	N_{100}	N_{150}	N_{200}
P0	x	x	x	x	x
P50		x	x	x	x
P100		x	x	x	x
P150			x	x	x
P200			x	x	x

Methods applied to determine the parameters: quantitative parameters (yield, plant height, TGW, hectoliter test weight) were simply measured by means of a grain counter and a HL-balance. For the flour quality examinations the Zeleny-index was determined according to the relevant Hungarian standard (MSZ ISO 5529:1993). The falling number was determined by means of a Perten system.

The results gained from the experiment were processed by means of Microsoft Excel spreadsheet application and assessed according to Sváb (1973).

Results and discussion

The basic meteorological data of the investigated growing season (2012/2013) are summarized in Table 2.

According to Bocz et al. (1992) the water demand of winter wheat in winter is 140-150 mm, while its total water demand is 400-450 mm. Birkás and Gyuricza (2001) quantified 280-340 mm water that is needed in the period from March to July for winter wheat.

On the base of these data it can be said that the investigated period was sufficient from the point of view of water supply with its 496.7 mm

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total amount of precipitation, of which 135.1 mm fell in winter and 302.3 mm in the period from March to July.

The yields have linearly increased parallel to the increase of the fertilizer doses, both in the cases of nitrogen and phosphorus. At the level of 50 kg ha^{-1} P-dose a slight decrease of yield was experienced compared to the plots not treated with P, but this difference was not significant (Fig. 1.).

The effect of fertilization on TGW was less considerable than in the case of yield. Ragasits (2001) had similar experiences on a Ramann type brown forest soil in Keszthely. The increase of TGW due to the higher doses of fertilization is so small that it hardly exceeds the SD level (Fig. 2.).

The highest value was measured at 200 kg ha^{-1} nitrogen and 0 kg ha^{-1} phosphorus level. Nevertheless it can be established that the increasing P-doses resulted in the decrease of TGW – significant difference could be figured out between the plots at P_0-N_{200} level.

Assessing the plant height data, it can be established that the higher fertilizer doses

Table 2. The basic meteorological data of the investigated growing season

	Oct. 2012.	Nov. 2012.	Dec. 2012.	Jan. 2013.	Febr. 2013.	Mar. 2013.	Apr. 2013.	May 2013.	June 2013
Avg.temp. (°C)	11.8	6.9	-0.7	-0.3	2.6	3.8	12.8	17.3	20.4
Precipitation (mm)	40.6	18.7	41.6	42.5	51.0	110.2	47.3	81.9	62.9

Source: Karcag Research Institute of University of Debrecen

Figure 1. Grain yields in the function of N and P fertilization

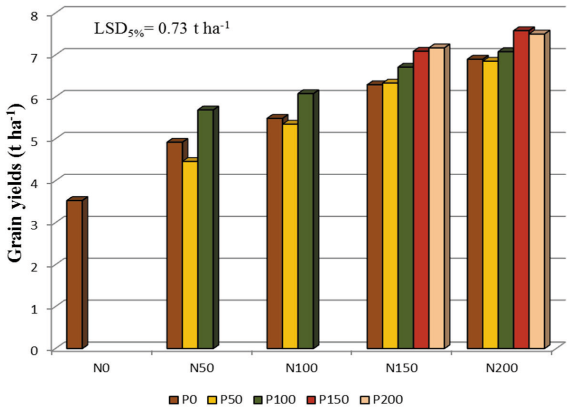


Figure 2. Thousand grain weights in the function of N and P fertilization

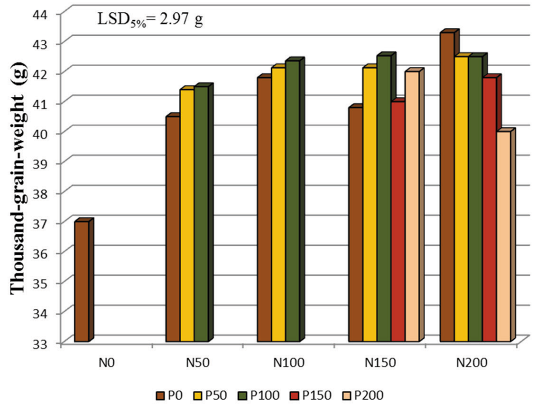


Figure 3. Plant height in the functionof N and P fertilization

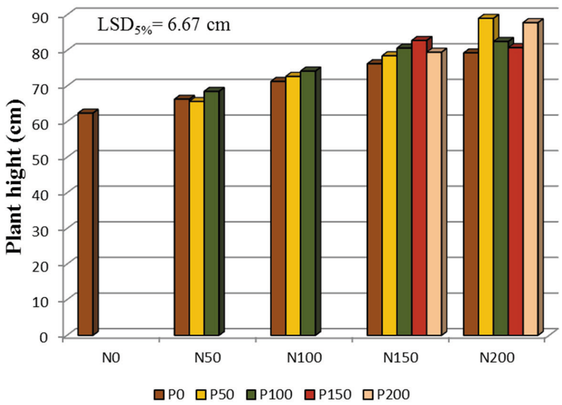


Figure 4. Test weights in the functionof N and P fertilization

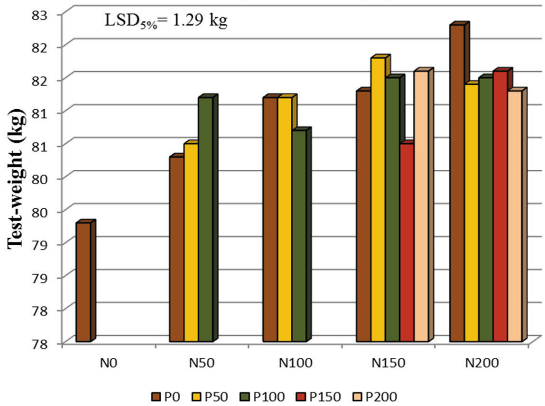


Figure 5. Zeleny value in the function

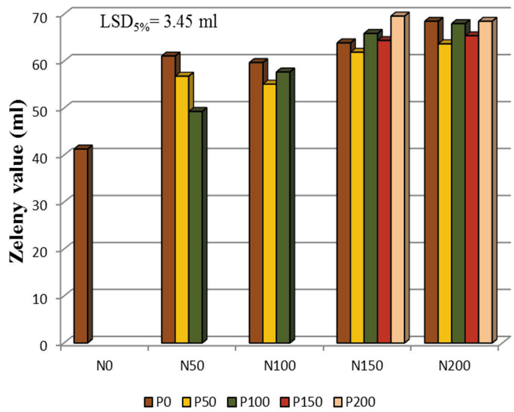
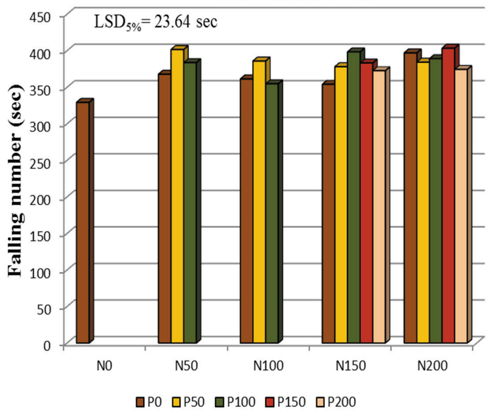


Figure 6. Falling number in the function of N and P fertilization



resulted in significantly higher plant height. The tallest plants were almost 50% taller than the shortest ones (Fig. 3.). Nevertheless the increase of plant height is not desired by the practice as it has several unfavourable effects (especially for the farmers who has no livestock and cannot utilize the higher amount of straw resulting in extra work and costs). The higher the plants, the higher is their sensitivity to lodging due to the less stem stability resulting in harder harvest and lower quality straw. The higher amount of straw also results in lower Harvest-index and drought tolerance.

The test weight of the various wheat varieties is a particularly important parameter for the mill industry as this value characterizes the milling yield very well. On the base of our results it could be established that there is no considerable effect of fertilization on this physical parameter, no correlation could be figured out. Though the higher N-doses resulted in a slight increase of the test weight of wheat, decrease was detected when phosphorus was added too (Fig. 4.).

In the case of the Zeleny sedimentation value definite improvement was detected due to N-fertilization (Fig. 5.).

The higher N-doses resulted in 50% higher values compared to the untreated control. At lower N-doses (50-100 kg ha^{-1}) our experience was the same as Ragasits's (2001), the increase of P-doses resulted in lower Zeleny values.

The Falling Number method is the most popular method for determining sprout damage. It is cited in several national and international papers that the value of falling number is mainly determined by the year effect, beyond the genetic variability of wheat.

Its explanation is that the sprouting germination is caused by damp or rainy weather conditions during the final stage of maturation of the crop; the germination causes an accelerated starch degrading. We also found that fertilization had not a significant effect on the α-amylase activity (Fig. 6.).

Conclusions

Plant nutrition is only one of the several factors of utilizing the genetic potential of wheat varieties. On the base of our experiment it could be established that extreme high doses of fertilizers (150-200 kg ha^{-1} substance) are needed to a considerable increase of the investigated parameters. Environmental protection and economic points of view must be taken into account beyond genetic potential and year effect.

The financial possibilities of the famers are limited, they consider the extra costs needed for the extra yield (marginal cost) when make their decisions about wheat production knowing the

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upper limit of fertilization costs that affordable and benefits in higher quantity and quality. Our goals are to specify these empiric and often inaccurate fertilizer doses applied in the practice and to provide a growing technology and plant nutrition recipe adequate to our winter wheat varieties used regularly by the farmers and producers. Due to the complexity of this issue, further examinations are needed to achieve these goals.

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THE WATER USE EFFICIENCY OF MAIZE DEPENDING ON ABIOTIC STRESS FACTORS IN FIELD EXPERIMENTS

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Abstract

There is little direct information about the effects of the abiotic stress factors such as low soil water content on the photosynthesis system of field crops. Some recent publications pay attention to this field of research. Water stress has significant effect on the yield and other agronomic parameters of maize. The aim of our work was to get more data about the relations between water supply and the assimilation parameters. The photosynthetic gas exchange parameters of maize are remarkably improved by nutrient supply in well watered conditions. The water stress through decreased stomatal conductance has significant negative effect on the assimilation parameters of the crops. The obtained results suggest that the water use efficiency of the maize is higher in dry conditions. In well water supply state maize uses up to 300 per cent more water for 1 g CO_2 assimilation.

Keywords: maize, water use efficiency, abiotic stress

Introduction

There are some articles dealing with photosynthesis system and the water use efficiency topics of maize published in the last decades (Raschke, 1970, Lu and Zhang, 2000, Hirashawa, 1999, Kutasy and Csajbók, 2009, Csajbók and Kutasy, 2012). Shangguan et al. (2000) wrote that the nutrient and water supply has significant effect on the photosynthetic gas exchange of the plant. The better nitrogen supply results in poorer water use efficiency comparing to the lower nitrogen supply conditions, due to the high rate decreasing in photosynthetic activity. Janda et al. (1998) studied the effect of temperature in the growing period on the net photosynthesis rate of inbred maize lines. They found, that at optimal temperature there were no significant differences between the maize lines in the net photosynthesis rate, but after cold treatment the net photosynthesis rate of the lines with lower cold tolerance reduced significantly. Kang et al (2000) implemented a two years study on the effect of water stress on the photosynthesis rate of maize leaf. They stated that the reduced photosynthesis of the water-stressed leaf recovered its previous level three days after irrigation applied. Ben-Asher et al. (2008) studied the transpiration and photosynthetic activity of sweet corn in climatic chambers. Their results show that increasing temperature causes higher transpiration and decrease the photosynthesis intensity (with 1 µmol m^{-2} s^{-1} by 1 °C temperature increasing).

Materials and methods

The measurements were carried out between 1999 and 2013 at the Látókép research site of the Debrecen University in small plot (15.4 m^2) experiments. The soil of the experimental area is calciferous chernozem. The soil specific plasticity index (KA) was 43; the pH value was nearly neutral (pH_{KCl}=6.46) and it has favourable water regime. The minimal water storing capacity is 808 mm in the 0-200 cm layer. The unavailable water content is 295 m in the 0-200 cm layer. The amount of available water in saturated state is 513 mm in the 0-200 cm layer of which 342 mm is readily available. The watertable is at 8-10 meters depth.
The set crop rotations: triculture (winter wheat – maize – pea), biculture (winter wheat – maize), monoculture: maize.

Fertilization levels:
control: $N_0P_0K_0$, $N_{120}P_{90}K_{90}$ kg ha^{-1}

Assimilation parameters were measured in the field by the LICOR LI-6400 portable photosynthesis device.

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It has two infrared gas analyzers to measure CO_2 and H_2O mole fraction in air. The light was controlled in the sample chamber, we used 2000 μmol photon m^{-2} s^{-1} PAR, with 90 % red (630 nm) and 10 % blue (470 nm) light. There is a contact thermometer in the leaf chamber to measure leaf temperature. We measured light adapted leaves, six times per leaf, in four repetitions. The water use efficiency parameters were calculated from the measured data (WUE g CO_2 kg^{-1} H_2O) and (1/WUE kg H_2O kg^{-1} CO_2). We analyzed and evaluated the data of experimental results with the SPSS 22.0 statistical software package. The accuracy of the statistical analysis was given at the level of $LSD_{5\%}$ according to the method of Sváb (1981). The results were evaluated with analysis of variance, and Pearson's correlation analysis.

