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Acta Agronomica Hungarica, Vol. 43 (1–2), pp. 3–9 (1994–95)

Soil science and agrochemistry

EFFECT OF HEAVY METAL LOAD ON SOIL AND CROP

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(Received: 16 December 1992; accepted: 4 May, 1993)

The purpose of this work is to evaluate the movement of some important contaminants in soil-plant system. The general research program based on experimental studies and in perspective has the following goals (food chain concept) for investigation:

– Behaviour of heavy metals in the soil (fixation, availability, leaching, volatilization, transformation, etc.)

– Effect of these elements on soil life (soil biological activity, recording of macro- and micro-organisms in soil, etc.)

– Absorption of these elements by plant roots and their transport within the plants (their accumulation in the shoots, leaves, stems/stalks and grains)

– Effect of these elements on the quantity and quality of the yield, the resistance against plant diseases and weediness

– Effect of these elements on the animals. The plant material derived from the field experiment is fed up by animals in feeding experiments led by the Institute for Animal Feeding of the University of Veterinary Science.

On the basis of the first year results seems, the movement of Al, As, Cu, Cr, Ni, Hg and Pb is rather limited in the food chain, their accumulation can mainly be detected in the roots. On the other hand, Ba, Cd, Mo, Sr, Se and Zn considerably accumulated in the vegetative plant parts. Whereas the generative organ (grain) is genetically protected, accumulation occurs only in essential elements Mo, Se and Zn.

Keywords: heavy metals, soil-plant, maize, soil contamination, food chain

Introduction

The natural circulation of elements on Earth is limited and life has adapted itself to this condition. The more mobile (partly harmful) fractions have disappeared from the soil; the concentrations of undesired elements in the soil solution and in natural waters are low. The situation may drastically change when the available harmful element content of the soil is increased by some orders of magnitude through sewage sludges of high metal content, etc., added to the soil. The composition and quality of the soil may be transformed, involving qualitative changes in the crop grown in it, and in the grazing or forage-eating animals. Contamination of the soils by harmful elements is one of the forms of chemical load on the environment, which is of basic sanitary, economic and ecological importance.

Man, like all terrestrial animals, depends on the soil for a basic source of food. The human metabolism is based on an enzyme system making use of the essential elements (Fe, Mn, Zn, Cu, etc.) while eliminating the harmful ones (As, Be, Cd, Hg, etc.). The

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human organism is not evolutionally prepared for adapting itself to the chemical load of the environment. The accumulating elements are relatively stable and may cause irreversible changes. According to literary data (Purves, 1985; Fergusson, 1991; Kádár, 1991) in the blood, urine, hair, tissues of the urban population the concentrations of lead (Pb) and cadmium (Cd) have increased by orders of magnitude.

The investigations were aimed at finding an answer to the question: to what extent can the major microelement contaminants accumulate in the nutrient chain? We attempted to follow the movements of elements in the soil-plant-animal system. The points studied experimentally were:

1. The behaviour of the elements in the soil (fixation, availability, leaching, volatilization, transformation, etc.).
2. Their effect on the life of soil (biological activity, macro- and microorganisms in the soil, etc.).
3. Absorption of elements by plant roots, and their transport within the plant (their accumulation in shoots, leaves, stalks, grains).
4. Effect of elements on the quantity and quality of yield, on disease resistance and weediness.
5. Their effect on animals. The plant material obtained from the field experiment is used for feeding experiments at the Department for Animal Feeding of the University of Veterinary Science.

The materials of the experiments (soil, plant and animal) were analysed by the ICP laboratory in the Agrochemical and Plant Nutrition Section of the TAKI. Our investigations are supported by the Ministry of Environment Protection and Regional Development. The small-plot loading experiment was set up in spring 1991 in Mezőföld (region in Transdanubia) at the Nagyhörcsök Experiment Station of the Institute. From the first year results, no far-reaching conclusions can be drawn, and the emphasis was laid on formulating the problem.

Materials and methods

The soil for the experiment was calcareous loamy chernozem formed on loess containing about 3% humus and 5% $CaCO_3$ in the ploughed layer. To ensure a satisfactory macroelement supply 100 kg/ha N, P_2O_5 and K_2O were given yearly as basic fertilization in the whole experiment. The 13 selected microelements were followed up on 4 levels of loading (in 52 treatments) and in 2 replications. The size of plot was 21 m^2, and the experiment was arranged in split-plot design. In the first year, maize was sown with the usual cultural practices employed. Harvesting was carried out by means of a plot combine. The treatments of the experiment and the forms of the compounds applied are contained in Table 1.

During the vegetation period, soil samples were taken twice. The average samples each represent 20 subsamples taken from the ploughed layer. The plant stand was also sampled several times, with 20 plants per plot taken from the net area: root + shoot at 4–6-leaf stage, leaves below the cob at the time of flowering, stalk and grains on harvesting. The preparation and the ICP analysis of the samples were made in the usual way. From the plant the total element content was determined after exposure in teflon bomb using cc HNO_3 + H_2O_2, while, in the soil samples, the available or mobile NH_4acetate + EDTA soluble element content was measured after Lakanen and Erviö (1971).

Table 1

Treatments in the field experiment
(Calcareous chernozem, Nagyhörcsök, 1991)

Sign of the element	Treatments, kg/ha, Spring 1991				Chemical used
	1	2	3	4	
Al	0	90	270	810	$AlCl_3$
As	30	90	270	810	As_2O_3–$NaAsO_2$
Ba	0	90	270	810	$BaCl_2$
Cd	30	90	270	810	$CdSO_4$
Cr	0	90	270	810	K_2CrO_4
Cu	0	90	270	810	$CuSO_4$
Hg	30	90	270	810	$HgCl_2$
Mo	0	90	270	810	$(NH_4)\,6Mo_7O_{24}$
Ni	0	90	270	810	$NiSO_4$
Pb	0	90	270	810	$Pb\,(NO_3)_2$
Se	30	90	270	810	Na_2SeO_4
Sr	0	90	270	810	$SrSO_4$
Zn	0	90	270	810	$ZnSO_4$

Note: Basal dressing in the whole experiment: 100 kg/ha N, P_2O_5 and K_2O

Results and conclusions

As shown by the data in Table 2, among the elements and compounds examined Al, Cr, Cu, Mo, Ni, Se proved phytotoxic at 4–6-leaf stage. The grain yield at the time of harvesting was demonstrably reduced by Cr, Mo, Pb, Se only, i.e. by 4 of the 13 salts applied. It seems first of all that in this calcareous, well-airated soil the anion-forming metals (chromate, molybdenate, selenate) may represent a problem of toxicity to maize. Increasing doses of Cr led to an almost total destruction of maize and weed plants.

The results of soil and plant analyses are shown in Table 3. In the case of no effect of the treatment, i.e. no element accumulation appeared in the different plant parts, we considered it unnecessary to record it. The error of the analyses (more correctly: the error of sampling) was very great in the first year of the experiment, since a single ploughing did not enable the fertilizers to mix properly with the soil. In spite of this, the trends shown here are convincing. As seen from the soil analysis data, the available supplies of most microelements generally increased by orders of magnitude. The Cr, Hg and Se content of the untreated control was below the limit of measuring, while As was 0.2 – 0.4 ppm, and Cd 0.1 – 0.2 ppm. As the changes reached orders of magnitude, the data of the soil and plant analyses are rounded out to whole ppm values. Values below 0.5 ppm are marked "0".

There were considerable Al pools both in the soil and in the plants. The Al concentration in the roots was only redoubled by fertilization. Concentrations of Cu, Ni, Pb increased several times. The next degree of accumulation was shown by As, Cr, Hg

Table 2

Dry matter yield and grain yield of maize in the field experiment
(Calcareous chernozem, Nagyhörcsök, 1991)

Sign of the element	Treatments, kg/ha Spring 1991				L.S.D$_{5\%}$	Mean
	0	90	270	810		
	Dry matter (DM) of the maise plants with 4–6 leaves, kg/ha					
Al	145	135	105	55		110
Cr	155	75	20	15		66
Cu	205	195	145	125		168
					60	
Mo	140	130	95	25		98
Ni	200	190	145	110		161
Se	145	140	90	75		113
	Grain yield at harvest, t/ha					
Cr	8.1	5.2	1.9	1.6		4.2
Mo	8.5	8.4	7.4	4.7	1.5	7.2
Pb	8.9	8.4	7.8	6.4		7.9
Se	8.9	7.6	5.7	4.3		6.1

which increased by orders of magnitude in the roots. On the other hand, no accumulation was observed in the aboveground plant parts. It seems thus, that the movement of Al, As, Cr, Cu, Ni, Hg anb Pb is inhibited in the plant, and therefore a possible accumulation in the soil of the above 7 elements shown in Table 3 does not interfere with the nutrient chain of soil–plant–animal.

With the soil analysis data of Table 3 also taken into consideration, it can be established that 3–10% of the Cr-, 10–20% of the Al- and As-, 20–40% of the Ba, Mo, Ni, Se, Sr-, 40–50% of the Zn, Pb, Cu- and 100% of the Cd applied, remained in the first year as ammonium acetate + EDTA soluble form in the ploughed layer.

Another part of the microelements examined moved from the roots to the aboveground parts. Barium, cadmium and strontium accumulated in the young shoots, and at the time of harvesting in the stalk. The grain, on the other hand, is genetically protected, shuts out the harmful elements, and only the essential trace elements (molybdenum, zinc, selene) are accumulated in it. The maximum element concentrations in the major organs of maize, as shown in Table 3 were: 294 ppm Cd, 990 ppm Mo in the root; 40–50 ppm Ba, Cd, Zn, 107 ppm Mo in the stalk; 14–41 ppm Mo, Se, Zn in the grain.

Since there are almost no literary data on exact field experiments, a false picture may be formed about the behaviour of the harmful elements.

On the basis of experiments in nutrient solutions and culture pots, it is generally accepted that Cd at higher than 10 ppm concentrations can be toxic both in soils and in plants. However, the actual problem is that this element may accumulate in the edible green plant parts without causing damage to the plant. In this way it may inperceptibly contribute to the Cd load on man and animal.

Table 3

Available element content of soil in plow layer and total element content of plant in the field experiment, ppm.
(Calcareous chernozem, Nagyhörcsök, 1991)

Soil/	Treatments, kg/ha, Spring 1991					
plant	0	90	270	810	L.S.D.$_{5\%}$	Mean
			Aluminium (Al)			
Soil	67	73	86	90	17	79
Roots	91	114	95	198	42	124
			Arsenic (As)			
Soil	0	7	18	66	81	23
Roots	0	7	8	23	46	10
			Chrome (Cr)			
Soil	0	2	6	30	30	10
Roots	4	24	77	158	95	66
			Copper (Cu)			
Soil	7	24	49	110	39	48
Roots	9	13	25	43	45	23
			Nickel (Ni)			
Soil	3	14	40	74	11	33
Roots	8	12	26	38	27	21
			Mercury (Hg)			
Soil	0	4	49	189	75	61
Roots	0	10	12	63	23	22
			Lead (Pb)			
Soil	5	29	56	158	181	62
Roots	4	6	8	24	24	11
Shoots	1	1	3	5	1	3
			Barium (Ba)			
Soil	20	29	41	100	15	47
Roots	27	21	38	114	22	50
Shoots	4	8	22	96	17	32
Stalks	5	7	19	52	39	21
			Cadmium (Cd)			
Soil	0	60	172	456	224	176
Roots	0	34	168	294	253	126
Shoots	0	1	4	12	2	4
Stalks	0	4	12	46	17	16
			Strontium (Sr)			
Soil	31	48	67	146	59	73
Roots	30	34	39	77	32	45
Shoots	19	27	29	42	21	29
Stalks	9	13	13	20	3	14

Table 3 *(cont'd)*

Soil plant	Treatments, kg/h, Spring 1991 0	90	270	810	L.S.D. 5%	Mean
			Selenium (Se)			
Soil	0	7	22	122	73	38
Roots	0	19	18	51	26	25
Shoots	0	9	24	60	24	24
Stalks	0	6	11	20	5	10
Grains	0	8	12	22	5	12
			Zinc (Zn)			
Soil	2	14	54	153	99	56
Roots	24	36	70	131	26	65
Shoots	19	51	76	126	38	68
Stalks	7	47	31	54	65	35
Grains	8	25	28	41	38	25
			Molybdenum (Mo)			
Soil	1	21	26	104	80	38
Roots	4	140	455	990	615	397
Shoots	3	107	284	781	83	294
Stalks	0	35	38	107	44	45
Grains	0	4	6	14	4	6

Note: Data are only given when an effect of the treatment or an accumulation of the element could be detected. Soil data are NH_4–Ac+EDTA soluble element contents, plant data are total element contents of the cc HNO_3 + H_2O_2 digestion. For calculation: 100 kg/ha given element is theoretically equal to 30 ppm in the ploughed layer.

Summary

In a field experiment set up in 1991 on a calcareous chernozem soil the effect of 13 microelements – including several heavy metals considered toxic – on the available element content of the soil, as well as on the yield and microelement uptake by maize plants was examined. The treatments of the experiment and the forms of salts applied are contained in Table 1; the air-dry yield of maize in Table 2; the available element content of the soil determined by ammonium acetate + EDTA method as well as the element concentrations of the plants are shown in Table 3. Soil and plant samples were taken from each plot; 20 subsamples, or plants and plant organs, respectively, formed the average sample of the plot. In the analyses, ICP technique was used. Major conclusions:

1. The grain yield of harvesting was demonstrably reduced by Cr, Mo, Pb, Se. Increasing doses of chrome caused an almost total destruction of maize and inter-row weeds.
2. Al, As, Cr, Cu, Ni, Hg and Pb did not move from the root to the aboveground parts. It seems therefore that these 7 elements cannot endanger the soil–plant–animal food chain, inasmuch as their movement in the plant is inhibited.
3. Cd, Ba, Sr accumulated in the vegetative parts, while the grain only accumulated the essential elements (Zn, Mo, Se) and shut out the toxic ones, respectively.
4. Cd, Ba, Sr as well as Zn, Mo, Se accumulate in the edible green plant parts without doing damage to the plant, thus contributing inperceptibly to the heavy metal load on man and animal.

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PREDICTION OF THE DISSOLUTION OF CALCIUM SULPHATE CONTAINING SOIL AMENDMENTS BY A COMPUTER MODEL

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In soils with an approximately same degree of sodium saturation but differing in the chemistry of their salinization, the quantitites of dissolved calcium sulphate may vary to a great extent. Therefore, when predicting the amendment dose, the chemical composition of the soil solution cannot be neglected.

A model – based on the equilibria of calcium sulphate dissolution – has been developed to compute the quantity of calcium sulphate dissolved in soil solutions or soil aqueous extracts, taking into account the influence of common and non-common ions on the solubility of amendments. In this model the soil solution is treated as a multicomponent electrolyte solution. The validity of the computerized model was proved by the good agreement between the computed and measured data obtained in aqueous salt solutions and soil saturation extracts saturated with calcium sulphate.

A significant difference (on 5% level) was found between the calcium and sulphate ion concentrations measured in soil saturation extracts saturated with activated fine-grain anhydrite and the ones computed by the model when the thermodynamic solubility product of anhydrite published in the literature was applied. This means that the activity of the solid phase of this material is probably not a constant value and is not equal to one. Nevertheless, the model is suitable, even in its present state, to predict the solubility of calcium sulphate containing amendments in the soil solution.

Keywords: salt-affected soils, soil solution, ion anotiations, solubility of gypsum

Introduction

For the chemical amelioration of salt-affected soils, calcium sulphate containing amendments, like gypsum or anhydrite, have been used with good results for a long time. There are several methods applied for the determination of the dose of these amendments. The most frequently used method (Antipov-Karataiev, 1953; Soil Survey, 1974) takes into account the quantity of exchangeable Na^+, the cation exchange capacity and the mass of the soil to be ameliorated. In the case of soils containing sodium salts of alkali reaction, the quantity of exchangeable Na^+, and that of Na_2CO_3 and $NaHCO_3$ are taken into account together at the calculation of the dose (Herke, 1957; Ábrahám and Bocskai, 1971; Soil Survey, 1974; Abrol et al., 1975; Chauhan and Chauhan, 1984).

However, it is usually neglected at these calculations and at the preliminary prediction of the effectivity of the amendment, that the liquid phase of the soil is a multicomponent electrolyte solution, whose concentration and chemical composition have a strong influence on the dissolution of inorganic mineral salts. The solubility of calcium sulphate present in the soil or applied as an amendment increases with the concentration of the soil solution, but it may decrease in spite of the increase of the solution concentration, if the electrolyte already previously contains the cation or anion of the dissolving salt.

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There are a number of publications dealing with the influence of common and non-common ions on the dissolution of gypsum in aqueous salt solutions, in soil solutions or soil aqueous extracts (Seidell and Linke, 1958; Denman, 1961; Dutt and Donen, 1963; Marshall et al., 1964; Tanji, 1968, 1969; Yeatts and Marshall, 1969; Nakayama, 1971a; 1971b; Bennett and Adams, 1972; Muratova et al., 1980; Redly et al., 1980; Szabolcs and Darab, 1980). Computer models have been also developed for the prediction of the solubility of gypsum in multicomponent electrolyte solutions: in aqueous salt solutions (Marshall and Slusher, 1968; Tanji, 1968, 1969; Nakayama, 1971b; Ponizovsky and Pachepsky, 1979) as well as in soil solutions and extracts (Dutt and Doneen, 1963; Tanji et al., 1967, 1972; Oszter and Mc Neal, 1971; Muratova et al., 1980; Mironenko et al., 1981; Sokolenko, 1986).

We are demonstrating in this work that at the determination of the dose of calcium sulphate containing chemical amendments, it is not enough to consider only the decrease of sodium saturation of the soil to be achieved. It is also necessary to take into account the quantity and quality of the soluble salts present in the soil and the actual saturation concentration of calcium sulphate in the soil solution. A model has been developed to compute the solubility of gypsum and different anhydrite materials in aqueous salt solutions, soil solutions and soil aqueous extracts by taking into account the influence of electrostatic interactions of ions, the effect of common and non-common ions on the dissolution of the amendments. The computed values were compared with the measured ones obtained in our experiments.

Materials and methods

The dissolution of soil chemical amendments containing calcium sulphate [analytical $CaSO_4 \cdot 2H_2O$ (gypsum) and previously physically treated mineral fine- and coarse-grain $CaSO_4$ (anhydrite)] were determined in the saturation extracts (Richards, 1954) of meadow solonetz soils. 2 g calcium sulphate was added to 100 ml of the soil saturation extract, and after four hours shaking, the solutions were filtered. The ion concentrations in the extracts before and after application of calcium sulphate were determined with usual analytical methods (Richards, 1954). The characterization of the soils, the detailed description of the experiment and the data of the analyses were published earlier (Darab et al., 1979).

A model – based on the equilibria of calcium sulphate dissolution – has been developed to compute the quantity of calcium sulphate dissolving in soil solutions or soil aqueous extracts calculating with the ion concentrations of the original solutions. The measured and computed quantities of dissolved calcium sulphate were compared to evaluate the applicability of the developed model. (The program has been written by István Pintér computer specialist on BASIC, PASCAL and PL/1 languages for ZX SPECTRUM, IBM PC and ESZR-40 computers, respectively.)

The input data are the concentrations of the dominant ions in the liquid phase of salt-affected soils ($Ca^{2+}, Mg^{2+}, Na^+, K^+, HCO_3^-, SO_4^{2-}, Cl^-$) the dissociation constants of the most important ion-pairs present ($CaSO_4^0, MgSO_4^0, NaSO_4^-, NaHCO_3^0, CaHCO_3^0, MgHCO_3^+$) and the thermodynamic solubility product of gypsum or anhydrite (Table 1). The quantity of dissolved calcium sulphate is computed with a quadratic equation using the method of successive approximation. Every step involves a subroutine (Csillag and Darab, 1985) to compute the electrostatic interactions of ions: the short-range interactions (ion-pair formation) and the long-range ones (characterized by ion activity coefficients). The computation ends when Ca^{2+} and SO_4^{2-} concentrations corresponding to the thermodynamic solubility product are reached, that is, when the solution becomes saturated with the amendment (Fig. 1).

Table 1

The inputs and output data of the program computing the dissolution of calcium sulphate

Input data

(1) Measured "total" ion concentrations of the original soil solution or extract (before application of calcium sulphate $Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, HCO_3^{-}, SO_4^{2}, Cl^{-}$)

(2) The thermodynamic solubility product of calcium sulphate ($K_{sp} = 2.55.10^{-5}$ for gypsum, for example)

(3) The thermodynamic dissociation constants of ion-pairs (pKd values from Sillén and Martell, 1964):

$CaHCO_3^+$: 1.26	$MgHCO_3^+$: 1.16	$NaHCO_3^0$: – 0.25
$CaSO_4^0$: 2.31	$MgSO_4^0$: 2.36	$NaSO_4^-$: 0.72

(4) Ion activity coefficients calculated with the second approximation of the Debye-Hückel equation

Output data

(1) The quantity of dissolved calcium sulphate (X), that is the increase of Ca^2 an SO_4^{2-} concentrations in the soil solution

(2) The electrolyte composition of the soil solution saturated with calcium sulphate

a/ free ion concentrations and activities

b/ ion-pair concentrations

Derivation of the quadratic equation computing the dissolution of calcium sulphate

The calcium and sulphate concentrations of a solution saturated with calcium sulphate are determined by the thermodynamic solubility product of the saturating compound:

(1)

$$K_{sp_{CaSO_4}} = (Ca^{2+})_{f,g} \cdot (SO_4^{2-})_{f,g} = \left[Ca^{2+}\right]_{f,g} \cdot \left[SO_4^{2-}\right]_{f,g} \cdot \gamma_{Ca^{2+}f,g} \cdot \gamma_{SO_4^{2-}f,g}$$

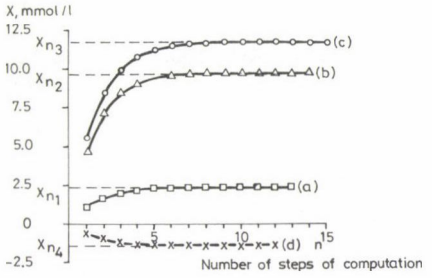

Fig. 1. The increase of the computed quantities (X) of dissolved or precipitated calcium sulphate during the successive approximation procedure.

Dissolution: Profile: Mezőtúr-102. (a) 40–60 cm; (b) 80–100 cm; (c) 120–140 cm depth. *Precipitation:* (d) Soil solution data from a soil column model experiment (Rédly and Darab, 1981). X_n: the quantity of dissolved or precipitated calcium sulphate in the n-th step of the computation.

where ( ) : ion activity, mol/l
[ ] : ion concentration, mol/l
γ : the activity coefficient of the ion
g : as an index denotes values after saturation with calcium sulphate
f : as an index denotes concentrations and activities of free ions (not involved in ion-pairs) (Csillag and Darab, 1985).

