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NAKVI National Agricultural Advisory, Educational and Rural Development Institute (NAERDI)

ECO-MOTIVE

Training-the-trainers for ecological small scale production

Cooperation for innovation and the exchange of good practises

1st September 2014 - 30th August 2017

The project will provide the training background, including technical knowledge and skills, as well as the knowledge transfer methodology (appropriate teaching methods, enhancing motivation and social sensitivity training) to provide user groups (primarily local authorities, and their programme staff) with course material and training modules for trainers in small-scale ecological production focusing on:

- organic small animal husbandry
- organic vegetable production
- organic fruit production
- direct local marketing of ecological produce

Expected results:

- Training-the-trainers teaching curriculum
- Trainig-the-trainers teaching material with teaching methods
- Course materials for end-users (disadvantaged rural people): 5 illustrated, easily understandable booklets

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NITRATE MOVEMENT IN THE SOILS OF HUNGARIAN FIELD TRIALS

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ABSRTACT

The leaching process of nitrate was studied in a long-term field experiment at Gödöllő on a brown forest soil started by Debreczeni in 1980. 0, 90, 180, 270, 360 kg ha^{-1} y^{-1} N doses have been broadcasted as basal fertilization in the form of ammonium-nitrate for 16 years. The test plant was maize in monoculture. In 1986 the soils of the treatments were sampled in 6 replications from 0 to 3 meters depth at every 20 cms. The nitrate content of the sample was determined.

The nitrate content of the soil samples in replications was rather different in spite of the nearly flat area, so the data were evaluated by the help of the average of 6 replications.

Increasing the dose of nitrogen fertilization nitrate content in the soil profile increased, as well. The 20-40% of the nitrogen fertilizer can be found in the 3 meter layer of the soil profile. The amount of this accumulated nitrate is equal to few hundred kgs of nitrogen. The maximum of nitrate accumulation front can be found at 160-260 cm. The place of maximum accumulation moves downward when increasing the dose of nitrogen fertilization. In the case of high nitrogen dose nitrate accumulation could be found below 3 meters depth, too.

Results prove the necessity of a suitable technology preventing nitrate losses. As the experiment shows 100 kg N ha^{-1} fertilizer nitrogen enough for the maximum crop yield in this area. At higher fertilizer rates the residual fertilizer nitrogen and the nitrate released from sol organic reserves are lost by leaching. Climatic conditions in experimental area are unfavourable for nitrogen fertilizer application is autumn. The yearly water surplus is 116 mm and this is enough for leaching the amount of nitrate exceeding plant demand into 1-3 meter depth of soil or into deeper horizons.

The reasons for differences in the nitrate-N profiles of the various locations included in the National Long-term Fertilisation Trials appeared to be primarily dependent on the different rainfall conditions and soil texture and on the N fertiliser rates.

In response to increasing rates of mineral fertiliser there was an exponential rise in the nitrate-N content of the soil, both in the root zone and in deeper soil layers. At annual fertiliser rates of 0 and 50 kg N ha^{-1}y^{-1}, no nitrate accumulation was observed, but the leaching of nitrate ions was already detectable at the 150 kg N rate, and increased considerably in the case of 250 kg ha^{-1}y^{-1}. Based on the exponential curve, a substantial increase in nitrate-N could be observed at an N fertiliser rate of 2000 kg/ha/20 years. In agreement with data from the literature, under the climatic and soil conditions in Hungary, nitrate accumulation can occur when fertiliser N rate more than 100 kgha^{-1}y^{-1}.

The depth of maximum nitrate-N accumulation after various rates of mineral fertilisation was nearer to the surface in heavier soils (greater values of K_A).

A comparison of the roles of soil texture and rainfall on the basis of correlation coefficients revealed that the depth of maximum nitrate-N accumulation was influenced to a greater extent by soil texture.

keywords: leaching, long term field experiment, nitrate, soil

PREVIOUS HUNGARIAN AND INTERNATIONAL RESULTS

Nitrate leaching was studied for a winter leaching period in a layered calcareous silt loam with tile-drains at about 1-m depth. Measured NO_3 leaching was 11 kg N ha^{-1} y^{-1} in the relatively dry, winter leaching period 1991-1992 (De Vos et al. 2000).

Paramasivam et al. (2001) evaluated NO_3-N distribution in soil solution at various depths in the vadose zone, and N leaching below the root zone for two cropping seasons. The treatments included 112, 168, 224, and 280 kg N ha^{-1} y^{-1}. At the 60- or 120-cm depths, the NO_3-N concentrations occasionally peaked at 12 to 100 mg L^{-1}, but at 240 cm NO_3-N concentrations mostly remained below 10 mg L^{-1}. The careful irrigation management, split fertilizer application, and timing of application contributed to the low leaching of NO_3-N below the root zone. Calculated NO_3-N leaching losses below the rooting depth increased with increasing rate of N application and the amount of water drained, and accounted for 1 to 16% of applied fertilizer N.

Results showed that well and moderately well drained

fields had consistently higher ground water NO_3^- compared to more imperfectly drained fields receiving comparable N inputs (Young and Briggs 2007).

A long-term (1982-2004) field experiment was conducted to investigate the effects of nitrogen fertilizers on accumulation of nitrate-N in the soil profile (0-210 cm). Annual applications of N fertilizer and manure for 23 successive years had a marked effect on NO_3-N accumulation in the 0-210 cm soil profile. Accumulation of NO_3-N in the deeper soil layers with application of N fertilizer and manure is regarded as a potential danger, because of pollution of the soil environment and of groundwater (Yang et al. 2006).

The magnitude of nutrient accumulation and its distribution in the soil profile varies with soil-climatic conditions. The objective of the study was to determine loading and distribution of manure-derived nitrogen in the soil profile as influenced by repeated manure applications. Lower crop removal and reduced leaching of NO_3-N due to drier conditions contributed to greater accumulation of nitrate-N in the top 60 cm. At large manure rates, excess N from the balance estimates could not be accounted for in soil organic N and was assumed to be lost from the soil-plant system. At the Dixon LHM site, deep leaching of NO_3-N was observed at the excessive rate up to the 150 cm depths compared to the control. To prevent loading, rates of applied manure nitrogen should be reduced when crop N removal potential is diminished by high frequency of drought (Stumborg et al. 2007).

A long-term fertilizer experiment on dry land of the Loess Plateau, northwest China, has been conducted since 1984 to study the distribution and accumulation of NO_3-N down to a depth of 400 cm in the profile of a coarse-textured dark loess soil after continuous winter wheat cropping. Annual N and P (P_2O_5) rates were 0, 45, 90, 135 and 180 kg ha^{-1}. After 15 successive cropping cycles, the soil samples were taken from each treatment for analysis of NO_3-N concentration. The application of fertilizer N alone resulted in higher NO_3-N concentration in the soil profile than the combined application of N and P, showing that application of P could greatly reduce the NO_3-N accumulation. With an annual application of 180 kg N ha^{-1} alone, a peak in NO_3-N accumulation occurred at 140 cm soil depth, and the maximum NO_3-N concentration in the soils was 67.92 mg kg^{-1}. The amount of NO_3-N accumulated in the soil profile decreased as the cumulative N uptake by the winter wheat increased (Fan et al. 2003).

Nitrate leaching occurs when there is an accumulation of NO_3-N in the soil profile that coincides with or is followed by a period of high drainage. Therefore, excessive nitrogen fertilizer or waste effluent application rates or N applications at the wrong time (e. g. late autumn) of the year, ploughing pasture leys early in the autumn or long periods of fallow ground, can all potentially lead to high NO_3-N leaching losses. N returns in animal urine have a major impact on NO_3-N leaching in grazed pastures (Di and Cameron 2002).

Nitrate leaching from agricultural soils can increase groundwater nitrate concentrations. Ammonium nitrate was applied only to the percolation lysimeters. Leachate from the lysimeters was extracted from a depth of 2.1 m and soil samples were collected from field plots in

0.3 m depth increments to 2.1 m on a periodic basis. Determining accurate yield expectations under deficit irrigation conditions, correct scheduling of irrigation and the use current best management practices for N management can help minimize nitrate losses in leachate (Tarkalson et al. 2006).

Very low NO_3 concentrations were found in the rooting zone at most sample positions, indicating that crop demand during recent growing seasons matched or exceeded supply. Accumulations of NO_3 below the rooting zone indicated that deep percolation of NO_3 has been an important process over the longer term throughout the upper and mid slope positions of the landscape. A lack of NO_3 accumulation in one lower-toe position and the depression indicated that excess NO_3 in these profiles may have been leached into the groundwater and/or removed via denitrification or simply may not have accumulated (Whetter et al. 2006).

A long-term (1982 to 2000) field experiment was conducted - under wheat - wheat-corn rotation to determine the effects of N, P, and K chemical fertilizers and farmyard manure accumulation on nitrate (NO_3-N) in the soil profile (0-180 cm).

Fertilizers (N, NP, and NPK) led to NO_3-N accumulation in most subsoil layers. Combined applications of fertilizers and manure reduced soil NO_3-N accumulation in soil compared with fertilizers alone. In conclusion, the findings suggest that it is important to use balanced application of chemical fertilizers and manure at proper rates in order to protect soil and underground water from potential NO_3-N pollution while also sustaining high crop production (Yang et al. 2003).

Four variants of a leaching experiment were conducted at 2 sites to parameterise and check the theory. The experiment involved the application of ammonium chloride to an area of 25 m^2, and then from 6 days to 5 months taking soil samples at 200 mm intervals down to 2 m depth and analysing them for chloride, ammonium, and nitrate. Background concentrations were obtained by contemporaneous sampling nearby. In one variant of the experiment 353 mm of rain in 6 days moved nearly half the applied nitrogen to below 400 mm depth (Banabas et al. 2008).

Drainage and nitrate leaching were simulated using the Water and Nitrogen Management Model (WNMM). Nitrate concentrations in the drainage water and nitrate leaching increased with increasing N application rate. Annual leaching losses ranged from 21.1 to 46.3 kg N ha^{-1} (9.5-16.8%) for inputs between 0 and 150 kg N ha^{-1}. Growth of oilseed rape decreased the nitrate concentration in the drainage water, but growing N fixing peanuts did not. Rainfall had a greater impact on nitrate leaching than crop uptake. The loss of nitrate was low during the dry season (October-February) and in the dry year (rainfall 17% below average) mainly as a result of reduced drainage (Sun et al. 2008).

Under fertilizer treatment, larger quantities of NO_3-N were present in the upper soil layer (0-40 cm) at 7 days after fertilization. From 7 to 37 days after fertilization, NO_3-N decreased, obviously because of the heavy rainfall together with the increase in the capacity of maize to accumulate N in this period and a significant decrease in NO_3-N stock was observed. There was a significant positive correlation between the quantity of NO_3-N stock decrease and the nitrogen fertilizer application rates during this period. And there was more NO_3-N accumulated in the lower layers under fertilization treatment at 76 days after N fertilizer application. Nitrogen fertilizer application increased NO_3-N concentration and stock in 0-100cm soil profile and changed NO_3-N distribution during maize cropping season. Nitrogen fertilizer application promoted movement of NO_3-N down the soil profile and increased N loss (Yin et al. 2007).

Numerous studies have shown that 54-72% of mineral nitrogen fertilizer applied is taken up by the plant, 8—21% is bound in the soil organic matter, 2—18% is lost to the atmosphere by denitrification and only 2-8% is lost by leaching (Owen and Jürgens-Gschwind 1986). In their opinion the major source of leached nitrate is the nitrogen mineralised from the soil organic reserves. However these values are valid at careful fertilizer application. In general, nitrate movement in the soil follows water movement. Increased leaching loss of nitrate can be resulted by rainfall, and irrigation between the growing seasons when soils are without plant cover. Less water percolates through heavy soils than through light soils, resulting in lower nitrate leaching losses from heavy soils. On average, nitrate leaching losses are 30-40 kg ha^{-1} from sandy soils and 20-30 kg N ha^{-1} from loamy soils. In the absence of a crop leaching losses are extremely high. High groundwater level also favours nitrate leaching from the soil. With very high fertilizer nitrogen application rates the proportion of the applied nitrogen taken up by the plant decreases, and the residual fertilizer nitrogen in the soil will be vulnerable to leaching. Approximately 300 mm annual drainage water at 100 kg N $ha^{-1}y^{-1}$ fertilizer rate has only little effect on nitrate 1eaching loss, but at higher fertilizer rate more than 100 kg N $ha^{-1}y^{-1}$ is the 1eaching loss from a sandy soil. Application of nitrogen fertilizer in spring rather than in autumn avoids leaching of fertilizer nitrogen.

The N_{min} method (Wehrmann and Scharpf 1979) is based on observations that cereals utilise the mineral nitrogen contents of deeper soil levels. To start with, the mineral nitrogen content of the 0-90 cm soil level was taken into account when applying nitrogen head dressing in spring. Later, wide-ranging studies proved that winter wheat is capable of efficiently utilising the mineral nitrogen content of the soil to a depth of 150 cm (Kuhlmann et al. 1989). The utilisation of the mineral nitrogen present in deeper soil layers was also confirmed in the case of other crops (barley, sugar beet, maize) (De Willigen and Van Noordwijk 1987). These nitrogen sources were found to be used by maize varieties with nitrogen requirements in the shoot and large root density in the soil layers (Wiesler and Horst 1994). The uptake of nitrate-N leached into deeper soil layers is taken into consideration by crop models and nitrogen submodels (SOILN) (Jansson et al. 1991).

The objective of this study is to monitor nitrate movement in the soils of some Hungarian fertilization trials effected by annual nitrogen fertilizer application.

FERTILIZATION EXPERIMENT IN GÖDÖLLŐ

The experiment was set up on a brown forest soil at the experimental station of Gödöllő University of Agricultural Sciences at Szárítópuszta in 1969. The physical soil type in the top 60 cm was sand, fol1owed by sandy loam, loam and, at a depth of 200-300 cm, clay or clayey loam. The thickness of the humus layer was 35 cm, with $CaCO_3$ appearing at a depth of 60 cm. The humus content was 1.3 % in the ploughed layer and less than 1 % in lower layers. The parent material was loess the groundwater level was below 4 m, with a layer of limestone in the soil profile at a depth of around 2 m.