Results and discussion

To present the water supply state of maize in the studied years we calculated the potential (PET) and actual evapo-transpiration (AET) (Szász, 1997) and their ratio. 2000, 2002, 2007, 2009, 2012 were very dry years, the PET:AET ratio was very low in these growing seasons. On the contrary the water supply in 2004, 2005,

Figure 1. Estimated PET and AET values, PET:AET ratio and the precipitation in maize growing season (Látókép, 1999-2013)

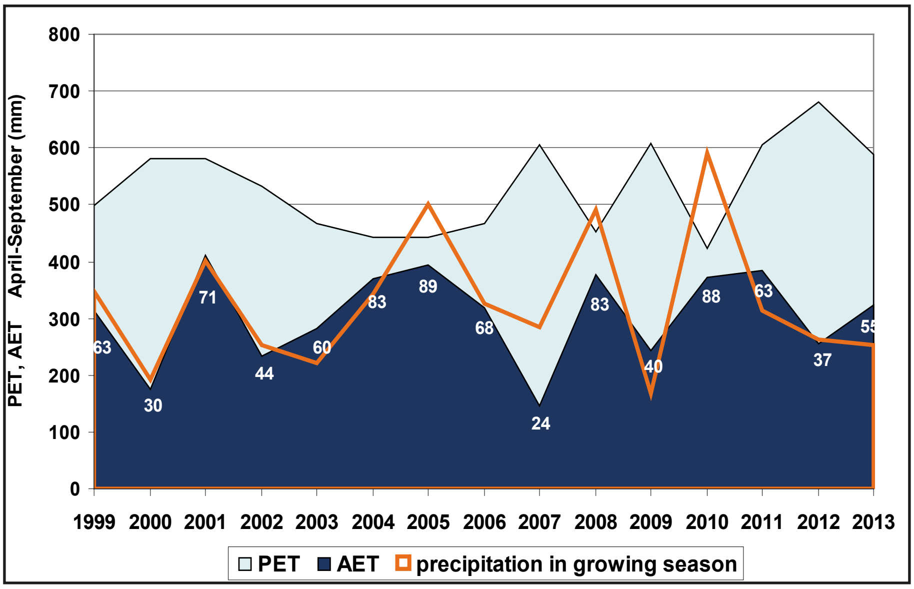

Figure 2. The estimated potential (PET) and actual evapotranspiration (AET) values and the difference between them in maize (Látókép, 2013)

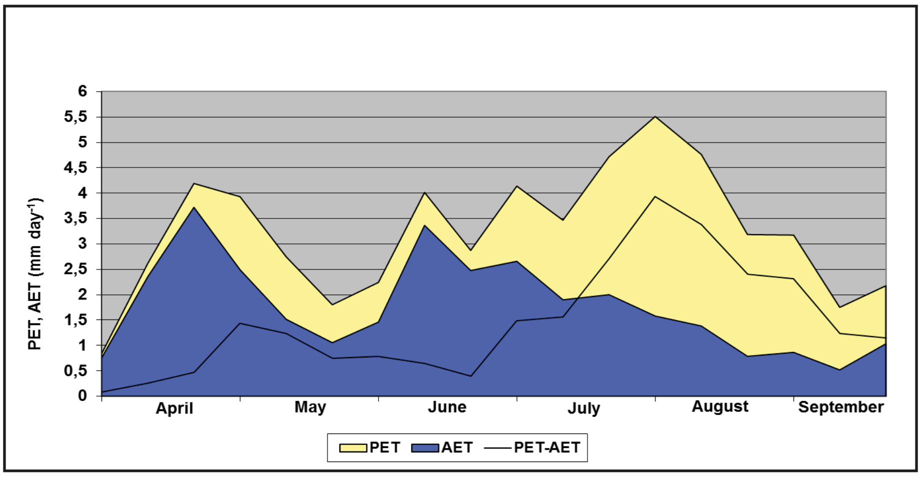

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Table 1 Correlations between the transpiration, the water use efficiency and the measured photosynthesis parameters of maize (r values of Pearson correlation) (Látókép, 04 07 2013)

		Cond	Trmmol	1/WUE	tair-tleaf
Monoculture	Cond(1)	1	0.990	-0.949	0.689
	Trmmol(2)	0.990	1	-0.950	0.599
	1/WUE(3)	-0.949	-0.950	1	-0.638
	tair-tleaf(4)	0.689	0.599	-0.638	1
Biculture	Cond(1)	1	0,971	-0.900	0.761
	Trmmol(2)	0,971	1	-0.935	0.598
	1/WUE(3)	-0.900	-0.935	1	-0.544
	tair-tleaf(4)	0.761	0.598	-0.544	1
Triculture	Cond(1)	1	0.980	-0.948	0.800
	Trmmol(2)	0.980	1	-0.961	0.683
	1/WUE(3)	-0.948	-0.961	1	-0.654
	tair-tleaf(4)	0.800	0.683	-0.654	1

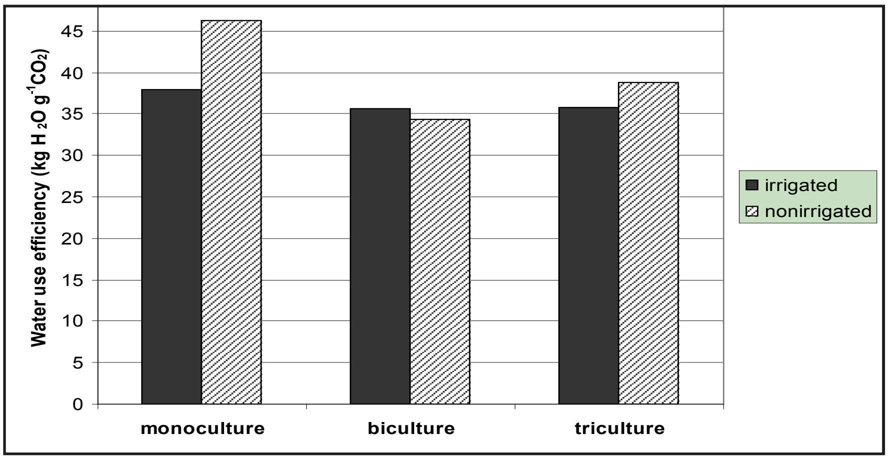
1: stomatal conductance (mol H_2O m^{-2} s^{-1}), 2: transpiration (mmol H_2O m^{-2} s^{-1}), 3: water use efficiency (kg H_2O $g^{-1}CO_2$), 4: air temperature – leaf temperature (°C). The correlation coefficient values are significant at P=5% level in every above cases.

2008 and 2010 was very good to maize (Figure 1). In 2013 the first half of the growing season was favourable regarding to water supply, but from July it was very dry, and the AET:PET ratio was only 27.5% in August (Figure 2).

We did analysis of water use efficiency of maize in different crop rotation varieties. Analyzing the assimilated CO2 value pro one liter transpired water is a useful method of characterizing the water use efficiency of the crops. Our data show that there are significant strong positive correlation between stomatal conductance and transpiration, significant strong negative correlation between transpiration and water use efficiency in every crop rotation variations (Table 1). The difference between air and leaf temperature shows significant positive correlations to the transpiration and the stomatal conductance and negative correlation to the water use efficiency.

The water use efficiency was higher in 2013 (38.14 g CO_2 kg^{-1} H_2O) than that of in wet 2010 (23.33 g CO_2 kg^{-1} H_2O). There were significant differences between the crop rotation varieties, monoculture: 42.09 g CO_2 kg^{-1} H_2O, biculture: 35.01 g CO_2 kg^{-1} H_2O and the triculture: 37.31 g CO_2 kg^{-1} H_2O ($LSD_{5\%}$=1.09). As monoculture means unfavourable water supply comparing to the biculture, this data

Figure 3 Water use efficiency of maize in different crop rotation variations (Látókép, 2013)



coincide with results of our previous researches under remarkably different water supply showing that maize use water with much less efficiency under favourable water supplying conditions than in dry years. Maize transpires 150-260% more water to one gram CO_2 assimilation than in dry years or in water stress (Figure 3). The irrigation had significant effect on the water use efficiency of maize in the experiment. The greatest effect we measured in monoculture (non irrigated: 46.27 g CO_2 kg^{-1} H_2O, irrigated: 37.91 g CO_2 kg^{-1} H_2O). The better water supply caused significantly lower efficiency in water use.The difference was lower in the triculture rotation (non irrigated: 38.84 g CO_2 kg^{-1} H_2O, irrigated: 35.62 g CO_2 kg^{-1} H_2O) and the lowest difference was in the biculture variation in water use efficiency (non irrigated: 34.39 g CO_2 kg^{-1} H_2O, irrigated: 35.62 g CO_2 kg-1 H_2O).

We also made measurements in experiments on nutrient supply relating to the water use of maize. In 2003 the plants in control plots transpired more water to assimilate 1 g CO_2 as did the plants in fertilized plots in the first 3 measurement dates (Figure 4). The adequate nutrient supply increased the efficiency of water use of maize. At the fourth

Figure 4. Effect of nutrient supply on the water use efficiency of maize (Debrecen, 2003)

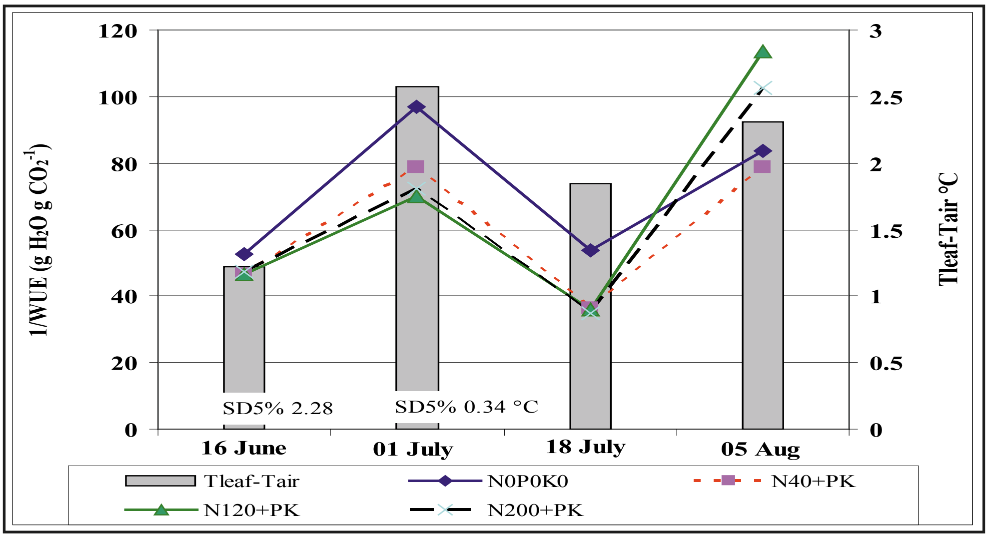


Figure 5. The effect of light intensity on the water use efficiency of maize, wheat and some weeds (Látókép, 2004)

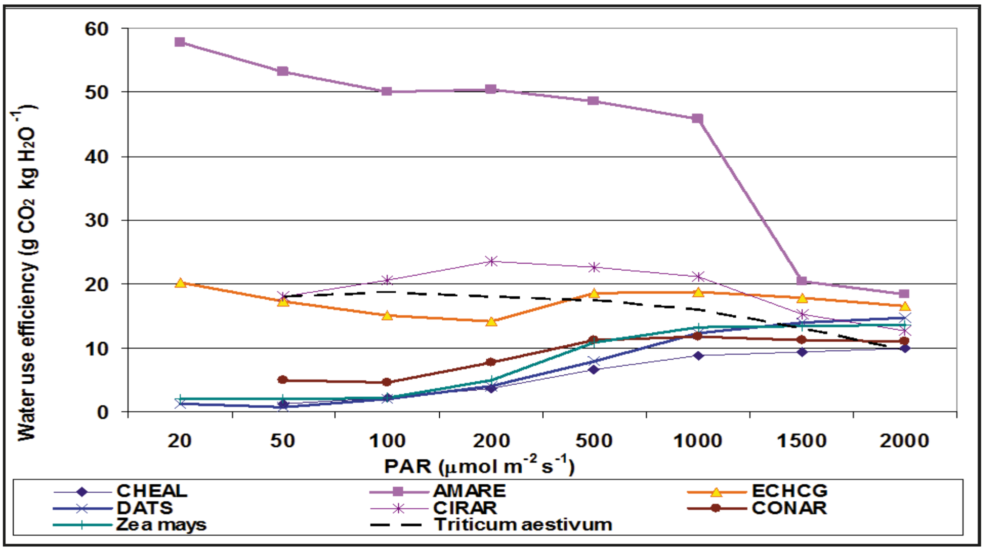


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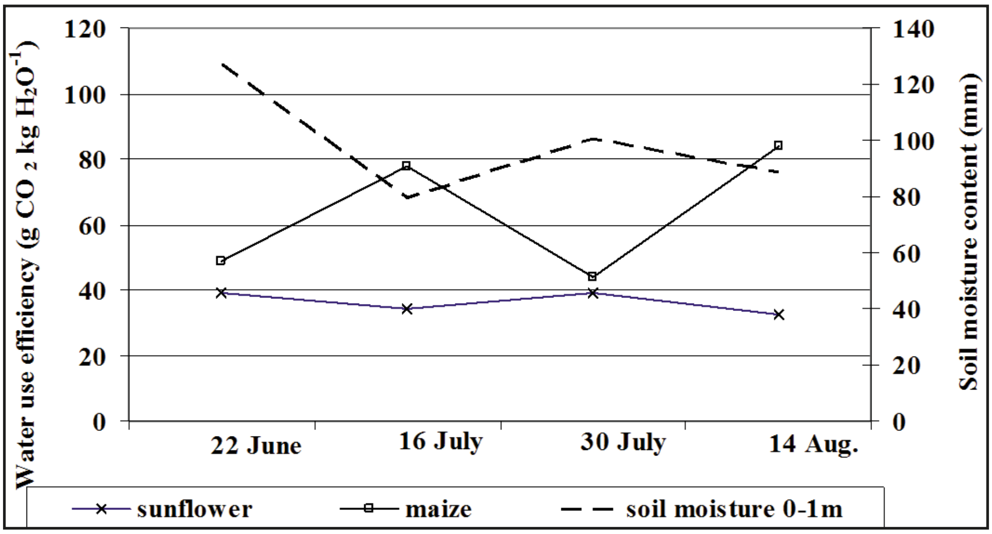
Figure 6 Water use efficiency of maize and sunflower in relation to soil moisture (Debrecen, 2007)



date the higher fertilization levels caused lower water use efficiency. The fertilization has significant effect on the water use efficiency in the monoculture only ($N_0P_0K_0$: 39.52 g CO_2 kg-1 H2O, $N_{120}P_{90}K_{90}$: 44.66 g CO_2 kg^{-1} H_2O). In 2004 we also recorded photosynthesis light curve of some crops and significant weeds. The plants with C4 photosynthesis way can be separated clearly at higher light intensity levels. Figure 5 shows the effect of light intensity on water use efficiency. Increasing light intensity resulted in better water use efficiency of maize. Below the 200 µmol m^{-2} s^{-1} PAR light intensity maize had the lowest net photosynthesis rate beside Datura stramonium and Chenopodium album. Amaranthus retroflexus plants used water much more effectively below 1500 µmol m^{-2} s^{-1} PAR light intensity comparing to other studied weeds or crops.