The total concentrations of ions include ion-pair concentrations because, during the usual analytical measurements, like complexometric titration of Ca^{2+}, or the determination of SO_4^{2-} ion-pairs dissociate. Therefore, the free Ca^{2+} and SO_4^{2-} concentrations in the solution saturated with calcium sulphate can be calculated by subtracting the concentrations of calcium- and sulphate-containing ion-pairs from the measured, total Ca^{2+} and SO_4^{2-} concentrations of the saturated solution:

(2)

$$\left[Ca^{2+}\right]_{f,g} = \left[Ca^{2+}\right]_{t,g} + \Sigma c_{ip Ca,g}$$
$$\left[SO_4^{2-}\right]_{f,g} = \left[SO_4^{2-}\right]_{t,g} + \Sigma c_{ip SO_4,g}$$

where t, g: as an index denotes the total Ca^{2+} and SO_4^{2-} concentrations of the solution saturated with calcium sulphate. They are the sum of ion concentrations of the original solutions and those dissolved from the added calcium sulphate (X; mol/l):

(3)

$$\left[Ca^{2+}\right]_{t,g} = \left[Ca^{2+}\right]_{t,o} + X$$
$$\left[SO_2^{2-}\right]_{t,g} = \left[SO_4^{2-}\right]_{t,o} + X$$

where t, o: as an index denotes the total, measured Ca^{2+} and SO_4^{2-} concentrations of the original solution (before application of calcium sulphate).

Combining equations (1), (2) and (3) the thermodynamic solubility product of calcium sulphate is:

(4)

$$K_{ipCaSO_4} = \left\{\left[Ca^{2+}\right]_{t,g} \Sigma c_{ipCa,g}\right\} \cdot \left\{\left[SO_4^{2-}\right]_{t,g} - \Sigma c_{ipSO_4,g}\right\} \cdot$$
$$\cdot \gamma_{Ca^{2+}f,g} \cdot \gamma_{SO_4^{2-}f,g} = \left\{\left[Ca^{2+}\right]_{t,o} + X - \Sigma c_{ipCa,g}\right\} \cdot$$
$$\cdot \left\{\left[SO_4^{2-}\right]_{t,o} + X - \Sigma c_{ipSO_4,g}\right\} \gamma_{Ca^{2+}f,g} \cdot \gamma_{SO_4^{2-}f,g}$$

The quantity of dissolved calcium sulphate can be expressed after multiplying the corresponding parts of equation (4). An equation quadratic for X is obtained, which can be reduced to a form of $aX^2 + bX + c = 0$. Its parameters are:

(5)

$$a = \gamma_{Ca^{2+}f,g} \cdot \gamma_{SO_4^{2-}f,g}$$
$$b = \left\{\left[Ca^{2+}\right]_{t,o} + \left[SO_4^{2-}\right]_{t,o} - \Sigma c_{ipCa,g} - \Sigma c_{ipSO_4,g}\right\} \cdot a$$
$$c = \left\{\left[Ca^{2+}\right]_{t,o} + \left[SO_4^{2-}\right]_{t,o} - \left[Ca^{2+}\right]_{t,o} \cdot \Sigma c_{ipSO_4,g} - \left[SO_4^{2-}\right]_{t,o} \cdot \Sigma c_{ipCa,g} + \Sigma c_{ipCa,g} \cdot \Sigma c_{ipSO_4,g}\right\} a - K_{ipCaSO_4}$$

Some of the constituents of the quadratic equation are known

$$\left(\left[Ca^{2+}\right]_{t,o} + \left[SO_4^{2-}\right]_{t,o}; K_{ipCaSO_4}\right) \cdot$$

The others are substituted in the equation in the first step of the successive approximation with the corresponding values of the original solution. Therefore, in the first step:

(6)

$$\Sigma c_{ip_{Ca,g}} = \Sigma c_{ip_{Ca,o}}; \qquad \Sigma c_{ip_{SO_4,g}} = \Sigma c_{ip_{SO_4,o}}$$

$$\gamma\, Ca^{2+}{}_{f,g} = \gamma\, Ca^{2+}{}_{f,o}; \qquad \gamma\, SO_4^{2-}{}_{f,g} = \gamma\, SO_4^{2-}{}_{f,o}$$

As every member of the quadratic equation, except X, are known, it can be solved.

The values given in expressions (6) and the computed X-value are modified in every step of the iteration. All of them converge to a corresponding value after a relatively few number of steps (not more than 20, according to our computations till now) (Fig. 1). The last values are characteristic to the composition of the calcium sulphate saturated solution. The model assumes that the change in the electrolyte composition of the solution during the successive steps results only from the increase of Ca^{2-} and SO_4^{2-} concentrations, as the concentrations of other ions are equal to the ones of the original solution.

The description of the algorithm

The flow-chart of the model computing the dissolution of calcium sulphate is given on Fig. 2.

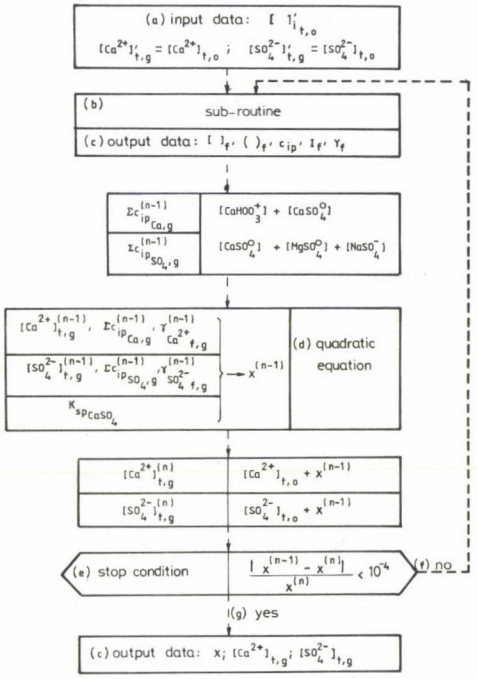

Fig. 2. Flow-chart of the model computing the dissolution of calcium sulphate in the soil solution

X : the quantity of dissolved calcium sulphate, mol/l;

[ ]t : measured "total" ion concentration, mol/l;

o : before; g: after application of calcium sulphate; K_{sp}: thermodynamic solubility product of calcium sulphate; [ ]': ion concentration in the 0^{th} cycle; i: i^{th} ion; [ ] and ( )$_f$: the concentrations and activities of "free" ions, resp., mol/l; c_{ip}: ion-pair concentrations, mol/l; I_f: ionic strength (calculated from "free" ion concentrations and charged ion-pair concentrations), mol/l; γ: ion acivity coefficient; n: See Fig. 1 (upper index due to technical reasons).

(b) : Computing the actual elektrolyte composition of the solution (Csillag and Darab, 1985)

At first, the electrolyte composition of the original solution, that is, free ion concentrations and activities and ion-pair concentrations in it are computed by the subroutine (Csillag and Darab, 1985). Then the quadratic equation is solved for a first X value by knowing the concentrations of calcium and sulphate containing ion-pairs

$$\left(\Sigma c_{\#Ca,o} = \left[CaHCO_3^+\right] + \left[CaSO_4^\circ\right]; \Sigma c_{\#SO_4,o} = \left[CaSO_4^\circ\right] + \left[MgSO_4^\circ\right] + \left[NaSO_4^-\right]\right),$$

and the calcium and sulphate ion activity coefficients

$$\left(\gamma_{Ca^{2+}f,*},\ \gamma_{SO_4^{2-}f,*}\right)$$

in the original soil solution. This X value as dissolved quantity of calcium sulphate is added to the measured, total Ca^{2+} and SO_4^{2-} concentrations of the original solution according to equation (3). By this way Ca^{2+} and SO_4^{2-} concentrations get nearer to the ones characteristic to the saturated solution (first step on Fig 1).

The $[Ca^{2+}]_{t,g}$ and $[SO_4^{2-}]_{t,g}$ values obtained by the first step are the input calcium and sulphate concentrations of the subroutine in the next step. In this step the electrolyte composition of the solution is computed as if a certain quantity of calcium sulphate had been already dissolved in it. With the output data of the subroutine, the quadratic equation is solved again for a new X value (second step on Fig. 1). Then, increasing with this X value the Ca^{2+} and SO_4^{2-} concentrations of the original solution, the electrolyte composition is computed again by the subroutine.

The quantity of dissolved calcium sulphate is increasing in every step of the model, but the degree of the value of increase decreases till an X_n value corresponding to saturation concentration and defined by the thermodynamic solubility product is reached (Fig. 1). The procedure is continued till the increase of X ends, that is, becomes negligible:

(7)

$$\frac{|X_{n-1} - X_n|}{X_n} < 10^{-4}$$

where the numerator is the difference of two X values obtained in the two last steps. The computation ends when this difference is less than ten thousandth (10^{-4}) part of the X value obtained in the last step. Then the state of saturation is reached, which is proved by the fact, that the product of Ca^{2+} and SO_4^{2-} activities computed in the last step is equal to the thermodynamic solubility product of calcium sulphate.

Results and discussion

The saturation extracts treated with gypsum have been obtained from the different horizons of the same soil profile (Mezőtúr-102). The chemistry of salt composition is determined by the prevalence of sodium and sulphate ions in every extracts. The total ion concentration varies in a wide range due to the variation of sulphate, sodium and partly calcium and magnesium ion concentrations (Table 2). The increase of sulphate ion concentration causes a sharp decrease of the dissolution of gypsum, despite the increasing ion concentration of the saturation extracts.

The same phenomenon can be observed treating saturation extracts with anhydrite. The saturation concentration of anhydrite decreases with the increasing concentration of sulphate ions (Table 2, profile Cegléd-16) if the chemistry of dissolved salts is sulphatic. The saturation concentration of calcium sulphate is remarkable higher in saturation extracts having sodium-bicarbonatic-sulphatic ion composition even in the case of relatively low total ion concentration of the electrolyte (Table 2, profile Cegléd-22). There are no differences in calcium sulphate concentrations of electrolyte saturated with fine- or coarse-grain anhydrite.

Table 2

The effect of the ionic strength (I) and chemical composition of the saturation extracts of salt-affected soils (Darab et al., 1979) on the dissolution of calcium sulphate.

Profile No. and depth of sampling, (cm)	pH	Cations			Anions			I	ΔCa^{2+} me/l	
		Ca^{2+}	Mg^{2+}	Na^{+}	HCO_3^-	SO_4^{2-}	Cl^-	mmol/l		
		me/l								
Mezőtúr-102									Gypsum	
40–60	7.3	16.84	33.43	97.83	0.87	131.60	14.70	238.6	6.10	
60–80	7.7	3.60	8.09	63.04	1.71	63.44	12.36	113.7	20.52	
80–100	6.8	3.76	8.42	63.04	0.95	63.12	13.80	114.2	20.92	
100–120	7.1	2.82	4.37	43.48	1.10	35.44	12.16	71.0	22.22	
120–140	7.1	2.74	3.19	36.96	0.67	30.60	11.36	61.0	21.30	
									Anhydrite	
Cegléd-16.									F	D
0–9	6.8	4.02	2.81	15.22	5.92	14.98	5.50	37.2	52.86	53.26
10–19	7.7	2.23	3.81	15.65	6.26	12.84	4.90	33.4	45.81	52.24
30–40	8.0	3.02	19.06	61.52	6.74	82.63	13.60	147.0	38.54	39.39
55–65	8.1	2.21	12.24	50.70	6.97	65.50	13.70	116.8	38.14	39.00
Cegléd-22.										
0–11	7.7	1.86	2.75	6.78	4.18	4.92	2.00	16.8	65.29	67.59
12–20	8.2	1.15	0.69	7.83	7.30	3.21	1.74	14.0	65.30	66.00
21–35	8.2	0.92	0.79	22.30	6.23	5.79	2.00	17.9	66.09	66.23
40–50	8.2	0.69	0.79	13.04	9.43	6.83	2.00	21.1	64.74	64.74

DCa^{2+}: the incerase of Ca^{2+} concentration due to the dissolution of gypsum, fine- and coarse-grain anhydrites
F: Fine–grain; D: Coarse–grain

Table 3

The decrase of SAR and ESP values with the dissolution of calcium sulphate in the function of the original SO_4^{2-} concentrations of soil saturation extracts

No. of profile and depth of sampling, cm	$\frac{[SO_4^{2-}]_{t,o}\ \Delta[Ca^{2+}]_t}{mol/1 \cdot 10^3}$		Dissolved calcium sulphate t/ha	SAR_o	SAR_g	ESP_o	ESP_g
1. Mezőtúr–102 40–60 ($CaSO_4 2H_2O$)	65.80	3.05	0.47	19.5	18.1	21.6	20.3
2. Mezőtúr–102 120–140 cm ($CaSO_4 \cdot 2H_2O$)	15.30	10.65	1.65	21.5	8.7	23.3	10.4
3. Cegléd–22 40–50 cm ($CaSO_4 D$)	3.41	32.37	3.97	15.2	3.6	17.4	3.9

$[\ ]_t$: measured "total" ion concentration; o: before; g: after application of calcium sulphate; ESP: calculated from the SAR value of the solution (Richards, 1954); D: coarse-grain anhydrite; Δ : the increase of Ca^{2+} concentration due to the dissolution of calcium sulphate (from measured data).

The decrease of the dissolution of calcium sulphate with the increase of sulphate salinization decreases the effectivity of calcium sulphate containing chemical amendments. It is demonstrated on Table 3 that in soils with an approximately same degree of sodium saturation but differing in the chemistry of their salinization, the quantities of dissolved calcium sulphate may vary even one order of magnitude. The SAR and ESP values decreased remarkable if the saturation extract contained sulphate ions in low concentration and they practically did not changed in case of soil having sulphatic salinization. The prediction of the dissolution of calcium sulphate in the soil liquid phase with known concentration and chemistry of salinization gives way not only to a better method of the calculation of the dose of chemical amendment but it enables an estimation of the efficiency of the chemical amelioration.

The validity of our computer model was proved by the close agreement between the computed and measured data obtained in aqueous salt solutions and soil saturation extracts saturated with calcium sulphate (Fig. 3 and Table 4). We used the published data of the dissolution of gypsum in salt solutions containing common and non-common ions and we computed the dissolved quantities of calcium sulphate knowing the original concentration and composition of the salt solution. The function of calculated and measured values of dissoluted concentration is significant on 0.1% level and the value of its slope is $0.931^{\pm}$ 0.024 (Fig. 3). In the case of soil saturation extracts, there is also no significant differences between measured and computed values of calcium sulphate saturation concentration on 5% level (Table 4).

Table 4

Comparison of the measured ($\overline{Y}_m$) and computed ($\overline{Y}_c$) values characterizing the dissolution of calcium sulphate

Soil profile	Soil chemical amendent	$K_{sp} \cdot 10^{-5}$	Characteristic values	n	$\overline{Y}_m$	$\overline{Y}_c$	$\overline{Y}_m - \overline{Y}_c$	L.S.D$_{5\%}$
					mol/l·10^{-3}			
Mezőtúr–102.	$CaSO_4 \cdot 2H_2O$	2.55	$[Ca^{2+}]_{t.g}$	5	12.08	12.05	0.03	1.03
			$[SO_4^{2-}]_{t.g}$	5	39.55	41.50	1.95	2.02
Cegléd–16 and Cegléd–22.	$CaSO_4D$	5.74	$[Ca^{2+}]_{t.g}$	8	29.03	29.34	0.31	2.85
			$[SO_4^{2-}]_{t.g}$	8	41.28	40.63	0.65	3.05
Cegléd–16 and Cegléd–22.	$CaSO_4F$	5.59	$[Ca^{2+}]_{t.g}$	8	28.30	28.64	0.34	3.22
			$[SO_4^{2-}]_{t.g}$	8	40.50	39.93	0.57	4.12
Cegléd–16 and Cegléd–22.	$CaSO_4$*	4.2	$[Ca^{2+}]_{t.g}$	8	$\frac{CaSO_4F}{28.30}$	$\frac{CaSO_4}{22.44}$**	5.86	3.30
			$[SO_4^{2-}]_{t.g}$	8	40.50	33.73	6.78	4.18

L.S.D$_{5\%}$: Significant difference. ** Solubility values computed with * the thermodynamic solubility product of anhydrite ($4.2 \cdot 10^{-5}$) published by Stumm and Morgan (1981); KSO: solubility product; F and D: fine- and coarse-grain anhydrite, respectively. $[\ ]_{t.g}$: total Ca^{2+} and SO_4^{2-} concentrations of the solution saturated with calcium sulphate; n: number of data; Y: mean value.

The value of ion activity products of fine- and coarse-grain anhydrites were somewhat higher than the solubility product of anhydrite published in the literature (Table 4). Applying the literature value ($4.2.10^{-5}$) (Stumm W. and Morgan, 1981) in our computation, a significant difference (on 5% level) was found between the calcium and sulphate ion concentrations measured in soil saturation extracts treated with fine-grain anhydrite and the values computed by our model. This means that the activity of the solid phase is probably not a constant value and is not equal to 1. This fact should be taken into consideration in the evaluation of the solubility equilibria of anhydrite. Nevertheless, the model is suitable, even in its present state, to predict the solubility of calcium sulphate containing amendments and the saturation concentration of calcium and sulphate ions in soil solution, with the knowledge of the original chemical composition of the liquid phase.

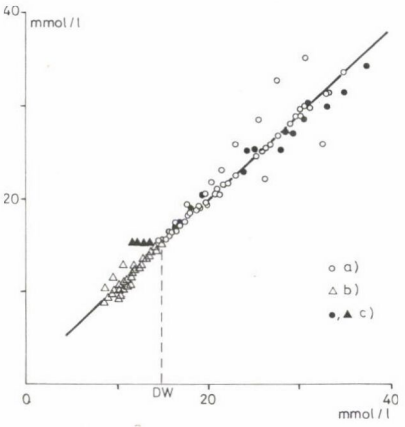

Fig. 3. Comparison of the quantities of dissolved gypsum computed by the model and measured in aqueous salt solutions ($MgCl_2$, KCl, NaCl, $MgSO_4$, K_2SO_4, Na_2SO_4, $CaCl_2$ and their mixtures, published in the literature: Bennett and Adams (1972); Denman (1961); Marshall et al. (1964); Nakayama (1971b); Seidell and Linke (1958); Tanji (1968); Yeatts and Marshall (1969).
Measured and computed solubility of gypsum in solutions containing a) non common ions; b) common ions; c) both non-common and common ions. DW: solubility in distilled water. Vertical axis: computed dissolved gypsum quantity. Horizontal axis: measured dissolved gypsum quantity. $y = 0.001 + 0.931x$; $r^2 = 0{,}968^{***}$; $n = 200$.

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Plant physiology and biochemistry

RECENT DATA ON FERTILIZATION OF PEAR VARIETIES

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The fertilization of 14 pear varieties was studied from 1984 till 1989 in Nagykanizsa region, where ecological conditions of subalpine climate are very favourable for pear production.

Purpose of this work was to obtain information about the tendency to parthenocarpy, ability of self- and open pollination and to determine the best variety combinations with good fertility of 14 world-wide pear varieties under the ecological conditions of Hungary.

Results confirm that pears have to be considered self-sterile as the rate of fruit set was low and characterized only a few varieties.

Parthenocarpy is not characteristic of any of the 14 varieties under the conditions of the study.

Three open pollination groups of the 14 varieties were made, based on fertility of free standing flowers (low-fruit set rate under 4%, medium – between 4.1 and 8%, high fruit-set rate above 8%).

Important differences were observed between fruit set rate of varieties, and also fruit set rate of the same variety in different years.

By testing 77 variety combinations, pollinizers with good and extremely good fertility were determined, and the seed number of fruits of different combinations was measured.

There is unilateral incompatibility between 'Passe Crassane' and 'Packham's Triumph'.

Keywords: pear, *Pyrus communis* L., varieties, fertilization, self-fertility, parthenocarpy, cross-pollination, inter-incompatibility

Introduction

In Hungary, there is a very poor variety assortment in commercial pear orchards and it is limited only to a few prominent varieties ('Williams', 'Doyenné du Comice', 'Conference', 'Abate' 'Fetel' etc.). One of the main purposes of the pear growing modernization is widening of the variety assortment and the introduction of new varieties in order to satisfy the market demand. The main standpoints of variety selection are the productivity, safe cropping, excellent fruit quality and storage quality. In the last ten years some new valuable varieties became cultivated ('Packham's Triumph', 'Beurre Durondeau', 'General Leclerc' etc.).: The fertility conditions of the above-mentioned varieties have not been clarified and they are deficient in certain respects.

Recently Máthé (1977, 1977a, 1979) reported on a method, according to which the flowering process of the herbaceous species *Adonis vernalis* L. can be numerically characterized. It seemed to be useful also in characterizing as well as comparing both the course of flowering and the rate of simultaneous flowering of *Matricaria chamomilla* L.

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(Máthé et al., 1985, Francz et al., 1985) and *Capsicum annuum* L. cultivars. The method could have advantages also in the comparative study of pear cultivars.

The purpose of this work was to examine the fertility conditions of traditional and new pear varieties under the favourable ecological conditions of the Hungarian pear growing. The main purpose of the research was to observe the genetically determined fruit setting and pollinizing ability of pear varieties.

Materials and methods

Studies were carried out in the virus free 14 variety model orchard of the Nagykanizsa State Farm between 1984 and 1990. The orchard was established in 1979 on pear seedling rootstock and trained to natural spindle. Data were collected from 12 trees per variety.

Detailed descriptions of methods used were published by Nyéki (1972, 1974, 1977).

Results

Self-fertility

The self-fertility of pear has been known since 1884 (Waite, 1884). Literature relating to self-fertility of pears was summarized by Nyéki and Soltész (1995): most of the pear varieties are self-sterile. Only a few self-sterile pear varieties are known in the world. The tetraploid pear varieties are self-fertile.

From the literature it has been noted that the rate of self-fertility was often inconsistent in the case of certain pear varieties. Most of the researchers fail to publish the data on the number of the germinative (viable) seeds being in the fruits which are developed from self-fertility. The number of viable seeds developed in the fruit are suitable for the characterization of self-fertility – beside the fruit set.

Because the natural parthenocarpy occurs often in pear, seed examinations are needed in the parthenocarpic fruits that may appear as self-fertile. Fruit set of caged, self-pollinated flowers was studied among 14 pear varieties in 1984 and 1990.

The results of 1984 were that 949 self-pollinated flowers gave 0% fruit set, but in 1990 from the 1417 self-pollinated flowers there developed 1.2% fruit.

These results confirm the data of Nyéki (1972). Accordingly these pear varieties have to be considered self-sterile under the Hungarian ecological conditions except some varieties (Madame Du Puis, Olivier de Serres, Pringalle, 'Beurre Bosc'). They have a chance – at a low percent – to develop fruit on the effect of artificial self-pollination.

For this reason need to be carefully chosen pollinizers for the pear varieties.

Natural (vegetative) parthenocarpy

When the stamens are removed and the possibility of self- and open pollination are precluded, it is called natural or vegetative parthenocarpy. In the case of parthenocarpical fruit set, the seedless fruit develops and ripens without ovule fertilization.