Nitrogen fertilizer was applied in the experiment from autumn 1969 onwards at rising rates of 0, 90, 180, 270, and 360 kg Nha^{-1} in the form of ammonium-nitrate. Phosphorus and potassium fertilizers were applied together in the rates of 0, 60, 120, 240 kg P_2O_5 $ha^{-1}y^{-1}$ and 0, 50, 100, 150, 200 kg K_2O $ha^{-1}y^{-1}$, respectively. The experiment was not irrigated except of 3 years when 100 mm of water were applied yearly. Maize was sown on the area in a monoculture for 20 years. Yields and the nitrogen content of stalk and grain were measured. In autumn 1989 fertilization was discontinued and alfalfa was sown on the experimental area. Each year the hay yield and the nitrogen content of alfalfa were recorded. In 1986, 1989 and 1994 soil samples were taken every 20 cm in 6 replications to a depth of 3 m from the various fertilizer treatments. Nitrate content of the soil samples was determined. Phosphorus and potassium were applied as basal fertilization in autumn.

NATIONAL LONG-TERM FERTILIZATION TRIALS

Data from the National Long-term Mineral Fertilisation Trials served as the basis of the analysis. These trials were set up using a uniform methodology in various parts of Hungary in 1967–68. The locations and soil types are representative of the conditions characterising crop production in all the major agricultural regions of the country.

The nitrate-N data from core sampling in the B rotation of the experimental series coded OMTK AB 018 were used in the calculations. In the first four cycles (between 1968/69 and 1983/84) the B rotation involved winter wheat–maize–maize–winter wheat, after which the crop sequence was changed to winter wheat–maize–sunflower–winter wheat. N fertiliser rates of 0-50-150-250 kg/ha were applied each year, amounting to a total of 0-950-2850-4750 kg N/ha over the 1968–1988 period. Phosphorus and potassium were each applied at uniform annual rates of 100 kg/ha active ingredients.

Samples were taken from the 0–3 m layer of soil in summer 1988. The nitrate-N data published by Németh and Buzás (1990) for each 20 cm of these profiles were used to determine how the N fertiliser applied over the course of 20 years altered the nitrate-N content of the 0–3 m layer. The crops included in the rotation are chiefly able to utilise the nitrate-N content of the 0–1 m layer, so the nitrate-N contents of the 0–1 and 1–3 m layers were also analysed separately.

The average annual rainfall quantities at the experimental locations ranged from 476 to 647 mm, as follows: Bicsérd: 647 mm, Iregszemncse: 614 mm, Hajdúböszörmény: 538 mm, Karcag:476 mm, Putnok: 564 mm, Nagyhörcsök: 548 mm, respectively.

The upper level of plasticity (texture) according to Arany had the following values at each location: Bicsérd: 45, Iregszemcse:37, Hajdúböszörmény: 54, Karcag: 47, Putnok: 41, Nagyhörcsök: 38, respectively.

RESULTS AND DISCUSSION

In a long-term fertilization experiment set up in 1969 on a brown forest soil in Gödöllő, the 0-3 m soil layer under a maize monoculture had accumulated a total of 130-2050 kg nitrate-N by 1986 (Table 1. and 2.) depending on the rates of nitrogen applied (Füleky and Debreczeni 1991). Between 1986 and 1989 the quantity of nitrate-N in the soil continued to increase due to the application of unchanged fertilizer rates. In long-term experiments in other parts of the country a similar extent of nitrate-N accumulation was observed (Németh and Buzás 1990). Considering the fact that the Gödöllő soil also contained several hundred kg nitrate-N, it was a natural thought to sow alfalfa, which is more deeply rooted than the crops previously used, and has a high nitrogen requirement, in order to utilise the nitrate-N to be found in the deeper layers of the soil.

The vertical distribution of nitrate is shown in Figure 1.

In the control plot the amount of nitrate-N is only a few kg N ha^{-1} in the 3 meter soil layer. Increasing the rate of nitrogen fertilization the amount of nitrate-N in the soil profile increases. The maximum of nitrate accumulation is found at about 2 meter. Nitrate distribution usually has a minimum at 40-80 cm depth. Nitrogen uptake of plants usually effects of the nitrate shift by 100 cm depth. Nitrate being in deeper horizons practically is lost for plant uptake and moves downwards with water movement. However

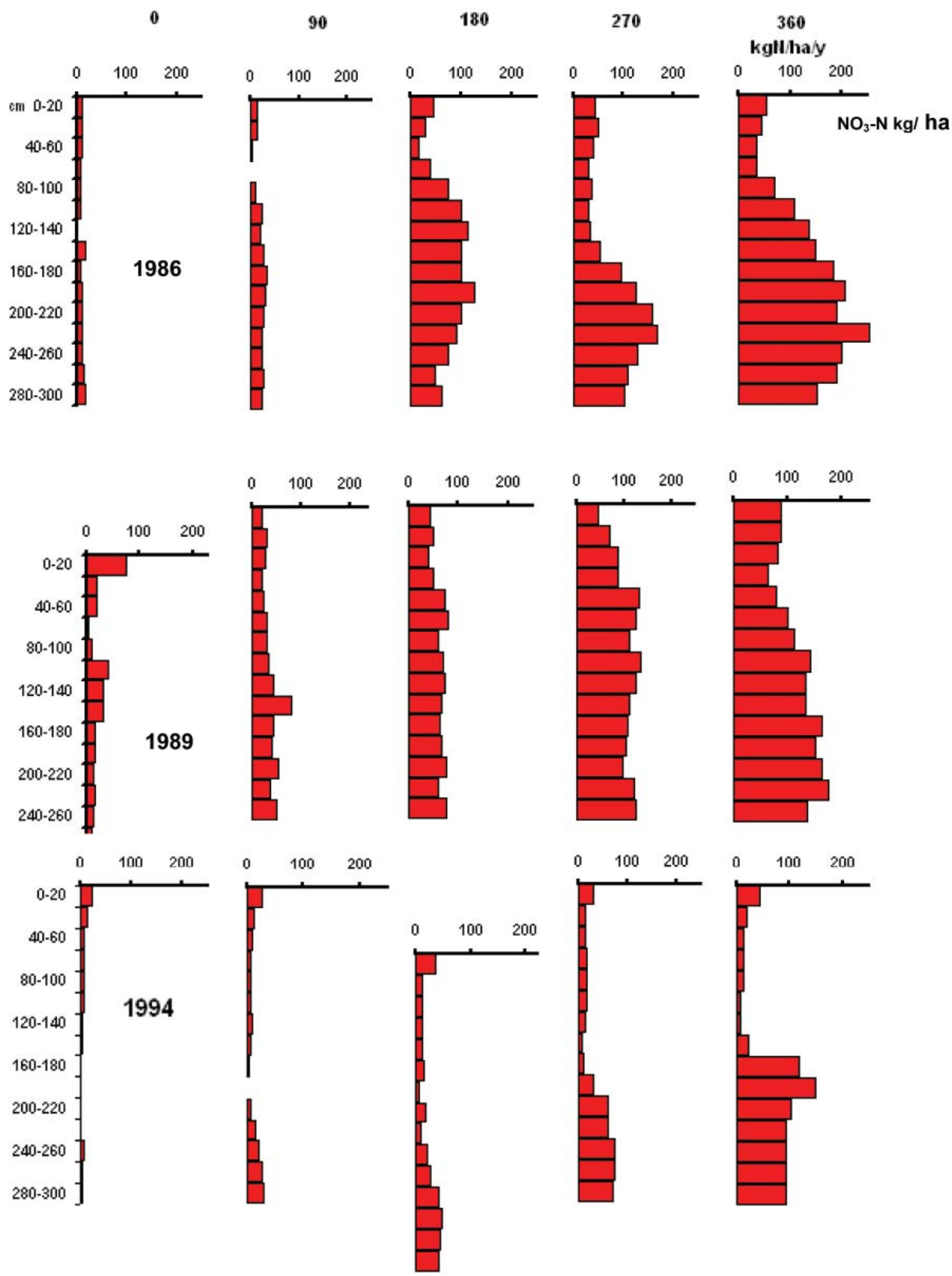

Figure 1: The vertical distribution of nitrate-N of Gödöllő in 1986, 1989 and 1994, respectively

TABLE 1: Yield and nitrogen content of maize and alfalfa plants in 1986, 1994, respectively					
Nitrogen application rate, N kg $ha^{-1}y^{-1}$	0	90	180	270	360
Average grain yield of maize t ha^{-1}	2.80	4.25	4.44	4.44	4.24
Average N content of grain, in %	1.30	1.40	1.44	1.45	1.48
Average N content of stalk, in %	0.61	0.70	0.74	0.77	0.77
Average N uptake by the crop N kg $ha^{-1}y^{-1}$	53	90	98	98	95
Average N content of maize (grain + stalk), N kg t^{-1}	19.1	21.0	21.8	22.2	22.5
Sum of the N uptake by alfalfa kgha^{-1}	587	716	778	809	828

TABLE 2: Nitrogen fertilizer rates and the amount of accumulated nitrate in 1986, 1989, 1994, respectively					
Nitrogen application rate, N kg $ha^{-1}y^{-1}$	0	90	180	270	360
Total amount of fertilizer nitrogen, N kg ha^{-1}	0	1440	2880	4320	5760
Total N uptake by the crops, N kg ha^{-1}	852	1436	1561	1574	1512
Nitrogen balance in 1986 N kg ha^{-1}	-852	+2	+1319	+2746	+4248
Nitrogen-N in 3 m soil layer, N kg ha^{-1}	134	298	1125	1189	2051
Nitrogen-N in 1 m soil layer, N kg ha^{-1}	41	43	205	198	235
Nitrate-N in 3 m soil layer in the % of nitrogen balance	-	>100	85	43	48
Nitrogen balance in 1989 N kg ha^{-1}	-1070	+14	+1666	+3432	+5294
Nitrogen-N in 3 m soil layer, N kg ha^{-1}	288	628	933	1634	1787
Nitrogen-N in 1 m soil layer, N kg ha^{-1}	82	132	256	424	397
Nitrate-N in 3 m soil layer in the % of nitrogen balance			56	48	34
Nitrogen balance in 1994 N kg ha^{-1}	-1710	-791	+842	+2525	+4371
Nitrogen-N in 3 m soil layer, N kg ha^{-1}	90	183	381	625	885
Nitrogen-N in 1 m soil layer, N kg ha^{-1}	56	61	85	87	95
Nitrate-N in 3 m soil layer in the % of nitrogen balance			45	25	20

further significant nitrate enrichment can be expected below 3 meter at 180 kg N ha^{-1} or higher rates of nitrogen fertilization.

Nitrate accumulation shown in Figure 1. can be compared to the nitrogen uptake of maize plants. Yield and nitrogen concentration of grain and stalk are given in Table 1. Data for nitrate accumulation in the soil can be seen in Table 2. Crop yield was found to be increased by90 kg N ha^{-1} in the field. Additional fertilizer rates increased only the N content of grain and stalk but not the yield. In the control treatment and uptake by stalk and grain together was about 53 kg yearly. In other treatments this is fluctuated about 100 kg N ha^{-1}. In control plots than only source of nitrate-N is nitrogen mineralised from the soil organic reserves (852 kg N ha^{-1} in 16 years). As it can seen from the nitrogen balance at 90 kg N ha^{-1} fertilizer rata the amount of nitrogen taken up by crop is equal to the nitrogen added in 15 years. Nevertheless, the 3 meter layer of soil profile has remarkable nitrate content (298 kg N ha^{-1}). For this the downwards movement of both the mineralised and fertilizer originated nitrate can be responsible. At 180 kg N ha^{-1} and higher fertilizer rates N balance becomes more positive so a considerable amount of fertilizer nitrogen has to remain after each harvest in the soil. Data for vertical distribution show very significant amount of nitrate accumulation in the 3 meter layer of soil$_1$ at higher fertilizer rates. At 0, 90, 180, 270, 360 kg N ha rates 134, 298, 1125, 1189, 2051 kg nitrate-N ha^{-1} are found in 3 meter soil 1ayer. From these amounts only 41, 43, 205, 198, 235 kg N ha^{-1} are in the upper 1 meter layer which is available for the plant roots. These data suggest that a big amount of nitrate practically lost for plant uptake and its fate is the leaching.

The annual rainfall surplus is 116 mm in the average of 16 years and it is enough for leaching the nitrate not taken up by plants. Regarding the nitrate content of 3 meter soil layer in relation to the total amount of applied fertilizer nitrogen, the 20-40% of fertilizer nitrogen can be found in this soil layer. If the amount of nitrate accumulated in the 3 meter 1ayer is related to the "nitrogen balance" .data, at 90 kg N ha^{-1} fertilizer rate the whole amount of nitrogen balance, at 180 kg N ha^{-1} rate 85%, at 270 and 360 kg N ha^{-1} rates 43 and 48% of the "nitrogen ba1ance" can be found in the 3 meter soil layer. As it can be seen in Figure 1 this nitrogen loss is probably not caused by nitrogen volatilization to the atmosphere but by leaching downwards with the drainage water into the deeper soil layers.

At increasing nitrogen rates nitrate accumulation in soil is due to the fact, that at higher fertilizer rates plants take up nitrogen mainly from the fertilizer, so nitrate mineralised from soil organic reserves is exposed to leaching. Application of nitrogen fertilizer in autumn

TABLE 3: Distribution of nitrate N as a function of increasing mineral fertiliser rates, nitrate-N kgha^{-1}

a) In the 0-3 m soil layer	N fertiliser rates (kg Nha^{-1}year^{-1})			
	0	50	150	250
Bicsérd	129	146	336	568
Iregszemcse	129	123	262	751
Hajdúböszörmény	131	143	300	576
Karcag	110	143	522	1008
Putnok	132	128	206	440
Nagyhörcsök	151	170	401	1219
Mean	130	142	338	674

b) In the 0–1 m soil layer	N fertiliser rates (kg Nha^{-1}year^{-1})			
	0	50	150	250
Bicsérd	55	59	57	102
Iregszemcse	60	56	54	143
Hajdúböszörmény	55	51	113	256
Karcag	37	43	110	211
Putnok	61	59	85	158
Nagyhörcsök	60	72	78	176
Mean	55	57	83	174

c) In the 1–3 m soil layer	N fertiliser rates (kg Nha^{-1}year^{-1})			
	0	50	150	250
Bicsérd	74	87	279	466
Iregszemcse	69	67	208	608
Hajdúböszörmény	76	92	187	320
Karcag	73	100	412	797
Putnok	71	69	121	282
Nagyhörcsök	91	98	323	1043
Mean	75	85	255	500

also can be contributory factor tor nitrate leaching into deeper soil horizons.