Table 2 Water use efficiency of maize in different cropyears (Látókép, 2007-2013)

Years	WUE ($g\ CO_2\ kg\ H_2O^{-1}$)	per cent
2010	23.33	100
2011	52.88	227
2012	36.19	155
2013	61.52	264
2007	77.82	334

We analyzed the soil moisture content and the difference of air and leaf temperature in maize and sunflower in 2007. The temperature difference follows the soil moisture change in case of maize, but in sunflower does not. Sunflower could cool its leaves with transpiration in opposite to maize. Sunflower transpirated more water to fix 1 g CO_2 and there was almost no connection between its water use efficiency and soil moisture. In case of maize the higher soil moisture content resulted in lower efficiency of water use, and the low soil moisture resulted in higher efficiency (Figure 6).

The water use efficiency data of maize in the last four years show that the lowest efficiency was in 2010, a year with very good water supply. In droughty years like 2012 and 2013 the efficiency was much better. And the data of very droughty 2007 prove this statement (Table 2).

Conclusions

We found significant, close positive connection between the difference of leaf and air temperature and the water use efficiency of maize. The warmer the leaf comparing to the air, the more the transpirated water to assimilate one unit CO_2. The nutrient supply caused significant difference in the water use efficiency of maize. The lowest efficiency was in the control ($N_0P_0K_0$) plots, the differences were 10-35%. The nutrient supply

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increased the efficiency of water use of maize. We proved negative connection between the water use efficiency of maize and the soil moisture content in the droughty 2007 year. The higher the moisture content of the soil, the lower the water use efficiency. The increasing light intensity resulted in better water use efficiency of maize up to 1000 µmol photon m^{-2} s^{-1} PAR level. In dry conditions maize uses water very effectively, while the good water supply results in lowering efficiency of water use. Maize transpires 150-300% more water to assimilate 1 g CO_2 in wet years, comparing to dry years or water stress state. The irrigation had significant effect on the water use efficiency of maize, the greatest effect we measured in monoculture.

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THE HISTORY OF BROOMCORN MILLET (*Panicum miliaceum* L.) IN THECARPATHIAN-BASININTHEMIRROROFARCHAEOBOTANICAL REMAINS I. FROM THE BEGINNING UNTIL THE ROMAN AGE

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Abstract

The domestication of broomcorn millet based on latest archaeobotanical investigations occurred in the arid areas of North China and in same time in Central Asia. The knowledge of cultivation of broomcorn millet the Carpathian Basin before the 6th thousand BC. Since this time in all archaeological ages are available but in different frequency. Broomcorn millet was a widespread and favoured cereal. Knowing the eating habits of nomadic and semi-nomadic people, this find is expected, as broomcorn millet is a favoured cereal with a short growing season and rapid development requiring relatively little tending. This is expected as broomcorn millet was a key crop for Hungarians in the Middle Ages. A significant amount was grown traditionally by Hungarians through time until the appearance of maize, the new gruel plant. In the first part will be present the history of broomcorn millet in the Carpathian Basin from the beginning until the Roman Age.

Keywords: broomcorn millet, archaeobotany, macroremains, Carpathian-Basin, prehistorical ages

Material and Method

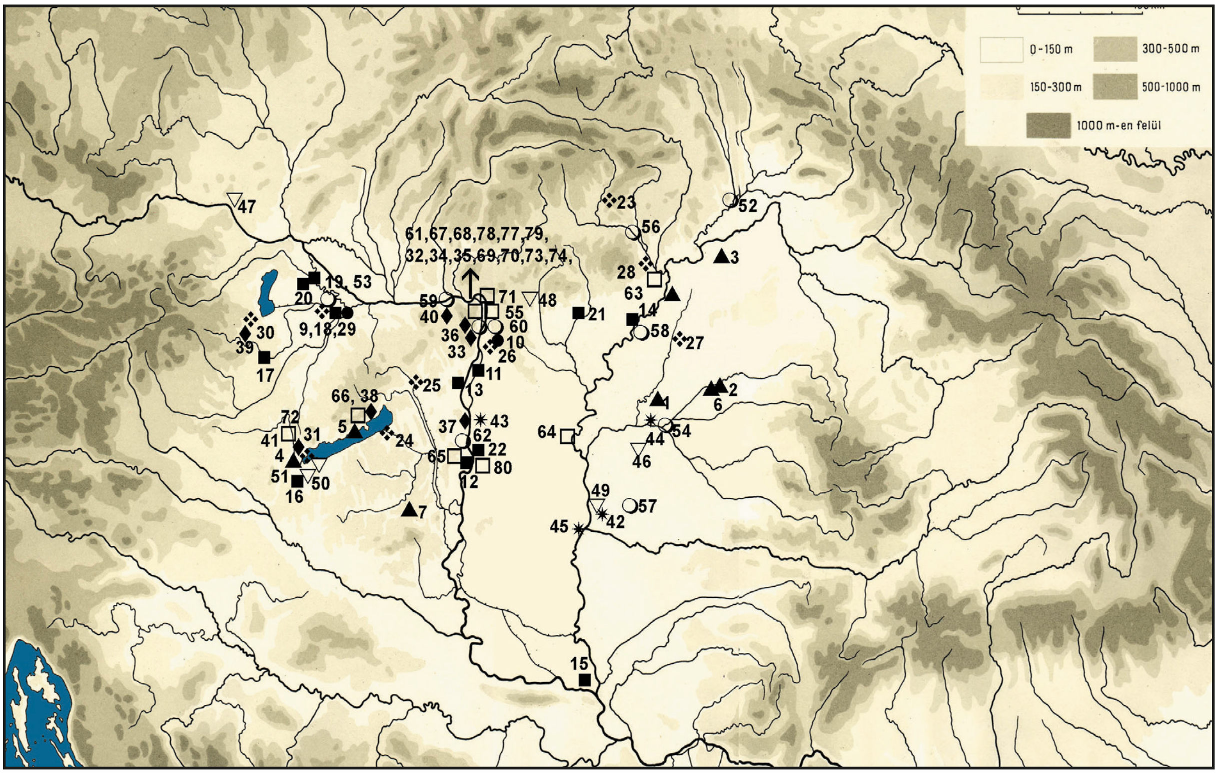
Archaeobotany or palaeo-ethnobotany is the science of the identification of plant remains and plant products. Its main area of investigation is the history of plant cultivation. It studies the relationship between human beings and flora especially as they relate to human economic activities. In addition to the identification of cultivated plant remains, it monitors the transformation of wild species into cultivated plants and the spread of plant cultivation and agriculture in general. Furthermore, it evaluates the images of plants originating from various eras, the decultivation of plant species and the data of the various social sciences associated with plants. Archaeobotany, as the study of plant macrofossils (seeds and fruits) obtained from archaeological excavations, becomes particularly important when there is very little or no archaeological artifacts related to agriculture, written or iconographical material available about the cultivation of plants found. This is particularly the case in relation to prehistoric cultures of the Carpathian Basin.

Archaeobotany is a branch of botany. All the elements of botany, i.e. morphology, taxonomy, anatomy and geobotany are used in the course of identification of the materials from archaeological excavations. In addition, it plays a „bridging" role within the system of sciences as it is intimately connected with the science of archaeology as well.

Archaeobotanical investigation has demonstrated convincingly that the Carpathian Basin is one of the longest inhabited parts of Europe. Cultivated plants arrived in the Carpathian Basin with the first Neolithic agrarian culture some eight thousand years ago. Archaeobotanical investigation has demonstrated convincingly that the Carpathian Basin is one of the longest inhabited parts of Europe. Cultivated plants arrived in the Carpathian Basin with the first Neolithic agrarian culture some eight thousand years ago.

Archaeobotany is an interdisciplinary scientific field, an important tool for understanding prehistoric ways of life, indeed the only source of agricultural history until the Roman Age. Plant remains allow us to infer botanical knowledge, methods of farming, dietary habits and environments of people of those periods.
Plant remains are rarely found in the excavation of archaeological sites. Such organic materials are unstable and in natural conditions they are decomposed very quickly by microorganisms. However, they may be preserved in extraordinary

Fig 1. Broomcorn millet sites in the Carpathian Basin



Neolithic (6000-4300 BC) ▲

▲ 1 Ecsegfalva
▲2 Berettyóújfalu Nagy Bócs-dűlő
▲3 Ibrány-Nagyerdő Huda-tábla
▲4 Alsópáhok-Kátyánalja dűlő
▲5 Zánka Vasútállomás
▲6 Berettyóújfalu-Herpály
▲7 Lengyel
▲8 Polgár-Csőszhalom

Eneolithic or Copper Age (4300-3000 BC) ●

● 9 Győr-Szabadrétdomb
● 10 Rákoskeresztúr-Újmajor

Bronze Age (3000-900 BC) ■

■ 11 Szigetszentmiklós Vízmű
■ 12 Bölcske-Vörösgyír
■ 13 Százhalombatta-Földvár
■ 14 Poroszló-Aponhát
■ 15 Mošorin-Feudvár
■ 16 Balatonmagyaród-Hídvégpuszta
■ 17 Gór-Kápolnadomb
■ 18 Börcs-Paphomlok
■ 19 Mosonmagyaróvár-Németbánya
■ 20 Mosonmagyaróvár-Németdűlő
■ 21 Ludas, Varjú-dűlő
■ 22 Solt-Tételhegy

Iron Age (900 BC-1st Century AD) ❖

23 ❖ Aggtelek-Baradla barlang
24 ❖Siófok-Balatonszéplak
25 ❖ Fehérvárcsurgó-Eresztvény erdő
26 ❖ Rákoskeresztúr-Újmajor
27 ❖ Ebes Zsong-völgy
28 ❖ Miskolc-Hejő
29 ❖ Mosonszentmiklós-Pálmajor
30 ❖ Sopron-Krautacker
31 ❖ Keszthely-Fenékpuszta

Roman Age (1st-5th Century AD) ♦

32 ♦ Budakalász-Luppa csárda
33 ♦ Budapest-Körte Street
34 ♦ Leányfalu-Móricz Zsigmond Street
35 ♦ Óbuda, Bécsi Street 38–42
36 ♦ Óbuda Corvin Square
37 ♦ Dunaújváros,
38 ♦ Nemesvámos-Balácapuszta
39 ♦ Sopron-Beloiannisz Square and Városház Street
40 ♦ Tokod
41 ♦ Keszthely-Fenékpuszta

Barbaricum (1st-5th Century AD) ✷

42 ✷ Hódmezővásárhely-Solt Palé
43 ✷ Szalkaszentmárton-Dögtemető
44 ✷ Endrőd No. 170.
45 ✷ Kiskundorozsma-Nagyszék

Migration Period (5th-9th Century AD) ▽

46 ▽ Eperjes-Csikóstábla
47 ▽ Devín (Dévény)
48 ▽ Szirák
49 ▽ Szegvár-Oromdűlő
50 ▽ Fonyód-Bélatelep
51 ▽ Zalavár-Vársziget

Hungarian Conquest time and Arpad-Age (9th-13th Century AD) ❍

52 ❍ Zemplin (Zemplén)
53 ❍ Lébény-Billedomb
54 ❍ Gyomaendrőd
55 ❍ Rákospalota-Újmajor
56 ❍ Edelény-Földvár
57 ❍ Kardoskút
58 ❍ Tiszaörvény
59 ❍ Esztergom-Kovácsi
60 ❍ Rákospalota-Újmajor
61 ❍ Budapest I. Hunyadi Street 22.
62 ❍ Solt-Tételhegy

Late Medieval Age (13th-17th C. AD) ☐

63 ☐ Muhi
64 ☐ Lászlófalva-Szentkirály
65 ☐ Dunaföldvár-Öregtorony
66 ☐ Nagyvázsony-Csepely
67 ☐ Budapest I. Dísz tér 10.
68 ☐ Budapest I. Úri utca 40.
69 ☐ Budapest I. Dísz tér 8.
70 ☐ Budapest I. Hunyadi utca 22.
71 ☐ Vác Piac utca
72 ☐ Sümeg-Sarvaly
73 ☐ Budapest I. Színház utca
74 ☐ Budapest II. Medve utca
75 ☐ Nagyvázsony-Csepely
76 ☐ Baj Öregkovács hegy
77 ☐ Budapest I. Military Headquarters (volt Honvédelmi Főparancsnokság)
78 ☐ Budapest I. Teleki palota
79 ☐ Budapest I. Kapucinusok utca
80 ☐ Solt-Tételhegy

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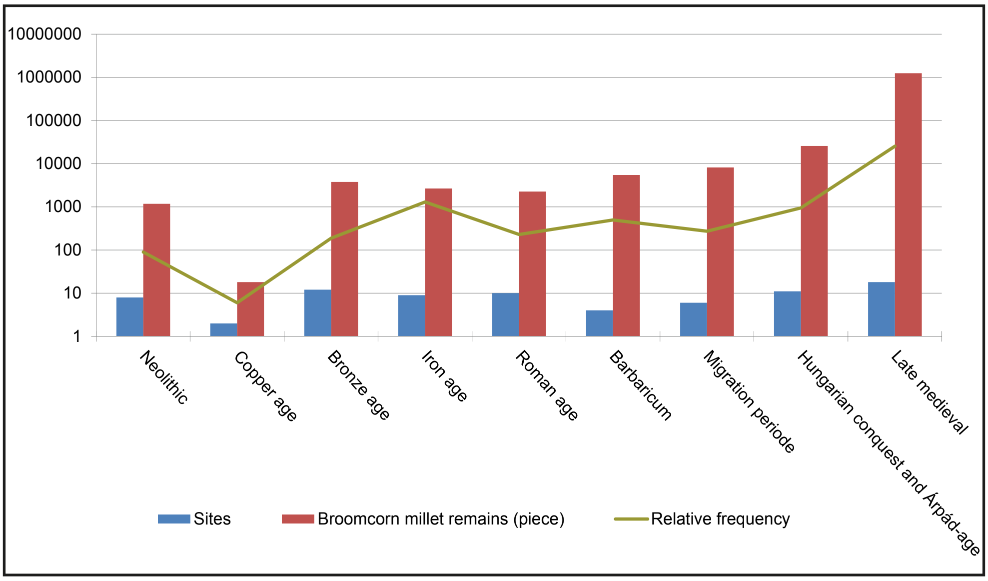
Fig. 2. The most important date of broomcorn millet in the Carpathian-Basin.



conditions (e.g., charring by fire, immersion in water, extremely dry microclimates). Furthermore, the recognition of such remains requires extensive experience, systematic sampling, accurate flotation and identification. The archaeobotanical research in Hungary more than 150 years goes back. During this long time 50 researchers were activ in this topic and 500 archaeological sites have been processed. 10 million piece seeds of 700 plant taxons (mostly species) were identified from the neolithic to the Middle Ages in the Carpathian Basin.