Table 1

Tendency to natural (vegetative) parthenocarpy of pear varieties
(Nagykanizsa, 1985–1990)

No.	Variety	Number of flowers emasculated	Fruit set (%) average of the years
1	Clapp Favorite	649	0.4
2	Jules Guyot	551	1.0
3	Williams	434	0.3
4	Williams B.13	432	0.5
5	Williams Bon Chretien	362	0.0
6	Packham's Triumph	568	1.7
7	Conference	591	1.2
8	Beurre Bosc	363	0.0
9	Beurre Bosc Typ. B.	348	0.0
10	Beurre Durondeau	521	0.5
11	Beurre d'Anjou	286	0.0
12	General Leclerc	433	1.4
13	Passe Crassane	495	0.0
14	Doyenné du Comice	162	0.0
Total		6195	
Average			0.5

Only a few pear varieties tend toward considerably and permanently natural parthenocarpy genetically. The 'Précoce de Trevoux' (4–69%), and the 'Arabitka' (21–51%) Hungarian local variety belong to the varieties mentioned above.

These varieties tend toward natural parthenocarpy so considerably and permanently that they can be grown in a separate block without variety combinations.

Other pear varieties – some tend toward parthenocarpy at a low per cent – have to be planted and grown in combination with different varieties (Nyéki, 1973, 1974).

Pear varieties were assigned to 6 groups by Nyéki (1974) on the basis of measurement of natural parthenocarpy: (1) unsusceptible varieties (fruit set rate 0%); (2) very low (0.1–1%); (3) low (1.1–5%); (4) medium (5.1–10%); (5) high (10.1–20.0%); (6) very high (fruit set rate above 20%).

The genetical determination and annual changing of natural parthenocarpy tendency of pear varieties was also examined by Nyéki (1973). He set up 4 groups of varieties: (1) genetically unsusceptible; (2) parthenocarpic fruitlets drop during the preharvest drop; (3) irregular; (4) regular tendency to parthenocarpy.

The new results of six years of examinations relating to tendency to natural parthenocarpy of 14 pear varieties are summarized in Table 1. During the 6 years there developed fruit from the 0.5% of 6195 radically emasculated (removing the stamens and sepals) flowers.

It was established that the examined pear varieties, which are important in the pear growing in Hungary and the world, show no tendency to parthenocarpy under the Hungarian conditions.

On the basis of tendency to natural genetical parthenocarpy, pear varieties can be ranked to the following groups: (1) genetically unsusceptible (0%) Williams Bon Chretien, 'Beurre Bosc', 'Beurre Bosc', Typ. B., Beurre d'Anjou, 'Passe Crassane', Doyenné du Comice; (2) very low (01.–1.0%) Clapp Favorite, Jules Guyot, Williams, Williams B.13, 'Beurre Durondeau'; (3) low (1.1–5.0%) 'Packahm's Triumph', 'Conference', 'General Leclerc'.

Fertilization of open-pollinated flowers

The fruit set following open-pollination is a genetically determined feature of the varieties (Nyéki, 1973). There are great differences among the varieties relating to fertilization of open-pollinated flowers. Fertilization ability of open-pollinated flowers and yield per tree are affected by the following factors: flower density; climatic factors during the bloom; circumstances of pollination and fertilization; variety composition; quantity, quality of pollen; the number, rate and placing of pollinizer varieties; effectivity of pollen transfer (pollinations by insect); rate of flower, fruitlet and pre-harvest drop; the quality of fruit on the tree; weight and size of fruit.

Researchers estimate that the rate of fruit set necessary in order to reach a great (30 t/ha) yield (Nyéki and Soltész, 1995) is different (3–20%). Nyéki (1973) determined 4–8% ripened fruit rate – depending on the measurement of blossom and fruit set conditions – under the Hungarian ecological conditions as being very favourable for pear production.

Table 2

Fruit set (%) of open-pollinated flowers and open fertility groups of pear varieties
(Nagykanizsa, 1984–1990)

No.	Variety	Number of flowers observed	Average of fruit set %	Open fertility groups
1	Beurre de Anjou	3691	0.5	1. low
2	Passe Crassane	3832	1.9	(fruit set
3	Doyenné du Comice	774	2.6	rate under
4	Packham's Triumph	6866	3.2	4%)
5	Clapp Favorite	2501	4.4	2. medium
6	Williams	3953	5.2	(4.1–8%)
7	Williams Bon Chretien	4010	6.2	
8	Beurre Bosc Typ. B.	2903	6.3	
9	Williams B. 13.	3659	6.9	
10	General Leclerc	2452	7.0	
11	Beurre Durondeau	4213	7.3	
12	Beurre Bosc	3200	9.5	3. high (fruit
13	Jules Guyot	2678	10.0	set rate
14	Conference	3594	12.1	above 8%)
Total		48326		
Average			5.9	

The results on 14 varieties studied over a period of 7 years are listed in Table 2 based on data registered in a commercial pear orchard. In 6 years out of the 7 years of the experiment, the average fruit set of varieties varied between 4.2 and 5.9%, but in 1990 it was 10.8% (it was the best year from the examined 7 years). The 14 varieties were ranged into 3 open-pollination groups (low, medium and high). According to our observations the measurement of open pollination was different in the varieties and there was significant difference in the same variety annually. For example, in the case of the very productive 'Conference' in 1987 0.8%, in 1990 23.9% fruit set was measured.

Genetically determined open-pollinated ability of Beurre d'Anjou flowers is low. It reached 0% fruit set in 3 years, between 0.4 and 0.5% in 2 years and between 1.1 and 1.3% in 2 years. This variety was able to reach 1.3% maximal fruit set during 7 years.

Annual regularity and equalized open-fertilization characterized the 'Beurre Bosc'. The fruit set of this variety was between 12 and 18% in 3 years. During 7 years fruit developed from the 5.9% of the observed 48 326 flowers.

Cross-pollination

In order to choose the right pollinizer varieties for the principal pear varieties the flowers were bagged, emasculated and the pollination was done artificially by hand during the experiment. Crossing tests of 77 variety combinations were carried out between 1984 and 1988 in Nagykanizsa. The results of cross-pollination – fruit set of variety combinations and the number of viable seeds per fruit – are shown in the Table 3. In the case of high fruit set viable seed number per fruit was also high, which means good sexual affinity of varieties.

On the basis of 20 years' experiment (Nyéki, 1989) and fertilization observation it is assertable that the weather conditions affect the fertilization differences more than the varieties themselves.

Important differences were observed (Nyéki, 1977) between fruit set of certain variety combinations yearly and also fruit set on the different trees.

The measurement of fruit set may indicate the sterility also in the case of varieties with good fertility (0% fruit set). A number of researches proved (Nyéki and Soltész, 1995), that cross pollination is not constant in certain variety combinations. Rather it depends on the place of growing and ecological conditions (effect of year). Cross-pollination conditions experiments need to be done because there are frequent inconsistencies in the different countries. Experimental results on pear were summarized by Nyéki (1977). A triploid pollinizer was not used in our experiment, as it is well-known these varieties are not good pollinizers. Cross combinations were put in 4 groups on the bases of fruit set (Table 4). In order to characterize parental varieties it is not enough to consider the fruit set rate, but also the seed content of fruit is necessary. The quality of fertilization may be characterized by the fully developed (viable) seed number in the fruit.

It is well known from the scientific literature (Nyéki and Soltész, 1995) if there is a high yield in the tree then fruit containing less than 3 seeds will usually fall to the ground. Fruits containing more (5–8) viable seeds – because of the better fertilization –

Table 3

Fruit set (%) and viable seed number of pear fruits in different cross combinations

♀ \ ♂	Beurre de Anjou	Beurre Durondeau	Beurre Bosc	Beurre Bosc TYP. B	Clapp Favorite	Conference	General Leclerc	Jules Guyot	Passe Crassane	Packham's Triumph	Doyenné du Comice	Williams B. 13	Williams	Williams Bon Chretien
1 Beurre de Anjou		3.2 7.0							3.8 7.5					
2 Beurre Durondeau	7.0 7.0				2.0 5.0	13.8 9.0		7.2 5.6	11.5 7.4	11.0 7.3	10.0 1.0		10.6 8.0	11.0 6.7
3 Beurre Bosc				19.0 8.9		4.4 7.6	7.7 8.4				12.0 9.2			
4 Beurre Bosc Typ. B			6.0 7.0			17.0 9.6	19.0 9.8							
5 Clapp Favorite		5.7 5.9				1.0 4.0		10.0 2.0		2.0 5.0		10.0 4.0	2.0 6.0	
6 Conference		7.8 3.3	17.1 5.8		5.0 7.8		20.4 6.0	12.0 6.0		14.0 6.2	7.5 3.6	15.0 2.7	14.0 7.2	16.0 6.5
7. General Leclerc			21.0 3.8			13.0 6.0			4.1 0		6.1 5.8		0.8 0	
8 Jules Guyot		10.0 6.1	11.5 5.5		15.2 8.3	17.6 8.0	9.3 3.0		33.0 7.6	12.1 6.2		3.0 2.0	12.0 10.0	
9 Passe Crassane	9.2 4.4	3.7 5.5								0 -				
10 Packham's Trimph	7.0 7.3	8.6 6.6			6.0 7.2	10.0 8.2	6.0 5.0	3.6 8.0	18.0 6.8		11.0 5.9	4.0 4.0	13.7 6.7	16.0 5.8
11 Doyenné du Comice		4.6 5.8	4.0 8.5		4.8 8.0					3.9 6.4				
12 Williams B. 13							9.0 9.8	10.0 4.0						
13 Williams		8.2 6.9				9.6 6.6	12.0 7.1	3.7 5.2	3.0 6.5	8.6 6.5				
14 Williams Bon Chretien					1.0 2.0	18.0 8.7		6.0 6.4						

Note: The table contains the maximal value observed in certain combinations

Table 4

Grouping of pollinizer varieties according to cross-pollinization results

Group number	Group name	Fruit set rate
1	incompatible	0%
2	badly fertile pollenizer	0.1– 8.0%
3	good pollenizer	8.1 – 15.0%
4	extremely good pollenizer	above 15 %

above 15% fruit set – are more resistant against the possible nutrition physiological disorders than the fruits containing less (3.5) viable seeds.

The results of resent investigations demonstrate that the number and quality of seeds developed in the fruit depend on the pollinizer varieties. The crossing combinations are assorted into 4 groups according to the number of viable seeds (Table 5). Table 6 summarizes the pollinizer varieties with good and extremely good fertility and the number of plump seeds per fruit in different crossing combinations.

Table 5

Group of cross combinations on the basis of seed content in their fruit

Group number	Group name	Viable seed number pieces/fruit
1	parthenocarp	0
2	small	0.1 – 4.0
3	medium	4.1 – 7.0
4	great	7.1 – 10

In selecting pollinizers, the following important requirement have to be considered: (1) tight simultaneous flowering with the receptor variety; (2) constantly good fertility ability year by year; (3) high (above 8%) fruit set; (4) good fruit set; (5) distant genetic relation with the pollinated variety; (6) tendency vegetative parthenocarpy; (7) the pollinizer and receptor varieties should fertilize each other reciprocally.

Inter-incompatibility

Inter-incompatibility of pear has been known since O*sterwalder* (1910). Inter-incompatibility was detected in many variety combinations (see summarized data Nyéki and Soltész, 1984). It is reported that there is unilateral incompatibility variety combination. In the 'Passe Crassane' x 'Packham's Triumph' combination gave 0% fruit set from 651 flowers during 5 years. In the reciprocal combination there are great yearly fluctuations between 0 and 18.0% during 4 years.

Table 3, remarkably, indicates that: self-fertility (6–19% fruit set and 7.0–8.9 plump seeds) was observed in the case of reciprocal pollination between 'Beurre Bosc' x

Table 6

Good and extremely good pollinizer varieties and the viable seed per fruit data in different cross combinations
(Nagykanizsa, 1984–1989)

No.	Varieties	According to the cross-pollination	
		good pollinizer (fruit set 8.1–15.0%) varieties	extremely good pollinizer (above 15%) varieties
1	Beurre d'Anjou*		
2	Beurre Durondeau	6:4; 9:4; 10:4; 11:2; 13:4; 14:3	
3	Beurre Bosc	11:4	
4	Beurre Bosc Typ. B.		6:4; 7:4
5	Clapp Favorite	8:2; 12:2	
6	Conference	8:3; 10:3; 12:2; 13:4	3:3; 7:3; 14:3
7	General Leclerc	6:3	3:2
8	Jules Guyot	2:3; 3:3; 7:2; 10:3; 13:4	5:4; 6:4; 9:4
9	Passe Crassane	1:3	
10	Packham's Triumph	2:3; 6:4; 11:3; 13:3	9:3; 14:3
11	Doyenné du Comice*		
12	Williams B.13	7:4; 8:2	
13	Williams	2:3; 6:3; 7:4; 10:3	
14	Williams Bon Chretien		6:4

Note: 6:4 – No. of pollinizer variety: group of seed content in the fruit

'Beurre Bosc' Typ.B', in 1987. In. 1990 both 'Beurre Bosc' types proved to be self-fertile ("Beurre Bosc" 4.8% and 'Beurre Bosc' Typ. B. 3% fruit set). Further experiments are needed to clarify the self-fertility of 'Beurre Bosc'.

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THE WEATHER EFFECT ON RIPENING PERIODS AT DIFFERENT GRAPE VARIETIES

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Continuing their previous studies the authors assumed that the point of intersection in graphic presentation of decreasing acid content and increasing sugar content has a high priority in the interpretation of ripening processes of grapevine. Ten years of data were investigated for two varieties of early-ripening ('Ezerjó' and 'Rizlingszilváni') and two varieties of late-ripening ('Olasz rizling' and 'Hárslevelű') grape.

The ripening process was divided into three periods. The first period begins at the time of flowering and ends when the refraction value reaches 5%, this value can be taken as the beginning of the rise of the curve. The second phase takes until the point of intersection of refraction (%) and acid content (g/l) curves. The third phase goes until harvesting.

Experimental data were gathered during a period of ten years from stocks spacing 3 x 1 meter grown by high cordon training system in the farm of the University for Horticulture.

Considering the date of flowering there were no significant differences among the varieties although this date altered year by year. The duration of the first phase of the ripening period was significantly different for the early-ripening and late-ripening varieties in Table 1 while the length of the second phase was longer only for the Ezerjó variety which is characteristic acidic type grape.

Quantitative values of refraction and acid content at the intersection were close together for the four varieties in Table 2, therefore it can be assumed that there is a general shape of the ripening processes.

The values of refraction are shown in Figures 1-4 as well as the values of acid content in Figures 5–8.

Considering the relationship between the development rate and the temperature the length of the phases can be calculated. (Eq. 2.)

In the first phase the effect of precipitation is also considered. Results are shown in Figures 9–16.

Considering the quality of the yield the variety of Olasz rizling is proved to be the most sensitive to the environmental conditions among the four varieties. The effect of weather on the quality of the yield was investigated by comparing a bad and a good year (Figures 17–19). The distribution of precipitation and the heat accumulation above 15 ^{o}C were significantly different for the bad and the good years.

Keywords: grapevine, ripening, cultivars 'Ezerjó', 'Rizlingszilváni', 'Olaszrizling' 'Hárslevelű'

Introduction

In spring, when the vegetation period begins, the grape-vine buds start growing as dwarf shoot primordia. The inflorescences on the bearing shoots elongate in a relatively short time and, in 40–50 days following bud burst, assume dimensions characteristic of the variety.

The environmental conditions and biological potentials of flowering largely determine the possibilities of pollination and fructification. After such complex preliminaries characterized by multiple interactions, from the time of fructification the development of grape fruit can be calculated up to the harvest. This period ranges from 60 to 120

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days, depending on variety and weather. Despite the fact that the formation of buds with the flower primordia in them (previous year's vegetation period) and the changes taking place during the period between budding and flowering are also organic parts of the development of the yield, the greatest interest still follows the changes taking place in the period between fructification and harvest. This is supposed to be connected with the fact that the most important physico-chemical and biological changes realized in the quantity and quality of yield occur in the cluster and berry, which also represent an economic value. These changes are built on the metabolic processes of the phases of growth and development, and take place in a relatively short time; on the other hand, they may be considerably modified by external environmental factors, and by internal hormonal factors that coordinate the intermediary metabolic processes of the plant. Thus, while the vine produces fruit every year according to the general rules of life processes, owing to changes in the external and internal factors modifying the process, the volume and quality of yield may considerably vary from year to year.

In previous studies we tried to find correlations between the change of the weight of cluster and the trend of the meteorological elements, as well as between changes in the dry matter content (refraction value) of the yield, and the trend of the meteorological factors (Polyák et al., 1992a and b). In essentials, varietal differences and peculiarities could be demonstrated; early and late varieties are sensitive to the meteorological elements in differenct phases of the ripening period.

In the present paper we wish to show a general rule in connection with all ripening processes. It concerns the so-called point of compensation occurring in the course of the opposite direction changes of must degree and total titrable acid.

Literary review

It is highly instructive to acquire a knowledge of the different authors' experimental methods and results related to the ripening processes of grapes. Some of the publications presenting the research results try to find correlations between the degree of must and the meteorological factors. Ichele (1965) consider it necessary to know the meteorological factors and the microclimate of the area before choosing the rootstocks, with the view of maintaining the desirable vigour. Sou-Zsu (1963) found a close correlation between the performance of the varieties and the characteristics of weather, but the varieties retained their fundamental hereditary features. Matsui et al. (1979) in the last phase of the ripening process observed rapid sugar accumulation in the berries, which they attributed to a process of transport from the leaves. This statement is not acceptable at present, since the reserve carbohydrates of ligneous organs also are mobilized. Their statement that, with the reduction of the foliage, the sugar content of the berries also decreases can be accepted with certain limits of value. We also agree with the results of experiments on cluster load, e.g. in the case of Calo and Jannini, when in consequence of cluster thinning the degree of must increased. Katarjan and Potapov (1963) published remarkable results; they found that the amplitude of the daily temperature of air substantially influenced the intensity of sugar accumulation in berries and the time of ripening. In their opinion, both the high and the low temperatures are unfavour-

able. They recommend soil mulching for its favourable effect on the process and time of ripening. Under the conditions of Hungary, Dunkel et al. (1981) consider the areas of good exposure, the slopes, to be outstandingly favourable for vine growing. Csepregi (1985, 1992), evaluating the results of his long-term experiments, draws a parallel between the degree of must at the time of harvest and the meteorological characteristics of the vegetation period, and thereby supplies valuable data for studies on the subject.

The ripening process is not restricted to the change of the degree of must, since during this period the berry performs independent metabolism on the one hand, and serves as the site of collecting the metabolites, on the other. By complex biochemical analyses, Koblet (1980) pointed out that a part of the organic acids present in the berries transformed into sugar too; and Drawert (1963) published the same much earlier with the important statement that, in the last phase of ripening the amount of amino acids, substances containing nitrogen also increased in the berries.

The correlation examinations serve as a basis for disclosing the causal relations between the characteristics of the ripening process and the environmental factors (Schneider and Staudt, 1980). These efforts are helped by preparation of models and their evaluation e.g. in the works of Gutterez et al. (1986), but mathematical equations were also set up (Maujean-Brun et al., 1981) for the description of the complex correlations between sugar accumulation and acid content reduction.

Researchers dealing with the ripening processes of grapes have long since had a notion that the ripening period of the berry consists of several phases.

According to Manzoni (1955), in the period between fructification and ripening there are two main development phases: the premature and the ripening period. Borogono-Taretto et al. (1984) discuss the course of ripening in detail from the aspect of choosing the right time of harvest, with special regard to the aromatic substances. Hungarian authors described three phases of this period: a) growth of green berries, b) ripening, c) over-ripening (Hegedüs et al., 1966). Katarjan – Potapov (1961) use the term of physiological ripeness too, giving the active heat sum required for reaching this stage. Today we accept the division of Alleweldt et al. (1975), which describes the functions of the processes, and separates four phases, as presented by Kozma (1991): phase 1: takes 6–10 days from fructification and is characterized by the slow growth of the pericarp; phase 2: the period of quick cell division over 18–42 days, identical with the phase known as the growth of the green berries; phase 3: the berry attains its final form, when growth hardly occurs for 10–20 days; phase 4: the elongation of the cell nucleus results again in quick growth, the sugar % rises, while the amount of total titrable acid decreases. This phase lasts to the physiological ripeness, as long as sugar flows into the berries. According to Alleweldt et al. (1975) in phases 1 to 3 auxins, in phases 2 and 3 organic acids dominate, while phase 4. is characterized by intensive sugar accumulation and acid reduction. According to Farmahan and Pandey (1976) in phase 4 abscisic acid (ABS) also accumulates besides the nitrogenous substances, delaying to some extent the ripening processes.

Materials and methods

For our examinations we used the measuring data of 4 vine-grape varieties (Olasz Rizling, Rizlingszilváni, Hárslevelű and Ezerjó) collected between 1975 and 1984 in the Model Farm of the University of Horticulture and Food Industry, Szigetcsép. The vine-stocks of the varieties examined, aged between 10 and 20 years were trained on high cordon. For the examinations, the lower clusters of shoots in the middle of the arms were collected, 15 clusters on each occasion. Measuring was started in the growth phase of the green berry and was repeated every 10 days throughout the ripening phase.

The cluster weight, berry weight, number of berries per cluster, the refraction value of must, the titrable acid content, etc., were determined with methods used in uvology. The analyses were performed in the laboratory of the Department of Vine Growing. The meteorological data were collected by the workers of the UHF Agrometeorological and Water Management Department.

In the course of evaluating the data, we are going to show the tendencies of physical and chemical changes taking place during the ripening of cluster and berry, and their correlations with the meteorological factors. This paper can be regarded as a continuation of the two previously published in 1992 (Polyák et al., 1992a, 1992b).

In our opinion, the time when the curves of the opposite direction changes of titrable acid (g/l) and refraction value (dry matter %) intersect plays an outstanding role in interpreting the ripening processes. For characterizing the ripening process, we used the trend in time of the quantity of titrable acid. We took the time of flowering as the beginning of our scope of time. The period from flowering to harvest was divided into three phases; the first phase lasted from flowering to the time when the degree of must began to rise; the second phase, to the point of time when the degree of must, i.e. the refraction value (dry matter %) and the titrable acid (g/l) assumed the same value; while the third phase covered the changes of the subsequent period up to the harvest.

We examined the variety dependent nature of the length of the individual phases, as well as the way the meteorological factors influenced the lengths of period and the degree of must and amount of titrable acid observed at the critical points.

Discussion

Characterization of ripening phases

Within the same year the varieties were in flower at almost the same period. However, the time of flowering varied from year to year. In 1979 and 1983 flowering set in early (1 June), in 1975 and 1977 it was medium early (10 June) and so was in 1981, 1982 and 1984 too, while in 1976, 1978 and 1980 flowering took place late (20 June).