Compared to the average 588 mm precipitation measured over many years, there was a deficiency of 199 mm in 1989-1990, 13 mm in 1990-1991 and 197 mm in 1991-1992, while a surplus of 55 mm was recorded in 1993-1994. Due to this water deficiency and the extremely high water requirements of alfalfa, the available quantity of water was presumably insufficient to allow a greater quantity of nitrate-N to migrate downwards, so the decline in the nitrate-N quantity in the lower soil layers after 4 years of alfalfa production can be attributed to the nitrogen uptake of the alfalfa. As shown by the data on the nitrate-N content of the 3 m soil layer, presented in the Table 2., there was a substantial decrease by 1994 compared with the 1989 figures. In the control treatment the nitrate-N quantity, which was initially already small, decreased to below a value of 10 kg N/ha/20 cm throughout the profile (Figure 1.). In plots previously given rates of 90 and 180 kg N/ha the nitrate-N quantity dropped below 10 kg N/ha/20 cm up to a depth of 220

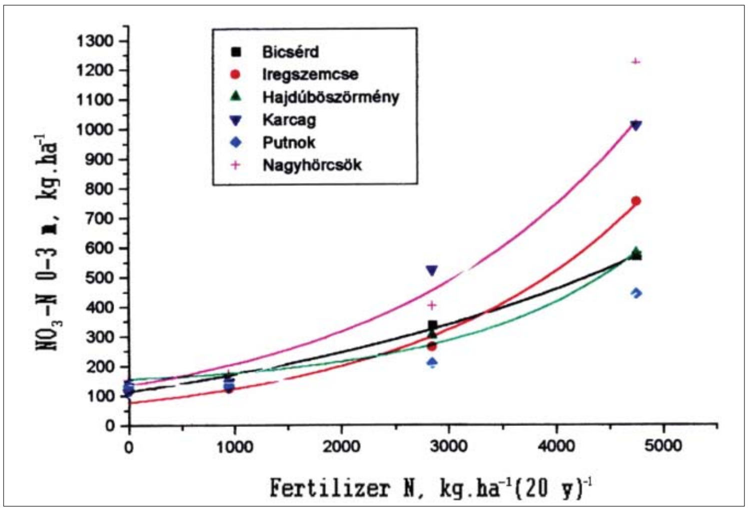

Figure 2: Nitrate-N content of 0-3 soil layer as the function of N fertilisation

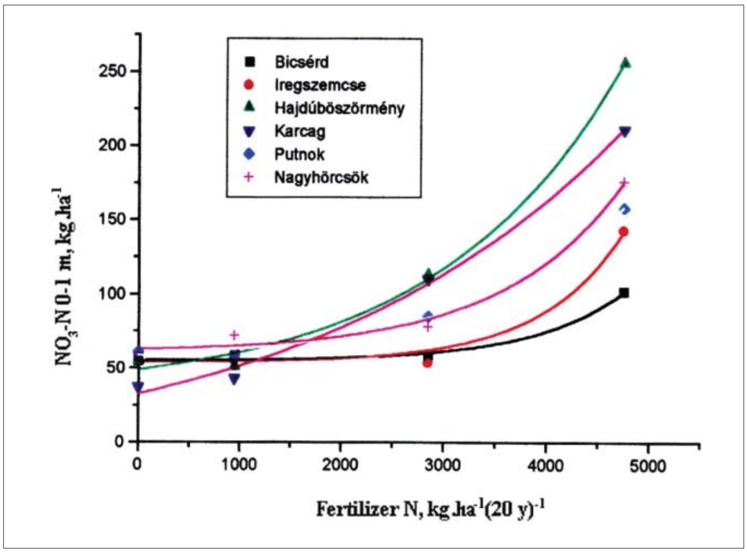

Figure 3: Nitrate-N content of 0-1 m soil layer as the function of N fertilization

cm, while this reduction was observed up to 200 cm in the 270 kg N/ha treatment. In the 360 kg N/ha treatment there was a substantial decline in the soil nitrate-N content up to 160 cm. In addition a considerable reduction in the nitrate-N level could be observed throughout the soil profile during the production of alfalfa. In the course of 4 years the alfalfa removed between 550 and 823 kg/ha N from the soil, depending on the original nitrogen rates. At the same time, the loss of nitrate-N content the 3 m soil layer ranged from 215 to 1040 kg N/ha. In the control plot the available nitrate-N was insufficient to satisfy N requirement of alfalfa, while in plots previously given lower rates of nitrogen fertiliser the quantity of nitrate-N removed from the 3 m soil layer over the 4-year period was roughly equivalent to the quantity of nitrogen absorbed by the alfalfa. In the case of the highest nitrogen fertiliser rates the reduction in the nitrate-N content of the soil exceeded the nitrogen uptake of the alfalfa plants. This fact, observed chiefly in the deepest soil layers after a very large accumulation of nitrate-N, suggests the further migration of nitrate-N to still deeper layers. This is confirmed by examinations on deep-lying roots, showing that alfalfa roots could be found up to a depth of 180 cm, though with a great reduction in mass at lower levels. A gradual reduction in root density is in agreement with the depth of the nitrate-N exhaustion zones previously established.

RESULTS OF LONG-TERM FERTILIZATION TRIALS

In response to N fertiliser rates in excess of plant needs, nitrate-N accumulates in the soil in various quantities and at different depths. If the nitrate not absorbed by the crop is to be prevented from reaching lower depths, it is essential to study the role of ecological and technological factors in the fate of nitrate-N in the soil.

On control plots, an average of 130 kg/ha nitrate-N was detected in the 0–3 m layer of soil (Table 3.). As the N fertiliser rates rose, there was a rapid increase in the nitrate-N quantity detectable in the soil. At the 150 kg/ha mineral fertiliser rate there was an average nitrate-N content of 338 kg/ha in the 0–3 m layer, with extreme values of 206 and 522 kg/ha. The regular application of 250 kg/ha N fertiliser over 20 years resulted in an average nitrate-N quantity of 674 kg/ha in the 0–3 m layer, ranging from 440–1219 kg/ha. From the point of view of plant uptake, the nitrate-N content of the upper 1 m soil layer is of prime importance. At depths available to the crops, annual rates of 0-50-150-250 kg N/ha led to nitrate-N contents of only 55-57-83-174 kg/ha in the 0–1 m layer, averaged over the six locations. The nitrate-N content of the 1–3 m soil layer is lost to the crops, and may reach the groundwater with downward water migration.

In the long-term experiments there was an exponential rise in the nitrate-N content of the 0–3 m soil layer as the fertiliser rates increased (Figure 2.).

In the control almost all the nitrogen was derived from the mineralisation of soil organic matter. The application of an annual 50 kg/ha N fertiliser (950 kg/ha/20 years) resulted in only a minimal rise in the soil nitrate-N content. When the rate was increased to 150 kg/ha a year (2850 kgNha^{-1}year^{-1}) the soil nitrate-N content was almost doubled. After a further rise in the fertiliser rate the curves separated, depending on soil properties, climatic conditions and plant N uptake. At rates of above 150 kgNha^{-1}year^{1} the relatively slow increase in nitrate-N content accelerated greatly. By treating the 0–1 and 1–3 m layers separately, a more differentiated picture of nitrate-N accumulation was obtained. There was an exponential rise in the nitrate-N content of the 0–1 m layer with increasing rates of mineral fertiliser (Figure 3.). Compared with the control, the addition of 950 kg/ha over 20 years (50 kgNha^{-1}year^{-1}) did not generally increase the soil nitrate-N content. However, even at rates of below 150 kg/ha/year N fertiliser, at around 100 kg/ha, the nitrate-N curves for the different locations began to separate.

The nitrate-N content of the 1–3 m soil layer below the root zone also changed exponentially, but in contrast to the 0–1 m layer, a rise in the nitrate-N content could be observed even at rates just above 50 kg N/ha. The nitrate-N content of the 1–3 m soil layer was hardly influenced by plant N uptake and depended primarily on the downward migration of surplus water.

Under the climatic and soil conditions in Hungary, the annual application of 100 kg/ha N fertiliser (2000 kg/ha/20 years) does not result in any great increase in the nitrate-N content. At higher rates, however, depending on

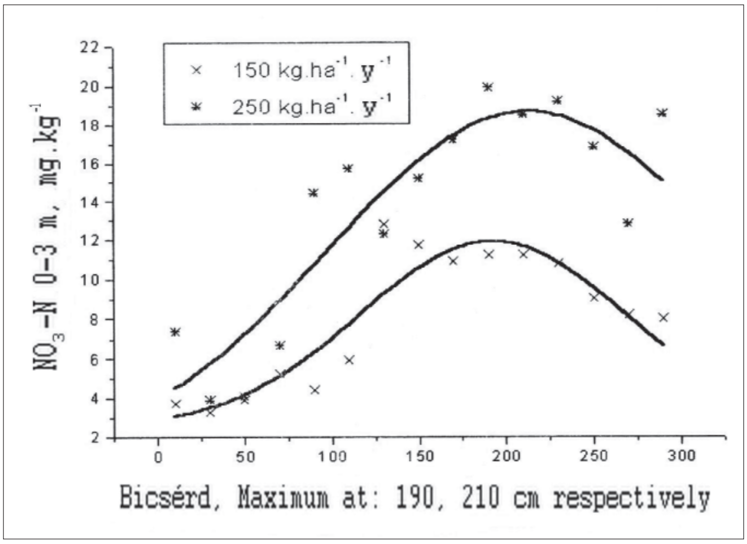

Figure 4: Gauss distribution of nitrate-N in 0-3 m soil layer at Bicsérd

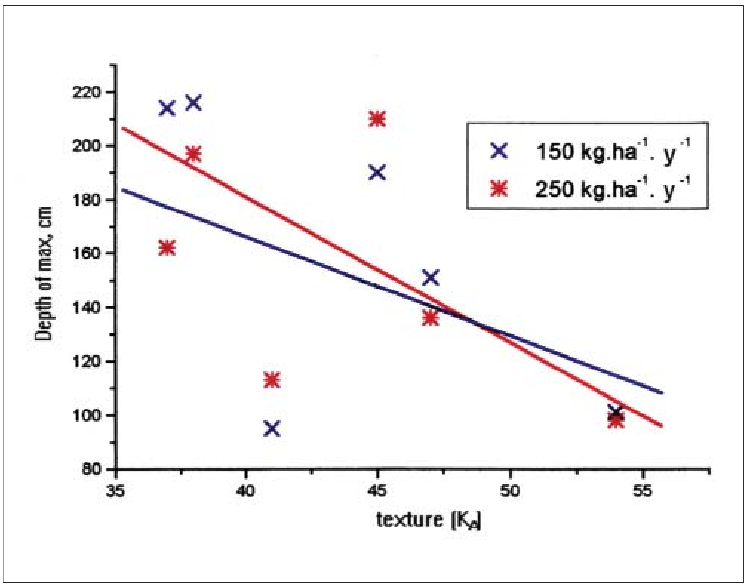

Figure 5: Correlation between soil texture (K_A) and the depth of maximum nitrate-N accumulation

TABLE 4: Depth of maximum nitrate-N accumulation, cm

	N fertiliser rate kgha^{-1}year^{-1}	
	150	250
Bicsérd	190	210
Iregszemcse	214	162
Hajdúböszörmény	101	98
Karcag	151	136
Putnok	95	113
Nagyhörcsök	216	197
Mean	161	152

the climatic and soil conditions, there is an exponential rise in the nitrate-N content in the 0–3 m soil layer, both in the 1 m root zone and in the 1–3 m layer below it.

Nitrate-N migration towards the deeper soil layers can be clearly characterised by the depth of maximum nitrate-N accumulation in the profile, which was determined using a Gauss distribution curve. The nitrate-N concentrations recorded every 20 cm in the 0–3 m soil layer were plotted as a function of depth, after which a Gauss curve was fitted (Figure 4.).

The maximum nitrate-N accumulation in the various soils was detected between 95 and 216 cm at the 150 kg/ha N fertiliser rate and between 98 and 216 cm for the 250 kg/ha rate (Table 4.).

The mean depth of maximum nitrate-N accumulation was 161 and 152 cm at the two N rates, indicating that the N fertiliser rate had no clear influence on the depth of maximum accumulation.

Linear correlation analysis was applied to determine the correlation between the depth of maximum nitrate-N accumulation and the soil texture or rainfall migration.

The depth of maximum nitrate-N accumulation after various rates of mineral fertilisation was closer to the surface in heavier soils (greater values of K_A). As the soil became finer (Figure 5.) the maximum nitrate-N accumulation was found at shallower depths. On soils with sandy texture the downward movement of nitrate-N was more intense, so the maximum accumulation was detected in lower soil layers. The steepness of the two curves confirmed that under Hungarian conditions the depth of maximum nitrate-N concentration was practically independent of the fertiliser rate applied.

The depth of maximum nitrate-N accumulation did not exhibit a close correlation with the rainfall (r = 0.405 and 0.541). There was, however, a clear tendency for higher quantities of rainfall to cause the nitrate-N to move to deeper layers and accumulate there (Figure 6.). A comparison of the roles of soil texture and rainfall on the basis of correlation coefficients revealed that the depth of maximum nitrate-N accumulation was influenced to a greater extent by soil texture.

The reasons for differences in the nitrate-N profiles of the various locations included in the National Long-term Fertilisation Trials appeared to be primarily dependent on the different rainfall conditions and soil texture and on the N fertiliser rates.