During this time up to now 1,294,209 piece broomcorm millet were found in 80 settlements from the Neolithic until Late Medieval (Fig. 1). But these distribution by ages are different. (Fig. 2).

Results and Discussion

Phylogenetics of broomcorn millet

The origin and place of domestication of broomcorn millet (*Panicum miliaceum* L.) are yet to be established. Its wild form has not been determined with certainty. It may have descended from the *P. spontaneum* Lyssev ex Zhuk. species that occurs in Kazakhstan, Mongolia, Northern China and Afghanistan (de Candolle 1894; Soó 1973).

Following Mansfeld (1986), the broomcorn millet genus is divided into three groups (in: Bányai 1971):

1. wide-clustered broomcorn millets (*Panicum miliaceum* L. convar. *effusum* Alef., -> *Panicum miliaceum* L. var. *effusum* Alef.
2. side-curving, banner-clustered broomcorn millets (*Panicum miliaceum* L. convar. *contractum* Alef.,
3. compact-clustered broomcorn millets (*Panicum miliaceum* L. convar. *compactum* Koern.

In addition the Multilingual Multiscript Plant Name Database distinguishes in the Broomborn Millet Group the following items:

- *Panicum miliaceum* L. convar. ‚*Aureum*' Alef.
- *Panicum miliaceum* L. convar. ‚*Sanguineum'* Alef.
- *Panicum miliaceum* L. subsp. *agricola Scholz & Mikolás*
- *Panicum miliaceum* L. subsp. *miliaceum* sensu Tsvelev.
- *Panicum miliaceum* L. subsp. *ruderale* (Kitag.) Tzvelev.
- *Panicum miliaceum* L. var. *flavum* Schur.

They can also be classified on the basis of the colour of their hulls: white, yellow, red, brown, grey.

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Broomcorn millet is a tetraploid (2n=36), self-fertilizing cultivated plant. Its growing season is very short (60–90 days). It is sown in the spring, but a second, summer sowing also ripens. It tolerates extreme conditions (heat, poor soils, drought) well. During threshing and cleaning, the buds often break off. It is characteristic of the seed that the socket of the scutellum is shorter than half the length of the seed (Schermann 1966). Today, broomcorn millet has lost much of its significance. It has more or less disappeared from Europe. It is primarily cultivated in Eastern and Central Asia, India and parts of the Middle East.

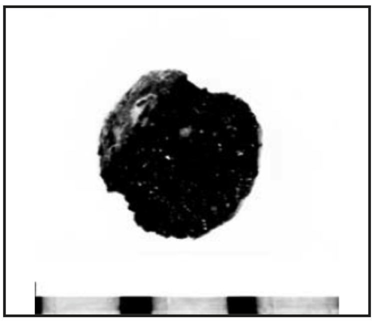
According to recent research in Cishan sites of Central Asian (9^{th} –7^{th} thousands BC) P. miliaceum phytolith residues were found (Hunt et al. 2008). Consequently the cultivation of millet can be started in northeastern China and the Loess Plateau before the beginning of 8^{th} thousand BC (Crawford 2009; Lu et al., 2009). Knowledge of their production spread around the Yellow River valley and mountain areas around 6th thousand BC (Zhao 2005; Crawford et al., 2006; Liu et al., 2009). Before 5^{th} thousand BC in China, Caucasus, Syria, Egypt, East- and Middle Europe already from 41 sites are know the remains of the genus Panicum (*P. miliaceum, P. cf. miliaceum, Panicum sp., Panicum type, P. capillare* (?), *P. turgidum*) (Hunt et al. 2008). Anachronistics that the appear of broomcorn millet in Middle-Asia and East- and Middle-Europe overlapping his appear in China. It is therefore likely that the domestication of millet occurred in the same time in Yellow River region and in Middle-Asia region (Lisitsina 1984; Zohary, Hopf & Weiss 2012).

The oldest remains of broomcorn millet are from Eastern and Central Europe: Chokl/Dagestan (beginnigs of 7–6th thousands BC) (Amirkhanov 1987), Arukhlo/Georgia (8000-7150 cal BP) (Lisitsina 1984), Sacarovka/Ukraine (Starčevo-Körös culture, 7600-7500 cal BP) (Janushevich 1984). Some millet grains were also found in Luca Vrublevecaja and Soroki/Ukrajna (Tripolje culture) (Janushevich 1976), Blahutovice/Czech Republic (Tempír 1979) and Eizenberg/Thuringia (5^{th} millennium BC) (Rothmaler & Natho 1957), Gomolava/Jugoslavia (4^{th} millennium BC) (van Zeist 1975), Northern Italy (3^{rd} millennium BC) (Villaret-von Rochow 1958). Broomcorn millet has been shown to have existed in Central Asia since the 3^{rd} millennium Bronze Age (Lisitsina & Prisepenko 1977). More recently it has been found at the Shortungha site in Afghanistan (end of 3^{rd}, beginning of 2^{nd} millennium BC) (Willcox 1991). Other uncertain finds may be broomcorn millet: Tepe Yahya/Iran (5^{th} millennium BC) (Costantini & Costantini-Biasini 1985), Georgia (Neolithic: 5^{th}–4^{th} millennium BC) (Lisitsina 1984), Northern China (Neolithic, Yang-Shao culture: 4^{th} millennium BC) (Ho 1977).

History of broomcorn millet from the beginning until Roman Age

In case of the early archaeobotanical founds the knowledge of cultivation of broomcorn millet reached the Carpathian-Basen at the beginning of 6th thousand BC. Evidence for this one that millet appear in the sites of Criş (Körös) culture in the beginning of 6th thousand BC (Glăvăneştii Vechi) and also be present in Vădastra (second half of 6^{th} thousand BC) (Comşa 1996). But the spread of millet towards Europe our country has played an important role. The Körös sites (6^{th} thousand BC) are the earliest occurrence of broomcorn millet in Hungary (Gyulai 2010a). Only one millet grain was found in Ecsegfalva (Békés county) in excavations campaign between 1999¬-2001 (Bogaard, Bending & Jones 2007). In Berettyóújfalu Nagy Bócs-dűlő Körös site two grain millet were available in the year 2004-2005 (Dani et al. 2006). In this case Ibrány-Nagyerdő Huda-tábla Körös sites was a surprise. Here were

Figure 3. Broomcorn millet grain from Ibrány-Nagyerdő Photo: Á. Kenéz



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found in 2008 10 piece millet grain (Domboróczki & Raczky 2010; Gyulai 2010b) (Fig. 3).

The material from the so-called Transdanubian group of Middle Neolithic Linearband Ceramic culture (LBK) is closely related to the cultural region covering Western and Central Europe (Füzes 1990). In 1966, during the construction of a new road connecting Alsópáhok and Felsőpáhok, in the part called the Alsópáhok-Kátyánalja dűlő, mud-flakes were collected as the construction cut into a pit from the more recent phase of the Transdanubian linear band pottery population (Bakay, Kalicz & Sági 1966, site 1/20). The surface of the mud-flakes from the bottom of the pit showed imprints of broomcorn millet. In 1964, during the reconstruction of the train station at Zánka, a number of Neolithic pits were unearthed (Bakay, Kalicz & Sági 1966, site 60/10) (Fig. 4). Unexpectedly large quantities of carbonised remains of many cereals including broomcorn millet were found (Hartyányi, Nováki & Patay 1967/68).

Figure 4. Broomcorn millet naked grains from the Linearbandceramic culture settlement in Zánka.
After Füzes 1990.

In addition to hulled wheats (einkorn, emmer), broomcorn millet was also found at the Middle Neolithic Berettyóújfalu-Herpály site (Nándor Kalicz and Pál Raczky's excavation 1978–82). According to 14C testing, the estimated date of the Berettyóújfalu-Herpály site is 6570–6270 BP (Hertelendi et al., 1997).

Late Neolithic sites in Hungary are relatively rich in broomcorn millet remains. These archaeobotanical finds are generally carbonised recovered from burnt-out houses and various waste or grain storage pits.

Plant remains from sites associated with the Lengyel culture in the Transdanubian region indicate that the population still cultivated plants, but less intensively than in the previous era. At the Lengyel site, which gave the culture its name, Mór Wosinsky conducted several excavations between 1885 and 1890.

The dating of the findings raised several subsequent problems (Hartyányi, Nováki & Patay 1967/68). In 1890, Imre Deininger himself collected seeds there. His identification of the seeds from the vicinity of fireplaces, pits and from pots was published in 1892. He found here broomcorn millet as well.

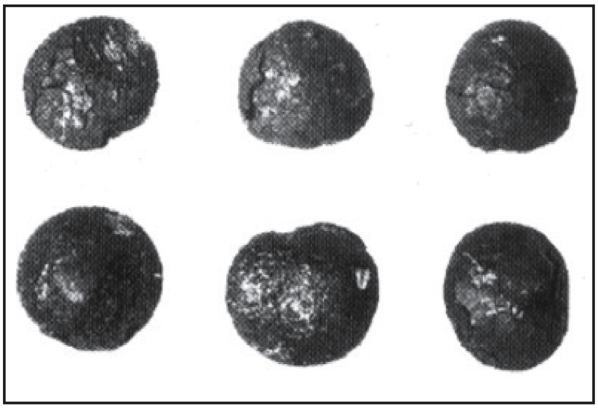
All botanical samples from the tell settlements of Tisza-Herpály-Berettyóvölgy contain broomcorn millet. At the 1995 excavation, led by Pál Raczky, we collected a large number of soil samples from the floor levels of houses and various pits found in the Late Neolithic tell and in the settlement around the tell. According to radiocarbon dating, the estimated date of the Polgár-Csőszhalom site is 6700–6370 BP (Hertelendi et al. 1997). Sporadic occurrences of broomcorn millet grains were also found.

During an archaeological survey between 1991 and 1994 prior to the extension of the M1 motorway around Győr, András Figler found a site containing material from several periods of the Copper Age (in: Gyulai 2010a). The samples from Győr-Szabadrétdomb of the Ludanice – Balaton-Lasinja culture contained plant remains indicating that the cultivation of cereals was restricted to two species during that era, barley and broomcorn millet. Material recovered from the Bolerazi layer of Győr-Szabadrétdomb contains many more cereal remains. Broomcorn millet was an important meal plant.

During the excavation of the Rákoskeresztúr-Újmajor site, the soil samples from the Ludanice culture pits only yielded a few dozen seeds (excavation by Zoltán Bencze and Zsuzsa M. Virág 1995–96). Yet barley and fast growing, spring-sown and rather modest broomcorn millet was still possible and important in those conditions.

The extreme, cool and rainy climate improved in the Late Copper Age. Probably for the bad climatic conditions of the Late Copper age broomcorn millet still missing in the Baden culture. As a result of recent research, we are now able to report more botanical finds associated with the Early Bronze Age Bell-Baker Culture (2600-2200 BC) population. In 1999, Anna Endrődi found a new site containing bell-shaped pottery in the area of the Szigetszentmiklós Waterworks. We flotated the soil samples and recovered grains of broomcorn millet (Gyulai 2003). Broomcorn millet only occurs in two Middle Bronze Age sites: Bölcske-Vörösgyír

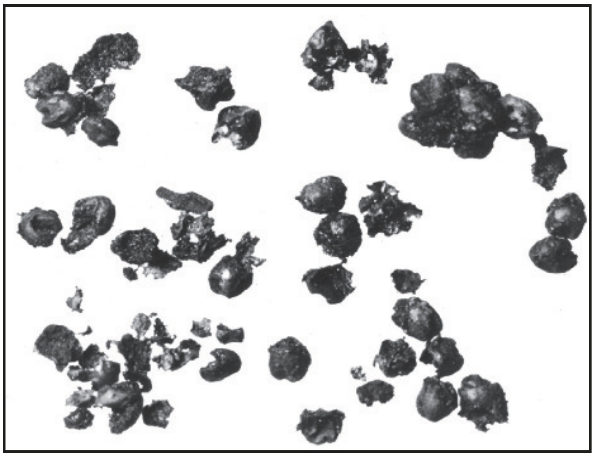
Figure 5. Broomcorn millet naked grains from the Bölcske-Vörösgyír Middle Bronze Age tell settlement. Photograph by T. Kádas.



and Százhalombatta-Földvár (Gyulai 1996a). That was the Vatya culture period in which the cultivation of broomcorn millet began to spread in the Carpathian Basin (Fig. 5).

The Late Bronze Age Urnfield culture was a cultural trend and a material culture that extended to a large part of Europe (Harding 1987). Agriculture was the foundation of an efficient production method. Plant species from the environment of the site indicate a warm, continental climate of the Subatlantic phases which is similar to today’s climate. People conducted self-sufficient crop production. As the flour of broomcorn millet is unsuitable for making bread, those cereals were primarily used to make meal.

Figure 6. Broomcorn millet naked grains from Poroszló-Aponhát, Inventory of the Hungarian Agricultural Museum, Budapest.



According to seeds found at the Poroszló-Aponhát site of the Gáva culture, hulled wheats (einkorn, emmer) and broomcorn millet continued to play an important role in the Great Plain (Hartyányi, Nováki & Patay 1967/68) (Fig. 6).