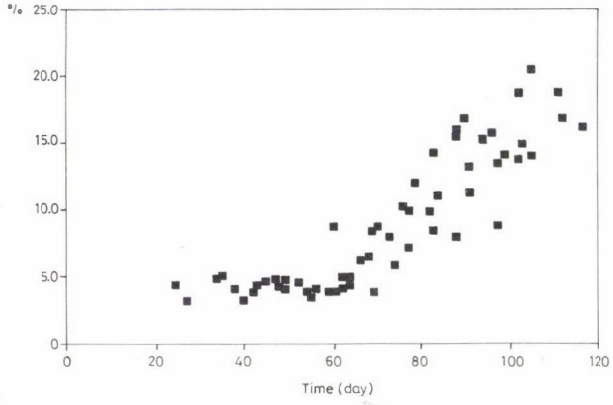

Fig. 1. Dry matter content of berry from flowering to harvest. 'Olasz rizling', Szigetcsép 1975–1984

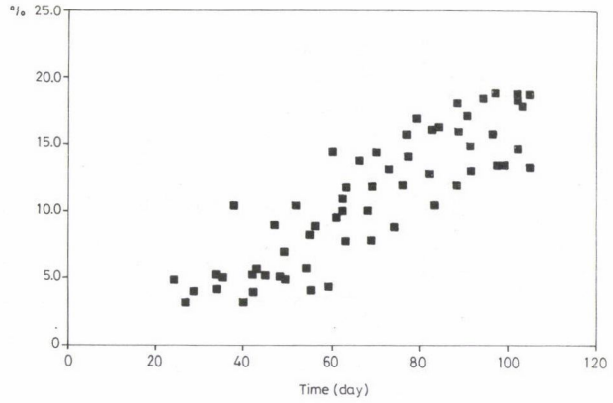

Fig. 2. Dry matter content of berry from flowering to harvest. 'Rizlingszilváni', Szigetcsép 1975–1984

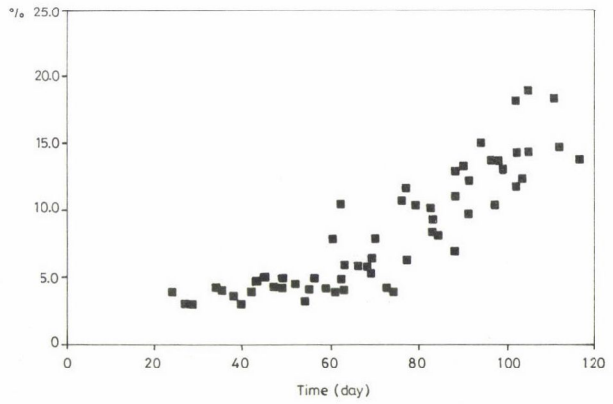

Fig. 3. Dry matter content of berry from flowering to harvest. 'Hárslevelű', Szigetcsép 1975–1984

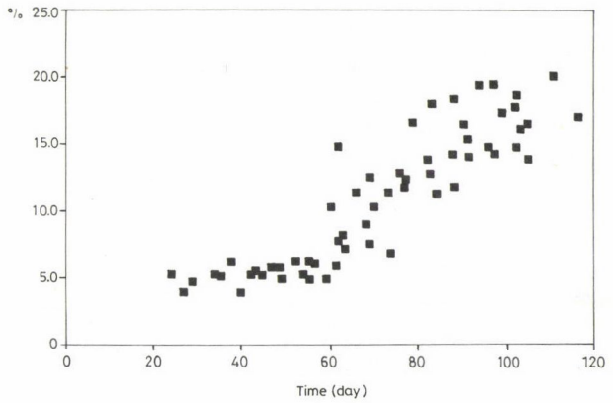

Fig. 4. Dry matter content of berry from flowering to harvest. 'Ezerjó', Szigetcsép 1975–1984

At the beginning of the measuring period, the degree of must hardly changed, then suddenly began to rise sharply with a declining tendency by the end of the measuring period (Figs 1–4) The change characterizable by a sigmoid curve unequivocally agrees with the literary data. As regards the change of the total titrable acid, again in agreement with the literary data, a slight rise can be observed at the beginning of the first phase followed by a sharp decline; then at the end of the period, the change slows down (Figs 5–6). The rise of the degree of must and the reduction of the amount of titrable acid begin from almost the same point of time, which was determined through interpolation by finding the day when the degree of must exceeded the value of 5 (MD=5). We examined for each variety the length of the period from flowering to the time when MD=5 was attained. This period was regarded as the first phase of the ripening process. The second phase lasts from MD=5 to the time when the degree of must and the titrable acid show the same value; this is the so-called point of compensation. Table 1 contains

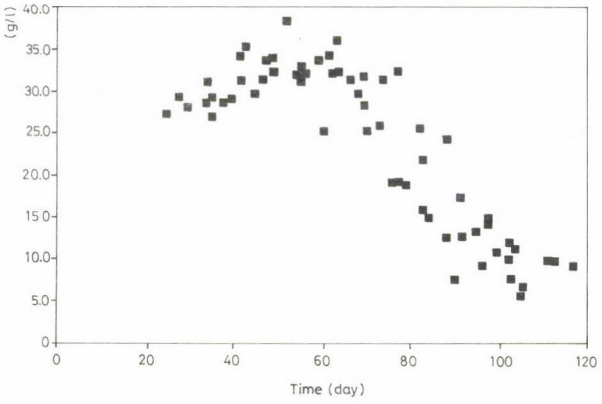

Fig. 5. Titratable acid content from flowering to harvest. 'Olaszrizling', Szigetcsép 1975–1984

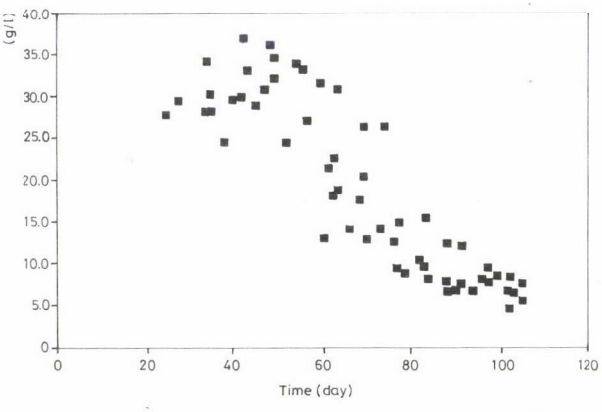

Fig. 6. Titratable acid content from flowering to harvest. 'Rizlingszilváni', Szigetcsép 1975–1984

Table 1

Lengths of ripening phases in days

Phases	Grape cultivars			
	Olaszrizling	Rizlingszilváni	Hárslevelű	Ezerjó
Phase I (MD=5)	62.6	41.0	63.4	33.1
scatter	5.1	7.8	6.6	5.8
CV%	8.1	18.9	10.5	17.6
Phase II (point of compens.)	30.0	35.4	36.0	51.9
scatter	6.4	4.3	6.2	8.0
CV%	21.5	12.2	17.2	15.4

the average length of time, scatter and variation coefficient per variety for the first (MD=5) and second (point of compensation) phase.

In respect of phase I the 4 varieties can be placed in two groups. In the late varieties of Olaszrizling and Hárslevelű an average of 62–63 days are required after flowering for the degree of must to rise; that is, for the ripening process to start. In the early 'Rizlingszilváni' and 'Ezerjó' varieties, the degree of must begins to rise after 33–41 days. According to the statistical t-test, there is a significant difference (P=0.1%) between the groups in this respect, while the varieties within the group do not differ significantly. The yearly variation is lowest (8.1%) with Olaszrizling, and is only 10.5% in the case of 'Hárslevelű' but it is slightly higher – 18.9 and 17.6% – in the varieties Rizlingszilváni and Ezerjó, respectively. As regards the tendencies of changes, our data agree with those of Matthews and Anderson (1988).

Table 2

g/l values of must refraction percentage and total titratable acid at the point of compensation

Year	Grape cultivars			
	Olaszrizling	Hárslevelű	Rizlingszilváni	Ezerjó
1975	12.6	13.5	14.0	12.8
1976	14.6	15.1	14.0	16.6
1977	14.8	14.8	14.0	15.7
1978	13.6	13.2	13.6	15.0
1979	14.9	14.7	13.4	14.0
1980	9.1	11.9	12.1	14.3
1981	13.7	15.7	14.3	16.0
1982	14.0	13.0	12.2	13.1
1983	13.5	12.8	12.3	13.8
1984	13.4	13.7	12.2	13.9
average	13.45	13.84	13.21	14.52
scatter	1.58	1.13	0.86	1.20
CV%	11.71	8.17	6.49	8.25

The length of phase II is shortest (30 days) in Olaszrizling, and significantly differs from that in the other varieties (P=5%).

In the case of 'Rizlingszilváni' and 'Hárslevelű' the length of phase II is 35–36 days on the average, and there is no significant difference between them. In the variety Ezerjó, this period is longer (52 days), supposedly in consequence of the stable acid metabolism characteristic of the variety, and the time difference from the other varieties is significant at 1% level. The yearly variation in the length of phase II is lowest (12.2%) with 'Rizlingszilváni', followed by 'Ezerjó' with 15.4%. 'Hárslevelű' with 17.2% and finally by 'Olaszrizling' with 21.5%.

The values of the degree of must and of the titrable acid observed at the point of compensation are shown in Table 2. The difference in average value is significant only between 'Ezerjó' and 'Olaszrizling' and between 'Ezerjó' and 'Rizlingszilváni' at 10% and 5% level, respectively.

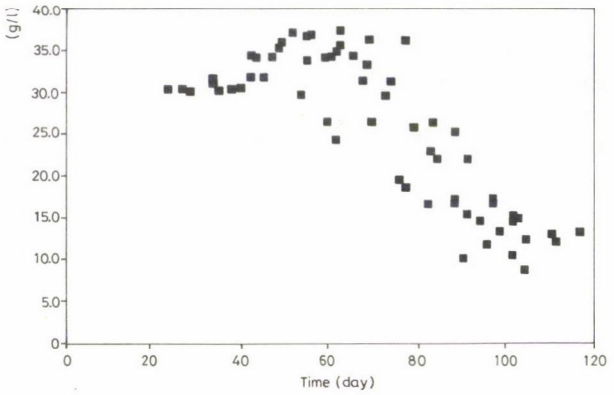

Fig. 7. Titratable acid content from flowering to harvest. 'Hárslevelű', Szigetcsép 1975–1984

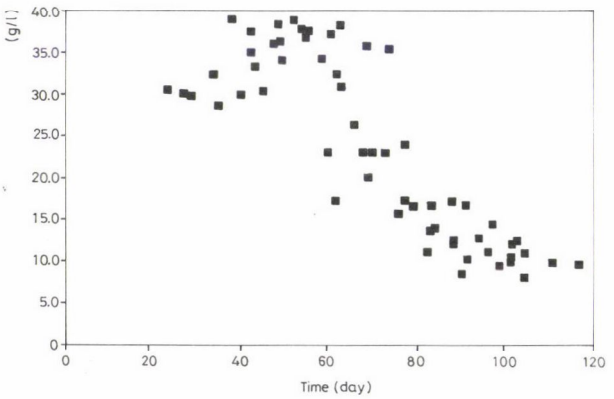

Fig. 8. Titratable acid content from flowering to harvest. 'Ezerjó', Szigetcsép 1975–1984

On the average of 10 years, the degree of must measured at the point of compensation is lowest with Rizlingszilváni (13.2%). Hardly different from it are the values of 'Olaszrizling' and 'Hárslevelű' (13.5 and 13.8%, respectively) while, in the case of 'Ezerjó', it is somewhat higher (14.5%).

It must be noted that the degree of must at the point of compensation was remarkably low in 1980, particularly for the variety Olaszrizling. The value of this parameter was the lowest in this year for Rizlingszilváni and Hárslevelű too, but this extremity was not observed in the case of Ezerjó. We wished to know whether there is any connection between the length of phase II and the degree of must at the point of compensation, but the very low value of the correlation coefficient for each variety excluded any connection between them.

From the point of compensation to the harvest, the degree of must rose by 3–4% on the average, except for the variety Hárslevelű, where in 4 of the 10 years no further increase occurred, which means that the rise of the degree of must and the reduction of the titrable acid reached the point of compensation only at the time of the last sampling. The year of 1980 was also an outstandingly bad year for all varieties examined from this standpoint. The greatest increase in three varieties ('Olaszrizling', 'Rizlingszilváni', 'Hárslevelű') appeared in 1983 (6.9%, 6.5% and 6.2%, respectively), while in 'Ezerjó' it occurred in 1979 (4,7%). The 10-year averages of the degree of must at the time of harvest were: 'Olaszrizling' 16.45%, 'Rizlingszilváni' 17.2%, 'Hárslevelű' 15.3%, 'Ezerjó' 17.3%. The difference of averages can be regarded as statistically significant only between 'Hárslevelű' and 'Ezerjó' and between 'Hárslevelű' and 'Rizlingszilváni'.

The amount of titratable acid decreased by an average of 3.5–6.0 g/l from the point of compensation to the harvest, except for the variety 'Hárslevelű' in which the average decrease was only 1.4 g/l. 1980 was an extreme year for each variety in respect to this parameter too. The amount of titrable acid at the time of harvest was 5.9 g/l for 'Olaszrizling', 7.25 g/l for 'Rizlingszilváni' 12.45 g/l for 'Hárslevelű' and 10.95 g/l for 'Ezerjó' on an average. The average showed significant differences in the case of each pair of varieties. We examined the correlation between the value of the degree of must and the concentration of the titrable acid. Contrary to our expectation, the correlations was statistically significant only for the variety 'Rizlingszilváni' (r=0.82); with the other varieties, it proved loose (r=0.3–0.4).

Connection between the length of the ripening processes and the environmental factors

Considering that the examinations took place at the same site during the ten years, the soil and the special microclimatic effects did not change. That is, as a changing environmental effect, the yearly varying conditions of weather can be taken into consideration. For a numerical characterization of the rather complex nature of weather, the measured values of the individual meteorological elements and the quantitative parameters obtained from them can be used. Among the meteorological elements we rely on the daily mean temperature, the sunshine hours and the amount of precipitation.

Table 3

Average temperature sums of the ripening phases

Phases	Parameters	Grape cultivars			
		Olaszrizling	Hárslevelű	Rizlingszilváni	Ezerjó
I (MD=5)		1327.6	1348.6	862.1	689.2
	scatter	70.9	116.9	131.9	91.4
	CV%	5.3	8.7	15.3	13.3
II (point		564.4	664.4	736.4	1060.4
of compesation)	scatter	96.1	91.6	81.4	136.9
	CV%	17.0	13.8	11.1	12.9

It is known that the biological and chemical processes are highly dependent on the temperature and can be illustrated with the so-called Ql0 curve.

Table 3 shows the average, scatter and variation coefficients of heat sums in phase I and II In the case of longer phases, the variation is smaller; that is, the necessary heat sum is rather stable, in phase I 1323.7 and 1348.6 °C/day for 'Olaszrizling' and 'Hárslevelű' with a variation of 5.9 and 8.7%, respectively, while for the varieties 'Rizlingszilváni' and 'Ezerjó' the average heat sum is 862 and 689.2 °C/day with a variation of 15.3% and 13.3%, respectively.

The average of the temperature totals in phase II. is the lowest for Olaszrizling, 564.4 °C/day, followed by Hárslevelű with 664.4 °C/day, then by Rizlingszilváni with 736.4 °C/day. Finally, it is 1060.4 °C/day in the case of 'Ezerjó'. The variation coefficients for the individual varieties are of a similar order, 13–17%.

The average lengths of the ripening phases develop under average temperature conditions. The value of the function attached to the development phases, here to phase I and II, is 0 on the initial day and 1 at the end of the phase. The growth between 0 and 1 is given by the *daily rate of development* or its accumulation. The average development rate is obtained as the invert of the average length of phase. The actual development rate is higher if the mean temperature of the day is higher than the average characteristic of the phase, and is lower if the daily mean temperature is lower than the average. From the

Table 4

Average temperature (T^*) and development rate (m_i^*) of ripening phases, and the "a" precipitation parameter

Phases	Parameters	Grape cultivars			
		Olasz rizling	Hárslevelű	Rizlingszilváni	Ezerjó
I (MD=5)	T^*	21.208	21.270	21.026	20.821
	m_i^*	0.016	0.016	0.024	0.030
	a	–0.015	–0.015	–0.01	–0.01
II (point of	T^*	18.812	18.455	20.802	20.431
compensation)	m_i^*	0.033	0.028	0.028	0.019
	a	0	0	0	0

average phase lengths and temperature totals, we determined the average temperature characteristic of the phases, T*, and the average development rate, m_i* for each variety (Table 4). The actual rate of development for a given day is expressed with the ratio of the mean temperature of the day to the average T*, supposing linearity:

$$m_i /T/ = (T/T^*)\ m_i^*$$

According to our observations the effect of precipitation can be so described and be taken into consideration that the lack of precipitation shortens the development phases, while a more than average amount of precipitation lengthens them. Considering that the moisture status depends not only on the amount of precipitation on a given day, but also on the precipitation conditions of the previous period, we take the average precipitation of the previous 5 days into account, when expressing the effect of precipitation. So the daily rate of development will be:

$$m_i /T,P/ = m_i /T/-m_i /T^*a^*\ sum/CP_i =5$$

where sum (Pi-5) is the sum of precipitation on the previous 5 days, the value of the a parameter may slightly vary with the variety (Table 4).

Equation 2 represents the basis of the simple model by which the lengths of the individual development phases can be calculated. Let us take r/0/=0 for the initial day of the development phase. From this day the development rates (my) are summed up:

$$r/n/ = sum/\ m_i\ /\ /i = 0\ ...n)$$

n is the value at which r/n > = 1 will be the initial day of the next phase.

In Figs 9–12. the time of MD=5 calculated with the above model and that of the observed MD=5 are shown for each year. The difference between the observed and the calculated values was 2.6 days with Olaszrizling, 4.3 days with 'Rizlingszilváni' and 'Hárslevelű' and 3.7 days with Ezerjó on the average.

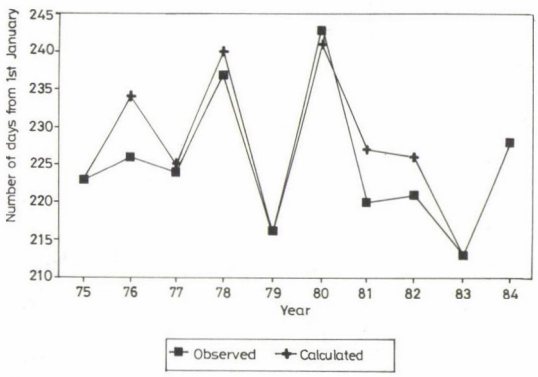

Fig. 9. Observed and calculated times when the dry matter content of berry is MD = 5 = 5%. 'Olaszrizling'

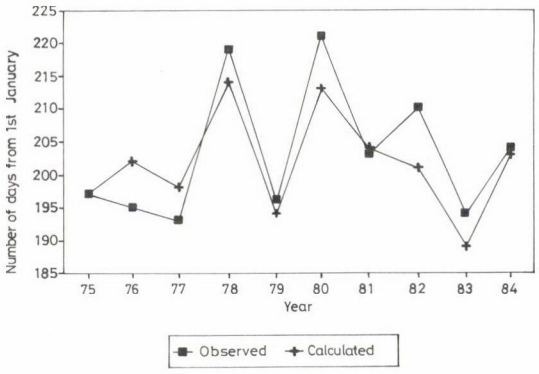

Fig. 10. Observed and calculated times when the dry matter content of berry is MD = 5 = 5%. 'Rizlingszilváni'

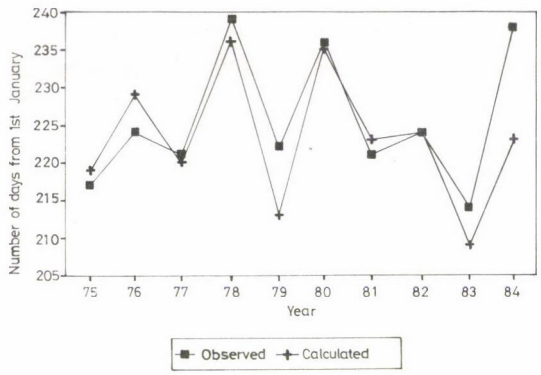

Fig. 11. Observed and calculated times when the dry matter content of berry is MD = 5 = 5%. 'Hárslevelű'

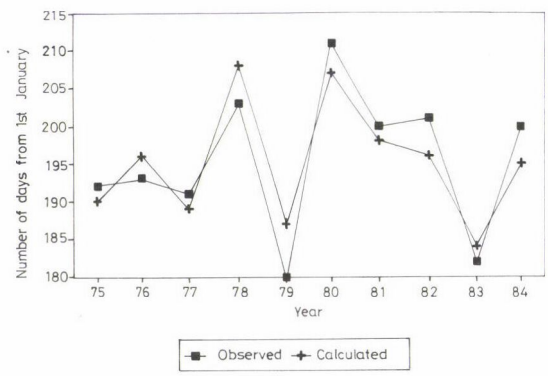

Fig. 12. Observed and calculated times when the dry matter content of berry is MD = 5 = 5%. 'Ezerjó'

In Figs 13–16. the calculated and observed values of the point of compensation are seen. The average difference between the two values was 4.3 days with the variety 'Olaszrizling' 3.6 days with 'Rizlingszilváni' 4.6 days with 'Hárslevelű' and 6.3 days with 'Ezerjó'. It must be noted that, when calculating the length of phase II, we did not take the effect of precipitation into account; that is, we took the value of parameter "a" for 0, since we found that it did not affect much the values calculated with the model.

The weather dependence of must degree and titrable acid is shown through the comparison of the worst and the best year, 1980 and 1983, respectively. In 1980 flowering set in late, in the third week of June, while in 1983 the vine was in flower already in the first week of June. When comparing the two years for meteorological conditions after flowering, we find that surplus of total sunshine hours only appears from about the 70th day after flowering, in favour of the year of 1983 (Fig. 17).

In accumulated precipitation (Fig. 18), the whole year of 1983 was drier, though the difference became remarkable from the 40th day after flowering; further, in 1983 there were longer dry periods more often than in 1980. A considerable difference is found between the two years in temperature conditions, especially when we consider the temperature total above 15 °C (Fig. 19). The curve of the temperature sum in 1980 reaches the section of saturation much sooner, some 75 days after the time of flowering, while in 1983 the saturation phase only follows about 100 days after flowering. Moreover, the temperature sum attained in 1980 remained below 400 degrees, while in 1983 it exceeded 700 degrees. In the development of the degree of must at the time of harvest, two factors thus play a role. One is the time of flowering, which is important because the subsequent potential ripening phase can be the longer, the sooner flowering has set in. A further difference that the varieties showed was, in the case of late flowering, a high degree of must in the varieties 'Olaszrizling' and 'Hárslevelű' cannot be reckoned with, even if the temperature, precipitation and sunshine conditions were favourable after flowering. However, with the varieties 'Rizlingszilváni' and 'Ezerjó' the time of flowering is less decisive, since in 1976 and 1978 the 18.5–19.4% must degree was reached even after late flowering.