In response to increasing rates of mineral fertiliser there was an exponential rise in the nitrate-N content of the soil, both in the root zone and in deeper soil layers. At annual fertiliser rates of 0 and 50 kg N/ha/year, no nitrate accumulation was observed, but the leaching of nitrate

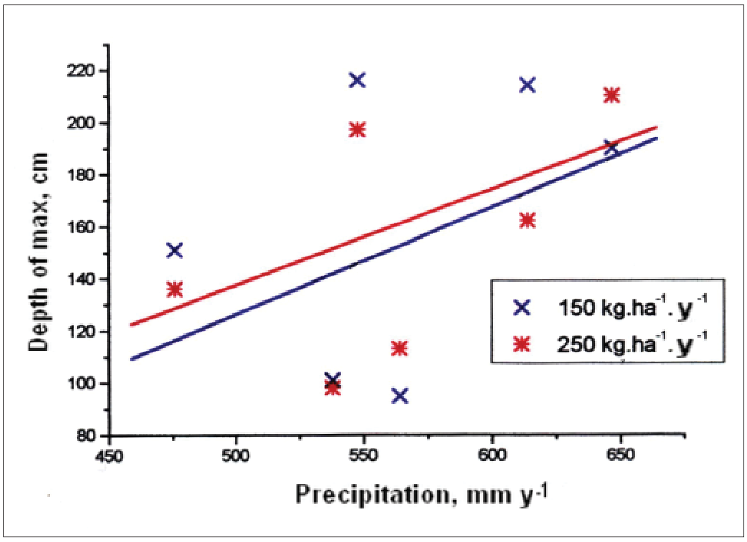

Figure 6: Correlation between precipitation and the depth of maximum nitrate-N accumulation

ions was already detectable at the 150 kg N/ha/year rate, and increased considerably in the case of 250 kg/ha/year. Based on the exponential curve, a substantial increase in nitrate-N could be observed at an N fertiliser rate of 2000 kg/ha/20 years. In agreement with data from the literature, under the climatic and soil conditions in Hungary, nitrate accumulation can generally be expected at fertiliser N rates in excess of 100 kgha^{-1}year^{1}.

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IMPROVE SPECIES COMPOSITION IN NORTHEN HUNGARIAN MOUNTAINS ON PLAINS BLUESTEM INFESTED SWARD BY OVERSEEDING

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ABSTRACT

The plains bluestem *(Bothriochloa ischaemum)* is a native element of the Hungarian flora, but due to disturbance monodominant spots may occur, which is not desirable to both grassland management and nature conservation. We have set our overseeding experiment on a 40-year-old fallow field left after a vineyard in Northern Hungarian mountains, which is utilized as a sheep pasture, to suppress this unpleasant species. We have utilized: *Lolium perenne, Poa angustifolia, Bromus inermis, Dactylis glomerata*. The experimental-plot size was 5×4 m. During the experiment

(3 years) we have failed to significantly suppress the plains bluestem bluestem at this extremely dry habitat, but managed to stop infestation. The *L. perenne* could be a useful component in optimal seed-mixture because of it's initial aggressive growth rate, but only in wet years due to high demand of water. The pure seed sowing however is risky on natural habitat of plains bluestem, which has typically dry and nutrient deficient soil conditions. *D. glomerata* as pure seed is recommended for forage production, it's ability of reducing the number of species on pasture is derived from it's high productivity and prolification. Applying in seed mixture could serve nature conservation as well. *P. angustifolia* has suppressed the plains bluestem and also had positive effect on species composition, therefore very useful both as pure seed or in mixture. *B. inermis* could not prevail under extreme conditions.

keywords: *Bothriochloa ischaemum*, overseeding, *Lolium perenne*, *Poa angustifolia*, *Bromus inermis*, *Dactylis glomerata*

INTRODUCTION

In Hungary the plains bluestem is a character species on dry upland- and rangeland-steppe grasslands (Festuco Brometea Br.-Bl. et Tuxera ex SOO 1947), but rarely can be found on semi-arid grasslands as well. It's mass scale spreading caused by agricultural disturbance on natural grasslands, like intensive grazing and erosion, (e.g. Virágh and Fekete 1984, Zólyomi and Fekete 1994), burning (Penksza et al. 1994), turf collection (Bartha 2007), former mining activity (Bauer 1998). The shrub clearing and abiotic stress (e.g. dry years) (Bartha 2007) may create ideal conditions for monodominant patches. In a well structured lawn, if the stress factor eliminated the adaptable grass species could suppress the plains bluestem easily. However, if the dominant true grasses disappear (e.g. micro-scaled limitation of propagulum),

the plains bluestem still flourishing even in non favourable environment. If the regular disturbance opens the sward, clear soil patches will appear. The local space-dominant plains bluestem can occupy the open spots fairly quickly, helping to close the canopy again, and reduce soil erosion (Illyés et al. 2007). It's mending role is part of the natural dynamics of the vegetation which is acceptable until not setting back the true grass immigration or not preventing sward regeneration (Horváth and Kovács 2008). The high coverage of plains bluestem evolves because the degradation of natural true grass composition (Virágh and Fekete 1984, Zólyomi and Fekete 1994, Kelemen 1997) or it is a phase in degraded pasture-, fallow regeneration process (Bartha 2007). Grasslands where plains blue-stem is dominant, can accumulate thick grass litter. This species has dense root system and forms tussocks. Between them significant amount of slow degrading grass litter builds up, which may prevents germination of plants (Illyés et al. 2007), immigration and survival of less competitive species (Bartha 2010). The plains bluestem's spread and growth of dominance have negative effect on physiognomian structure of grasslands. Induces biodiversity degradation, reduces compositional diversity (Virágh 2002, Virágh and Somodi 2007, Szabó et al. 2008, Szentes et al. 2011, 2012). It's nitrogen content is less than the C_3 species (Yuan et al. 2007), therefore the protein content and feed value is lower as well. The morphological characteristics are adverse too, so livestock usually doesn't graze at all, which increases the accumulation of grass-litter. The expansion of this species is undesirable for both grassland management and nature conservation. Our aim was to examine whether possible to hold back plains bluestem and improve grass species composition with the help of agro-technology.

TABLE 1: Plot recording dates

Year	Cronological recording
2009	10. 8.
	5. 7.
2010	7. 8.
	10. 10.
	5. 5.
2011	7. 5.
	–
	5. 9.
2012	7. 3.
	–

MATERIAL AND METHODS

The experimental plot is at Kisfüzes, border slopes of North Hungarian Mountains. The average annual temperature is 9 °C, while the average temperature is 16 °C during the

vegetation period. The average annual precipitation is 560 mm, 360 mm falls in the vegetational seasons. The soil type is luvisol. The trial has been set on a sheep farm, on 100 ha of pasture in 5 paddocks, with 150 Texel ewes and lamb. The inspected sward is situated in a North-North West and South-South East directional valley on a 40-year-old vineyard fallow. Hard to classify the plant composition, dominant species are *Bromus inermis* and *Poa angustifolia*. Common species are *Achillea nobilis*, *Plantago lanceolata*, *Verbascum phoeniceum* and the plains bluestem *(Bothriochloa ischaemum)*. The average coverage is about 40-70% depending from phenological state. The experiment has been set up in a 20 ha paddock, South-South Western steep slope 246 to 247 m above sea level. Because of the exposure and sloping area, dry and warm microclimate has evolved, beside strong erosion, which slows down natural succession. We set up 30 5x4 m plots in the autumn of 2009. Plots were randomly distributed. We analized 15 of these for this paper. Four treatment and one control in three repeat. The treatments were: overseeding of *Lolium perenne, Poa angustifolia, Bromus inermis, Dactylis glomerata*. This method was chosen because of the significant risk of erosion due to the steep slope. We locked down the paddock after overseeding and

sheep grazing, till November of 2010. We left sufficient time for growth of the renewal. The recording dates are shown in Table 1.

In 2011 and 2012 we skipped the conological recording due to the serious drought. We set the coverage value in percentage.

RESULTS

At the first two years *Lolium perenne* overseeding was successful (Figure 1, 2). It had more than 40% coverage by the end of 2010, and it would not let plains bluestem spread, but in 2012 entirely disappeared, so the total coverage of true grasses decreased to the 2009 level. The legumes had the highest coverage in 2010 with 34%. The dicotyledons' coverage fluctuated, but in July 2011 they well compensated the decreasing true grasses. The coverage of plains bluestem reduced from 6% to 3-4% by the end of the trial. The gross coverage at the Lolium-overseeding has been grown from 60% to 97%, followed with this cultivar's total disappearance (short lifetime) ending with the original composition.

The overseeding with *Poa angustifolia* resulted top coverage in October 2010, then slowly declined, but in 2012 was still double of the initial coverage (Figure 3, 4). *B. inermis* coverage has been increased in the rainy periods. Plains bluestem was steady at about 3-4%. True grasses' coverage increased to 50.8% by October 2010 as a result of overseeding. This year had rainy spring and summer therefore legumes came up (*Trifolium campestre, Medicago minima*). The dicotyledons' coverage in July 2011 was the largest. The diversity in grasslands is important as associations respond more effectively to extreme stressors (e.g. : weather) and also handy in feeding and nature conservation. Other species also grown the best in this period. The changes in grass-canopy reduced to the initial level, effected by stolon type (long lifetime) species overseeding in 2012. This method has performed less efficient than overseeding with short lifetime species. This perfectly represents the primary effect of drought on grass composition.

Dactilys glomerata coverage has continuously increased in 2010. The highest coverage (70%) was in the spring of 2011 (Figure 5, 6). In July 2012 coverage has remained over 20%. The plains bluestem's coverage was about 2-8%. The higher values were in dry periods. The overseeded *Dactylis* did not let the plains bluestem spreading, but also suppressed other species.

Bromus inermis plots has reached maximum coverage at 27% in autumn 2010 (Figure 7, 8). *P. angustifolia* coverage was similar, however coverage was only 10%. The plains bluestem and other species spread well. The coverage of other dicotyledonous species was also relatively high.

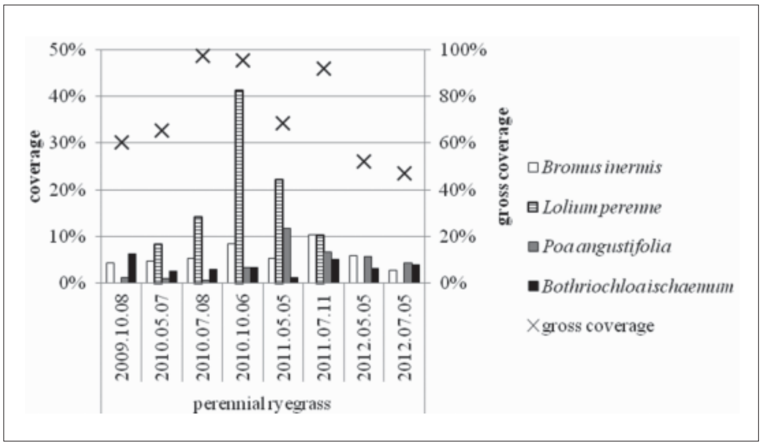

Figure 1: The average coverage of the most common true grasses in the perennial ryegrass overseeded plots.

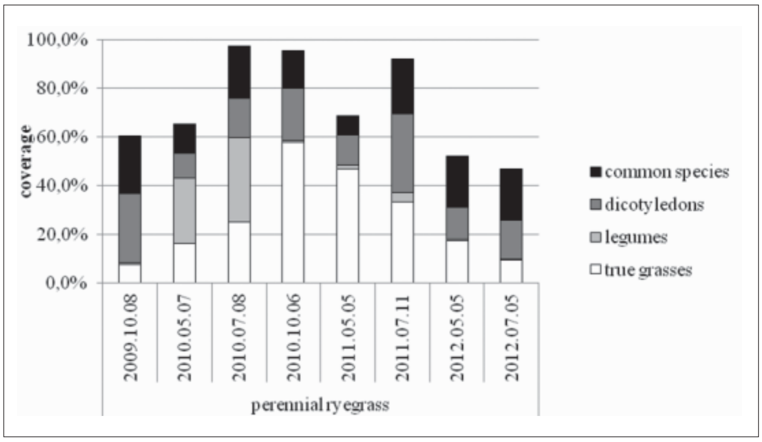

Figure 2: The average coverage of the different plant groups in the perennial ryegrass overseeded plots.

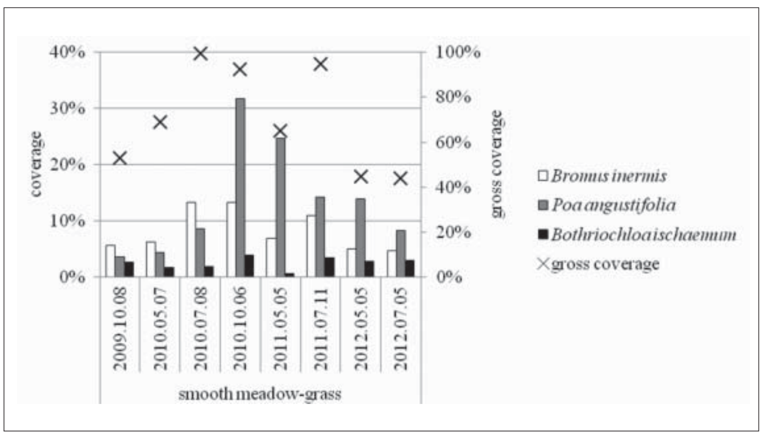

Figure 3: The average coverage of the most common true grasses in the smooth meadow-grass overseeded plots.

Plains bluestem has grown the best in **control** plots (Figure 9, 10). It has reached 9-10% of coverage. The common true grasses has reached their maximum coverage in May 2011. However the gross coverage in autumn of 2010 was the highest. *Bromus inermis* was the dominant species.

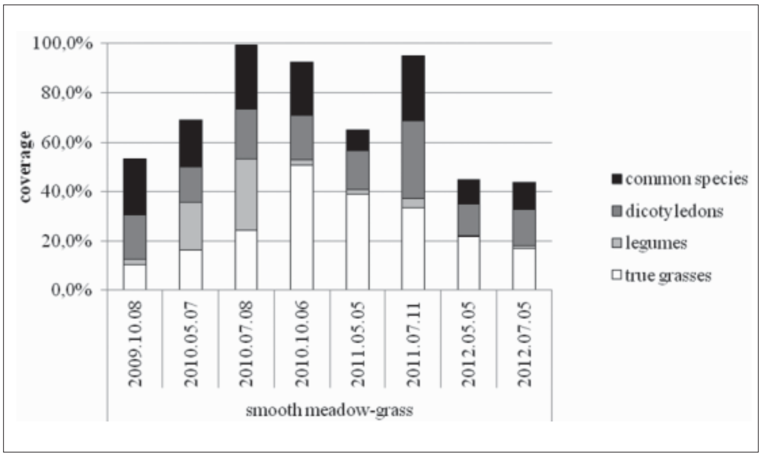

Figure 4: The average coverage of the different plant groups in the smooth meadow-grass overseeded plots.