Seeds, found in the middle Urngrave layer (12th to 9th centuries BC) of the Mošorin-Feudvár tell settlement near the Tisza River, provide an excellent picture of the plant cultivation culture of the Late Bronze Age – Early Iron Age (Kroll 1990). In addition to barley, rye, wheat, broomcorn millet were also cultivated. In 1987, during the excavation of the Tumulus culture settlement of Balatonmagyaród-Hídvégpuszta, organic remains, seeds, including broomcorn millet grains fragments were found at the bottom of a refuse pit. Beside this, millet gruel remains and gruel crumbs consisting of a few naked, i.e. husked, millet grains were found. Based on recovered pottery fragments, the archaeologist László Horváth dated the remains to around 1200 BC. Organic materials floated from the Late Bronze Age refuse pit were identified as leftover foods including broomcorn millet meal remains (Gyulai 1996b) (Fig. 7).

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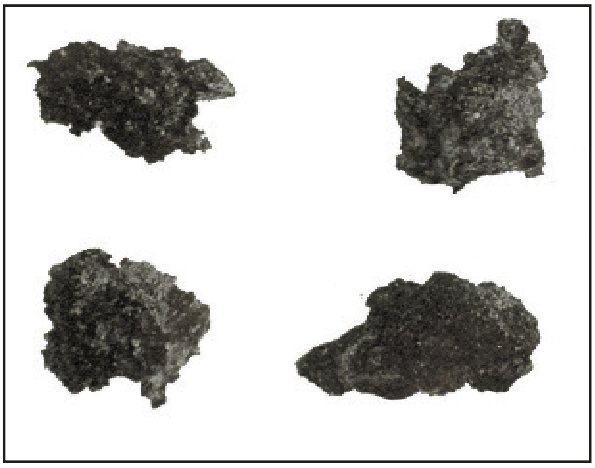
Figure 7. Millet porrige from a waste pit of the Late Bronze Age settlement of Balatonmagyaród-Hídvégpuszta. Photograph by T. Kádas.



During processing of the finds we found several slightly porous carbonised fragments of variable size from a single larger food item.

Archaeobotanical processing, the macroscopic analyses of Max Währen and the microscopic studies of Benno Richter as well as the instrumental analytical investigation performed by János Csapó (macro- and trace elements, amino acids and fatty acids) drew the conclusion that the fragments are remnants of a wild strawberry cake made using baking industry standard bread wheat and broomcorn millet flour with the addition of pig fat.

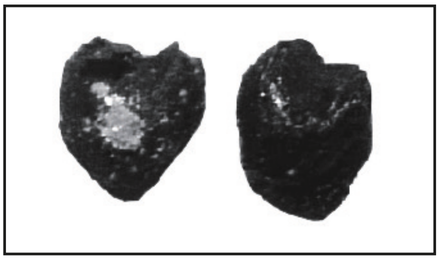
From 1989 to 1993, we floated a significant quantity of Late Bronze Age botanical material at an excavation by Gábor Ilon at Gór-Kápolnadomb (Gyulai & Torma 1993). The inhabitants of the settlement lived in a varied environment and conducted lively agricultural activities. A small number of broomcorn millet seeds indicates that it was not a very significant cereal. Naked, i.e. hulled broomcorn millet grains indicate that it was also processed.

Between 1994 and 1996, during an archaeological survey preceding the extension of the M1 motorway near Győr, several Late Bronze Age sites were excavated under the management of András Figler: Börcs-Paphomlok, Mosonmagyaróvár-Németbánya, Mosonmagyaróvár-Németdűlő. Quantity of cereal grains occurred in the following ranking: emmer, six-rowed barley, broomcorn millet, bread wheat. Broomcorn millet in Late Bronze Age settlements occurs in the largest quantity, but barley is also present. Excavation of a prehistoric settlement covering several tens of hectares began in 2001 at the Ludas, Varjú-dűlő site (Gyulai 2012a). The majority of objects found during the excavation led by László Domboróczky, Csilla Ács, Károly Tankó and Simon Gall belong to the Late Bronze Age Kyjatice culture. Fewer samples were taken from fireplaces. Obviously, broomcorn millet and emmer grains found there must have fallen down the side of the fireplaces during cooking and were slowly carbonised by the radiating heat. Millet gruel fragments consisting of a few pieces of grains.

Cereal remains from Early Iron Age Hallstatt sites in Hungary indicate that plant cultivation culture was by no means uniform. They cultivated six-rowed barley, emmer, spelt, common and club wheat and broomcorn millet. They grew significant quantities of broomcorn millet. The exact ages of archaeological layers explored at different places in the Aggtelek-Baradla cave system between 1876 and 1877 are almost impossible to determine. According to Hartyányi, Nováki & Patay (1967/68), graves in the "Bone house" and "Corridor" and the seeds found therein are from the Early Iron Age. Their opinion is based on radiocarbon tests of broomcorn millet: 2560 BP ± 75 years (a report by Mebus A. Geyh from 1967 in: Hartyányi, Nováki & Patay 1967/68). Imre Deininger (1881) classified seeds these contexts into ten cultivars; such as, common bread wheat, einkorn, broomcorn millet, naked barley. Miklós Füzes collected a few grains of broomcorn millet from the Early Iron Age burnt layer at Siófok-Balatonszéplak (in: Hartyányi, Nováki & Patay 1967/68). Éva F. Petres, Béla Jungbert and Tibor Kovács excavated nine Early Iron Age barrow graves in the territory of the Fehérvárcsurgó-Eresztvény forest between 1983–87. Earth samples collected there were processed in 1996 (Gyulai 2012b).

The burnt graves contained mainly carbonised grains of cereals: six-rowed barley, emmer bread wheat and broomcorn millet found likewise characterise Early Iron Age culture in Hungary. The broomcorn millet is in all scythian settlements available. The Rákoskeresztúr-Újmajor site excavated in 1996 by Anna Endrődi, has thus far provided the Scythian botanical findings from the Carpathian Basin (in: Gyulai 2010a). The most important cereal identified was six-rowed barley followed by broomcorn millet. It must be noted that the dominance of barley and broomcorn millet appears to be a typical feature of migrating, quickly moving nomadic peoples dealing with animal husbandry. Several soil samples were collected in 2003 from settlements of the Scythians in Ebes Zsong-völgy was excavated by János Dani. The number of broomcorn millet grains were three times more that of wheat grains. The same location also yielded fragments of milletgruel (Gyulai unpublished data). Melinda Hajdú conducted an excavation by Miskolc-Hejő in 2012. According to radiocarbon dating, the estimated date of the settlement is 490–390 BP. In the mud of one well broomcorn millet grain was found as well (Pósa et al. in print). All this demonstrates that the Scythians, although they did use wheats and grew them in a kind of ancient mixed grain, were preoccupied with the production of barley and broomcorn millet that better suited their way of life and traditions (Fig. 8).

Figure 8. Broomcorn millet naked grains from the Scythian settlement of Rákoskeresztúr-Újmajor. Photograph by the author.



It was also stated with regard to the botanical finds from Mosonszentmiklós-Pálmajor (András Figler's excavation 1993–94) that the composition of grain crops changed in the Celtic Period. Hulled wheat varieties were „neglected", and aside from six-rowed barley, common bread wheat and broomcorn millet are the staple crop. The samples taken from one of the farm buildings and a grave in Sopron-Krautacker from the Late La Tène (LT/C2-D) proved to be extremely rich in species. Sporadically, broomcorn millet is also found (Gyulai 2010a). Also Celtic samples collected and floated by Miklós Füzes in 1983 at Fenékpuszta, on the site excavated by István Erdélyi, containing a significant amount of carbonised grains with some broomcorn millet grains (Gyulai & Lakatos 2013).

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THE HISTORY OF BROOMCORN MILLET (*Panicum miliaceum* L.) IN THE CARPATHIAN-BASIN IN THE MIRROR OF ARCHAEOBOTANICAL REMAINS II. FROM THE ROMAN AGE UNTIL THE LATE MEDIEVAL AGE

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Abstract

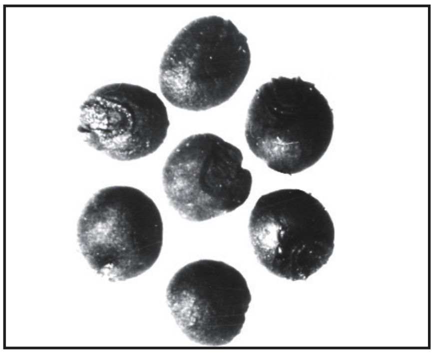
The domestication of broomcorn millet based on latest archaeobotanical investigations occurred in the arid areas of North China and in same time in Central Asia. The knowledge of cultivation of broomcorn millet the Carpathian Basin before the 6th thousand BC. Since this time in all archaeological ages are available but in different frequency. Broomcorn millet was a widespread and favoured cereal. Knowing the eating habits of nomadic and semi-nomadic people, this find is expected, as broomcorn millet is a favoured cereal with a short growing season and rapid development requiring relatively little tending. This is expected as broomcorn millet was a key crop for Hungarians in the Middle Ages. A significant amount was grown traditionally by Hungarians through time until the appearance of maize, the new gruel plant. In the second part will be present the history of broomcorn millet in the Carpathian Basin from the Roman Age until the Late Medieval Age.

Keywords: broomcorn millet, archaeobotany, macroremains, Carpathian-Basin, historical ages

History of broomcorn millet from the Migration Period until the Late Medieval

Broomcorn millet was also eaten in roman Pannonia (1st-4th Century AD) albeit its amount and significance fell behind those of wheat and rye. The majority of carbonised grains come from Late Roman barbicans and other military settlements (canabae): Budakalász-Luppa csárda, Budapest-Körte Street, Leányfalu-Móricz Zsigmond Street, Óbuda, Bécsi Street 38–42, Óbuda Corvin Square, but it can be found at other places as well: Dunaújváros, Nemesvámos-Balácapuszta, Sopron-Beloiannisz Square and Városház Street, Tokod (Hartyányi, Nováki & Patay 1967/68) (Fig. 1).

Figure 1. Broomcorn millet naked grains found on the peel of an oven in a Late Roman building, Budapest, Körte utca 29. Inventory of the Hungarian Agricultural Museum, Budapest.



According to the investigations made by Miklós Füzes in 1970-72 and in 1974, cereals account for the overwhelming majority of seed remains coming from the Late Roman Period in Keszthely-Fenékpuszta, naked barley occupying first place among them. There is somewhat less of common bread wheat and rye followed by the rarely seen broomcorn millet with common oat least. In 1993, at the western fortress gate of the Keszthely-Fenékpuszta fortress, at the excavation led by Róbert Müller, a substantial amount of Roman botanical material was collected (Gyulai & Kenéz 2009). Significant amounts of broomcorn millet as well gruel fragments were also found in by Orsolya Heinrich-Tamáska conducted excavations 2009 (Gyulai, Kenéz & Pető 2013).

While the high level crop production practices of the inhabitants in Pannonia are supported by considerable evidence, relatively little is known of the plant growing habits of the „barbarians" who lived in the Great Hungarian Plain. Addressing this issue will substantially improve our awareness of the culture and lifeways of these peoples.

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Their cropping culture must have differed from that of the Romans to a great extent, particularly in terms of the cultivars as no changes had taken place since the prehistoric ages (Hartyányi, Nováki & Patay 1967/68). Its naked grains were identified in the sites of Földeák, Garadna. Contemporary literature offers little information of the Sarmatian Period. Ptolemaeus wrote of their cities in the middle of the 2nd Century AD (in: Párducz 1971), while Plinius Secundus (Nat. hist. 18, 100) reports that their staple food was millet porridge mixed with horse milk and blood. According to archaeobotanical findings from the Late Sarmatian Period so far (Hódmezővásárhely-Solt Palé, Szalkaszentmárton-Dögtemető) their main crop was broomcorn millet, yet hulled emmer wheat was also cultivated (Hartyányi, Nováki & Patay 1967/68).

Botanical macrofinds, analysed between 1987 and 1990 by Dénes B. Jankovich, gave similar results to the pollen analysis at Endrőd No. 170. Botanical samples came from Sarmatian (4–5th Centuries) houses and pits of different uses. The relatively great number of barley and broomcorn millet finds suggest the survival of nomadic dietary patterns (Gyulai 2011).

Further significant results of Sarmatian archaeobotany were obtained by Csaba Szalontai and Katalin Tóth in 1998 and 1999 at the Kiskundorozsma-Nagyszék site dated to the 3rd–4th centuries AD. Their most important cereals were six-rowed barley and broomcorn millet, reflecting doubtlessly a continuation of their nomadic traditions (Gyulai 2003).

Botanical finds from the Carpathian Basin and surrounding countries indicate that in the Migration Period a much more modest crop production system replaced Roman agriculture. Broomcorn millet is dominant, a characteristic type of cereal for quickly moving nomadic people (Wasylikowa et al., 1991).

It is well known that the Huns, who settled in during the middle of the 5th century, were nomadic, warfaring people. Unfortunately, no botanical material has been recovered to date associated with their settlement in the territory of Hungary. We know from Priscos rhetoris' contemporary description that their food was made of broomcorn millet, their drinks of barley (in: Harmatta 1952).

Caches associated with the Gepids, a people who lived in the area beyond the Tisza, are explicit evidence of a farming lifestyle (Müller 1982). Cereals that were found in remains in Eperjes-Csikóstábla (excavations by Csanád Bálint 1976–77) in earth samples taken from the floor of a house destroyed by fire must have been grown locally. Most are broomcorn millet, the rest are common bread wheat and six-rowed barley grains (in: Gyulai 2010).

In 1986 excavations were carried out in Devín (Dévény), near Bratislava, Slovakia, along the limes at the time, at a settlement dated to the 5th century AD, populated by Danube Germans or maybe Kvads (Pieta 1988; Pieta & Plachá 1989). The composition of the cereals obviously stocked for kitchen use shows an advanced level of agriculture: 66% rye, 21% common bread wheat, 11% barley, 1.6% broomcorn millet.

We do not know much of the lifestyle of the Avars, the people moving in to the Carpathian Basin in 568 AD and again later in 670. It is thought that the main crop of the Avars was broomcorn millet, yet this does not mean that they continued to be nomads, but rather that they insisted on their historical tradition with respect to eating habits. One can assume for certain that by the 7–8th Centuries, the Avars have changed their way of living. They settled down and conducted a farming system mixed with livestock husbandry (Kollautz & Miyakawa 1970).