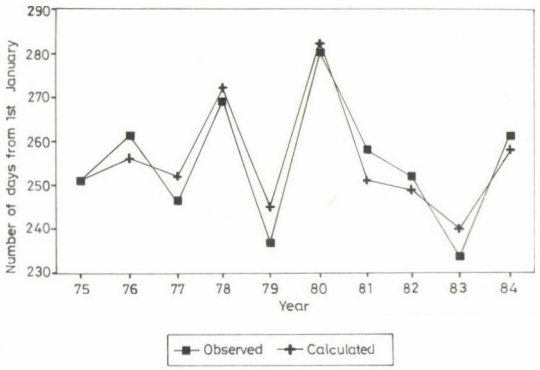

Fig. 13. Calculated and observed time of intersection of changes in dry matter and acid content. 'Olaszrizling'

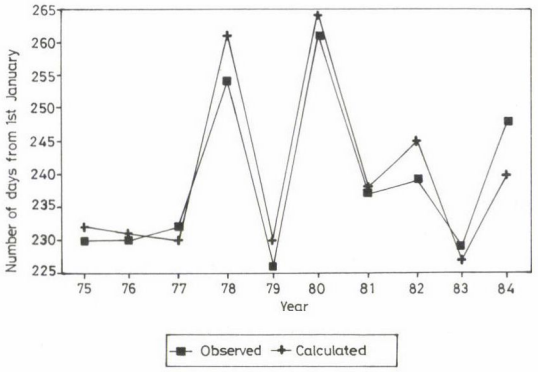

Fig. 14. Calculated and observed time of intersection of changes in dry matter and acid content. 'Rizlingszilváni'

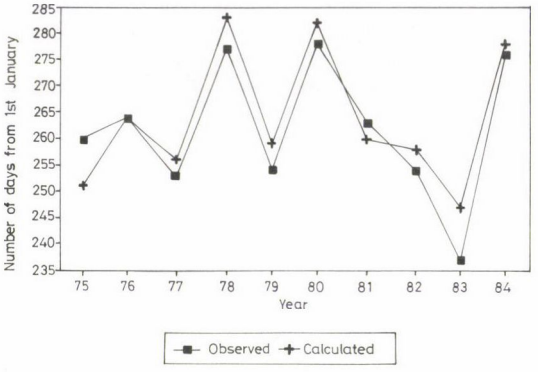

Fig. 15. Calculated and observed time of intersection of changes in dry matter and acid content. 'Hárslevelű'

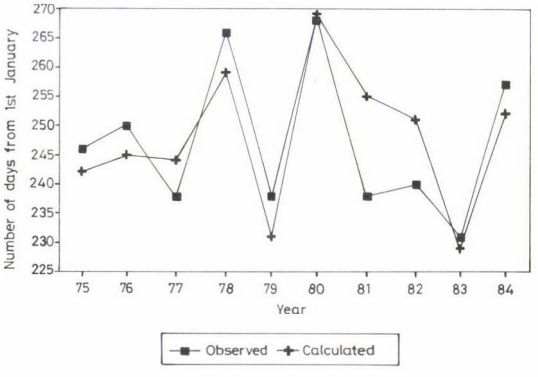

Fig. 16. Calculated and observed time of intersection of changes in dry matter and acid content. 'Ezerjó'

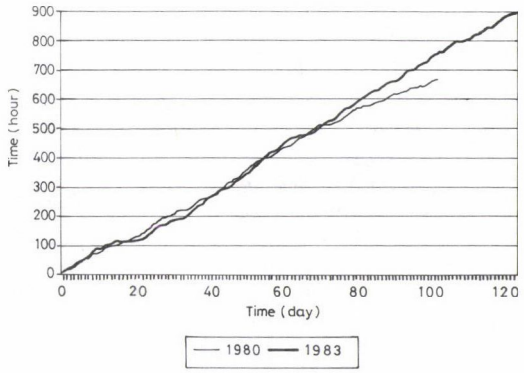

Fig. 17. Accumulated sunshine hours from flowering to 1 October

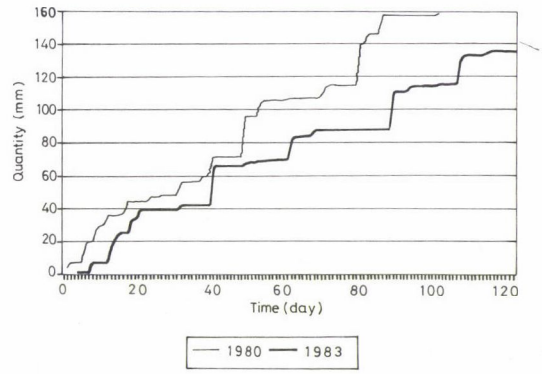

Fig. 18. Accumulated precipitation from flowering to 1 October

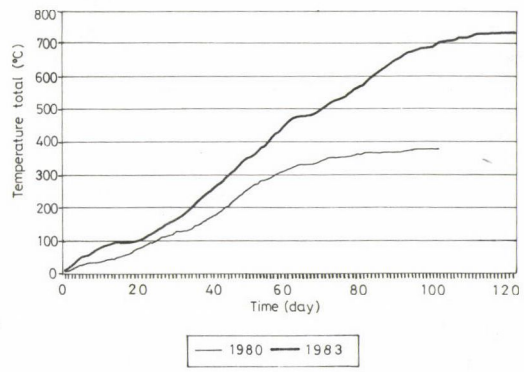

Fig. 19. Effective temperature sum from flowering to 1 October

Summary

It can be established that the length of the ripening phases of grapes and the dynamics of the course of ripening depend on the weather, and above all on the variety. As regards the time of ripening, the varieties do not essentially differ. The rapid increase in the degree of must begins soonest in the variety 'Ezerjó' followed by 'Rizlingszilváni' and the process starts last in the varieties 'Olaszrizling' and 'Hárslevelű'. From the time of flowering a conclusion can be drawn on the time when the degree of must (dry matter %) begins to rise rapidly, with an average deviation of about 5–6 days. On the basis of meteorological data, the estimation can be made more precise only by 1–2 days with the above described method. The period from the time when the degree of must begins to rise to the time when the point of compensation is reached is also variety dependent. This period is shortest in the variety 'Olaszrizling' somewhat longer in 'Rizlingszilváni' and 'Hárslevelű' and longest in the variety 'Ezerjó'. With the time of MD=5 known, this point of time can be estimated with some 6–8 day reliability on the basis of the average length of phase. With the meteorological data taken into consideration, the estimation can be improved by 2–3 days.

At the point of compensation the degree of must (dry matter %) and the amount of titrable acid (g/l) showed nearly the same value in the varieties included in the experiment. In the varieties 'Olaszrizling' 'Rizlingszilváni' and 'Hárslevelű' it was an average of 13.5 in 'Ezerjó' 14.5, supposedly reflecting therefore a general regularity.

After the point of compensation had been reached, the refraction increased by about 3–4% until harvest in the case of three varieties, except Hárslevelű, where the point of compensation very often was only reached at the time of harvest. The average value of the degree of must at the time of harvest was the highest (17.3 and 17.2%) in the early varieties of 'Ezerjó' and 'Rizlingszilváni'. In this respect, these varieties do not statistically differ from one another. On the average of the 10 years, the value of refraction at the time of harvest was 16.5% for 'Olaszrizling' and 15.3% for 'Hárslevelű'. The most extreme values in the 10-year data series were found for the variety 'Olaszrizling' (9.1 in 1980 and 20.4 in 1983), which suggests that this variety is highly sensitive to the weather conditions, concerning the trend of the sugar and acid-content, rather than the length of the ripening phases.

As regards the value of titrable acid at the time of harvest, the varieties showed significant differences. On the average of 10 years, the lowest value was obtained in 'Rizlingszilváni' (7.3‰) followed by the variety 'Olaszrizling' (9.5‰) and 'Ezerjó' (10.95‰); it was the highest (12.5‰) in the variety 'Hárslevelű'. The highest yearly variance in this respect characterizes the variety 'Olaszrizling'. Between the final degree of must and titrable acid content, only a loose correlation was found in most of the varieties, but it was relatively close in the single case of 'Rizlingszilváni'.

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EFFECT OF NaCl ON ENZYMES IN SALT-TOLERANT AND SALT-SENSITIVE RICE CULTIVARS

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The effect of NaCl on the *in vitro* activity of twelve enzymes involving various metabolism was studied in the salt-tolerant and salt-sensitive rice cultivars. The salt-tolerant CSCl exhibited less stimulated activities of starch phosphorylase, α-ketoglutaric dehydrogenase, succinic dehydrogenase, pyruvic dehydrogenase, acid phosphatase, protease and highly stimulated activities of invertase, ascorbic acid oxidase, polyphenolase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase than did the salt-sensitive TKM 4 in their leaves, in response to saline environments. The variation in the levels of these enzymes, involving different facets of metabolic machineries, could be the speculative reactions and could act as possible indicators in salt-tolerance mechanism of rice cultivars when the growth and development were affected by threshold level of salinity.

Keywords: rice, salt tolerance, enzymes

Introduction

The mechanism of salt tolerance of cultivated crop species, that differ considerably in the tolerance to salinity, range from restricted ion uptake and translocation into the shoot to structural metabolic changes that decrease salt injury (Rathert, 1983). Differences in salt tolerance occur not only between crop species but also between varieties. The latter are attractive objects. The high cytoplasmic sodium and chloride concentration and the lower osmotic potential may affect the structure and function of enzyme proteins (Flowers et al., 1977). It has been proposed that salt-tolerant species are better able to regulate the cytoplasmic ionic status and thus avoid interactions between enzyme and salt, which may be responsible for salinity damage in other species (Greenway and Osmond, 1972). However, selectivity of ion uptake and translocation and the metabolic reactions of the plants vary with stress intensity and with the plant species and line. In our earlier reports, similar adaptations of the metabolic response of rice cultivars have been proposed to account for salt tolerance with organic acids (Krishnamurthy et al., 1987a), enzymes particularly amylase, ATPase and nitrate reductase (Krishnamurthy et al., 1987b), chlorophyll (Krishnamurthy et al., 1987c), amino acids (Krishnamurthy et al., 1987d), polyamines (Krishnamurthy and Bhagwat 1989) and growth and yield (Krishnamurthy et al., 1987e). It was of interest, therefore, to examine the effect of NaCl on the *in vitro* activity of a number of enzymes involved in

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different facets of metabolisms and to identify the levels of enzyme variation towards salt tolerance and salt sensitivity of rice cultivars, when the growth and development were affected by threshold level of salinity.

Materials and methods

The seeds of rice (*Oryza sativa* L.) cultivars viz., CSC 1 (salt-tolerant) and TKM 4 (salt-sensitive) were procured from Tamil Nadu Agricultural University, Coimbatore, India. The salt-tolerant and salt-sensitive groups were classified in our earlier reports on the basis of growth and grain yield (Krishnamurthy et al., 1987e). The pot culture experiments were carried out in earthen pots filled with 7 kg of soil under net house conditions during the wet season. Salinization was imposed on three-week-old seedlings grown under nonsaline conditions by the addition of sodium chloride solution of EC 10 m mhos/cm in the pot once a week, with a combination of normal watering on other days, for seven weeks. Care was taken to avoid drainage of solution of water by slow addition. The volume of water or solution used was slightly less than the full field capacity of the soil, and the addition was withheld at the slightest indication of seepage. Control plants received only tap water. Similar method of salt addition had been found to be efficient in our earlier studies to test the salt tolerance of rice cultivars (Krishnamurthy et al., 1987a–1987e). The shoot system was harvested six weeks after initial salinization and the third leaf from the top was selected for the assays of enzyme activities. All the assays were done from three replicates (i.e. three independent experimental earthen pots) each containing three separate plants.

The invertase (EC 3.2.1.26) was extracted from one gram of fresh leaf material with 55 ml of 0.01 M acetate buffer (pH 4.6). The homogenate was centrifuged at 8,000 r.p.m. for 15 minutes at 0° to 2 °C. The supernatant was used as the source of enzyme. The method of assay of enzyme was that of Malik and Singh (1980) using 3.5-dinitrosalicylic acid reagent. Glucose was used as a standard to calculate the reducing sugar released.

Phosphorylase (EC 2.4.1.1) enzymes were extracted with 10 ml of ice-cold distilled water from one gram of fresh leaf material and centrifuged at 6,000 r.p.m. for 15 minutes at 0° to 2 °C. The reaction mixture contained in a total volume of 2.5 ml; 0.2 ml of 5% starch solution, 0.5 ml of 0.5 M citrate buffer (pH 6.5), 1.0 ml enzyme and water. The mixture was then incubated at 35 °C for 5 minutes and 0.1 ml of 0.1 M glucose-l-phosphate was added. The mixture was then incubated at 35°C 10 minutes before terminating the reaction by the addition of 5 ml of 5% TCA. The reaction mixture was centrifuged and the supernatant was used for the determination of inorganic phosphate by the method of Fiske and Subbarow (1925).

The peroxidase (EC 1.11.1.7) was prepared by homogenizing 3 ml of phosphate buffer (0.01 M, pH 6.0) with 500 mg of fresh leaf material, squeezed through two layers of cheese cloth, and centrifuged at 5,000 r.p.m. for 15 minutes at 0.4 °C. The supernatant served as the enzyme source. The assay system contained 6 ml substrate (1 ml of the stock containing 30% H_2O_2 in 100 ml, and was diluted to 100 ml with phosphate buffer, pH 6.0), 0.1 ml enzyme, 0.05 ml of 5% 0-dianisidine in methyl alcohol and distilled water to make the final volume to 7 ml. The reaction was initiated by the addition of enzymes and the colour development was read at 460 nm.

The extraction of ascorbic acid oxidase (EC 1.10.3.3) followed Dawson and Magee (1955), using 5 ml of 0.15 M citrate phosphate buffer (pH 5.7) and one gram of fresh leaf material. The homogenate was centrifuged for 15 minutes at 10,000 r.p.m. The entire operation was carried out at 4 °C. The reaction mixture consisted of 1 ml of the enzyme, 2 ml of 0.15 M citrate phosphate buffer (pH 5.7), 1 ml of ascorbic acid (one mg/ml) and 1 ml of distilled water. The reaction was terminated by the addition of 1 ml of 3% metaphosphoric acid. The ascorbic acid in the aliquots was determined as described by Jayaraman (1981), using 2,4-dinitrophenylhydrazine reagent.

The polyphenol oxidase (EC 1.10.3.1) was prepared by homogenizing 500 mg of fresh leaf material in 3 ml of phosphate buffer (0.05 M, pH 6.7) and centrifuged at 10,000 r.p.m. for 15 minutes at 0°–4 °C. The supernatant served as a crude enzyme extract. The method of enzyme assay is essentially that of Taneja and Sachar (1974), using catechol as substrate.

α-ketoglutaric dehydrogenase (EC 1.2.4.2), pyruvic dehydrogenase (EC 1.2.4.1) and succinic dehydrogenase (EC 1.3.99.1) were extracted with 7 ml of 0.1 M potassium phosphate buffer, pH 7.2 and 250 mg of fresh leaf material and centrifuged at 6,000 r.p.m. for 15 minutes at 0° to 2 °C and the supernatant served as an enzyme source. The assays of enzymes were carried out by the method of Kusunose et al. (1956) with modifications. The system contains in 6 ml (in μ moles): potassium phosphate buffer, pH 7.4, 200 ; Mg^{2+}, 20; KCN,

20; substrate, 150 (sodium salt of succinic acid or pyruvic acid or α-ketoglutaric acid); in addition 0.1 ml of dye (DCPIP, 0.0025% for α-ketoglutaric dehydrogenase; potassium ferricyanide, 0.03% for succinic and pyruvic dehydrogenase) and 1 ml of enzyme extract. The reduction of DCPIP was measured at 600 nm and potassium ferricyanide at 420 nm. The dye reduction was calculated from the standard curve prepared by DCPIP and potassium ferricyanide.

The method employed for the extraction and assay of acid phosphatase (EC 3.1.3.2) is essentially that of Hasson-Porath and Poljakoff-Mayber (1971). The enzyme was extracted with 7 ml of Tris-Maleate buffer (0.1 M, pH 7.0) and one gram of leaf fresh material, and assayed using p-nitrophenyl phosphate as substrate.

The extraction and assay of protease (EC 3.4.2.2) activity followed the Prisco et al. (1975) method. The method followed for the extraction and assays of glutamate oxaloacetate transaminase (GOT) (EC 2.6.1.1) and glutamate pyruvate transaminase (GPT) (EC 2.6.1.2) is essentially that of Harper and Paulsen (1969). The enzymes were extracted with 5 ml of Tris-HCl buffer (pH 7.2) and 500 mg of fresh leaf material and assayed by using 2,4-dinitrophenylhydrazine reagent. The protein content of all the enzyme extracts was determined by the modified Lowry et al. method (Hartree, 1972).

Results and discussion

In our previous paper we have reported that the salt-tolerant CSC 1 accumulated less sodium and chloride in the shoot system than the salt-sensitive TKM 4, in response to sodium chloride salinity (Krishnamurthy et al., 1987a). The salt-tolerant CSC 1 also showed high levels of total soluble and reducing sugars, less reduction in starch content (Krishnamurthy et al., 1986) and low promotion of amylase (Krishnamurthy et al., 1987b). Lower promotion of starch phosphorylase and more pronounced increase of invertase activities in the leaf of CSC 1 than in the salt-sensitive TKM 4, under saline condition when compared to their respective controls, were observed (Table 1). Rathert (1985) reported that the salt-tolerant cultivars of soybean accumulated higher levels of soluble sugars and highly stimulated activities of invertase and less stimulated activity of starch phosphorylase than did the salt-sensitive cultivars to NaCl salinity. These findings are in agreement with the present results. The markedly low increase of phosphorylase activity, particularly in stressed leaves of CSC 1, correlates in a way with the lesser reduction of starch content, which was reported in our earlier paper (Krishnamurthy et al., 1986). However, since the role of phosphorylase in starch metabolism is uncertain (Fekete and Viewed, 1973) and no clear results were obtained in previous experiments (Rathert, 1983; Rathert and Doering, 1983), the interpretation of NaCl effects on this enzyme in respect to tolerance mechanisms of the two investigated rice cultivars remains speculative. Salinity lowered the photosynthetic productivity by inhibiting the activity ribulose – 1,5-biphosphate carboxylase in crop plants (Seemann and Sharkey, 1986; Plant et al., 1989).

In addition, osmotic adjustments via accumulation of sugars to a certain extent in the leaves of tolerant CSC 1 seem to be typical for extremely tolerant plants, such as barley (Gauch and Eaton, 1942). The correlation between NACl-induced accumulation of organic acids (Krishnamurthy et al., 1987a), polyamines (Krishnamurthy and Bhagwat, 1989) and proline (Krishnamurthy, 1987d) and salt tolerance have been reported on the same CSC 1 and TKM4. How the osmoregulation mechanisms and variations in the enzymic adaptation of the two different rice cultivars differ is not known, but differences are to be expected.

Table 1

Effect of NaCl on the activity of different enzymes from the third leaf of rice cultivars six weeks after initial salinization

Enzymes	Cultivars	
	CSC 1	TKM 4
	Control	Control
Invertase (EC 3.2.1.26) (μ moles of reducing sugar produced hour/mg protein)	3.6±0.60 (+142)	17.4±0.40 (+128)
Phosphorylase (EC 2.4.1.1.) (μ moles of phosphate released/ 10 minutes/mg protein)	4.58±0.58 (+58)	7.06±0.56 (+141)
Peroxidase (EC 1.11.1.7) (Units increased/minute/mg protein)	83.3±3.6 (+44)	25.0±3.4 (+244)
Ascorbic acid oxidase (EC 1.10.3.3) (μ moles of ascorbic acid oxidised/hour/g fresh weight)	57.0±2.2 (+242)	77.0±4.1 (+71)
Polyphenolase (EC 1.10.3.1) (Units increased/minute/mg protein)	16.0±2.3 (+138)	12.0±2.4 (+67)
α-Ketoglutaric dehydrogenase (EC 1.2.4.2) (μ moles of DCPIP)	0.42±0.06 (+57)	1.07±0.13 (+150)
Succinic dehydrogenase (EC 1.3.99.1) (μ moles of $K_3Fe(CN)_6$ reduced minute/mg protein)	21.9±1.4 (+34)	43.2±1.7 (+100)
Pyruvic dehydrogenase (1.2.4.1) (μ moles of $K_3Fe(CN)_6$ reduced/minute/mg protein)	21.9±1.4 (+34)	43.±1.7 (+100)
Acid phosphate (EC 3.1.3.2) (μ moles of p-nitrophenol released 30 minutes/mg protein)	3.6±0.70 (+22)	2.9±0.70 (+141)
Protease (EC 3.4.2.2) (μ moles of glycine released/30 minutes/mg protein)	3.2±0.30 (+31)	6.6±0.70 (+132)
Glutamate oxaloacetate transaminase (EC 2.6.1.1) (n moles of oxaloacetate formed/30 minutes/mg protein)	1346±40 (+140)	2293±87 (–35)
Glutamate pyruvate transaminase (EC 2.6.1.2) (n moles of pyruvate formed/30 minutes/mg protein)	568±22 (+90)	688±63 (–26)

Each value is the mean ± SD for three replicates each containing three plants separately; figures in parenthesis indicate saline treated values expressed as percentage of changes over the control; + : increase; – : decrease.

There was a considerable variation in the levels of ascorbic acid oxidase and polyphenolase activities, as they showed a tremendous increase in salt-tolerant CSC 1 over that of the salt-sensitives TKM 4, when compared to their respective controls. The peroxidase activity was increased in both cultivars and this enzyme did not show any variation between salt tolerance and salt sensitivity under saline, over the control conditions (Table 1). The increase in the levels of ascorbic acid oxidase, polyphenolase and peroxidase is similar to the earlier observations in maize, lucerne and cotton exposed to Na_2SO_4 and NaCl salinity (Azizbekova, 1964). We have reported that CSC 1 exhibited less reduction of shoot growth than did the TKM 4 under saline conditions (Krishnamurthy et al., 1987e). These results show that salinity of the growth medium adversely influences the growth of shoot and also the activity of enzyme peroxidase,

polyphenolase and ascorbic acid oxidase. It is inferred that the changes of these three enzyme levels could be related to reduced growth, as observed earlier (Nieman, 1965).

The NaCl treatment promoted the enzymes of tricarboxylic acid cycle in varying degrees in the salt-tolerant and salt-sensitive cultivars. Salt-tolerant CSC 1 had low increased activities of α-ketoglutaric dehydrogenase, succinic dehydrogenase and pyruvic dehydrogenase, whereas it was highly increased in salt-sensitive TKM 4 under saline treatment, when compared to the control (Table 1). The stimulation of dehydrogenase indicated stimulation of the tricarboxylic acid cycle, and thereby respiration to salinity. The salinity treatment stimulated the activities of α-ketoglutaric dehydrogenase, succinic dehydrogenase and pyruvic dehydrogenase in barley, sunflower and tomato (Zhykovskaya and Lyakhova, 1969). Also, a direct correlation was noticed between salt tolerance and total dehydrogenase in pea varieties (Gupta and Parmil Kaur, 1970). Since the physiological basis of reactions on the carbohydrate metabolism in respect to salt tolerance is not known in details, and interference of other physiological process under these stress conditions is likely i.e. respiration (Nieman, 1965), the interpretation of the metabolic interactions with consideration of reduced growth of the plants remains speculative. The consensus in the literature suggests it highly improbable that there would be enzymic adaptation to function at high ionic strengths in the salt-tolerant CSC 1.