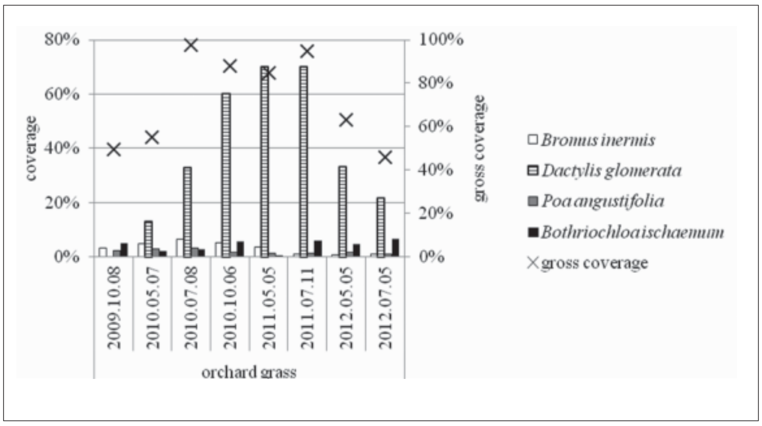

Figure 5: The average coverage of the most common true grasses in the orchard grass overseeded plots.

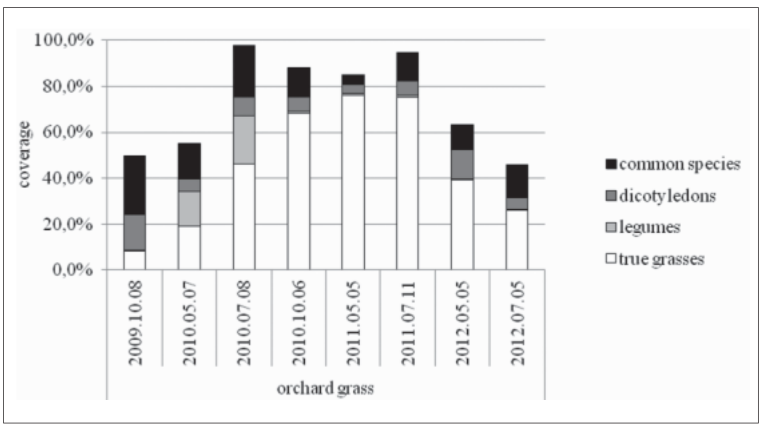

Figure 6: The average coverage of the different plant groups in the orchard grass overseeded plots.

DISCUSSION

In 2010 good weather conditions (plenty and continuous rainfall) made very effective the overseeding. Compare to 2009, the total grass coverage has increased significantly. In 2011, only one-third of last year's precipitation fell. Even in the first half of the year, very few (212.9 mm) from the end of July till December, a total of 6.6 mm rain fall. The plots' species composition changed and the total coverage reduced in every parcel. 2012 was also the year of drought, which further strengthened this process.

The smallest yield was in the *Lolium perenne* parcel. At the end of summer in 2011, due to the drought completely disappeared from the sward, but a year earlier there was over 40% coverage. The disappearance effected on its plains bluestem controlling effect as well.

The *P. angustifolia* was the most effective species to control plains bluestem but couldn't roll back completely, still did not effect on other grasses too.

D. glomerata has grown the best for forage production. It tolerates drought and has high productivity (Ecker, 1972; Gruber, 1942). It is important to note, that the coverage of *P. angustifolia* and *B. inermis* decreased in these plots. The plains bluestem's coverage was constant, but the true grasses' gross-coverage was the highest also. The other species' cover declined the most and was the smallest too in these parcels.

The *B. inermis* overseeding was successful, however by the spring of 2011 the coverage rapidly decreased and a year later the amount was the same as the beginning. This is probably caused by the longterm drought in this period. In these plots *P. angustifolia* and plains bluestem's growth increased too. There was also significant coverage of other species.

The coverage of useful true grasses in the control plot also showed fluctuations, demonstrated the dominant roll of weather. The cover of legumes also increased in 2010 similarly than in the treated plots. The other dicotyledonous species and coverage of other species are also well followed rainfall distribution.

CONCLUSIONS

At the extremely dry habitat we could not significantly reduce the plains bluestem neither of treatments during the experiment. The overseedings had positive effect on species composition. Because of the aggressive initial growth of *L. perenne* it may be useful components of swards in wet years to roll back plains bluestem. The pure seeding however is very risky at plains bluestem's typically dry and nutrient deficient habitats. *D. glomerata* in pure seeding, utilized in plains bluestem control, primarily recommended for forage production because of it's high productivity, accumulation and it's efficient species reductive effect. Therefore could be very effective in mixture to serve nature conservation purposes. *P. angustifolia* did not let overgrown the plains bluestem and had positive effect on species composition as well. It suits both for pure or mixed seeding. *B. inermis* did not get on under extreme conditions.

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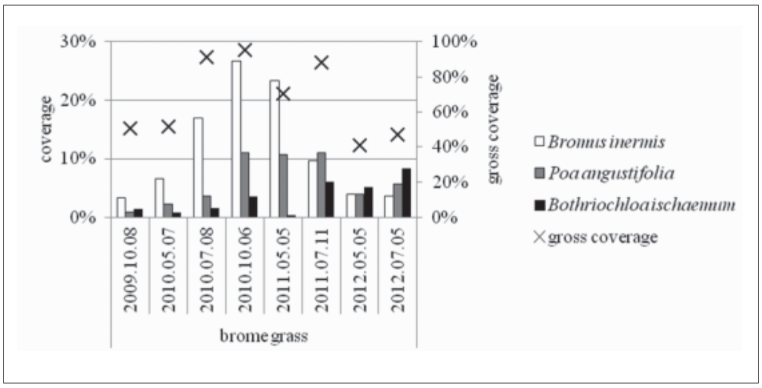

Figure 7: The average coverage of the most common true grasses in the brome grass overseeded plots.

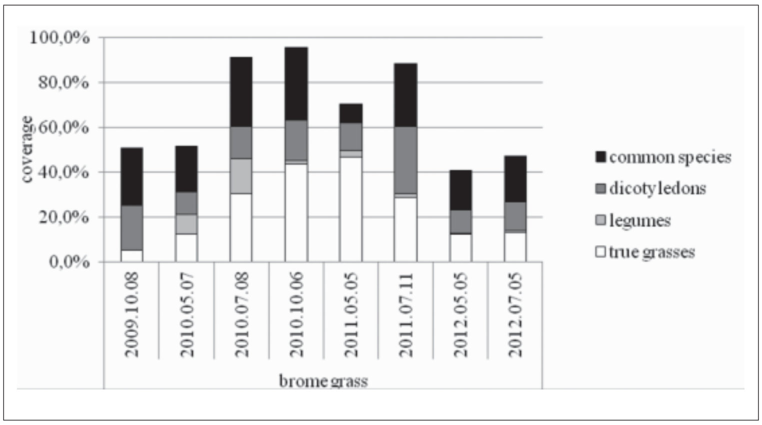

Figure 8: The average coverage of the different plant groups in the brome grass overseeded plots.

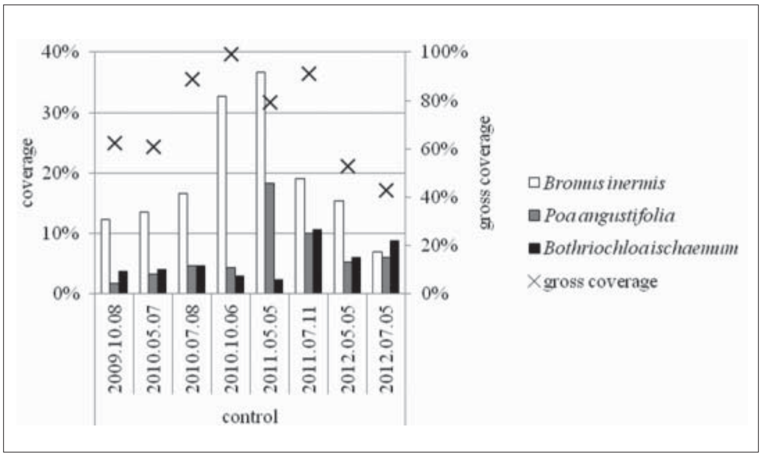

Figure 9: The average coverage of the most common true grasses in the control plots.

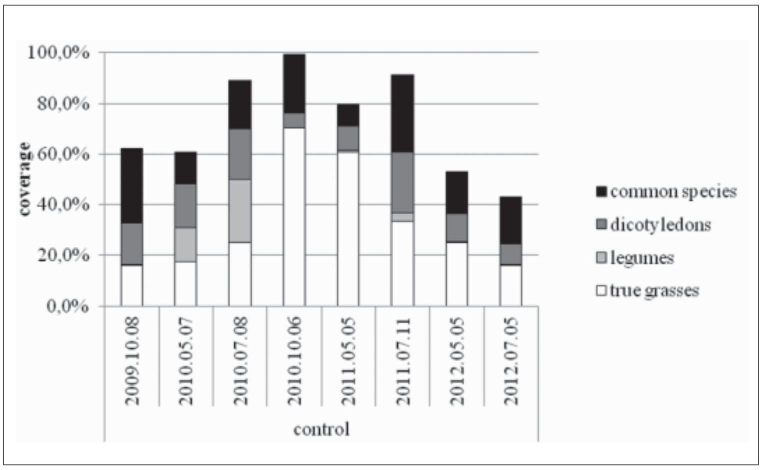

Figure 10: The average coverage of the different plant groups in the control plots.

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AFRICAN SWINE FEVER: AN INCREASING THREAT

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INTRODUCTION

African swine fever (ASF) is a devastating, highly contagious viral disease of pigs causing a variety of clinical signs, among them haemorrhagic lesions that resemble another wide spread disease, namely the classical swine fever. ASF is mostly fatal in domestic pigs but the virus can also infect European wild boars where the symptoms may occasionally be milder. Humans or species other than swine are not susceptible. ASF has a significant economic impact and currently there are no vaccines available to control the disease. It is among those swine diseases that are notifiable to the World Organization for Animal Health (Office International des Epizooties, OIE).

A BRIEF HISTORY OF THE DISEASE

The disease was first observed and later described in Kenya, Africa (Montgomery 1921). R. Eustace Montgomery, after whom the disease was originally named, conducted experiments with the virus in 1910 and on. His initial belief that the newly discovered disease of domestic pigs was a hyperacute form of the already well known classical swine fever was shortly proven otherwise. The East African cases were subsequently observed in South Africa (Steyn 1928) and later in Central and West Africa (Penrith et al. 2004). Today it is enzootic in a number of African countries of the Sub-Saharan region.

From Africa the virus spread to other parts of the world. The first such reported event was the 1957 outbreak in Portugal (Ribeiro and Azevado 1961), where the source of infection was waste from airline flights fed to pigs close to the airport. Since then, outbreaks have occurred in other countries of Europe including Spain, Italy, France, Belgium, The Netherlands and also again Portugal. Most of these outbreaks were quickly taken care of and the countries regained their ASF free status. In Portugal (1960-1993; 1999) and Spain (1960-1995) however the eradication process took longer and it was unsuccessful in Sardinia (Italy), where the virus is still present as an enzootic infection. The American continent was first reached by the infection in 1971 when an outbreak of ASF was recognized in Cuba. Over 400 thousand pigs were destroyed by the end of a successful eradication process, although later in 1980, Cuba was reinfected. Through trade and tourism ASF appeared in Brazil in 1978 and during the next year in Haiti.

The global ASF situation changed significantly during the past years. Before the year 2007 ASF spread significantly in many African countries reaching previously free areas. This phenomenon together with increased global trade and tourism led perhaps to the re-introduction of the disease to continental Europe, this time at the eastern part. An outbreak was reported in the Caucasus region during 2007 affecting Georgia, Abkhazia, Armenia, Azerbaijan and later ASF virus was detected by polymerase chain reaction in dead wild boars in the Chechen Republic of the Russian Federation (Gulenkin et al. 2011). The presence of the virus in wild boars showed that these animals could cross borders and carry ASFV to neighboring areas like in this case through the Caucasus probably along the Shatoy gorge. Although rigorous quarantine measures were introduced in order to stop further virus spread within Russian territories, the disease had become epizootic during the following years, affecting both wild boars and domestic pigs. During these years local outbreaks were identified around Orenburg and Saint Petersburg both over a thousand km away from the main area of the first outbreaks, indicating that the control measures were insufficient. Trade of contaminated pork products was responsible for the sudden spread to these directions. According to OIE data, by 2012 the disease spread northward and established itself around Moscow and also appeared in form of sporadic outbreaks near the EU border. In July of the same year an ASF outbreak was reported in backyard domestic pigs in the southeastern part of Ukraine (OIE 2012). By 2013, ASF reached Belarus and in 2014 the virus crossed the EU border and appeared in Lithuania, Poland, Latvia and Estonia.

The economic impact of the epizootic can only be estimated, but the graveness of the situation is shown well by the significant decrease of pig numbers in the initially affected countries like Georgia (70%) and Armenia (56%), or the virtual disappearance of pigs in Azerbaijan (FAOSTAT 2012).

VIRUS, VECTORS AND HOSTS

The causative agent is a non-enveloped arthropod born (arbo) virus with a double stranded DNA genome, belonging to the *Asfivirus* genus of the *Asfarviridae* family. African swine fever virus (ASFV) is the only member of this family and also the only known DNA arbovirus (Wardley et al. 1983). The virion is organized in a complex icosahedral structure of approximately 200 nm (well above the average size of most viruses) and it is recognized for its physical stability in the environment. It can remain infectious for over a year at room temperature and for much longer at 4°C. Destroying the virus requires harsh disinfectants. The viruses, based on serological and genetic differences, are grouped, currently 22 distinct and well characterized genotypes are recognized. Genotype I is the largest group and viruses belonging here are the ones that appeared in Portugal in 1957 (Bastos et al. 2003) and later in other countries of Europe or the Caribbean. Members of this genotype are present in Central and West Africa, and they show a great homogeneity in their genetic makeup. This uniformity makes it difficult to distinguish them from each other or to trace the origin of a fresh outbreak to the point of origin of a type I virus. All of the 22 genotypes are present in South and East Africa, with variable geographic locations within these regions (Lubisi et al. 2005; Boshoff et al. 2007). Some of the genotypes seem to be restricted to certain geographic areas, even to countries, whereas others do not show such restriction. Within the genotypes of these regions, viruses exhibit high genetic variability that is linked to the sylvatic cycle (see later) of virus maintenance. The viruses of the 2007 outbreak in Europe, according to molecular analysis all fall within genotype II (Malogolovkin et al. 2012).