It is certain, however, that a major staple food was bread (gruel) from broomcorn millet, as indicated by carbonised grains from the Szirák Avar graveyard (Hampel 1897).

The botanical remains in Szegvár-Oromdűlő are typical for a settled population growing tillage crops and garden produce (Gyulai 2010).

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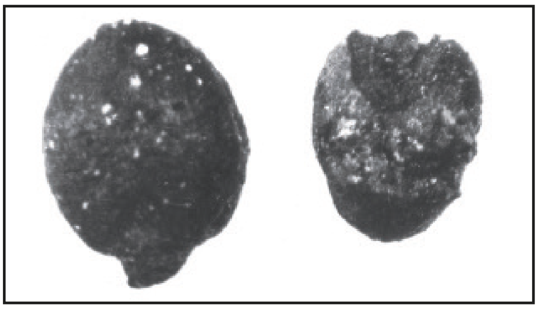
One of the favourite plants of those living here was doubtlessly broomcorn millet. Both their food and drink were made of it. During the flotation of a sample coming from grave ceramics, two pieces of husked broomcorn millet grain was also observed, burnt together with – or rather burnt into – food scraps. They lacked the germ portions that are usually broken away when husked.

One of the most important botanical findings in Hungarian archaeobotanical research was uncovered from the late Migration Period Fonyód-Bélatelep site, the excavation of Béla Horváth in 1964 (Gyulai, Hertelendi & Szabó 1992). Based on 14C tests, the age of the settlement, made up of lake dwellings, can be dated from the second half of the 7th century to the end of the 9th Century. The main crops of the inhabitants were barley, common bread wheat, club wheat, broomcorn millet, rye and common oat (Fig. 2).

Figure 2. Broomcorn millet naked (husked) grains with and without the germ disk from Fonyód-Bélatelep Late Migration Period lake dwelling. Photograph by the author.



Decades of excavations, led by Ágnes Ritoók and Miklós Béla Szőke, of a parking lot exposing the 9th Century site of Zalavár-Vársziget, initiated collection and flotation of soil samples for botanical remains in 1994 (Gyulai 1998). According to historical data, the Carolingian court centre was populated by common people of mixed ethnic composition. Most seeds and fruit remains come from the fill of a „well", which was probably a planked storage pit. The botanical material is dominated by cultivated plants and their weeds.

The most important crops were broomcorn millet, six-rowed barley and common bread wheat. Only a very few seed finds are available from the conquest age which relates to crop production (Hartyányi, Nováki & Patay 1967/68). An exception is a conqueror belonging to the elite, whose grave was located within the current country boundaries in Zemplén, which contained broomcorn millet grains. Consequently the most important food was gruel (Rapaics 1934; Gaál 1978).

The main Hungarian botanical find from the age of the conquest (beginning of the 10th Century) comes from Lébény-Billedomb, the 1993 excavation of Miklós Takács (Gyulai 1997). Hulled wheat types, typical in prehistoric ages, were not grown at all, only the more advanced naked grain common wheat are found. An important gruel plant was broomcorn millet, as unearthed carbonised broomcorn millet gruel pieces show.

Similar remains were found from the Early Árpádian Period site of Gyomaendrőd (excavation by Dénes B. Jankovich 1987–90) and from the Árpádian Period site found at the exploration of the M0 motorway in Rákospalota-Újmajor (excavation by Zoltán Bencze 1995–96). These gruel-like foods prepared from broomcorn millet imply the survival of nomadic eating habits (in: Gyulai 2010).

When exploring Edelény-Földvár in 1992–2001, Mária Wolf found clay pots placed on their sides near the oven of a burnt house from the 10th Century. One remain consisted of many carbonised fragments with no contamination as it was homogenous. Embedded in fine groats, husked millet grains were also observed (Gyulai 2014).

Relatively more evidence has been recovered from the period (10th–11th Centuries) after the conquest (Hartyányi, Nováki & Patay 1967/68). In the early period, broomcorn millet continued as the primary grain crop. In Kardoskút, during excavation of a 10th–13th Century village, cereal grains were found among burnt straw under an oven. Numerically, the most important grain was broomcorn millet followed by bread wheat and rye. In Tiszaörvény, during excavation of an 11th–13th Century village, broomcorn millet awn remains were observed, apparently stored in considerable amounts in a corner of a house.

Dominance of broomcorn millet remains supports the contention that, in the Great Plain after the conquest, nomadic lifestyle and nomadic pasture rotating large livestock husbandry continued to a significant degree. Broomcorn millet, to be grown more easily and ripening more quickly when compared to wheat, was the typical crop of nomadic and semi-nomadic husbandry.

In contrast to this, in Transdanubia along the river Danube and northern Hungary examining finds from the 10th–11th Centuries, common bread wheat, a crop assuming much more advanced production skills than broomcorn millet, appears, although only sporadically.

The same can be said about the remains coming from the northern part of the country: more valuable kinds of crops were grown here as well (Hartyányi, Nováki & Patay 1967/68). Adjacent to the Roman church at Esztergom-Kovácsi, 11th century graves provided botanical materials characterised by common bread wheat and rye, both having longer growing seasons.

When one compares the earliest finds of the period after the conquest, the conclusion is that broomcorn millet played an important role mainly in the Great Plain, while common bread wheat and rye did the same in Transdanubia. The finds from the Plain support the notion of limited nomadic patterns in the period after the conquest. The finds from Transdanubia and from the northern part of the country suggest a sedentary lifestyle and a more advanced level of agriculture.

The Danube River, which is a historical as well as floristic boundary, also divided the country into two major areas of different crop production: the Great Plain, producing more archaic plants (see production of emmer below) and Transdanubia, a more advanced region integrating the traditions of Roman agriculture.

Grain finds from later excavations in the 12th–13th Centuries in the Plain start to show similarities with those found in Transdanubia both in terms of species composition and their relative importance (Hartyányi & Nováki 1973/74). Apparently, the population in the Great Plain became settled only a century later, by the 12th–13th Centuries. The alteration of sowing seeds, representing a quality change in crop production, was completed by this time. Growing high nutrient common bread wheat and rye became customary. The significance of broomcorn millet declined but it was retained in production up to the Modern Period as an aftercrop providing gruel dishes.

In 1996 at the Rákospalota-Újmajor site, most probably another segment of Sikátor village, was excavated by Anna Gyuricza. This time, a well, dated to the 13th–14th Centuries, rich in plant residues was also identified. Expansion means, when compared to the Early Árpádian Period, club wheat and common oat. The number of club wheat grains, like that of broomcorn millet grains, is low, both of them being typical for the Middle Ages in Hungary (Gyulai 2010).

The objects explored in Solt Tételhegy in 1999 contained three culture layers (Late Bronze Age, Árpád-Age and Late Medieval). In the Bronze Age objects only one broomcorn grain was found. Much more, near by 400 pieces were available in the Árpád-Age objects (pits, fireplaces and houses). In the Late Medieval (15th Century) ashy layer not only dozen broomcorn millet grains were found but also charcoal cerealgruel (Gyulai in press).

Compared to other sites, the culture layer explored in the cellar under Hunyadi Street 22 in Budapest, first district in 1973, is unusually rich in cultivated plant remains. Here, a series of locally grown and gathered plants were obtained from a pot dated to the 13th century, but a number of broomcorn millet remains and several weed species were also encountered. Seeds were

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identified by István Skoflek and Mrs. Hortobágyi (in: Hartyányi & Nováki 1973/74).

Written sources (Diploma-Archives from the Sigismund-period) mention wheat six times, broomcorn millet three times, oat and hemp once between 1387–1399. Likewise, between 1400-1410 references occur to millet three times (in: Gaál 1978).

Important evidence for a uniform medieval crop production culture is seen in the late medieval botanical material of Muhi (in: Gyulai 2010). Although the samples collected and locally floated in 1995 at the Muhi medieval excavation site led by József Laszlovszky and Tamás Pusztai are still not completely processed, the cereals identified so far (common bread wheat, club wheat, rye, six-rowed barley, broomcorn millet) confirm the level of farming typical for the age.

Led by András Horváth Pálóczi, archaeologist at the Agricultural Museum, several wells were explored at the late medieval Cuman settlement in 1984–87 at Lászlófalva-Szentkirály. It is not known whether six-rowed barley, a grain crop that occurred most frequently in the finds, was grown as fodder or was intended for human consumption. However, broomcorn millet, common bread wheat and rye necessarily must have served for human consumption (in: Gyulai 2010).

Half of the archaeobotanical material found at Dunaföldvár-Öregtorony site (ruins of a 17th century house) consist of rye, somewhat less of common wheat and even less of broomcorn millet (Hartyányi & Patay 1970) (Fig. 3). A favourite gruel plant was broomcorn millet in the Hungarian Middle Ages.

It can be found at almost all medieval sites: for instance, in the destruction layer of a 15th–16th Century housing estate in Nagyvázsony-Csepely (in a 40 cm^3 mass of mostly hulled aggregate) and in the 17th Century dwelling- house at Székesfehérvár-Palotai Street 5 (Hartyányi, Nováki & Patay 1967/68).In the course of the exploration and reconstruction of Buda castle over the past fifty years, archaeologists found

Figure 3. Broomcorn millet naked grains from Dunaföldvár-Öregtorony, beginning of the 17th century. Inventory of the Hungarian Agricultural Museum, Budapest.

a number of wells. The first archaeobotanical examinations were made at the well of Dísz Square No. 10, led by Imre Holl. Seeds from the well, dated to the 14th century, were identified by Zoltán Zsák (in: Holl 1966). In 1955 Győző Gerő explored a 13th–14th century well at Úri Street 40. (Hartyányi, Nováki & Patay 1967–68). In 1966, at Dísz Square No. 8., yet another well with seeds was found, dated by Katalin I. Melis to the 13th–14th Centuries based on associated ceramics (Hartyányi & Nováki 1973/74). Compared to other contemporary sites, an unusually large quantity of garden produce remains were identified in a medieval well filling explored in a cellar of Hunyadi Street 22 in 1971. A wide variety of produced and gathered plants were unearthed from a pot dated to the 13th century surrounded by a burnt layer. Broomcorn millet and a number of weeds also occurred (Seeds were identified by István Skoflek and Mrs. Hortobágyi (Skoflek & Hortobágyi 1973).

Carpological remains (seeds and fruits) from the two Buda castle 14th–15th Century wells are all direct botanical finds. Water created anaerobic conditions that in turn prevented microbes from decomposing the diaspores. Although no broomcorn millet grains were found, husking refuse in forms of one thousand husks occurred in a non-carbonised form. The endospore fell out of the grains. This is evidence of cleaning as the eye often breaks out during threshing.

Glumellae coming from the cleaning procedure did not carbonise (Gyulai 2010). In Vác, Piac utca 14th-15th Century settlement (excavation by Orsolya Mészáros 2008–2009) were also some broomcorn millet grains found but in special way by iron salts conserved forms (Kenéz, Pető & Grynaeus in print).

Devastation layers from the Turkish Period are relatively well researched from a botanical point of view. Between 1969 and1974 Imre Holl and Nándor Parádi led the excavation of a village razed during the Turkish era in the 16th century in Sümeg-Sarvaly. Botanical finds from six houses devastated and burned during the Turkish Period and adjacent debris were processed by István Skoflek (1984–85) and Borbála Hartyányi (in: Nováki 1984/85). Beside grains and seeds of carbonised common bread wheat, rye, broomcorn millet and weeds, fruit remains were also encountered.

In Színház Street, which is situated in an area where the Buda castle existed in the Turkish Period (excavation by István Feld 1995) and in Óbuda, Medve street (excavation by András Végh 1995) common bread wheat and six-rowed barley grains were also found, but the numbers indicated that they must have not been very significant. Much more important were broomcorn millet and common oat (in: Gyulai 2010).

Cereals (common bread wheat, rye, broomcorn millet) mentioned in the tithe census were found without exception in the houses of Nagyvázsony-Csepely dated to the 15th–17th Centuries (Júlia Kovalovszky 1957–58 in: Hartyányi, Nováki & Patay 1967/68) (Figs. 4, 5).

In 1998 near Baj Öregkovács-hegy in the forest, excavated by Sándor Petényi, were also dated to the Middle Ages. Two pot fragments contained unusually large millet grains, incremented due to burning into the wall of the pot. Burnt grains were stuck together in smaller or larger clumps, obviously solidified in this way during cooking (Gyulai 2010). The millet gruel found here characterised Hungarian food culture in the Middle Ages. Millet gruel used to be a traditional staple food for Hungarians, which can be traced back to the times before the conquest. It is known from a number of sources that in the life of Hungarians, just as in the case of any other European people, the consumption of gruel made of husked millet played a decisive role.

Figure 4. Broomcorn millet naked grains from Nagyvázsony-Csepely (15th–16th centuries). Inventory of the Hungarian Agricultural Museum, Budapest.

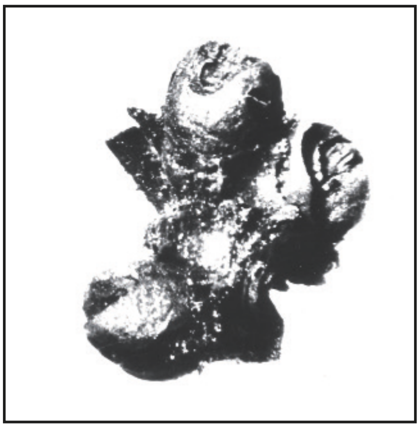
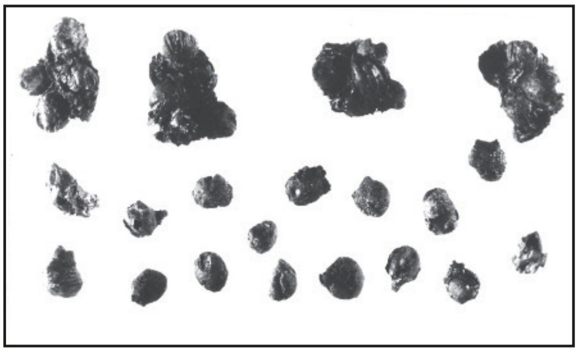


Figure 5. Broomcorn millet naked grains and gruel fragments from Nagyvázsony-Csepely (15th–16th centuries). Inventory of the Hungarian Agricultural Museum, Budapest.