The salt-tolerant CSC 1 had increased levels of total protein content (Krishnamurthy et al., 1988) and low increased activities of protease and acid phosphatase, while salt-sensitive cultivars showed decreased levels of total protein and highly increased activities of protease and acid phosphatase in their leaves, when compared to the control (Table 1). The protein breakdown and turnover was delayed by sodium chloride treatment in *Vigna sinensis*. Salinity decreased the protein synthesis and increased its hydrolysis, as was observed in grape leaves (Saakyan and Petrosyan, 1964). The nitrate reductase activity was stimulated under saline conditions in rice (Krishnamurthy et al., 1987b). The acid phosphatase activity was found to increase with salinity in wheat (El-Fouly, 1972) and barley (Dzhanibekova, 1972). However, there is no detailed studies on salt tolerance of cultivated crop species with protease and acid phosphatase enzyme activity. Therefore, the interpretation of these enzymes are rather important and worth trying for salt tolerance studies. ATPase and pyrophosphatase activities are generally found to be stimulated in glycophytes under saline conditions (Randall and Sze, 1989; Leach et al., 1990). It is to be noted from the present results that the glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activities were significantly increased in salt-tolerant CSC 1, whereas it decreased in the salt-sensitive TKM 4, in their leaves under NaCl treatment.

Therefore, it is evident that the relative salt tolerance of CSC 1 is characterised by exhibiting high levels of sugars and total protein, less reduction of starch content, less stimulated activities of starch phosphorylase, α-ketoglutaric dehydrogenase, succinic dehydrogenase, pyruvid dehydrogenase, acid phosphatase and protease, and highly stimulated activities of invertase, ascorbic acid oxidase, polyphenolase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase than in the salt-sensitive TKM 4, in their leaves under saline conditions. The variation in the levels of different enzymes involving various metabolisms could be speculative metabolic tolerance mechanisms and

could act as possible indicators for salt tolerance of rice cultivars under saline conditions. Even though the described metabolic interactions are not certain in all facts, they indicate possible mechanisms of metabolic stress resistance, besides our reported adaptations of the metabolic machinery of rice cultivars.

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IN VITRO REGENERATION AND PROPAGATION OF *PLATYCERIUM BIFURCATUM*

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The culture was initiated from pieces of leaf, the initiatory culture media contained 0.1-1.0 mgl^{-1} kinetin and naphthyl acetic acid. The culture medium was at the same time suitable for propagation, on the surface of the pieces of leaf in contact with the culture medium GGB (green globular body) differentiation was observed in 12 weeks. The best result was obtained with the culture medium containing 0.1 mgl^{-1} kinetin and naphthyl acetic acid. On a culture medium containing or not containing active carbon without hormones the plants regenerated from the GGBs, and in half a year reached the size suitable for rooting. On a culture medium containing active carbon and 2 mgl^{-1} naphthyl acetic acid rooting took place for 8 weeks. On the surface of the GGBs first meristems and then shoot primordia differentiated. The leaves were first one-cell row thick, then gradually reached the multicell-row form of leaf. The visible juvenile leaves were egg-shaped.

Keywords: *Platycerium bifurcatum,* micropropagation, GGB (green globular body) kinetin, plant regeneration

Introduction

The Platycerium bifurcatum is an epiphytic fern species originating from tropical Australia. With two kinds of leaf type (heterofilia) its appearance is particularly exotic. The shape of the assimilating leaf resembles a buck's-horn, the spores produced at the end of the leaf segments in a brown continuous layer increase the resemblance. With the round covering leaves the plant clings to the trees of the tropical forest. At the beginning these leaves are green, then wither and turn brown; new larger leaves spread over them while the necrotized leaves inside form humus.

Owing to its peculiar appearance the fern is an ornamental plant much liked all over the world, traditionally propagated by spores or offsets. These two methods of propagation are slow, because the plant develops few offsets, while raising marketable plants from spores takes 2 years (Nagy, 1986):

The micropropagation of ferns began in the seventies (Knauss, 1976) and its primary aim was to get rid of the pathogens. Varieties of *Nephrolepis exaltata* were grown in the largest quantities and even the technology of micropropagation was first elaborated for these plants (Loescher and Albrecht, 1979).

By the early nineties *in vitro* propagation of 16 various fern species was already carried on, and a number of complete propagation techniques too were elaborated (Sagawa and Kunisaki, 1990). The micropropagation of Nephrolepis species and varieties was made easy by the fact that from the tip of the stolon the plant was relatively easily initiated and regenerated. With the other fern species shoot and rhizome tips had to be used as explant, and they are more difficult to sterilize.

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In the course of experiments carried out with *Nephrolepis cordifolia* Higuchi et al. (1987) found that starting from the tip of stolon on a culture medium free from auxin but containing 0.5 mgl BA GGBs (green globular bodies) formed, from which on a hormone-free culture medium a multitude of plantlets regenerated. The experiments were later continued by Amaki and Higuchi (1990) studying the GGB formation of 5 fern species. The experiments were started from rhizome; on Murashige and Skoog (1962) (MS) culture medium GGB differentiation was observed in each species. With the GGBs cut into pieces and placed into a hormone-free culture medium the plants regenerated.

On the micropropagation of *Platycerium* only a few literary data were found which contained little and deficient information.

The *in vitro* propagation of *Platycerium stemaria* was initiated from rhizome tip by Hennen and Sheehan (1978). After removing the hairs and sterilizing, they placed the tips on a MS-based culture medium supplemented with 80 mgl^{-1} adenine sulphate and 15 mgl^{-1} indolyl acetic acid (IAA). Two months later, primordia differentiated from the explants shoot from which plantlets developed, and these were rooted.

Thentz and Moncousin (1984) initiated the *in vitro* propagation of *P. bifurcatum* from spore. They obtained the largest number of plantlets on 1/5 MS basic culture medium using 0.1 mgl^{-1} IAA and 0.5 mgl^{-1} benzyl adenine (BA).

Our work was aimed at elaborating the technology of *P. bifurcatum* micropropagation, whereby to produce a large amount of pathogen-free propagation material within a time shorter than when sowing spores.

Another purpose of our work was to follow by electron microscope the differentiation of GGBs developing in the course of propagation, and the regeneration of plants from them.

Materials and methods

In our experiments we used juvenile leaves of sterile plantlets as the initial object. We cut the leaflets into 0.5 cm^2 pieces and set them onto the inductional propagation culture medium, respectively, with their cutting surface downward.

The composition of the culture media used for culturing is shown in Table 1. As basic culture medium for the propagation and elongation the macroelements of the MS culture medium were used at half concentration, and the microelements and vitamines at full concentration. For rooting the Jámbor-Benczúr and Márta-Riffer (1990) macroelements (BM) and Heller (1953) microelements (HE), MS vitamines were used. The propagation was carried out with the combination of kinetin (KIN) and naphthyl acetic acid (NAA).

The elongation was carried out on the culture medium free from growth regulators, with active carbon added, and without. For rooting NAA and AC were used. To each culture medium the same quantity – 20 gl^{-1} – saccharose was added. The culture medium was sterilized in autoclave at 120 °C, under 10^5 Pa overpressure for 35 minutes.

Culturing was carried out in 100 ml Erlenmeyer flask with 25 ml culture medium poured to the base. The flasks were closed with gas permeable adhesive plastic film.

The cultures were kept at 16/8-hour photoperiod, 30 $\mu M.m^{-2}.s^{-1}$ illumination, at an average temperature of 25 °C.

The experiments were set up in 4 replications with 8 pieces of leaf or GGB each. The following data were recorded: length and width of GGB colonies (in 12 weeks), diameter of runner colonies (mm), number (n) and length (mm) of leaves, number (n) and length (mm) of roots. The data were evaluated by variance analysis (one-factor, random block design method).

Table 1

Composition of culture media used for the propagation, elongation and rooting of Platycerium bifurcatum

Culture medium	Macro-elements	Micro-elements	Supplements KIN mgl^{-1}	NAA mgl^{-1}	AC gl^{-1}	Agar-agar gl^{-1}
P1	1/2 MS	MS	0.1	0.1	–	7
P2	1/2 MS	MS	0.1	1.0	–	7
P3	1/2 MS	MS	1.0	0.1	–	7
P4	1/2 MS	MS	1.0	1.0	–	7
P5	1/2 MS	MS	–	–	–	8
P6	1/2 MS	MS	–	–	2	8
P7	BM	HE	–	2.0	2	8
P8	BM	HE	–	4.0	2	8

For the electron microscope examinations, the samples were taken from the GGBs, then from the differentiated cultures.

The samples were first fixed, then, after the fixative was washed out, dehydrated; then dried and coated with an Au layer of about 30 nm. The samples were examined with a BS 300 type scanning electron microscope, and photographed.

Results

Twelve weeks after the experiments had been set up GGB colonies developed on the surfaces of the pieces of leaf in contact with the culture medium. The length and width of the colonies varied with the culture medium used (Fig. 1). The largest GGBs were found to develop on the P1 medium, the result was significantly better than with the other 3 media.

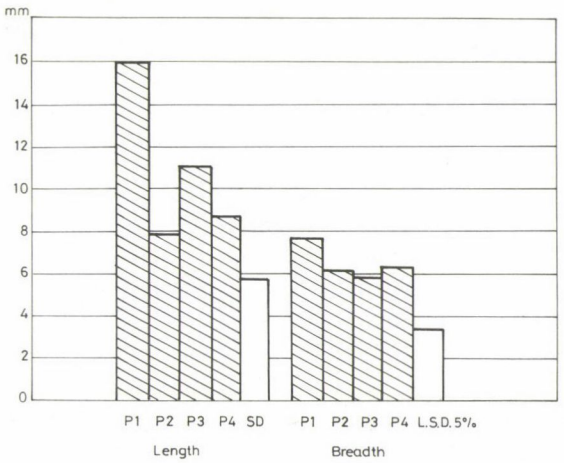

Fig. 1. Trend of length and breadth of GGB colonies differentiating on the leaves in the course of the micropropagation of *P. bifurcatum* on P1, P2, P3 and P4 culture media following 12 weeks of incubation

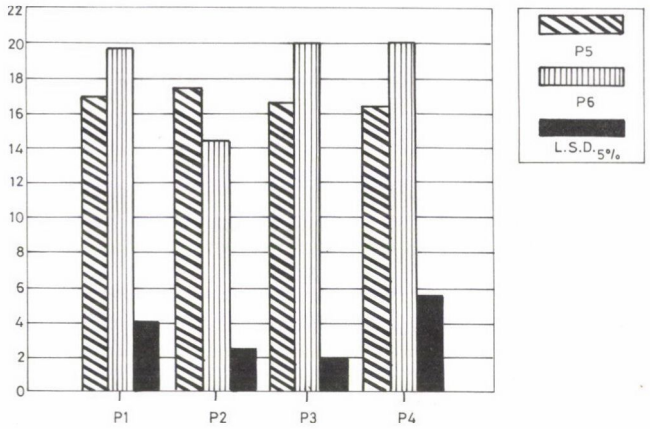

Fig. 2. Trend of the diameter of runner bunches at the end of the elongation phase on P5 and P6 media

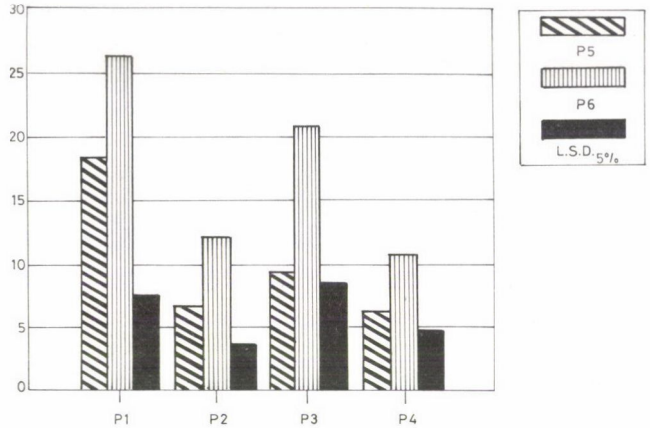

Fig. 3. Leaf number of runner bunches in the end of the elongation phase on P5 and P6 media

After the evaluation the GGB colonies – about 2–3 mm large by then – were separated from one another and placed onto elongation culture media, but the marks of the propagative culture media were kept in order to be able to study the after-effect of the growth regulators. On the two kinds of elongation medium – with repeated passages – the regenerated plantlets reached the size required for rooting in 6 months.

The diameter of the runner colonies developing from the GGBs was not greatly influenced by the hormone concentration of the propagative culture media (Fig. 2). A comparison of the two elongation media, on the other hand, shows better results in 3 cases on the P6 medium containing AC, in the case of plantlets propagated on the P3 medium significant difference was even pointed out. An examination of the number of leaves gives more conspicuous differences (Fig. 3). The largest number of leaves formed on the propagative culture media P1 and P3; the best result – 26.7 leaves – was given by

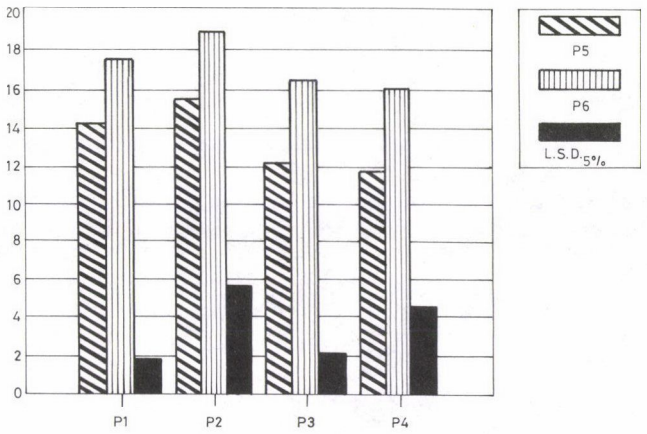

Fig. 4. Leaf lengths of runner bunches in the end of the elongation phase on P5 and P6 media

the plantlets originating from the P1 and raised further on the P6 culture medium. The result of the P4 medium was the worst. Further, out of the elongation culture media the AC-containing P6 medium gave significantly better result in every case. Figure 4 shows the data of leaf length. The longer leaves developed in the case of runner bunches originating from the P1 and P2 propagative culture media. As regards the length the medium containing AC proved again better – in two cases even significantly better.

Rooting took place for 8 weeks. The number of differentiated roots was hardly different in the two culture media, they were 7.3 and 8.2, respectively. The roots were 11.5 and 13.8 mm long on an average, the difference was not significant. Thus, the two rooting media did not essentially differ in effect, though the one containing less – 2 mgl^{-1} – NAA was by all means more suitable for rooting.

Figure 5 is intended to show the surface of GGB and the fully developed shoot apex. In part "A" on the surface of GGB 5 meristem tips of different size (1, 2, 3, 4, 5) can be observed with differentiating leaf primordia, which at the beginning are one-cell row thick. The long hairs covering the surface of GGB (6, 7, 8) can be easily distinguished from the leaf primordia.

The one-cell row leaves are incurved, characteristically of ferns, and bend over the meristem tip. In the course of subsequent development they differentiate into leaf-blade.

In part "B" (1, 2, 3, 4, 5) the fully developed shoot tip of *P. bifurcatum* can be seen with embowering leaves which totally cover the meristem and possess well-developed leaf-blade, though their tips are still one-cell row thick.

Discussion

Thentz and Moncousin (1984) used 0.1 mgl^{-1} IAA and 0.5 mgl^{-1} BA for the micropropagation of Platycerium. We solved the propagation with KIN, using the data of other ferns. In our experiments the lowest concentration – 0.1 mgl^{-1} KIN + 0.1 mgl^{-1} NAA – proved the best for the propagation.

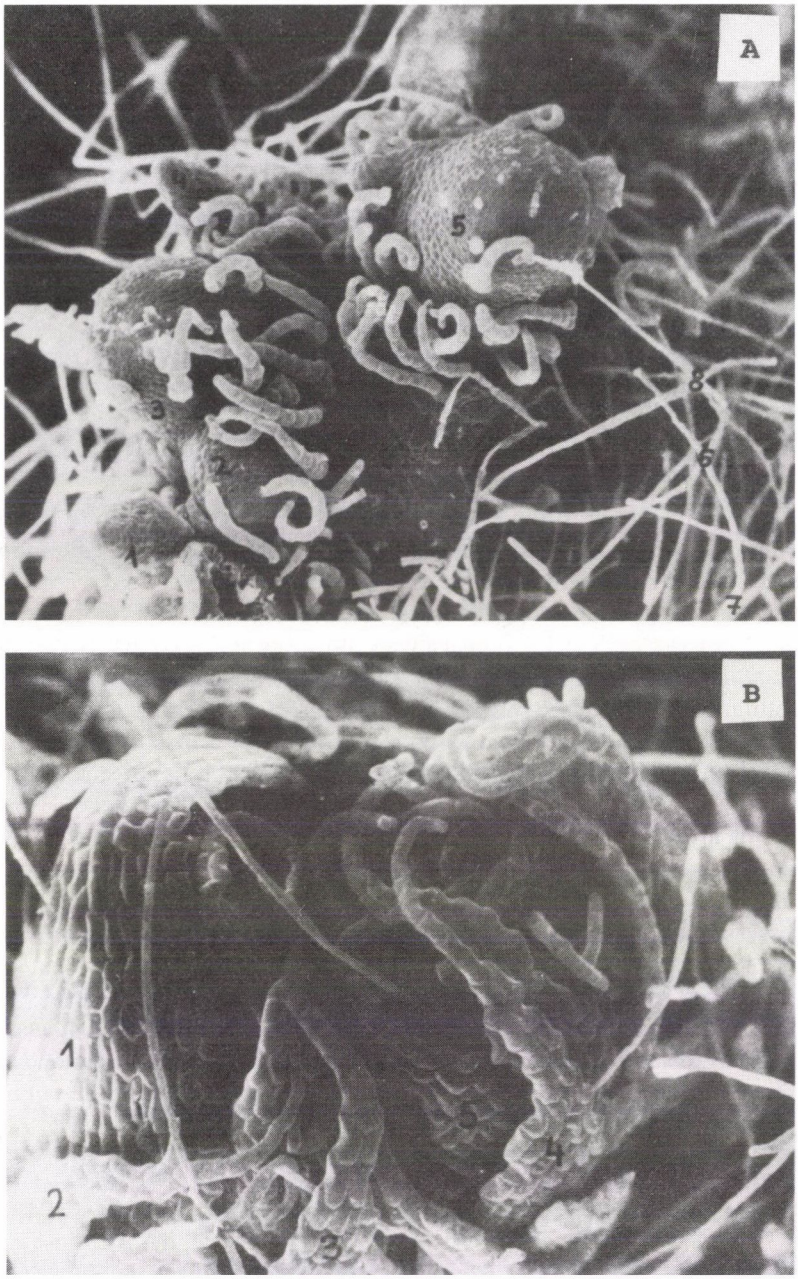

Fig. 5. Part "A": GGB surface of P. bifurcatum with shoot growth tips (1, 2, 3, 4, 5) and differentiating leaf primordia easy to distinguish from the long hairs (6, 7, 8) covering the surface of GGBs. On P1 culture medium (1/2 MS + 0.1 mgl^{-1} KIN + 0.1 mgl^{-1} NAA) after 10 weeks of incubation (SEM × 60)
Part "B": Shoot tip of P. bifurcatum with embowing well-developed leaf primordia (1, 2, 3, 4, 5) possessing leaf-blade, on P6 culture medium (1/2 MS + 2 g/l AC), following 4 weeks of incubation (SEM × 85)

As inoculum juvenile leaf pieces were used, their use in the case of Platycerium has not been reported yet. We found that along the cutting surface of the leaves groups of GGB developed in 12 weeks, in the same way as in the case of 5 fern species in the experiments of Amaki and Higuchi (1990) who started from rhizome. The regeneration of the plantlets from GGBs was carried out on hormone free 1/2 MS culture medium, in the same way as in the case of Nephrolepis, Asplenium and Pteris used by the above authors. To shorten the regeneration period we added 2 gl^{-1} active carbon to the culture medium. From the GGBs plantlets fit for rooting developed in 6 months, on the culture medium containing AC the regeneration proved better. In the course of regeneration the worst result was obtained with plantlets originating from the culture medium containing the largest amount of KIN and NAA. The long period of regeneration indicates the increased sensitivity of ferns to hormones. On a culture medium containing AC rooting took place for 8 weeks. Here too, the medium containing less – 2 mgl^{-1} – NAA proved better. We found that, with ferns, the use of AC was important for both elongation (regeneration) and rooting.

The regeneration of plants from GGBs was studied by electron microscope as well. The surface of GGBs is similar to what was described by Amaki and Higuchi (1990). At the beginning we also observed the differentiation of a great number of shoot meristems on the surface of GGB. This was followed by the leaf differentiation, first with a single cell-row, then the leaves gradually broadened into leaf-blades to assume finally the usual multi-cell layer structure.

In the course of our experiments we found that the micropropagation of *P. bifurcatum* may start from leaves as well. In the propagation phase – which here involves the differentiation of GGBs – the use of small KIN and NAA quantities is advisable. Elongation (regeneration) is very long – it takes half a year. By the end of the elongation phase the plantlets develop ovate juvenile leaves unlike the leaves characteristic of the species. Rooting took place in 8 weeks. Propagation continued – though to a low extent – even in the period of root development.

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SIMULTANEOUS FLOWERING OF PEAR VARIETIES AND OVERLAP OF FLOWERING CURVES IN DIFFERENT VARIETY COMBINATIONS

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Flowering phenology of 14 pear varieties was observed through 7 years (1984–1990) in the region of Nagykanizsa where the subalpine climate is ideal for pear growing.

The rate of simultaneous flowering, and overlap of flowering curves was evaluated based on 182 variety combinations.

According to our results combinations were ranked into three groups:

1. tight overlap: overlap of flowering curves is usually more than 70% and not lower than 50% in any years;

2. rate of overlap is inadequate; less than 50% in most of the years;

3. unstable overlap: in most of the years the overlap is more than 70%, but sometimes lower than 50%.

Using a pollinizer, only varieties of the first group assure a safe overlap. The rate of overlap of pollinizers can be higher among several (2–3) pollinizer varieties. When 2 pollinizers are used it is advisable to choose one variety flowering 2–3 days earlier and another variety flowering 1–2 days later than the variety to be pollinized. With this method, 90–100% overlap is provided for the variety to be pollinized.

According to our results, pairs of varieties with the high rate of overlap may be well-determined, and assure the optimal pollinizer for a certain variety.

Keywords: pear, *Pyrus communis* L., cultivars, characterization of flowering, simultaneous flowering

Introduction

The pear variety assortment of main producing countries and of Hungary is based on traditional varieties. Growing and market value of new pear varieties produced by improvement are continuously examined. The optimal pollinizer varieties are chosen during the blossom and fertilization examinations.

From the literature (Nyéki and Soltész, 1995) we came to the conclusion that blossom period observations and fertilization data observed in other countries are not valid (adaptable) for Hungary. In order to find optimal pollinizer varieties one must examine both the blossom date and the simultaneous flowering rate of pear varieties for several years, in Hungary.