Beside the genetic diversity of ASFV a high variability of virulence also characterizes the known isolates. As a result of this ASF may appear with different severity leading to diseases from rapidly developing hyperacute cases to slowly progressing and long lasting chronic manifestation.

Hosts of ASFV are domestic pigs and European wild boars and these are the species where after a successful infection the disease usually develops. ASFV in its natural geographic area, Africa, infects other wild pigs like the warthog (*Phacochoerus aethiopicus*), bush pig (*Potamochoerus porcus*) or the giant forest hog (*Hylochoerus meinertzhangeni*) but without clinical signs. During the symptomless infection of these animals the virus has several opportunities to enter a sylvatic cycle that involves arthropods. Soft ticks of the *Ornithodoros* genus (Plowright et al. 1974) serve as vectors. ASFV not only replicates in the soft ticks but through trans-stadial, trans-ovarian or sexual transmission it may remain present for generations in the ticks, meaning that the virus can be reinjected to wild pigs or domestic swine any time. Ticks and wild pigs maintain a constant cycle of virus replication in these areas.

Several studies have been conducted to assess the possible vector role of hard ticks, and most recently (de Carvalho et al. 2014) *Ixodes ricinus* and *Dermacentor reticulatus*, two common tick species in Europe were investigated. Researchers concluded that neither of these two serve as real vectors for ASFV, they found no evidence of virus replication, although ASFV DNA could be detected for up to 8 weeks in some instances.

The role of warthogs in the transmission of ASF in Africa had already been suspected by Montgomery (1921) and his observation was soon confirmed (Steyn 1928; De Kock

et al. 1940) proving that the reservoir of the virus was warthogs and in some areas bushpigs. Warthogs do not exhibit signs of the disease but can be infected and serve well for virus replication (Thomson 1985). According to serological surveys, warthog populations of Sub-Saharan Africa have significant but variable rates of infection. In certain areas, 90-100% seropositivity is not unusual whereas other groups of these animals may show much lower, down to 4% seroprevalence (Heuschele and Coggins 1969, Penrith et al. 2004). Transmission by ticks occurs in burrows, typically when warthogs, born uninfected, become infected when bitten by *Ornithodoros moubata* in the burrow. Shortly after they develop viraemia (for 2-3 weeks) and serve as fresh sources of virus for the ticks (Thomson 1980). Horizontal or vertical transmission is virtually unknown in warthogs; the tick-warthog cycle is essential for the survival of the infection (Plowright, 1981; Thomson, 1985). According to data, without *O. moubata* the cycle cannot be maintained, other *Ornithodoros* species in Africa are unsuitable for that purpose. Domestic pigs living in such areas are usually infected by ticks, since direct transmission from warthog to domestic pig, due to the low level of virus load in the former, is unlikely. Other African wild pigs, as bushpigs or the giant forest hog, may also play a role in ASFV transmission but it is certainly a limited one. In Europe another soft tick species, *Ornithodoros erraticus,* plays a similar vector role as *O. moubata* when maintaining the cycle between ticks and domestic pigs within tick infested farms (Sanchez Botija 1963). Unlike in *O. moubata,* only trans-stadial transmission has been observed in *O. erraticus* (Sánchez-Vizcaíno 2006). The long term virus maintenance in *O. erraticus* was demonstrated (Sanchez Botija 1982) showing that the virus could persist for up to 8 years post infection in the ticks.

The role of European wild boars and feral pigs in ASF epidemiology is rather complex. They are widely distributed around the world, also present in North Africa, and were introduced to some countries in the South of the continent. The situation in Sardinia shows how feral pigs can influence the success of an ASF eradication program. Traditional pig farming is common in Sardinia, backyard pigs often mingle with wild pigs, and maintain a cycle of ASF replication without the presence of invertebrate vectors.

THE DISEASE

Depending on age and susceptibility of the domestic pig, the dose of infective particles, the route of entry and most importantly the virulence of the infecting ASFV strain, the disease may develop rapidly or slowly and be 100% fatal or manifest in a long lasting illness or even remain an inapparent infection (Martins and Leitao 1994).

Infection via ticks is the most efficient way of virus entry and the number of virus particles required for the success is several magnitudes lower than in case of an oral or airborn infection. Depending on the route of infection, the virus enters a primary replication cycle in the nearest lymphoid tissues, for example the tonsils after an oral uptake. From these sites the virus breaks into the blood stream and spreads throughout the body through viraemia, during which the virus particles are usually associated with red blood cells, lymphoid cells and granulocytes. Virus spread is rapid, in experiments with newborn piglets, the virions were already present in the blood 8 hours post infection and by 72 hours, maximum titers were detected in tissues (Colgrove et al. 1969). The virus replicates mostly in cells of the mononuclear phagocytic system but it also targets a variety of other cells including the endothel of the blood vessels (Wilkinson and Wardley 1978). It was suggested that the haemorrhagic nature of the disease is due to the direct damage of the endothel (Sierra et al. 1987) but the release of different mediators causing blood vessel lesions may also contribute to this manifestation of the disease.

According to experimental infections and observations of natural cases (Plowright et al. 1994; Penrith et al. 2004) the disease may develop in different forms. Highly virulent viruses may induce a hyperacute form of the disease when after a swift and atypical introductory phase, the animals may die without ever showing clinical signs of ASF. Post mortem findings are scarce; a general congestion of organs, with some fluid exudation into body cavities may sometimes be observed.

More often these virulent strains cause an acute disease, with fever, anorexia, reddening of the skin, diarrhea and vomiting, haemorrhagic lesions on the body surface. Pregnant sows abort without exception. Animals usually die within a week or more and death toll can reach 100% in domestic pigs, although some may survive. Pathological findings include haemorrhages in organs and mucosal surfaces, fluid in body cavities, oedema, enlarged spleen and pneumonia. Viruses of moderate virulence induce subacute ASF, that may last for over a month and is characterized by intermittent fever and the animals may recover from the disease. Pneumonia, pericarditis and haemorrhagic lymph nodes are usually seen after dissecting the body.

The chronic cases are caused by ASFV strains of low virulence and these cases can easily be mistaken for other chronic illnesses as the clinical signs are not characteristic to ASF. Signs like reddening of the skin, growth retardation, loss of weight, respiratory problems, hairy coating are not unusual in other prolonged diseases. Several months are needed for the manifestation of the chronic form, and the similarity of these ASF cases to other chronic diseases lies in the common pathogenesis, characterized by the accumulation of complexes formed by virus particles and antibodies that eventually provoke a hypersensitivity reaction. The result of the reaction is wide spread tissue damage, most obviously in the lungs, kidneys, the skin and the blood vessels. Mortality rate is generally low, although pregnant sows usually abort, and pigs recovering from the disease never catch up with the uninfected ones. The post mortem lesions include arthritis, pneumonia, nephrosis and pericarditis.

Clinical signs or pathological findings of ASF resemble a number of other diseases (Penrith et al. 2004), in order to diagnose ASF correctly and as early as possible, diagnostic laboratories must be involved in the process.

ASF PREVENTION AND CONTROL

Currently there is no vaccine available against ASF, therefore control is based on basic epidemiological rules and strict biosecurity that serve the prevention of disease introduction to ASF free areas. Where prevention fails, a rapid stamping out of affected swine is essential in order to stop further spread (Sánchez-Vizcaíno 2006). Several efforts have been made in order to develop ASF vaccines since the first outbreaks in Portugal. Unfortunately they were not successful. The variability of ASFV and the lack or low level of antibodies that could neutralize the virus in the infected animals make ASF a difficult subject in vaccine development. Live, inactivated and subunit vaccine candidates have all been tested without much success (Escribano et al. 2013).

Early recognition of newly introduced cases in ASF free areas (Sánchez-Vizcaíno et al. 2009) is essential in order to block the spread of the disease from animal to animal and from one farm to the other. It is equally important to break the possible routes that link wild boars to domestic pigs, and where suitable arthropod vectors are present, it is essential to thwart the entry of the virus into them. Improvements in pig housing to prevent tick and wild animal contacts is a useful tool in breaking those routes. The Spanish eradication program has shown that in large farms with strict biosecurity measures swine could be rid of the virus within a reasonable time, whereas in small units the eradication program suffered significant delay.

The legislative background to handle the ASF situation present in the Eastern part of the EU is Council Directive 2002/60/EC (http://eur-lex.europa.eu, June 27, 2002), and the latest regionalization measures taken with respect to the development of the ASF cases in the EU are included in the Commission Implementing Decision of 2014 October 9, 2014 (2014/709/EU). Commission Decision 2011/78/EU set out measures to prevent ASF entry into the European Union from Russia, but after the confirmation of an outbreak of ASF in Belarus (2013) the Commission adopted Implementing Decision 2013/426/EU, which replaced Decision 2011/78/EU. The previous measures were revised and throughout audits, it was revealed that the cleaning and disinfection of trucks transporting feed could not fully be implemented, and authorities of the Eastern Member States have already started the practice of additional biosecurity measures to increase the level of awareness.

The Hungarian authorities, namely the National Food Chain Safety Office (NFCSO) fully complying with EU regulations initiated nationwide coordinated ASF control programs; increased surveillance near the borders, training programs for veterinarians, hunters and farmers. General public is notified of the necessary awareness and the importance of control and prevention using posters and information boards at the borders.

Despite the efforts and the full implementation of classical biosecurity measures, it seems at the moment that the threat of ASF spreading further to the West cannot be completely ruled out. A major tool is missing, as there are no vaccines that could efficiently slow the epidemic down and help stamping out procedures.

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NUTRITIVE VALUE OF PAULOWNIA (*PAULOWNIA SPP.*) HYBRID TREE LEAVES

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ABSTRACT

Paulownia is a very adaptable, fast growing and multi-purpose agroforestry tree species. *Paulownia* and its hybrids become much more interesting globally in the last few decades, because of many uses including timber (for construction, doors, furniture, kitchens, boats etc.), intercropping (with wheat, maize, grass or other crops), CO_2 and dust absorption etc. Additionally, *Paulownia* leaves are used for animal feed in some countries of the World, as well. According to that, this paper is dealing with the evaluation of nutritive value of *Paulownia* hybrid tree leaves.

For determining the nutritive value of *Paulownia* containing dry matter (DM) 286.4 g/kg forage, crude protein 177.5 g/kg DM; neutral detergent fibre (NDF) 415.2 g/kg DM; acid detergent fibre (ADF) 372.6 g/kg DM; acid detergent lignin (ADL) 94.7 g/kg DM. In terms of the crude protein contents, this trial reported that the leaves of investigated *Paulownia spp.* hybrid could be used as forage for the ruminant nutrition.

keywords: fresh *Paulownia* leaves, fatty acids, ruminants

INTRODUCTION

Paulownia is a very adaptable, fast growing and multi-purpose agroforestry tree species. It is a tree with a genus of between 6–17 species (depending on the taxonomic authority) from the monogeneric family *Paulowniaceae*, related to and sometimes included in the *Scrophulariaceae*. *Paulownia* is a genus of Asian hardwood trees native of China and which have been cultivated there for the past 3000 years. They are native to much of China, south to northern Laos and Vietnam, and long cultivated elsewhere in eastern Asia, notably in Japan and Korea.

They are deciduous trees 12–15 m (40–50 ft) tall, with large, heart-shaped leaves 15–40 cm across, arranged in opposite pairs on the stem. The flowers are produced in early spring on panicles 10–30 cm long, with a tubular purple corolla resembling a foxglove flower. The fruit is a dry capsule, containing thousands of minute seeds. The genus was named in honour of Queen Anna Pavlovna from The Netherlands (1795–1865), daughter of Tsar Paul I of Russia. At least six species of *Paulownia* are currently recognised. Such species include *P. elongata, P. fargesii, P. fortunei, P. galbrata, P. taiwaniana* and *P. tomentosa*. Others report 6-17 species of *Paulownia* including *P. catalpifolia* and *P. kawakamii* (Woods 2008).

Paulownia plays a very critical role in providing timber, fuel wood, fodder and food in many countries of the World. Several research projects have been done in the last decades due to the adaptation, utilization, production etc. of *Paulownia* species and hybrids around the World (Ates et al. 2008; Kalaycioglu et al. 2005; Rafighi and Tabarsa 2011). According to these researches, one can conclude that *Paulownia* species and hybrids can produce and show extremely good results in growth rates (Ayrilmi and Kaymakci 2013), biomass production (Woods 2008) and CO_2 (and dust) absorption, as well.

Interest in *Paulownia* is gaining momentum around the world, due to its fast growing nature, the ability to take up nutrients and the potential for intercropping (Wang and Shogren 1992; Jianbo 2006). With optimal conditions in terms of light and moisture, *Paulownia* is reported to be one of the fastest growing trees in the world (U.S. Forest Service 1990). *Paulownia* timber has many uses, including timber for construction, doors, furniture, kitchens etc. The deep rooting system of *Paulownia* in combination with the rapid rate of growth enables it to take up more nutrients than other species.

Environmental conditions

It is mainly suggested to use hybrids of *Paulownia* species as the basic trees for forestation and intercropping systems (Guo 1990). Several kinds of hybrids have been selected during the last few decades around the World, according to the environmental conditions and local circumstances (e.g. temperature, salinity, water supply etc.). The minimum requirements of planting Paulownia hybrids are shown in Table 1.