Millet gruel was a common dish, irrespective of social rank and privileges, and it continued to be so up to the 18th–19th Centuries until the spread of maize „Turkish wheat" coming from the New World provided a new gruel plant.

Modern examinations of ancient millet grains

The current technology of plant biotechnology and genetics makes it possible to regenerate

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plant individuals from only one cell (Dudits & Heszky 2000). If only one cell of the seed tested remained intact and untouched over the centuries, plant regeneration is technically possible (Gyulai et al., 2001). Plants can be developed from cells of surviving callus, provided the DNA content of the nucleus is not impaired. For this purpose broomcorn millet was taken first from the well dated to the beginning of the 15th Century, found under the building of the former Military Headquarters, Teleki Palace in Buda Castle, (excavation by Zoltán Bencze, Dóra B. Nyékhelyi, András Végh 1998–99), as well as from the well dated also to the beginning of the 15th century in Budapest I., Kapucinusok Street (excavation by András Végh 2000). The seeds were incubated using the same procedure as with recent plants, on culture medium supplemented with growth promoting hormones.

Germinating tissues were found only in broomcorn millet, muskmelon, cantaloupe and watermelon cells, unfortunately however the „resurrected" cells were soon killed by endogen infections (mycoplasms, phytoplasms).

DNA was isolated based on the methods developed by Gyulai et al. (2000) for molecular genetic studies only from material suitable for the extraction of the genetic material (broomcorn millet). Comparative genetic assessment of the material was tested using the PCR method (Williams et al., 1990). The genetic tests carried out confirmed that medieval broomcorn millet theoretically might contain intact surviving cells. Even though the plant regeneration experiment was not successful this time, the plants still contained a large amount of extractable DNA. The PCR method confirmed that the DNA extracted is of plant origin and does not come from the decomposing bacteria and fungi. PCR reactions verified easy to reproduce DNA, suitable for genome analysis and cultivar comparison. It seems to be an important result that DNA extracted from the nearly 700 year old seeds has a pattern different from those in the current broomcorn millet varieties. Genetic material of broomcorn millet recovered from the 15th Century well in the yard of the Buda castle Teleki palace (Gyulai et al., 2004). Further molecular genetic analyses might reveal the genetic relationships between today's and 15th century broomcorn millet, their heritage and genealogy as well as the origin of today's varieties (Lagler et al., 2006). Results in the longer run could be used for genetic improvement aiming at resistance.

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CRYOPRESERVATION OF SPERM OF THE ADRIATIC GRAYLING (*Thymallus thymallus*) AND THE MARBLE TROUT (*Salmo marmoratus*) FROM THE SOČA RIVER IN SLOVENIA

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Abstract

A cryopreservation protocol was tested on the sperm of genetically distinct Adriatic grayling (*Thymallus thymallus)* and the Natura2000-listed marble trout (*Salmo marmoratus*) from the Soča River basin in Slovenia. Sperm was frozen in an extender composed of 200 mM glucose, 40 mM KCl, 30 mM Tris (pH 8.0) and 10% methanol as a cryoprotectant in the vapour of liquid nitrogen. Sperm dilution ratios of 1:1, 1:4 and 1:9 were tested. The cooling rate was 57 ± 1°Cmin-1 in all cases. In the grayling, the highest ratio of eyed eggs (74 ± 4 % vs. 69 ± 6 % in the control) and the highest hatch percentage (63 ± 6 % vs. 56 ± 10 % in the control) was observed with a dilution ratio of 1:1. In the marble trout, the highest percentage of eyed eggs (84 ± 4 % vs. 88 ± 3 in the control) and the highest hatch rate (70 ± 3 % vs. 76 ± 2 % in the control) was again found using the 1:1 dilution ratio of the sperm. In both species, individual sperm samples had a significant effect on the results. Sperm-to-egg ratios ranging from 2.5 to 5.7 × 10^4 spermatozoa per egg yielded satisfactory hatch rates (67 ± 7 % to 73 ± 7 %) in the grayling.

Keywords: grayling, marble trout, sperm, cryopreservation, conservation

Introduction

The Adriatic grayling indigenous to the northern part of the peri-Adriatic river system including the Soča river basin in Slovenia is a phylogenetically distinct lineage within the species *Thymallus thymallus* (Sušnik et al. 2001). Due to intense stocking of a non-native grayling lineage from the Sava River, and successive introgression between the two lineages, at present only hybrids with different amounts of the original "Adriatic" genes exist in the Soča river basin. Based on genetic analysis the Angling Club of Tolmin (http://www.flyfishing.si/) that manages the Soča river basin has, conducted extensive selection of individuals with the highest amount of "Adriatic genes" and carries out systematic spawning and stocking of these fish into the river (Sušnik et al. 2004; Jesenšek & Šumer 2004). Another species of the same river system, marble trout (*Salmo marmoratus*), has also been severely affected by the introduction of non-native brown trout (*Salmo trutta m. fario*) from the Danubian drainage and subsequent hybridization (Povž 1995).

Marble trout is a Natura 2000- as well as Habitats Directive-listed (Council Directive 92/43/EEC 1992) species. Although most rivers in the Soča river basin are inhabited by hybrids, pure marble trout populations remained in the upper sections of several streams that were inaccessible to introduced brown trout (Fumagalli et al., 2002). Broodstocks consisting of individuals of several pure populations are cultured at the facilities of the Angling Club of Tolmin as a live gene bank. Grayling are due to their sensitivity to aquaculture conditions (e.g., sensitivity to infections and diseases, nutritional deficits and territorial and aggressive behaviour) extremely difficult to rear, particularly adult (sexually mature) animals. In order to diminish these technological inconveniencies, an efficient system for long-term sperm preservation, such as cryopreservation,

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should be established enabling drastic reduction of broodstock size. This system would also enable storage of sperm obtained from wild males, until it is genetically tested.

In case of the marble trout, cryopreservation of sperm from individuals of the remaining pure populations could ensure their survival. These populations that inhabit the headwaters of rivers are severely limited in numbers of fish and are continuously threatened by natural hazards such as earthquakes, landslides or floods (Povž et al., 1996).

Although the cryopreservation of salmonid sperm (particularly of the rainbow trout *Oncorhynchus mykiss*) has been studied extensively (for a review see Lahnsteiner 2000a), only a few reports have been published on the sperm of the grayling (Lahnsteiner et al. 1992, 1996a) and several issues concerning fertilizing capacity of frozen sperm, optimal sperm-to-egg ratio, lesions of preserved cells due to freezing and thawing remain to be solved. Fine structural changes in spermatozoa as a result of cryopreservation were investigated using electron microscopy systems (SEM and TEM) (Lahnsteiner et al., 1992). After thawing only 10-20% of sperm cells displayed intact morphology, although fertilization percentages of up to 80 % were observed. In the second study (Lahnsteiner et al. 1996a), a method originally developed for the rainbow trout was adapted to the grayling and the Danube salmon (*Hucho hucho*). Fertilization rates of 90 – 100 % of the control were observed using 10 % methanol as cryoprotectant, cooling at 1.5 cm above the level of liquid nitrogen and thawing at 25°C for 30 seconds. To the best of our knowledge there has been no report on the cryopreservation of marble trout sperm.

In order to develop a protocol for cryopreservation of the Adriatic grayling and the marble trout, the objective of this study was to experimentally investigate the effect of sperm dilution ratio on the fertilizing capacity of sperm after thawing. A further objective of the work was to optimize the sperm-to-egg ratio with respect to fertilization success using thawed sperm in the grayling.

Materials and methods

Gamete collection

In case of the grayling, approximately 30 spawning males and four spawning females kept in the fish farm of the Angling club of Tolmin were included in the investigations.

Before collecting sperm and eggs, all individuals were anesthetized using 2-phenoxyethanol at a dose of 0.8 ml l-1. Anesthetized fish were laid on a towel, their urogenital openings were wiped dry and sperm were stripped into 12-ml test tubes applying a silicone catheter (1 mm internal diameter, 1.5 mm external diameter) according to the protocol described by Glogowski et al. (2000). Females were inspected for the presence of ovulated eggs and eggs were stripped into a plastic bowl by gentle massage of the abdomen.

Milt was successfully collected from 12 individuals: three of them, having a sufficient milt volume (samples 10, 11, 13), were selected for individual cryopreservation, while three more samples were due to small milt volumes pooled before freezing. The remaining six samples were excluded from cryopreservation experiments due to poor motility.

Sperm motility of each specimen was estimated before cryopreservation and after thawing (post-thaw motility) under a light microscope at 100 × magnification by mixing 1 µl of sperm with 19 µl of hatchery water on a microscope slide.

Marble trout males were collected by electrofishing at three sections of the river Trebuščica. Collected fish were anesthetized on collection site using 2-phenoxyethanol at a dose of 0.8 ml l-1. Fish were laid on a towel, their urogenital pores were wiped dry and sperm was collected by aspiration through an elastic pipe into a dry test tube. At each sampling site pooled sperm was collected from 5-8 individuals, thus, they were numbered 1, 2 and 3 according to the sampling site. Sperm samples were then transported into the hatchery at 4°C and motility

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estimation was conducted as described with the grayling. Eggs from females of the broodstock maintained at the fish farm were collected during a routine spawning work and also transported into the hatchery at 4°C.

Sperm cryopreservation

Conditions of freezing and thawing were identical for both species. Before freezing, sperm were mixed at a ratio of either 1:1, 1:4 or 1:9 with an extender composed of 200 mM glucose, 40 mM KCl, 30 mM Tris (pH 8.0, set with concentrated HCl) and 10% methanol adjusted to have a final concentration of 10% following dilution with sperm. All chemicals used in the experiments were purchased from Reanal Laborvegyszer Kft (Budapest, Hungary). Diluted sperm were loaded into 0.5 ml straws (Minitüb, Tiefenbach, Germany) and frozen in the vapour of liquid nitrogen in a polystyrene box. Straws were placed on a 3-cm high polystyrene frame floating on the surface of liquid nitrogen and allowed to cool for 3 minutes. After cooling straws were plunged into liquid nitrogen. Frozen sperm samples were stored in a Statebourne BIO 10 storage dewar (Statebourne Cryogenics, Washington Tyne & Wear, UK). Sperm samples were used for fertilization after two to four days of storage. Samples were thawed in a 40°C water bath for 13 seconds.

Cooling rates were measured using a K-type thermocouple inserted into a straw filled with sperm of marble trout diluted with the extender and cryoprotectant at a ratio of either 1:1, 1:4 or 1:9 as described above. The thermocouple was connected to a Digi-Sense DualLogR thermometer (Eutech Instruments, Singapore). The straw was placed onto a 3 cm high polystyrene frame which was floating on the surface of liquid nitrogen in a styrofoam box and temperature readings were logged with the interval of 2 seconds. Cooling rate measurements were conducted in three replicates. Average cooling rates were calculated using the initial temperature values and the values at 3 minutes and the time elapsed.

Effect of dilution ratio on the fertilizing capacity of cryopreserved sperm

To investigate the effect of sperm dilution ratio on the fertilizing capacity of sperm after thawing, pooled eggs from two females were used in the grayling and from three females in the marble trout. Batches of 13g (N = 837 – 1029) eggs were used for fertilization with a single straw of thawed grayling sperm, whereas, in the marble trout batches of 20g (N = 209-224) of eggs were fertilized with one straw, in triplicates for both species. Sperm samples 10, 11 and the pooled sample were used in the grayling, while in the marble trout the pooled samples collected at the three sampling sites were used. Freshly stripped sperm served as a control in both species. Contents of a straw were released onto the eggs and gametes were activated using a DIA523 fertilizing extender (in 1 liter of water: 2.42 g Tris, 3.76 g glycine, 5.5 g NaCl prepared according to the protocol of Billard (1977)). Simultaneously, post-thaw motility of sperm was observed under a light microscope at 100× magnification by diluting 1 μl of thawed sperm in 19 μl of the above mentioned fertilizing extender on a microscope slide. After incubation in the fertilizing extender for approximately 1 hour, the extender was replaced by water and eggs were distributed into experimental incubators with a water flow of 90 l min-1. Fertilization percentages were calculated at eyed stage, and hatch percentages were calculated at the stage of hatching.

Effect of sperm-to-egg ratio on the fertilizing capacity of cryopreserved sperm

To optimize the sperm-to-egg ratio using thawed sperm of the grayling, again, pooled eggs of two females were divided into the batches of 10 g (N = 622 – 743) eggs in triplicates and fertilized with the cryopreserved sperm of sample 13 diluted at a ratio of 1:4 prior to cryopreservation. Fertilization was carried out using 200, 100 or 50 μl of thawed sperm (meaning 40, 20 or 10 μl of pure sperm). Details of fertilization, incubation and determination of fertilization and hatch percentages were the same as described in the previous experiment. Sperm concentration was determined by counting spermatozoa in a Bürker-type counting chamber at 100 × dilution (in the extender used for cryopreservation) in order to calculate the sperm-to-egg ratio. Finally, sperm-to-egg ratio was calculated by dividing the number of spermatozoa in the volume of fresh sperm used for fertilization with the number of eggs in the given batch.

Statistical analyses

Post-thaw motility, fertilization and hatch percentages were subjected to two-way analysis of variance (ANOVA) with Bonferroni's post test at $P < 0.05$ to investigate the main effects of individual sperm samples and dilution ratios. One-way ANOVA with Tukey's Multiple Comparison test at $P < 0.05$ was used to investigate the effects of the used sperm-to-egg ratios on fertilization and hatch rates. All statistical analyses were conducted using the statistical software GraphPad Prism 4.0 for Windows.