A numerical approach to the characterization of the flowering process was first reported by Máthé (1977, 1977a, 1979) on the example of herbaceous medicinal species, e.g. *Adonis vernalis* L., The Index of Flowering (*Index-V*) proved to be useful not only in detecting differences in the flowering process of *Matricaria chamomilla* L. but also in establishing correlation between flowering and the essential oil content of camomile

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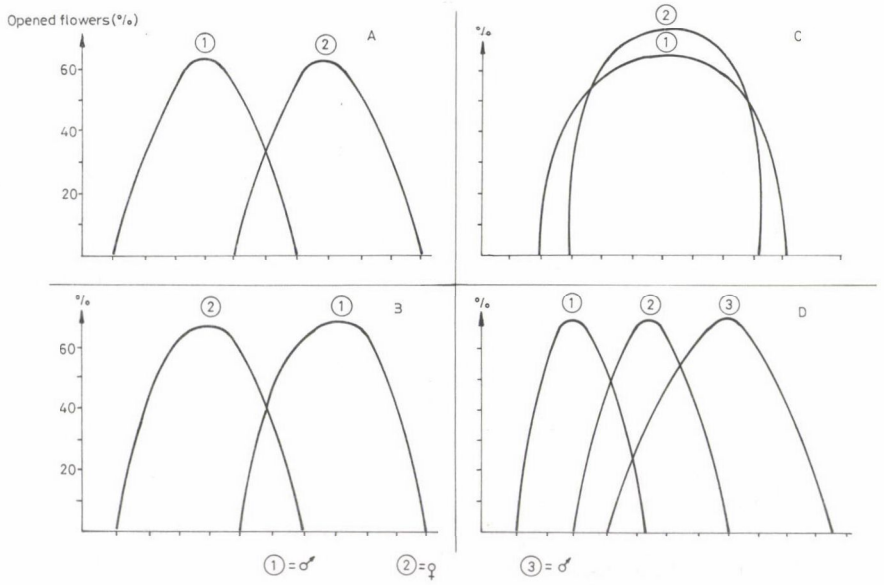

Fig. 1. Types of the overlap of flowering curves (Nyéki, 1989)

(Máthé et al., 1985, Franz et al., 1985). Under controlled environmental conditons, the Index of Flowering rendered possible the comparison of paprika cultivars grown in a phytotron (Máthé and Bahadli, 1989).

The method worked out by Nyéki (1989) was used for choosing simultaneously flowering varieties based on the overlap of flowering curves.

Materials and methods

Studies were carried out in the virus free 14 variety model orchard of the Nagykanizsa State Farm between 1984 and 1990. The orchard was established in 1979 on pear seedling rootstock and trained to natural spindle. Data were collected from 12 trees.

There are different methods to determine the bloom date.

The best-known method is the estimation of the beginning, main and end of bloom. Herbst and Rudloff (1939) and Weger et al. (1940)'s method gives an exact representation from the blossom course when the number and rate of flowers in different stages (anthesis, fully opened and petal fall) are determined each day during bloom.

Daily and hourly observation of sexual organs on numbered flowers (Nyéki, 1973) enable us to follow up the blossom course exactly.

During the bloom, 4 phenological stage were discerned: pink bud, beginning of bloom, blossomed, over blossomed.

The rate of flowers in different stages was represented on flowering phenogram and the overlap of flowering curves was determined according to Nyéki (1989). The possible overlap types of the flowering dates are shown in Fig. 1. According to "A" type figures the pollinizer variety blooms earlier, and the "B" type figure show that the pollinizer variety blooms later than the variety to be pollinized. Blossom dates do not overlap at all. These varieties cannot be planted together. The overlap of "C" type blossom date is desirable. In this case the simultaneous flowering and pollination is optimal between the two varieties. In the case of "D" type, safe pollination is assured by two pollinizer varieties (one early and one late bloomer) with decent blossom date overlap.

Results

Simultaneous flowering of varieties

The most important utilization territory of the blossom phenological observations is determination and choosing of the simultaneously flowering varieties. In planning orchards, the most important objective – mainly in case of self-steril varieties – is to assure continuous pollen during the blossom time in the orchard.

In order to reach this aim, the simultaneous flowering of varieties has to overlap in the highest degree. The main condition of cross pollination is the simultaneous flowering. The simultaneous flowering of two varieties is extreme, if their main blossom periods completely overlap and their blossom durations cover each other. At the beginning and the end of bloom relatively few flowers open so this period is less valuable than the main flowering from the standpoint of pollen supply and pollination. If there is a high temperature during the blossom (above 25 °C), all varieties (except late and very late bloomers) blossom closely or almost in the same time. In some years, when the beginning of blossom is warm but it is followed by a cool, rainy period, the blossom period of varieties is very long. In these years the early bloomers may get over the effective pollenization period before they could have been pollinated by others.

When bloom of pollinizer and pollinated variety does not overlap, a great crop loss may occur. This disadvantage cannot be compensated for by deploying bees. Safety of pollination is possible to increase if the self-sterile variety is combined with two pollinizer varieties. In this case it is practical, if one pollinizer blossom 2–3 days earlier and the other 1–2 days later than the self-steril variety, because the pollens of flowers blossomed during the beginning and end of blossom period are inferior biologically.

Groups of blossom period

Pear varieties are ranged in 3 (early, medium, later) or 4 (very early, medium, medium late, late) groups according to the beginning of blossom (1–5% of the flowers opened) and the date of main blossom (the rate of opened flowers are above 50%) occurrence. An abstract of special literature was made from the period of pear varieties blossom by Brózik and Nyéki (1971). Because these pear varieties are self-sterile, they need simultaneously flowering pollenizers which can fertilize each other reciprocally well. When choosing pollenizers it varieties of the same or adjacent blossom period should be considered.

Brózik and Nyéki (1971) demonstrated that in the long blossom period years mostly those varieties could assure the right overlap – with our blossom period – which were included in the same blossom group. In the medium long blossom period years the varieties included in the neighbour group can assure the overlap. In the short, fast blossom period years the varieties belonging to any groups can assure the right overlap and fertilization.

Based on 21 years of observation, the pear varieties were ranged into the above mentioned 4 groups of blossom period. 12.5% of the pear varieties' belong to the very early, 43.8% to the medium early, 37.5% to the medium late and 6.2% to the very late group.

Table 1

Simultaneous flowering rate of pear varieties
(Nagykanizsa, 1984–1990)

Variety combinations	Overlap of the territory under the flowering curves (%)							
	1984	1985	1986	1987	1988	1989	1990	average
1. Unsatisfactory overlap								
Beurre d' Anjou x Beurre Bosc	11	41	70	55	11	66	21	39
Beurre Boscx Beurre d'Anjou	36	72	74	48	15	79	28	50
2. Tight overlap								
Williams B. 13× Packham's Triumph	75	59	100	94	88	61	91	81
Packham's Triumph × Williams B.13	81	51	84	52	94	74	96	76
Williams × Conference	59	52	92	50	50	99	21	60
Conference × Williams	41	100	92	91	90	72	28	73
Williams B.13 × Beurre Durondeau	91	70	93	98	78	86	100	88
Beurre Durondeau × Williams B.13	40	59	66	59	88	90	66	67
Williams Bon Chretien × General Leclerc	–	64	93	100	100	75	17	75
General Leclerc × Williams Bon Chretien	–	73	72	84	79	63	15	64
3. Temporary unstable overlap								
Passe Crassane × Williams Bon Chretien	46	70	97	86	94	95	72	80
Williams Bon Chretien × Passe Crassane	77	78	83	93	91	89	83	85
Clapp Favorite × Beurre Bosc Typ B.	70	60	70	93	28	97	17	62
Beurre Bosc Typ B. × Clapp Favorite	70	77	99	79	46	80	13	66
Beurre Bosc × Doyenné du Comice	–	–	67	51	86	59	78	68
Doyenné du Comice × Beurre Bosc	–	–	69	57	82	90	75	75

The overlap of flowering curves in different variety combinations

In this study the overlap of flowering curves of the examined 14 pear varieties was determined (Table 1). The simultaneous flowering of varieties was considered unsatisfactory if the overlap of territory under the curves was under 50% in the majority of years. The simultaneous flowering of varieties was evaluated to be unstable if the simultaneous flowering was tight in most years but it had a very low measurement in some years.

Beurre d'Anjou and Beurre Bosc demonstrated very low simultaneous flowering (Fig. 2). The overlap of territory under the flowering curves was greater than 50% in 3 years from the examined 4 years.

The simultaneous flowering of two varieties is demonstrated in Fig. 3. The blossom of Williams B.13 and Packham's Triumph coincided in a large measure in every year, the beginning of blossom and main blossom period were in the same time in the majority of years.

Williams Bon Chretien and the General Leclerc are good examples of the unstable simultaneous flowering (not being safe in every year). On 5 occasions out of 6 observed years they blossomed close together, but 1990 the overlap of territory under the blossom curves was very low (17 and 15%). When selecting pollinizers, data of the worst year has to be considered. Safety of simultaneous flowering and pollination in variety combination may increase using more pollinizer varieties.

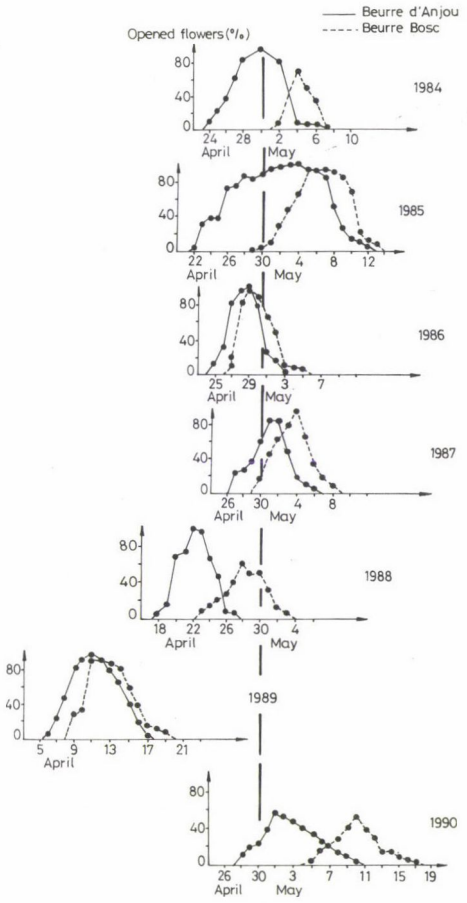

Fig. 2. Overlap of flowering curves of Beurre d'Anjou and Beurre Bosc pear varieties

Table 2

Simultaneous flowering rate of pollenizers blooming at different times
(Nagykanizsa, 1984–1990)

Variety combinations		Overlap of flowering curves (%)								
		1984	1985	1986	1987	1988	1989	1990	average	min–max.
Williams' Bon Chretien	Beurre d' Anjou.	85	100	100	89	96	100	89	94	85–100
	Beurre Bosc	42	48	67	44	17	81	18	45	17–81
Williams	Beurre Bosc Typ B.	33	52	71	56	25	80	17	48	17–80
	Packham's Triumph	84	84	97	96	70	75	80	84	70–97
Williams B.13	Beurre Bosc	36	86	79	85	18	83	20	58	18–86
	Passe Crassane	88	45	85	90	83	86	86	80	45–90
Beurre Bosc	Passe Crassane	–	–	50	47	28	50	49	45	28–50
	Doyenné du Comice	–	–	68	50	92	56	88	71	50–92

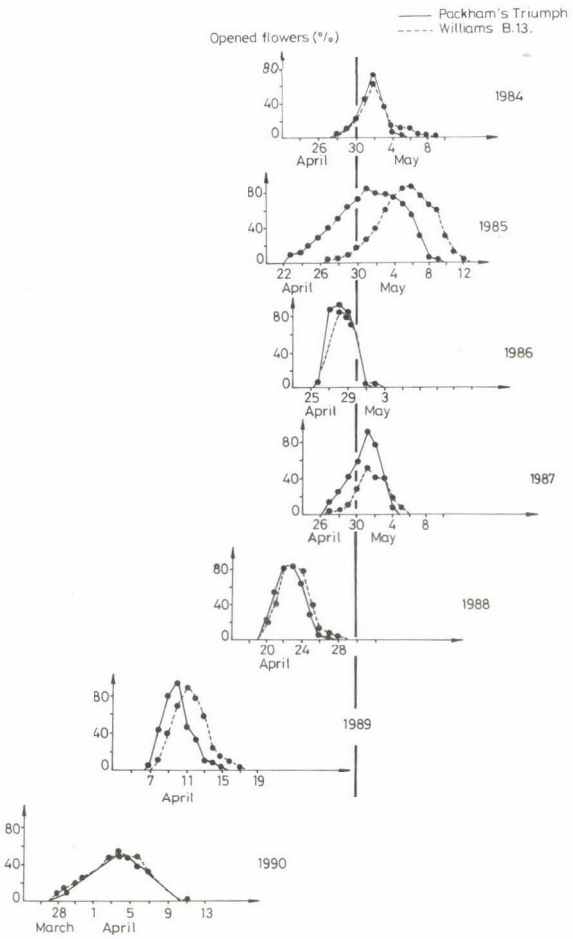

Fig. 3. Overlap of flowering curves of Williams B.13 and Packham's Triumph pear varieties

In this case we consider important that the pollenizer varieties must coincide in the full length of principal variety blossom period. Table 2 shows the simultaneous flowering levels of 4 receptor and 2-2 pollenizer varieties. Pollenizers blossoming earlier and later than the principal variety can completely cover the blossom period of the principal variety. Figure 4 demonstrates that the blossom period overlap of the principal variety may be assured completely using two pollenizer varieties (Passe Crassane, Beurre Bosc). Passe Crassane blossomed very tightly with the examined Williams B.13 variety on the 6 occasions from the observed 7 years. Three times from the 7 years the Beurre Bosc blossomed very late so the level of simultaneous flowering was not acceptable.

Only the Passe Crassane was acceptable from the observed two pollinizer varieties for the Williams B.13 variety, because their simultaneous flowering measurement was high.

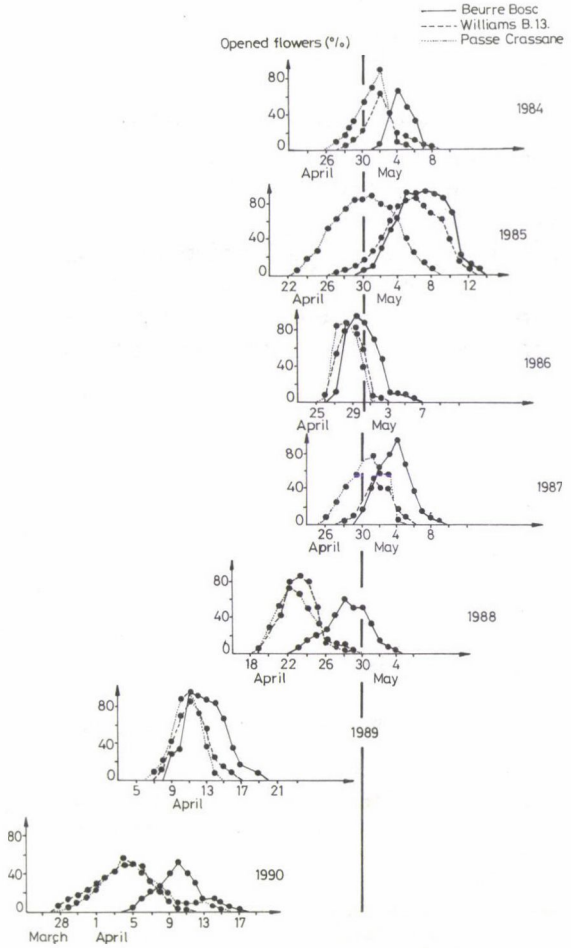

Fig. 4. Overlap of flowering curves of Beurre Bosc, William B.13 and Passe Crassane pear varieties

Discussion

Nyéki (1989) determined that simultaneous flowering measurement of self-sterile stone fruits must be higher than 70% in order to assure the safe pollination with optimal overlap of varieties blossom. Over 70% simultaneous flowering level was assured by the varieties including in the same blossom periods if the varieties had been ranged into the groups of blossom period.

The simultaneous flowering of two varieties is unsatisfactory if its rate is under 50%. These variety combinations of stone fruits may not be planted together.

The simultaneous flowering level which provides acceptable pollen supply and safe pollination is reachable only with the common plantation of several (2–4) pollinizer varieties.

In variety combinations one must consider the measurement of simultaneous flowering of varieties (at the number, rate and placing determination of the pollinizer variety).

The overlap of pear varieties' blossom period was evaluated on the basis of simultaneous flowering which was illustrated on phenograms. The simultaneous flowering measurement may be: (A) tight – above 70%; (B) unsatisfactory (rate of overlap is not adequate) – under 50%; (C) unstable overlap.

According to our results we came to the conclusion that, despite the determined variety combinations, the overlap of varieties blossom period is very unstable. The safe pollination of pear varieties may increase with two (or three) pollinizer which blossom at different times (earlier, in the same time and later than the pollinated variety).

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Plant cultivation

RESULTS OF TEN-YEAR N AND P NUTRITION OF IRRIGATED POOR ALKALI NATIVE *FESTUCA-PSEDOVINA*

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NP treatments of an irrigated *Festuca pseudovina* type of grass growing in meadow solonetz soil ensured 32–75% yield surplus on the average of 10 years: with 43 kg/ha N 32–47%, with 87 kg/ha N 47–56% and with 130 kg/ha N 63–75%. N applied in itself gave 14–31%, P 13–20% surplus; the highest rates of fertilizer caused yield depression. According to the regression calculations, the N fertilizer determined the yield to a proportion of 52%, the P fertilizer to 48%. In the N 130 treatment the grass attained the level of the 10 year average yield by the 3rd year, in the other treatments only by the 4–5th year. Under the influence of irrigation and nutrition the composition of the grass successively changed.

Keywords: grass, irrigation, N, P, nutrition (fertilization), yield, long-term experiment

Introduction

In Hungary there are about 160.000 hectares of limeless alkali grasses where mostly *Festuca pseudovina* is the dominant species of the association. Owing to the structure, water regime and poor nutrient status of the alkali soil, furthermore, due to the dry, occasionally droughty weather the yield of these native grasses is very low, about 0,40 t/ha dry matter.

Attempts to improve the alkali grasses started in Hungary after the turn of the century, first of all by irrigation (Gyárfás, 1902; Röszler, 1909; Sigmond, 1923; Herke, 1933) and from 1929 by soil amelioration, or later by soil amelioration and fertilization (Herke, 1953; Pettenhoffer, 1954). Between 1955 and 1960 in other soil types of Hungary the importance of grass fertilization was proved (Takáts, 1957; Schummel, 1958). In the literature, only works published until the beginning of the experiment are taken into account.

Since on alkali grasses with limeless soil without soil amelioration, wider fertilization experiments were not conducted until 1959, we started detailed examinations with the aim of attaining larger yields in *Festuca pseudovina* grasses through irrigation and an adequate nutrient supply. Of the first three-year results of the experiment, we published a preliminary report (Milkovich and Bánszki, 1962); the ten-year results are given below.

Until the beginning of the experiment, longer-term fertilization experiments with grasses, and with alkali grasses in particular, are hardly known in Hungary and abroad.

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Materials and methods

Between 1960 and 1969 we carried out fertilization experiments with irrigated poor fescue grass in Hortobágy-Halastó. The height above sea level of the native grass was 89.3 m.

The meteorological data of the experimental years are contained in Table 1. The annual amount of precipitation – but particularly its amount in the vegetation period – was much below the 50-year average in most experimental years. In the region of Hortobágy the growth season was dry or even droughty in several years; only 1965, 1966 and 1968 were exception during ten years.

The soil of the experiment was meadow solonetz. The major soil analysis data prior to the beginning of the experiment were: pH (KCl) 5,3; y_1 9.4; total salt content 0.03%; hy 1.46; humus % 3.1; nutrient status in ppm: NO_3 + NO_2 2,00 Al-soluble P_2O_5 25, K_2O 406. Thus, the humus content of the soil was good, but its phosphorus content was very low its potassium content very high. Therefore, besides the N treatments, a higher rate of P fertilization was planned and K was not supplied at all.

The experiment was set up in the autumn of 1959 in random block design with 6 replications in plots of 58 m^2. The grass belonged to the *Achillea-Festucetum pseudovinae* plant association.

The treatments of the experiment are given in Table 2. We tested 43, 87 and 130 kg/ha N in itself, as well as 47, 94 and 141 kg/ha P_2O_5 used one-sidedly, and several NP combinations at the above rates of fertilizer. Since the soil was very poor in phosphorus, we studied the effect of 188 kg/ha P_2O_5 given in addition to the amounts of P indicated above beside N 43 kg/ha, reckoning with a phosphorus effect. Because of the very high potassium status of the soil, we did not carry out K fertilization.

Half of the N fertilizer was distributed in spring, in the middle of March. The other half was applied on two occasions, in equal proportions in June and August. Three-quarters of the P fertilizer was given in autumn, November, and one-quarter of it again on two occasions, in equal parts, together with the N application in June and August. For N fertilization 25% ammonium nitrate with lime, for P fertilization 18% granular superphosphate, was used.

In the experiment trickling flood irrigation was used on 3 to 5 occasions a year at rates of about 80-100 mm, so we gave about 300–400 mm irrigation water a year. Besides the irrigation – which was a replacement of water – the annual and seasonal amount of precipitation played an influential, determinative role in the annual trend of yield.

The yield was determined by weighing after cutting, and on the basis of samples converted into dry matter. Two harvests were carried out every year, at the end of May and August.

Table 1

Meteorological data of the years of experiment
(Hortobágy-Halastó)

Years	Amount of precipitation, mm		Mean temperature annual average °C
	Annual	In growth season	
1960	561	262	10.4
1961	390	220	10.6
1962	432	206	9.4
1963	501	277	9.6
1964	556	301	8.9
1965	687	423	8.9
1966	646	360	11.4
1967	464	239	10.0
1968	534	361	10.0
1969	505	301	9.3
Average	528	295	9.8
50-year average	583	340	10.0
Difference	–55	–45	–0.2

Table 2

Treatments of the experiment

Number and symbol of treatments		Fertilizer active agent, kg/ha		
		N	P_2O_5	Total
1.	0	–	–	–
2.	N1	43	–	43
3.	N2	87	–	87
4.	N3	130	–	130
5.	P1	–	47	47
6.	P2	–	94	94
7-	P3	–	141	141
8.	N1P1	43	47	90
9.	N1P2	43	94	137
10.	N1P3	43	141	184
11.	N1P4	43	188	231
12.	N2P1	87	47	134
13.	N2P2	87	94	181
14.	N2P3	87	141	228
15.	N3P1	130	47	177
16.	N3P2	130	94	224

Soil samples had been taken from the 0–20 cm soil layer before the experiment was set up, and a soil profile was exposed. The soil analyses were performed by the laboratory of the Department of Soil Science of the Agricultural College.

The botanical survey of the association was made with Klapp's method on the 1st growth.

The yield and other experiment results were evaluated by statistical methods, variation analysis and multivariable regression.

Results and discussion

Dry matter output and efficiency

In response to irrigation and fertilization, the dry matter output of the treatments gradually increased in the first years of the experiment compared to the original yield of the native grass (about 0,40 t/ha), and the composition of the plant stand also changed successively, which appeared in the level of yield, too (Table 3). Under the influence of irrigation, the dry matter output of the control rose from 0.88 t/ha in the first year to 3.19 t/ha on the average of the 10 years, and in rainier years attained 4–6 t/ha. In treatment 16, in response to 130 kg/ha N + 94 kg/ha P_2O_5 the yield increased from the 1st year's 2.04 t/ha to 6–8 t/ha on dry matter basis; the ten-year average was 5.58 t/ha. The yield of the other treatments was between these values.