Table 1: Minimum requirements of planting Paulownia hybrids

Parameters	Values
Soil pH	5 – 8.5
Soil salinity	under 1%
Level of ground water	-2 m
Maximum height above sea level	700 – 800 m
Average yearly temperature	13 – 25 °C
Maximum temperature	+50 °C
Minimum temperature	-30 °C

Paulownia can adapt to a wide range of temperatures. Due to previous Australian researches, optimal temperatures for growth are reported to range from 24-29^{0}C and all species of *Paulownia* growing have been reported to withstand temperatures ranging from -10^{0}C to +40^{0}C. Australian researchers reported that *Paulownia* grows best in temperatures ranging from 24-30^{0}C but that mature *P. tomentosa* can endure temperatures as low as -200C. These trees require full light for best growth but *P. fortunei* and *P. fargesii* can tolerate shade to a limited extent as they have been grown in China in such conditions. *Paulownia* trees are not suited to areas with strong winds but thrive well in areas that are sheltered (Guo 1990).

Paulownia trees are very hardy, can tolerate a range of temperatures and have been reported to grow at altitudes up to 2000 m and latitudes 400 N and 400 S. The *Paulownia* tree grows 5-6 m tall during the first growing season and adds 3 to 4 cm in diameter annually if optimal growing conditions are present.

In trials that were conducted under biomass conditions with WPI Georgia and North Carolina State University (NCSU), trees that were cut off at the stump had grown 12-18 feet (3.6-5.5 m) in 16 months, with yields of 84 tonne DM/ha/annum. If we assumed a yield equivalent to one third of this figure for local conditions, then we would predict a yield of 28 tonne DM/ha/annum.

The root system of the *Paulownia* tree is unique, in that the roots grow deep in the earth and its crown develops a loose structure. Roots have been reported to reach 0.8-1.5 m or even 2 m in length and in sandy and other soils, 76% of the absorbing roots reach a depth of 40-100 cm,

One year old irrigated Paulownia spp. hybrid trees (Bodnár 2013)

Two years old Paulownia spp. hybrid trees (Bodnár 2014)

with only 12% of the roots within 0-40 cm. In comparison, almost 80% of wheat roots and 95% of maize roots are generally distributed 40 cm into the soil. Root development is dependent on soil structure, where *Paulownia* thrives in a loose, well drained sandy soil.

Whilst *Paulownia* can tolerate a variety of soil conditions, they are adversely affected by water logged soils. Heavy peat or sandy soils are favourable for the growth of *Paulownia*, with the converse applying for clay and rocky soils. The ideal pH of the soil for *Paulownia* is between 5 and 8. It is reported that *Paulownia* will not thrive in soils that are podsolic with heavy clay subsoils (Guo 1990; U.S. Forest Service 1990).

Australian researchers (Lyons 1993) reported that the soil requirements for successful growth of *Paulownia* were:

1. A deep soil with a porosity exceeding 50%, with sandy, volcanic and deep alluvial soils being best. *Paulownia* will also grow on soils that have naturally low humus content and with low fertility, providing drainage is good and trees are fertilised.

2. Soil pH is 5-8.

Soil and air humidity are very important for *Paulownia* growing. Additional watering is necessary if the annual rainfall drop under 100 mm per month. In comparison 10 mm of rain delivers 10 liters of water. Watering is needed in the following years if the monthly rainfall is under 50 mm. Insufficient watering slows the growth but does not kill the plant.

For optimal growth during the first months of development is crucially important to water them with 20 l per plant weekly. The quantity is good to be divided in two equal times and added by drip irrigation system. The establishment of drip irrigation is recommended technology when develop commercial plantation for *Paulownia* (Guo 1990).

Feeding possibilities of *Paulownia* leaves

Paulownia leaves are reported to have a similar feeding value to lucerne and are suitable for combining with wheat straw or hay for feeding to cattle, sheep or goats (World Paulownia Europe).

World Paulownia Europe state that if trees are planted at 540 trees/ha, *Paulownia* will produce 1220 kg DM/ha with 20% protein and 60% digestibility. A *Paulownia* tree that is 8-10 years old is reported to have 100 kg fresh leaves, with 2.8-3% Nitrogen (N) and 0.4% potash. Data detailing the chemical composition of the *Paulownia* leaf are (%)

ash (7.8), protein (22.6), organic matter (91.4), phosphorus (0.6), calcium (2.1), iron (0.6), zinc (0.9), metabolisable energy (MJ/kg) (15-18) (El-Showk and El-Showk 2003). The potential of the leaves as an ensiled fodder crop for Northern Ireland may warrant investigation, perhaps in mini-silos. When the leaves fall, they can be a valuable source of organic matter and nutrients for the soil (Wang and Shogren 1992) and can also be used for compost (Lyons 1993).

Aim of this study was evaluate *Paulownia* hybrid tree leaves chemical and fatty acid composition.

MATERIALS AND METHODS

Experimental design

Nutritional values of fresh *Paulownia spp.* hybrid leaves (*P. elongata, P. fortunei and P. tomantosa*) were measured in this project, with special regard to the amino acid and fatty acid composition. This hybrid is highly appropriate for timber production in temperate climate zone. Five samples (5-6 fresh leafs in each) were taken randomly from a 1 ha plantation area at Szerencs, Hungary.

Chemical analysis

The forage samples (n=5-5) were analyzed for dry matter, crude protein, crude fat, crude fibre and crude ash according to the procedure of the Hungarian Feed Codex (2004).

The forage fat was dissolved in a sodium hydroxide-methanol solution and re-esterified to methyl-esters according to the AOAC (1990) method using boron trifluoride (BF_3). Methyl esters of fatty acids were determined by gas chromatography using a Shimadzu GC 2010 apparatus (Japan) with a flame ionization detector (FID) and column (CP-SIL-88, 100 m × 0.25 mm × 0.2 μm).The split injection ratio was 50:1. The column oven temperature was held at 80°C for 0 min, then programmed at a rate of 2.5°C/min up to 205°C and held for 20 min and then increased again to 225°C at 10°C/min, and held for 5 min. The injector and detector temperatures were 270°C and 300°C, respectively. Helium was used as the carrier gas, applying a flow rate 28 cm/s. Peaks were identified on the basis of the retention times of standard methyl esters of individual fatty acids (Mixture Me 100, Larodan Fine Chemicals AB, Sweden). The proportions of the individual acids were calculated by the ratio of their peak area to the total area of all observed acids.

New Paulownia spp. hybrid plantation (Bodnár 2014)

Statistical analysis

Statistical analysis was processed by the SPSS 21.0 software package (Shapiro-Wilk test for normality distribution, F test for equality of Variances, t- and Welch's corrected t-test).

RESULTS AND DISCUSSION

The composition of the experimental plant samples are shown in Table 2.

The data shows that the dry matter of *Paulownia* leaves is relatively low (286.4 g/kg dry matter, DM). It is lower than the values reported for tree leaves (46-66 % of kg DM) by Azim et al. (2001). The crude protein content was medium high (117.5 g/kg DM) for a fresh forage, and similar than that reported for *Paulownia spp.* (160-200 g/kg DM), by Mueller et al., (2001). This value compare favourably with other values reported by Addlestone et al. (1998) for leguminous browse species such as *Robinia pseudoacacia*.

It is also the case of the low crude fibre content (124.3 g/kg DM) what is lower to the value given by Scmidt et al. (2000) for other green forages (200-300 g/kg DM) for ruminants.

Paulownia leaf NDF (415 g/kg DM) and ADF (372 g/kg/DM) values are concordance with previous data (Mueller et al. 2001). The NDF and ADF contents were similar the results of Azim et al. (2001) for Robinia leaves, but were higher than Salix, Populus and Mulberry tree leaves. The ADL content was higher than other leaves' content.

In the trial reported here the plant *Paulownia* could be used as forage for the ruminant nutrition, similar with China, the leaf of the tree *Paulownia spp.* is collected in autumn and fed to cattle, sheep and pigs (Zhaohua 1991).

The fatty acid compositions of the experimental plants are shown in Table 3.

TABLE 2: Chemical composition of forage

Chemical composition	Unit	Mean	SD	Min.	Max.
dry matter (DM)	g/kg forage	286,44	22,57	267,5	317,5
crude protein	g/kg DM	177,52	4,92	173,6	185,4
crude fat	g/kg DM	28,40	1,57	26,2	30,1
crude fibre	g/kg DM	124,28	13,11	112,5	145,2
crude ash	g/kg DM	105,08	11,52	93,4	121,2
NDF	g/kg DM	415,22	31,00	372,7	441,7
ADF	g/kg DM	372,58	20,80	349,8	400,1
ADL	g/kg DM	94,70	5,68	87,6	100,7

TABLE 3: Fatty acid profile of plant (%)

Fatty acids	Mean	SD	Min.	Max.
C12:0	0,28	0,11	0,12	0,35
C14:0	0,56	0,13	0,36	0,69
C14:1	0,74	0,00	0,74	0,74
C16:0	21,58	1,13	19,89	22,59
C16:1	2,75	0,70	1,91	3,48
C18:0	2,64	0,22	2,47	3,03
C18:1n-9c	10,27	1,49	9,07	12,51
C18:2n-6	13,32	1,24	12,38	15,07
C18:3n-3	24,61	1,26	22,51	25,5
C20:0	1,50	0,26	1,26	1,84
C20:3n-3	0,29	0,06	0,2	0,38
C20:4n-6	2,60	1,36	1,11	3,9
C22:0	0,76	0,60	0,11	1,29
C22:1n9	0,70	0,18	0,49	0,81
C24:0	0,69	0,25	0,52	1,13
SFA	33,86	1,23	32,51	35,83
MUFA	16,89	0,52	16,38	17,49
PUFA	49,25	1,55	46,68	50,72

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

The main fatty acids were palmitic acid (C16:0), oleic acid (C18:1n9c), linoleic acid (C18:2n6) and linolenic acid (C18:3n3). In our study, the green plant α-linolenic acid concentration in total fat was 24.6 %, the n-6/n-3 ratio was less than 1. The α-linolenic acid concentration of green maize reached 30 % (Pajor et al. 2013), grass is also rich in α-linolenic acid n account for about 50% of the total fat in grass (Cabiddu et al. 2005). In terms of the n-3 fatty acids composition was suitable for improving the contents of milk health promoters.

CONCLUSIONS

The nutritive value of the fresh *Paulownia* is relatively high (177.5±4.9g DCP/kg DM) and it could be considered as good PUFA source as grass for the ruminants. In terms of the crude protein contents, particularly in terms of the fatty acid composition was suitable for use as feeding by sheep, goat or cattle.

However, in practical feeding situations, sheep on low crude fibre content forage crops must have access to a source of roughage (straw, hay) adequate for maintenance of proper rumen activity.

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HEART RATE VARIABILITY AS NON-INVASIVE MEASURE OF STRESS IN CATTLE – FIELD STUDIES ON HUNGARIAN DAIRY HERDS

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ABSTRACT

Heart rate (HR) measurements have been used to determine stress in farm animal species since the beginning of the 1970's. However, according to the latest studies in veterinary and behaviour-physiological sciences heart rate variability (HRV) proved to be more precise for studying the activity of the autonomic nervous system. In dairy cattle, HR and HRV indices have been used to detect stress caused by social interactions, routine management practices such as castration and disbudding, or by machine milking. This review provides the significance of HR and HRV measurements in dairy cattle by summarizing current knowledge on measurement methods and data analysis. The biological background of the autonomic regulation of cardiovascular function and HRV are discussed. Equipment and methodological approaches developed to measure interbeat intervals (IBI) and the assessment of HRV in dairy cattle are described. Finally, selected results of our recent field studies are presented.

keywords: heart rate variability, stress, dairy cow, field studies, methods and recent results in Hungary

INTRODUCTION

In the past, housing technologies and management practices for dairy cattle were mainly assessed by descriptive behavioural methods. In the last decades – complementary to behavioural and physiological stress measures – heart rate (HR) and heart rate variability (HRV) enable continuous data recording by monitoring autonomic nervous system activity (Marchant-Forde et al. 2004; von Borell et al. 2007). Rapid changes in HRV parameters allow a precise identification of stressors therefore HR and HRV are often viewed as more detailed and immediate stress indicators than HPA measures (Stewart et al. 2008a; Mialon et al. 2012).

Even though the suitability of using HRV parameters to estimate sympathetic nervous system activity is still controversial, vagal tone indices are reliable indicators of both technological stress and the level of the animal's activity. As supported henceforth, recent HR and HRV studies proved that stress responses can be quantified by the relative changes in vagal activity which allows the monitoring of stress levels and adaptation mechanisms in different situations of dairy management and the comparison of technologies and handling methods in terms of animal welfare. In dairy cattle, an increasing number of studies used parameters of HRV to indicate stress caused by diseases, routine management practices, milking and painful procedures in calf rearing. In this paper, next to a summary of current knowledge in technique, data processing, analysis and assessment of HRV in dairy cattle we present a series of results of our field studies concerning dairy cow's cardiac activity under different stressful conditions.

PHYSIOLOGIACAL BACKGROUNG OF HEART RATE VARIABILITY

Heart rate is defined as the number of heart beats per min (bpm) and used widely to explore inner short-term events occurring parallel to behavioural changes (Lefcourt et al. 1999). Interpretations have often been based on the assumption that HR reflects the activity of the sympathetic branch of the autonomic nervous system and therefore it is an indicator of the stress response (Hopster et al. 1995; Sgoifo et al. 1999) and the animal's emotional reactivity (Wolf 1970; Obrist 1981).

However, it provides only little information on the underlying physiological mechanisms that govern its modification in many behavioural situations (Sayers, 1973). The complex interplay of the two branches of the autonomic nervous system is not always comprehensible when cardiac activity is measured only by HR (Marchant-Forde et al. 2004), as a rise in HR is due to an increase in sympathetic activity (Hainsworth 1995), the decrease of vagal tone or the simultaneous changes in both regulatory systems (von Borell et al. 2007).

In a physiologically and neurally intact heart, successive cardiac cycles (R-R intervals) are non-uniformly separated in time domain (Lewis et al. 2007). This beat-to-beat variability is referred to as HRV. With certain parameters of HRV the sympathetic and vagal activity can be monitored separately at the same time (Malik et al. 1996), moreover it is possible to measure the balance between the two (Porges 2003; von Borell et al. 2007). Besides the non-additive activity originating from the individual branches of the autonomic nervous system and the tonic changes in the central nervous system activity (mainly from the medulla oblongata), HRV is also affected by a series of control and feedback mechanisms that can provide quick reflexes (von Borell et al. 2007).