Results

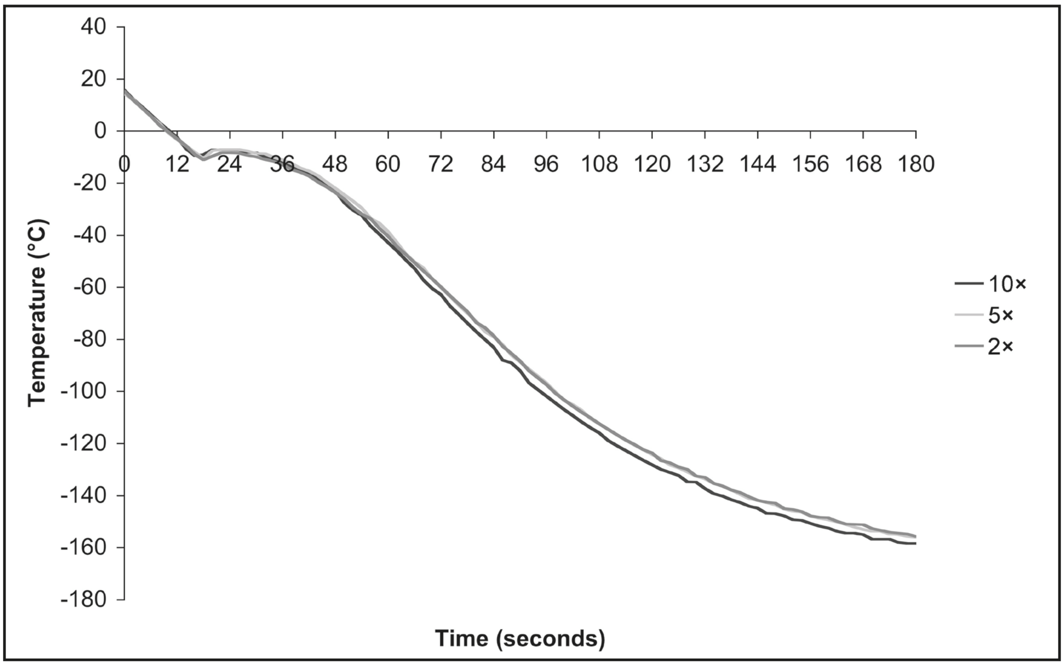
Sperm collection from the grayling through a silicone catheter was only partly successful – of the 12 stripped males sperm was successfully collected only from three individuals. Motility of grayling sperm immediately after collection was 78 ± 10% (N = 12), while that of the marble trout was 83 ± 6% (N = 3).

According to our measurements the cooling rates for the three dilution ratios were 57 ± 1 °Cmin^{-1} for 1:1, 57 ± 0 °Cmin^{-1} for 1:4 and 58 ± 1 °Cmin^{-1} for 1:9 (Figure 1). The highest post-thaw motility (50 ± 0 %) of grayling sperm measured in the first experiment corresponded to the pooled sperm sample with 1:1 dilution ratio (Table 1). In general, significant main effects of dilution ratios ($P < 0.0001$) and sperm samples ($P = 0.0090$) were observed on post-thaw motility of cryopreserved grayling sperm.

In case of the marble trout (Table 2) the highest post-thaw motility (23 ± 6%) was observed in sperm sample 1 at a dilution ratio of 1:9. Significant main effects of the dilution ratio ($P = 0.0110$) as well as the individual sample ($P = 0.0003$) on the post-thaw motility of marble trout sperm were found.

In case of fertilization percentages in the grayling at eyed stage (Table 1.), significant main effects of dilution ratios ($P = 0.0002$) and sperm samples ($P = 0.0285$) were found, however, only dilution

Figure 1. Cooling rates used in the experiments at sperm dilution ratios of 1:1, 1:4 and 1:9 (N=3).



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ratios had an effect (P = 0.0008) on the hatch rates. The highest fertilization (74 ± 4 %) and hatch (63±6%) percentages were observed in sample number 10 using the 1:1 dilution ratio. A gradual decrease of fertilization and hatch rates was observed only in sample number 10, whereas, in sample number 11 or in the pooled sample, this decrease was not significant or was not observed at all.

Table 1. Post-thaw motility (a), fertilization at eyed stage (b) and hatch (c) percentages observed using cryopreserved sperm of the Adriatic grayling. All data are given as mean ± SD (N = 3). Values sharing a superscript letter within a column are not significantly different at P < 0.05. Control fertilization: 69 ± 6 %, control hatch: 56 ± 10 %.

Dilution ratio	Sperm sample number		
a	Pool	10	11
1:1	50 ± 0[a]	43 ± 6[a]	37 ± 6[a]
1:4	43 ± 6[a]	40 ± 10[a]	30 ± 0[ab]
1:9	20 ± 0[b]	4 ± 2[b]	18 ± 13[b]
b.			
1:1	63 ± 13[a]	74 ± 4[a]	70 ± 2[a]
1:4	66 ± 11[a]	62 ± 2[a]	70 ± 4[a]
1:9	67 ± 3[a]	29 ± 13[b]	53 ± 10[a]
c.			
1:1	53 ± 12[a]	63 ± 6[a]	61 ± 4[a]
1:4	52 ± 12[a]	53 ± 0[a]	59 ± 4[a]
1:9	53 ± 6[a]	27 ± 12[b]	46 ± 8[a]

In case of the marble trout (Table 2.), the highest percentage of eyed eggs (84 ± 4%) and hatched larvae (70 ± 3%) was observed with sperm sample 3 and dilution ratio of 1:1. A significant main effect of individual samples was observed on the percentage of eyed eggs and hatched larvae (both: P<0.0001), yet, no main effect of the dilution ratio was found.

Sperm-to-egg ratio significantly affected both, fertilization (P = 0.0330) and hatch (P = 0.0455) percentages of grayling eggs. The use of 200 µl of thawed sperm gave the highest fertilization (78 ± 7 %) and hatch (73 ± 7 %) results, although, no significant difference was observed between the 200-µl and 100-µl groups (Table 3).

Table 2. Post-thaw motility (a), fertilization at eyed stage (b) and hatch (c) percentages observed using cryopreserved sperm of marble trout. All data are given as mean ± SD (N = 3). Values sharing a superscript letter within a column are not significantly different at P < 0.05. Control fertilization: 69 ± 6 %, control hatch: 56 ± 10 %.

Dilution ratio	Sperm sample number		
a	1	2	3
1:1	4 ± 6[a]	3 ± 2[a]	12 ± 8[ab]
1:4	13 ± 6[a]	4 ± 2[a]	20 ± 0[a]
1:9	23 ± 6[b]	5 ± 5[a]	10 ± 0[b]
b.			
1:1	40 ± 25[a]	50 ± 10[a]	84 ± 4[a]
1:4	67 ± 6[a]	15 ± 12[b]	80 ± 1[a]
1:9	60 ± 20[a]	22 ± 7[ab]	66 ± 17[a]
c.			
1:1	32 ± 20[a]	41 ± 10[a]	70 ± 3[a]
1:4	57 ± 6[b]	12 ± 9[b]	66 ± 3[a]
1:9	48 ± 16[ab]	17 ± 5[ab]	54 ± 13[a]

Sperm concentration was 9.131 ± 0.415 × 10^8 spermatozoa per ml, which resulted in 5.687 ± 0.181 × 10^4 spermatozoa per egg using 200 µl of thawed sperm, 2.545 ± 0.063 × 10^4 spermatozoa per egg using 100 µl of thawed sperm and 1.256 ± 0.026 × 10^4 spermatozoa per egg using 50 µl of thawed sperm.

Table 3. Fertilization at eyed stage and hatch percentages using different sperm-to-egg ratios observed using cryopreserved sperm of the Adriatic grayling. All data are given as mean ± SD (N = 3). Values sharing a superscript letter within a row are not significantly different at P < 0.05.

	Sperm-to-egg ratio (spermatozoa egg^{-1})		
	5.69 ± 0.18 × 10^4	2.54 ± 0.06 × 10^4	1.26 ± 0.03 × 10^4
Fertilization	78 ± 7[a]	72 ± 7[ab]	60 ± 4[a]
Hatch	73 ± 7[a]	67 ± 7[ab]	57 ± 5[b]

Control fertilization and hatch results were not counted in this experiment due to human error.

Discussion

In this study, we showed that different sperm dilutions have significant effects on the fertilizing capacity of thawed sperm in the grayling. Dilution ratio 1:1 appears to be the most effective one in this respect, moreover, the highest post-thaw motility and hatch percentages also corresponded with this dilution. This effect was most vividly expressed in post-thaw motility rates, whereas in case of fertilization and hatch percentages the results were more balanced and strongly depended on the individual sample. According to the results, pooling of sperm samples resulted in similar fertilization and hatch rates regardless of the dilution ratio of sperm and observed post-thaw motility. In contrast to our results, a previous study on salmonid species recommended the use of dilution ratios higher than 1:1 (Lahnsteiner et al., 1996b). Only dilution ratio of 1:3 was previously used grayling (Lahnsteiner et al., 1996a).

To the best of our knowledge, this is the first report on the cryopreservation of marble trout sperm. Fertilization and hatch results of marble trout eggs fertilized with cryopreserved sperm show great variation according to the sample used. Drastic reduction of fertilizing capacity of cryopreserved brown trout sperm has been observed by several authors (Labbe & Maisse 2001; Martínez-Páramo et al., 2008) and authors found no correlation between fertilization rates and motility, viability, plasma membrane lipid profile, ATP content, DNA damage, membrane integrity or membrane resistance to osmotic shock.

Nevertheless, effective on-site sperm quality evaluation methods would be necessary to determine the suitability of sperm sample for cryopreservation.

The results obtained in this study also suggest that sperm-to-egg ratio has a significant effect on fertilization and hatch rates. The importance of sperm-to-egg ratios in maximizing fertilization success has been described previously for several salmonid species (Lahnsteiner et al., 1995, Lahnsteiner et al., 1996b). It has to be considered that sperm concentration in the sample used in this experiment was low, i.e. more then 3-6 times lower than that reported for the grayling (Lahnsteiner 2000b). Such a low concentration can in part be explained by the fact that the experiment was conducted relatively late in the spawning season. In a previous study on the grayling, sperm-to-egg ratios of $1.2\text{-}1.6 \times 10^6$ spermatozoa per egg (Lahnsteiner et al. 1996a) were recommended. Results of the present study suggest that much lower sperm-to-egg ratios (as low as 2.5×10^4 spermatozoa per egg) can yield satisfactory hatch percentages. Due to the deficiency of literature on the cryopreservation of grayling sperm our results can contribute to the better understanding of problems associated with fertilization with cryopreserved sperm.

Acknowledgements

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ANNIVERSARY

PROFESSOR JÓZSEF ANTAL, A PROMINENT SCHOLAR OF CROP SCIENCE IS 95 YEARS OLD

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Professor József Antal (photo: M. Birkás)

Crop production is one of the most ancient activities of mankind. All scientific disciplines in the World are dedicated to the development of human race, however all of them are highly influenced by the people contributing results of mental power to that. Professor József Antal, a most prominent scholar of crop production research in Hungary, is one of those, whoever has done it. His life, his intellectual behaviour has been performing an everlasting record in the field of crop science.

He was born in a nobleman's family in Marosbogát, Transylvania in 1919. He was educated in the János Bólyai Protestant College at Marosvásárhely. Following agricultural studies at the Agricultural Faculty of the Kolozsvár University from 1937. He graduated with an excellent degree in 1943. Also, he got his first teaching experiences at the alma mater. As a young teacher he had to join the army. From 1943 until the end of the World War he was a soldier int he Hungarian army. On one of the last days of the war he was captured by the Russian troops, and as a prisoner of war he was kept in a lager in the Soviet Union until 1948. From 1948 to 1952 he was employed by the agricultural administration of Csongrád County in Hungary. His was engaged in various fields of education and public administration. In a long period between 1952 and 1974 he was a researcher in the Agricultural Research Institute of the Southern Great Plain, which was succeeded by the present Cereal Research Institute in Szeged. In this institute he has experienced all levels of a scientific career; from research assisstant to the position of the director of research.

In 1974 he was appointed to be a professor at the Gödöllő University of Agricultural Sciences. As an experienced and renowned scientist he was invited to be the head of the Crop Production Institute of the university. Since that time his personality labels the scientific and educational activities of Gödöllő in crop science. He had been the professor of crop production, head of department and then that of institute, and today he is a highly appreciated professor emeritus, - simply a father of all crop production scientists.

A brief summary of his exceptional scientific work. In the beginning he has taken part in the mapping of the highland pastures in the area of the Eastern Carpathian Mountains. Later he was engaged in scientific research related to potato and sunflower production. Most of his active life was dedicated to the research of sandy soil's agronomy; he used to be a forerunner in cultivation of sandy soil areas as well as a succesful breeder of bean varieties adapted to such conditions.

He had run extensive crop rotation tials as well as green manure field experiments. There were a wide range of field crops he worked with; legumes, fodder crops, medicinal plants and potato secondary cropping. His results in the field of oil seed radish are of international importance. Also he has contributed plant nutrition practices with his research results. In the field of ago-ecology he has established a series of basic principles.

The practical implementation of scientific results. Apart from his basic and applied research activities he has been involved in extension and innovation projects. Crop production technologies developed by him were widely used by small holders and large estates and by various extension services and government authorities.

He has been a diligent and succesful writer all along his life. Just a short bibliographic budget: Professor Antal has written eleven books, over hundred scientific papers and numerous other publications. His main works are the „Növénytermesztés homokon" (Crop production on sandy soils) and the „Növénytermesztők zsebkönyve" (Crop production vademecum) – the „antal" – as it is labelled by generations of agronomists and farmers.

The crown of his lifelong work is the last edition of the national handbook „Növénytermesztés" (Crop production), which serves the education of four universities and six agricultural colleges, and thousands of farmers.

He defended his CSc dissertation in 1956, and he was given the DSc title in 1974. He possesses many national and international scientific awards. Some of the most important ones: Westsik Vilmos insignum (1989), Beethoven-Brunswick plaquette (1989), Gisevius prize (1994), Baross László insignum (1994), and last but not least the highest award of crop production of Hungary, the Surányi insignum (2005).

Professor József Antal is 95 years old by now. He is active as he has always been during his life. A whole society of colleagues, students, theoretical and practical agriculturists surround him, and he broadcasts miracles of his personality and rudiments of a wisdom he possesses.

In the name of the scientific community, we would like to express our thanks to his work and results, and at the same time to wish him good health and further prosperous years.

Source of the graphics

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

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

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Lucius Junius Moderatus Columella

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