METHODS OF HEART RATE MEASUREMENT IN FIELD RESEARCH

Heart rate measurement is based on electrocardiography or the count of the arterial pulse, as in human medicine. Different types of Holter recorders and fixed or telemetric systems as well as portable HR monitors have been used to investigate HRV in dairy cattle (Kovács et al. 2014a). The latter ones were originally developed for human athletes and sport medicine research (von Borell et al. 2007) but these models do not record and store the whole electrocardiogram, only the interbeat intervals (Hopster and Blokhuis 1994). It was shown that a portable HR monitoring system is valid and reliable in measuring HR in animals compared to electrocardiography (Essner et al. 2013). 24h recordings are also possible with the newest models (Polar R–R Recorder and Polar Equine) in field studies (Marchant-Forde et al. 2004; Kovács et al. 2014b) (Figure 1 and 2).

In dairy cattle practice, the recording of interbeat intervals is carried out with two separate electrodes with a specific transmitter and a HR monitor (Figure 3). It is recommended to place one of the electrodes next to the sternum, on the left side of the chest (cardiac area) and the other one on the right scapula (von Borell et al. 2007). In some cases, electrode sites were shaved prior to attaching the electrodes (Després et al. 2002; Mohr et al. 2002), while others did not consider this important (Hagen et al. 2005; Schmied et al. 2008; Kovács et al. 2012b). The contacting surface was cleaned prior to attaching in every case. In one experiment electrode sites were dampened with 38°C salt water for better conductivity (Janžekovič et al. 2006). However, especially in experiments longer than 2 h using ample electrode gel is essential (Kovács et al. 2012b) not only because of the optimal electrode-skin contact, but for the adhesion of the electrodes to the skin surface (Després et al. 2002; Hagen et al. 2005; Schmied et al. 2008). In calves, electrode belts can be easily fixed around the thorax with an elastic strap (Després et al. 2002; Mohr et al. 2002; Clapham et al. 2007; Stewart et al. 2008a,b), while in cows strong girths must be used (Minero et al. 2001; Hagen et al. 2005, Gygax et al. 2008; Kovács et al. 2012b).

When interaction of animals and crowding cannot be avoided, especially in the milking parlour and in the pre-milking waiting area in loose housing systems, girths have to be set tight enough to prevent the shifting of electrodes (Kovács et al. 2012a) because, checking the device's position and fastening is difficult to implement further on. However, sudden pressure on of the cattle's chest can also induce bradycardia (Clabough and Swanson 1989). Therefore an adaptation period of one hour is needed for the animals to get accustomed with the equipment (Rushen et al. 2001; Mohr et al. 2002; Wenzel et al. 2003; Waiblinger et al. 2004; Stewart et al. 2008a), while others recommended 5 days in calves (Clapham et al. 2007) or 7 days in cows (Janžekovič et al. 2006). However, after fixing the girth, visible reactions (Figure 4) occur only occasionally for about 5 to 10 min (Mohr et al. 2002) and cows show no obvious signs of being hampered by the equipment (Kovács et al. 2014a). To eliminate biases in results, it is also recommended that preparations on animals shall be done by the same person each time to avoid stress that might influence results (Minero et al. 2001; Mohr et al. 2002). On working farms, the protocol should fit into the daily operations of the farm and it is important to avoid conditions that would overly distort data recordings (Kovács et al. 2012a).

The periodicity of HR must also be taken into consideration during recording. Several authors found that HR shows a circadian rhythm. A lower peak in the morning hours (between 6:00 and 8:00 p.m.) and a higher peak in late afternoon (between 16:30 p.m and 18:00 p.m.) were demonstrated (Yamamoto 1989; Aharoni et al. 2003; Brosh 2007). Others reported HR increase in the early evening hours (Wenzel et al. 2003; Hagen et al. 2005) which was most likely caused by increased metabolic activity in the evening. These findings suggest that it is advised to record baseline HR parameters close to (30 min) the event to be studied or calculate an average HR value at rest from data recorded at different times of the day.

The movements of free ranging animals has to be controlled – especially when elastic belt is used – to prevent the shifting of the electrodes on the body surface, as much as possible (Rushen et al. 1999; Mohr et al. 2002), since the non-sufficient skin-electrode conductance may cause errors in IBI signals (Minero et al. 2001; Hagen et al. 2005), most often due to the electrodes getting dry (Gygax et al. 2008). HRV parameters are highly sensitive to measurement

Figure 1: Observation of experimental cows

Figure 2: A focal animal with the HR-girth (photos 1,2,3,4,6 by Levente Kovács)

Figure 3: HR monitor after an experimental day

errors (Marchant-Forde et al. 2004) therefore selection of IBI data and correction criteria must be rigorous. Each 5-min time window of the recordings involved in HRV analysis must be inspected separately for artefact correction (Kovács et al. 2014a). It is recommended to exclude data segments if the error rate is larger than 5% or it contains three or more consecutive erratic R–R intervals (von Borell et al. 2007). Data correction is usually done automatically by special algorithms of the software installed. R–R data are transmitted wirelessly in an encoded form, for undisturbed recording of data deriving from different animals. The IBI signals are stored by the HR monitor and can be downloaded onto a computer wirelessly for later analysis (Niskanen et al. 2004).

Figure 4: Animals with HR devices

Figure 5: Data downloading in the barn using IrDa POLAR infrared interface (photo by Fruzsina Luca Kézér) Luca

ANALYSIS OF HRV

Following measurements data used to be downloaded to a computer for later HRV analysis (Figure 5). The activity of the sympathetic and the parasympathetic nervous system can be monitored separately with parameters of HRV (Task Force 1996). The effects of technological factors on the autonomic nervous system have been reported in dairy cattle using parasympathetic and sympatho-vagal indices of HRV (Mohr et al. 2002; Hagen et al. 2005; Kovács et al. 2012b). Several methods have been proposed for the assessment of HRV as reviewed by von Borell et al. (2007) in farm animals and by Kovács et al. (2012a) in dairy cattle, specifically.

Power spectral analysis of HRV helps to identify components that characterize the sympatho-vagal balance. The low frequency component (LF) depends on the baroreceptor modulation of both vagal and sympathetic impulses, and has been used as an indicator of stress in dairy calves (Stewart et al. 2008a) and cows (Mohr et al. 2002; Hagen et al. 2005; Konold et al. 2011). The high frequency (HF) component reflects the vagal control of the heart (Akselrod et al. 1985) and has been used in studies involving dairy cows (Hagen et al. 2005; Konold et al. 2011). Measuring the HR in itself does not allow us to distinguish the effects of the individual branches of the ANS (Hainsworth 1995), because an increase in HR may originate from reduced vagal, or increased sympathetic activity, or possibly from simultaneous changes in the activity of both branches (von Borell et al. 2007). Non-linear Poincaré measures and time domain parameters of HRV – especially root mean square of successive interbeat interval differences (RMSSD) – have recently been used in dairy cattle studies (Minero et al. 2001; Kovács et al. 2012b) and found to be successful in assessing the vagal regulation of cardiac dynamics, which plays a key role in response to stress (von Borell et al. 2007).

COW'S CARDIAC ACTIVITY DURING CONVENTIONAL MILKING

We involved 36 multiparous cows in this study for assessment of stress associated with milking in a parallel milking parlor with non-voluntary exit (Figure 6). HRV parameters measured during the morning resting (baseline period) were compared with those measured during the different stages of the entire milking process (Table 1).

No differences were found in HRV parameters between baseline period, preparation and main milking (Table 2).

TABLE 1: Studied periods of measurement involved in HRV analysis (Kovács et al. 2013)

Phase	Definition	Number of analyzed 5 min time intervals/cow per milking	in total
Driving	Time lag between letting out of the stable's gate and entering the holding area	1-2	3-5
Being in the holding area	Time interval between entering the holding area and stepping into the milking parlor	1-3	5-9
Preparation	Admission time + udder preparation time Admission time: time interval between entering the milking stall and the beginning of udder preparation Udder preparation: first contact between animal and milker until attachment of all teat-cups	1	4-6
Main milking	Time interval between the attachment and the removal of the last teat cup	1	4-6
Waiting after milking	Time interval between the removal of the last teat cup and stepping out of the milking stall with all four legs	1	4-6
Baseline period (morning resting)	Time spent lying after the morning milking. Bouts started from 5 min after the cow had lain down.	2-5	7-12

During the movement of the cows to the milking parlor (driving) and being in the holding area a considerable reduction in the vagal activity was detected. Parasympathetic measures of HRV (RMSSD, HF_{norm} and SD1) decreased, while SD2/SD1 representing the sympatho-vagal balance increased, compared with baseline. The same pattern was observed regarding the stage between removing the teat cups and leaving the milking parlor (waiting). No differences in any sympathetic measures (LF_{norm} and SD2) were observed between the baseline period and any of the milking stages.

Figure 6: Focal animals during milking

The results suggested that the milking process itself (preparation and main milking) is not stressful for cows. Decreased parasympathetic activity during driving was possibly a result of the physical activity of the cows, while waiting in the holding area and in the milking stall after milking caused stress for animals.

CARDIAC RESPONSES TO TRANSRECTAL EXAMINATION

In this study we evaluated HR and parasympathetic HRV parameters to monitor cardiac stress responses to palpation per rectum in lactating (n=11) and non-lactating (n=12) dairy cows. HR and HRV were recorded from 40 min prior to the examination until 120 min after it was completed. HR, RMSSD and HF were analyzed by examining 5 min time windows. To compare cardiac responses to the examination between groups, changes in HR and HRV parameters were calculated as area under the curve for lactating and non-lactating cows.

There was an immediate increase in HR during the exam in both lactating (+21.4 ± 2.4 beats/min) and non-lactating cows (+20.6 ± 2.3 beats/min), however, no differences were found between groups. The increase in HR in both groups along with a parallel decrease in RMSSD (lactating cows: –5.2 ± 0.4 ms, non-lactating cows: –5.1 ± 0.4 ms) and HF (lactating cows: –10.1 ± 0.8 nu, non-lactating cows: –16.9 ± 1.2 nu) during palpation indicate an increase in the sympathetic, and a decrease in the parasympathetic tone of the autonomic nervous system. The increase in RMSSD (lactating cows: +7.3 ± 0.7 ms, non-lactating cows:

TABLE 2: Heart rate variability parameters during lying and milking stages (Kovács et al. 2013)

Phases involved in HRV analysis	HRV parameters (Marginal means ± SD)					
	RMSSD (ms)	LF_{norm}	HF_{norm}	SD1 (ms)	SD2 (ms)	SD2/SD1
Baseline period (morning resting)	34.19 ± (4.61)	56.58 ± 5.48	42.15 ± 5.91	44.20 ± 3.27	69.07 ± 10.72	3.13 ± 0.43
Driving	19.61 ± 7.32***	89.29 ± 23.17	13.71 ± 5.33***	13.88 ± 5.18***	54.24 ± 23.20	6.13 ± 1.26*
Being in the holding area	23.68 ± 8.90**	75.74 ± 19.67	23.87 ± 9.11**	16.76 ± 6.30***	61.79 ± 26.79	5.39 ± 0.97*
Preparation	29.57 ± 11.04	47.80 ± 12.39	44.09 ± 16.83	30.94 ± 7.82	72.79 ± 31.18	4.48 ± 1.62
Main milking	34.80 ± 8.18	47.87 ± 7.89	48.45 ± 11.38	44.65 ± 5.79	87.41 ± 24.46	4.38 ± 1.03
Waiting after milking	25.11 ± 4.46*	68.66 ± 17.85	26.95 ± 6.35**	17.80 ± 6.71***	59.39 ± 25.26	7.97 ± 2.97**

Differences between milking stages and the baseline period are marked by NS: $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Descriptive statistics are based on individuals' means of log-transformed data. Ms = millisecond; SD = standard deviation.

+17.8 ± 2.2 ms) and in HF (lactating cows: +24.3 ± 2.6 nu, non-lactating cows: +32.7 ± 3.5 nu) immediately after the exam indicated a rapid increase in parasympathetic activity which decreased under the baseline values 10 min following the examination. The amplitude and the maximum RMSSD and HF values were greater in non-lactating cows than in lactating animals suggesting a higher short-term cardiac responsiveness of non-lactating cows. However, the magnitude and the duration of the stress response were greater in lactating cows, as indicated by the analysis of area under the curve parameters (area under HRV response curve, time to return to baseline).

Cow's response to the acute stress experienced during and following palpation was more prominent in parasympathetic HRV measures than in HR. Based on our results, the impact of the transrectal examinations on the cows' cardiac stress responses may have an impact on animal welfare on dairy farms, and investigating the effect of lactation on the cardiac stress reactions could prove useful in modeling bovine stress-sensitivity.

CONCLUSIONS

We found no acute stress during the milking process that reflected on the cardiovascular system, but after milking more restlessness was found with regard to stress during waiting for being let off the milking parlour detectable also in HRV. Since HR indicating only a slightly increased stress level during milking, we believe that the absolute size of this difference is so minor that no serious impairment of the welfare of dairy cows milked by parallel milking system can be testified.

Lactating cows exhibited lower short-term cardiac responsiveness to transrectal examination than non-lactating animals, whereas in terms of magnitude and duration, cardiac responses mirrored by PNS indices of HRV were more intensive in lactating cows than non-lactating ones. Further research is needed to clarify these differences in autonomic nervous system activity between non-lactating and lactating animals.

In summary, HRV may have benefits when investigating acute responses to environmental challenges constituting an immediate and detailed index of stress and welfare in dairy cattle. Focusing on several aspects of stress occurring in dairy farming systems that are most relevant to the animal's welfare may provide valuable information on how dairy cattle handling and housing can be improved in the near future.

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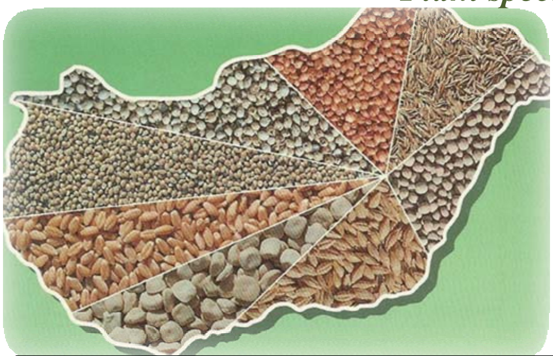

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Millet: *Lovászpatonai pirosmagvú, Maxi*
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