On the average of 10 years (Table 4), 43, 87 and 130 kg/ha N applied in itself gave 22, 31 and 14% yield surpluses, respectively, compared to the control. That is, with the highest rate N fertilization, yield depression occurred compared to the former N level. The one-sidedly used P active agent ensured 13% yield surplus in a quantity of 47 kg/ha, 20% and 16% surplus at a rate of 94 and 141 kg/ha, respectively; the largest quantity of P caused depression here, too.

Table 3

Yield results of NP fertilization of an irrigated poor fescue-type grass with alkali soil
(1960–1969)

Treatment			Dry matter output, t/ha										
Number	N kg/ha	P_2O_5 kg/ha	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1960–1969
1.	–	–	0.88	2.19	1.90	2.63	3.07	4.09	5.99	3.94	3.80	3.36	3.19
2.	43	–	1.17	2.63	2.34	3.21	3.94	5.11	7.01	4.67	4.53	4.38	3.90
3.	87	–	1.31	2.77	2.48	3.36	4.38	5.40	7.30	5.11	4.96	4.67	4.17
4.	130	–	1.17	2.34	2.04	3.07	3.80	4.82	6.42	4.23	4.38	4.09	3.64
5.	–	47	1.02	2.34	2.04	2.92	3.36	4.82	6.72	4.38	4.09	4.23	3.59
6.	–	94	1.17	2.48	2.19	3.07	3.65	5.11	6.86	4.53	4.67	4.53	3.83
7.	–	141	1.17	2.48	2.04	3.07	3.65	4.67	6.42	4.53	4.67	4.23	3.69
8.	43	47	1.31	2.77	2.63	3.50	4.09	5.26	7.45	5.40	4.96	4.67	4.20
9.	43	94	1.46	2.92	2.77	4.09	4.53	5.55	7.74	5.69	5.55	5.40	4.57
10.	43	141	1.61	3.07	3.07	4.23	4.67	5.69	7.74	5.84	5.69	5.26	4.69
11.	43	188	1.46	2.92	2.77	3.65	4.53	4.96	7.15	5.55	5.55	5.26	4.38
12.	87	47	1.61	3.21	3.07	3.94	4.67	5.69	7.74	5.99	5.69	5.26	4.69
13.	87	94	1.75	3.36	3.36	4.38	4.96	5.99	8.03	6.28	5.99	5.69	4.98
14.	87	141	1.75	3.36	3.65	4.53	4.82	5.84	8.03	6.28	5.84	5.55	4.97
15.	130	47	1.90	3.50	4.09	4.82	5.11	6.28	8.18	6.42	5.99	5.84	5.21
16.	130	94	2.04	3.80	4.38	5.11	5.55	6.72	8.61	6.72	6.57	6.28	5.58
$L.S.D._{5\%}$			0.75	1.02	1.05	0.37	1.25	1.69	1.19	0.57	0.42	0.61	1.06

Table 4

Ten-year yield average and efficiency of NP fertilization of irrigated poor fescue grass type in alkali soil

Treatment			Dry matter output			Yield surplus	Fertilizer active agent used for 1 t yield surplus, kg		
Number	N	P_2O_5	t/ha	%	D	per 1 kg active agent kg	total	N	P_2O_5
	kg/ha								
1.	–	–	3.19	100	–	–	–	–	-
2.	43	–	3.90	122	0.71	16.5	60.6	60.6	-
3.	87	–	4.17	131	0.98	11.3	88.7	88.7	-
4.	130	–	3.64	114	0.45	3.5	288.9	288.9	-
5.	–	47	3.59	113	0.40	8.5	117.5	–	117.5
6.	–	94	3.82	120	0.64	6.8	146.9	–	146.9
7.	–	141	3.69	116	0.50	3.5	282.0	–	282.0
8.	43	47	4.20	132	1.01	11.2	89.1	42.6	46.5
9.	43	94	4.57	143	1.38	10.1	99.3	31.6	68.2
10.	43	141	4.69	147	1.50	8.0	125.3	29.3	96.0
11.	43	188	4.38	137	1.19	5.2	194.1	36.2	157.9
12.	87	47	4.69	147	1.50	11.2	89.3	58.0	31.3
13.	87	94	4.98	156	1.70	9.9	101.1	48.6	52.5
14.	87	141	4.97	156	1.78	7.8	128.1	48.9	79.2
15.	130	47	5.21	163	2.02	11.4	87.6	64.4	23.2
16.	130	94	5.58	175	2.39	10.7	93.7	54.5	39.2
L.S.D. $_{5\%}$			1.06	33					

The various NP combinations resulted in surplus yields between 32 and 75%. P doses combined with 43 kg/ha N produced a yield surplus of 32–47%, the largest P dose (188 kg/ha) caused yield depression. The P quantities used with 87 kg/ha N ensured 47–56% surplus yield, but the 141 kg/ha P did not increase the yield above the level ensured by 94 kg/ha P. The P doses combined with 130 kg/ha N gave 63–75% yield surplus. Significant yield differences, compared to the control were practically obtained with the NP treatments. Yield differences between the treatment were not reliable.

The partial correlation determined by the multivariable regression analysis was 0,816 with the N and 0.690 with the P fertilizer. The total regression was 0.847. On the basis of the determination coefficients the yield was determined in 58% by the N- and in 42% by the P fertilizer, on the average of the experiment. The regression equation is:

$$y = 3.263+0.00108N+0.00579P$$

As regards efficiency: the increasing fertilizer doses showed decreasing efficiency. The smaller quantities were the most efficient. The yield surplus per 1 kg fertilizer active agent ranged from 3.5 to 16.5 kg in the treatments of the experiment. With the per ha yield surplus also taken into consideration, the combined application of 130 kg/ha N and 47 kg/ha P (treatment 15) was of the highest efficiency (11.4 kg). In this treatment was the quantity of fertilizer active agent used to produce 1 t dry matter output surplus the lowest (87,6 kg).

Table 5 and Fig. 1. show the yield surpluses of treatments during the years of the experiment. We examined when, during the ten years, the individual treatments attained a yield surplus equal to the 10-year average. In other words: by which year the successive

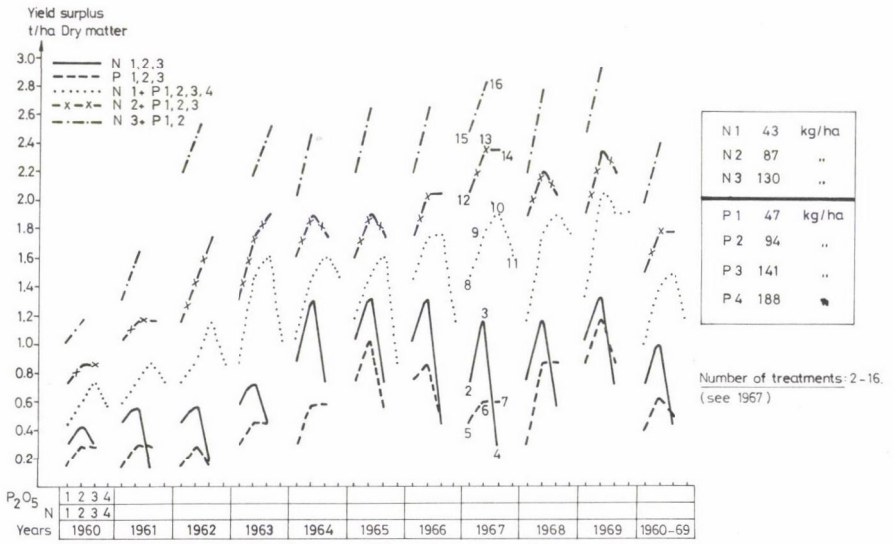

Fig. 1. Results of NP fertilization of irrigated Festuca pseudovina type grass

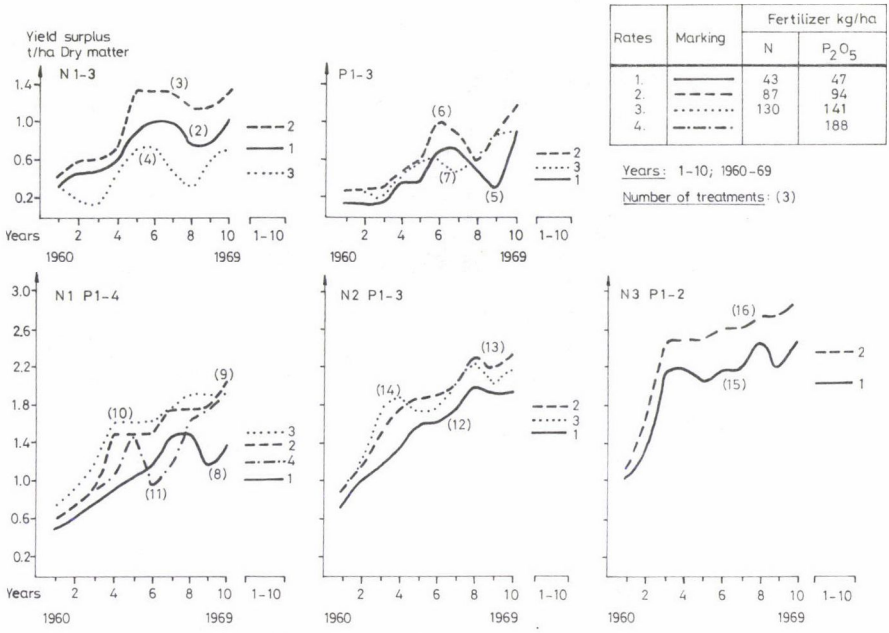

Fig. 2. Ten-year trend of yield surplus in the treatments

change of the treatments in response to irrigation and fertilization was completed, and the average yield level attained, respectively, from the beginning of the experiment. In Table 5 the underlined numbers indicate the fertilizer doses and the years. In the NP treatments the average yield was attained by the 3rd year with 130 kg/ha N, between the 3rd and 5th year with 87 kg/ha N, and by the 4th to 5th year in the case of 43 kg N applied per ha; in the one-sided N treatments by the 4th to 5th- and in the P treatment by the 5th to 6th year.

Further, the 10-year results of the experiment (Table 5 and Fig. 2) show the following tendencies: the depressive effect of larger quantities of N applied in itself appeared in the yield in the drier years and in the droughty vegetation periods (1961, 1962, 1967); the depressive effect – compared to the earlier yield level – of one-sidedly used P doses and of larger P quantities combined with smaller N doses appeared in rainier years and wet vegetation periods (1965, 1966, 1968), when the better water supply was, though, favourable for the efficiency of the P fertilizer, but as a negative effect the absolute and relative N deficiency increased. So, after all, a considerable yield depression occurred.

Conclusions: 1. The larger doses of N- and P fertilizers used one-sidedly caused yield depression. 2. In the NP combinations the N fertilizer promoted the efficiency of the P fertilizer, and the availability of the P content of soil, respectively. 3 In the case of the potassium-deficient grass, a soil overdosage of P – either one-sidedly or in NP combination with inadequate N:P ratio – caused yield depression.

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Table 5

Yield surpluses of NP fertilization of irrigated poor fescue grass type in alkali soil
(1960–1969)

Treatment			Dry matter surplus output, t/ha										
Number	N	P_2O_5	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1960–
	kg/h		1	2	3	4	5	6	7	8	9	10	1969
2.	43	–	0.29	0.44	0.44	0.58	0.87	1.02	1.02	0.73	0.73	1.02	0.71
3.	87	–	0.43	0.58	0.58	0.73	1.31	1.31	1.31	1.17	1.16	1.31	0.98
4.	130	–	0.29	0.15	0.14	0.44	0.73	0.73	0.43	0.29	0.58	0.73	0.45
5.	–	47	0.14	0.15	0.14	0.29	0.29	0.73	0.73	0.44	0.29	0.87	0.40
6.	–	94	0.29	0.29	0.29	0.44	0.58	1.02	0.87	0.59	0.87	1.17	0.64
7.	–	141	0.29	0.29	0.14	0.44	0.58	0.58	0.43	0.59	0.87	0.87	0.50
8.	43	47	0.43	0.58	0.73	0.87	1.02	1.17	1.46	0.46	1.16	1.31	1.01
9.	43	94	0.58	0.73	0.87	1.46	1.46	1.46	1.75	1.75	1.75	1.04	1.38
10.	43	141	0.73	0.88	1.17	1.60	1.60	1.60	1.75	1.90	1.89	1.90	1.50
11.	43	188	0.58	0.73	0.87	1.02	1.46	0.87	1.16	1.61	1.75	1.90	1.19
12.	87	47	0.73	1.02	1.17	1.31	1.60	1.60	1.75	2.05	1.89	1.90	1.50
13.	87	94	0.87	1.17	1.46	1.75	1.89	1.90	2.04	2.34	2.19	2.33	1.79
14.	87	141	0.87	1.17	1.75	1.90	1.75	1.75	2.04	2.34	2.04	2.19	1.78
15.	130	47	1.02	1.31	2.19	2.19	2.04	2.19	2.19	2.48	2.19	2.49	2.02
16.	130	94	1.16	1.61	2.48	2.48	2.48	2.63	2.62	2.78	2.77	2.92	2.39

Table 6

Percentage composition of grass and of major grass species in the 1st growth at the beginning and end of the experiment

Treatment number	Composition of grass, %					Cover by major grass species, %			
	symbol	grass[x]	legume[x]	weed plants[xx]	empty area	*Festuca pseudovina*	*Poa pratensis* ssp. angustifolia	*Alopecurus pratensis*	*Agropyron repens*
				Initial condition, 1960					
1.	0	52	1	31	16	40	5	4	2
				At the end of the experiment, 1969					
1.	0	66	1	29	4	44	10	4	4
2.	N1	75	1	22	2	47	15	3	4
3.	N2	77	1	21	1	48	14	2	5
4.	N3	74	1	24	1	53	10	2	6
5.	P1	69	1	26	4	50	9	3	4
6.	P2	73	2	20	5	52	11	4	5
7.	P3	71	3	22	4	51	9	3	4
8.	N1P1	83	1	15	1	60	16	2	2
9.	N1P2	85	1	13	1	60	17	3	2
10.	N1P3	82	2	15	1	55	20	3	3
11.	N1P4	78	3	17	2	52	16	3	2
12.	N2P1	85	1	13	1	59	20	2	3
13.	N2P2	86	2	11	1	56	22	2	3
14.	N2P3	80	2	17	1	50	24	2	2
15.	N3P1	83	1	15	1	52	24	3	3
16.	N3P2	85	1	13	1	50	25	4	4
L.S.D.$_{5\%}$		11	1	6	2	8	7	2	2

[x] Grasses, legumes valuable for feeding
[xx] Worthless, weed plants

Change of composition

In response to irrigation and fertilization, the native grass underwent successive changes during the 10 years (Table 6). The grasses reached a 71–86% cover compared to 52% in the first year. Legumes occurred in minimum numbers (1-3%), among them *Trifolium angulatum, Trifolium repens* and *Lotus corniculatus* deserve mentioning; P fertilization somewhat increased their share. The number of weeds fell to about half in the 10 years. Several weed plants disappeared (*Poa bulbosa f. vivipara, Eryngium campestre*), or were driven out from the association (*Koeleria gracilis, Capsella bursa pastoris, Lepidium species*). At the same time, the number of Carex species (*Carex stenophylla, Cares praecox*) slightly increased under the influence of irrigation. The same applies to the proportion of the Cyperaceae and Juncaceae species. The total cover of the grass increased to 98–99% in most treatments, compared to 84% in the first year. The number of species was reduced from the initial 70 to 28.

Out of the major grass species, the proportion of *Festuca pseudovina* increased from 40% in the first year to 47–60%, while the share of *Poa pratensis* ssp. angustifolia from 5% to 9–25%. It is interesting that the proportions of *Alopecurus pratensis* and *Agropyron repens* did not practically change.

Summary

In a 10-year experiment at Hortobágy-Halastó (Hungary) we examined an irrigated *Festuca pseudovina* type grass in meadow solonetz for the effects of 43, 87 and 130 kg/ha N- and 47, 94, 141 and 188 kg/ha P_2O_5 active agent used in themselves and in combinations, respectively. Owing to the very high potassium content of the soil, K fertilization was not carried out.

Compared to the original yield of the native grass (about 0.40 t/ha dry matter) the yield surpluses of the treatments gradually increased in the 1st to 5th year as a response to irrigation and fertilization, then became more or less steady at the yield potential of the successively changed grass, between a dry matter output of 3 and 6 t/ha.

The one-sidedly used N doses resulted in a 14–31% yield surplus, the P doses ensured yield surpluses between 13 and 20%; the largest quantities caused yield depression. The NP treatments gave surpluses between 32 and 75%. The equation of the multivariable regression is: y=3.263 + 0.00108N + 0.00579.P. On the basis of the determination coefficients the N fertilizer determined the yield in 58%, the P fertilizer in 42%, on the average of the experiment. The most efficient treatment was: N 130, P_2O_5 47 kg/ha.

In the NP treatments with the N 130 kg/ha dose the grass attained a yield equal to the 10-year average by the 3rd year, but in the other treatments and with other N doses this happened between the 3rd and the 6th year.

Under the joint influence of irrigation and fertilization, the share of *Festuca pseudovina* increased by some one-third, while the proportion of *Poa pratensis* ssp. angustifolia became 2-5-times the original one.

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EFFECT OF PRESOWING LIGHT-TECHNIQUE TREATMENT ON THE YIELD OF VEGETABLES

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Special light-stimulation technique (STIMOKOMPLUX) was used for the presowing irradiation of different vegetable seeds (tomato, red beet, radish, cucumber, red pepper). The investigations were carried out on small parcels for study of the stimulation effect on the yield.

Keywords: light-technique treatment, presowing irradiation, stimulation, yield increase, tomato, red beet, radish, cucumber, red pepper

Introduction

It is known that the low dose presowing radiation treatment – if the dose is in optimum range – has a positive influence on the yield (Berezina, 1975; Simon and Bhattachariya, 1977; Luckey, 1980).

In recent years, many investigations were carried out for the determination of the yield-increase of various plants growing from non-ionizing radiation technique (polarized light, laser etc.) treated seeds (Kőrösi, 1992).

The STIMOKOMPLUX system – as a special form of the AGROLUX technique – is a biotechnological method manifoldedly utilizing the characteristic features of different living organisms to increase their cellular activity, and thus change vitality as well, when exposed to an optimal luminous energy influence. Probably the luminous energy impulses generate resonance on the surface of the cell-membrane, and mobilize the enzyme systems, inducing intensive growth (Gáspár and Simon, 1988).

An earlier experiment was designed to explore how polarized light treatment of the seed, combined with supplementary illumination with intermittent light of various wavelengths of the seedlings obtained from light-treated seeds, affected growth and development of the seedlings. A favourable effect was observed in plant development, including the number of leaves, assimilating area of leaves at flowering, and rate of assimilations. The greatest surplus in wet and dry mass was in the stage of flowering. The results suggest that the best treatment was a combination of seed treatment + intermittent light of various wavelengths (Pál, 1987).

In 1990, field experiments with STIMOKOMPLUX stimulated sowing seeds were performed in different plants in Syria. The increase in yield at optimal dose was 13–14% for cucumber, 23.8% for potato, 8.3% for sunflower, 15–28% for maize, 31% for barley, 36% for tomato, 21.5% for pepper and 31.6% for eggplant. The most interesting

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phenomenon was that in some crops (barley, tomato, cucumber, eggplant, pepper and potato) the irradiation was done 15–30 days before sowing, which means that the light energy could be stored for a long period (Al-Oudat, 1992).

In this paper information is given about the stimulative effect of the STIMOKOMPLUX light-technique treatment on the yield of the investigated vegetables. Some results were reported in ESNA conferences (Szabó et al., 1989, 1991; Mednyánszky et al., 1990).

Materials and methods

For the treatment by the AGROLUX (STIMOKOMPLUX) technique, the experiments were carried out on small parcels wih tomato (*Lycopersicon esculentum* L. cv. K-815), red beet (*Beta vulgaris* L. cv. Bíbor henger), radish (*Raphanus sativus* L. cv. Jégcsap), cucumber (*Cucumis sativus* L. cv. Nimbus) and red pepper (*Capsicum annuum* L. cv. KM-622 and cv. Kalocsai). The seeds were disinfected with Cerezan. The experimental plots were designed as Latin square (29.6 × 11.5 m), the size of the small parcels was 4 × 5, or 7 × 9.5 m. Four variously treated samples were sown into the parcels, and 64 pieces per plant were examined in every treatment.

The STIMOKOMPLUX technique was applied for presowing treatments. As a radiation source, we used special light technique equipment (ASI-01) with polarized light. The doses were proportional to the duration (max. 120 s) of the treatments with approximately 10^4 lux, because the geometry and parameters of the light sources and other conditions were the same in all experiment. The photometric data were measured and they could be converted to radiometric units (Thimijan and Heins, 1983).

Results and discussion

The results of the effect of light-technique treatment on the yield increase are shown in Tables 1, 2, 3, 4 and 5.

Evaluating the data, we can establish that the treatments with optimum or nearly optimum doses have positive effects on the yields.

It should be mentioned that, in the case of red beet, more than 300% yield-increase was registered after the 20 s treatment, in comparison with the control. This increase-ratio is obviously improbable, indicating that this measured ratio would be the result of some unknown factors influencing the yield. The effect of presowing treatment can be evaluated as very effective if the yield-increase exceeds 20% in field conditions.

As a conclusion, it can be established that the light-stimulation has favourable effects on the yield of vegetables, so the application of the STIMOKOMPLUX non-ionizing radiation technique gives the possibility to increase the yield significantly. Besides the yield increase, the advantageous effect manifests in the more homogeneous and powerful germination and stronger resistance against freeze, illnesses etc., and in some cases in the better chemical composition.

Table 1

Effect of light-technique irradiation on the yield-increase of tomato (K-815)

Year	Duration (s)	Mass/piece (g)	Yield/parcel (kg/m^2)	Yield in % of control
1986	0	36.19±2.12	3.63±0.31	100.0
	15	40.08±1.87	4.46±0.39	122.8
	60	41.13±4.06	4.60±0.44	126.7
	120	36.24±4.11	3.87±0.40	106.6
	0	52.41±4.89	11.21±1.02	100.0
	15	62.39±5.91	13.81±1.45	123.1
1987	60	56.53±6.02	14.26±1.38	127.2
	120	56.19±6.21	11.85±1.21	105.7

Table 2

Effect of light-technique irradiation on the yield-increase of red beet (Biborhenger)

Year	Duration (s)	Yield/parcel (kg/m^2)	Yield in % of control
	0	3.616±0.311	100.0
	20	11.904±1.214	329.2
1986	120	4.784±0.426	132.3
	240	3.984±0.328	110.2
	0	2.590±0.276	100.0
	20	4.480±0.510	172.9
1987	120	2.910±0.257	112.3
	240	2.590±0.237	100.0

Table 3

Effect of light-technique irradiation on the yield-increase of radish (Jégcsap)

Year	Duration (s)	Mass/piece (g)	Yield/parcel (kg/m^2)	Yield in % of control
	0	37.47±3.22	2.39±0-24	100.0
	20	49.37±4.86	3.16±0.29	131.7
1986	60	38.42±4.22	2.46±0.26	102.5
	120	37.80±3.98	2.42±0.21	100.9
	0	41.96±5.01	1.68±0.27	100.0
	20	55.29±6.13	3.54±0.31	131.8
1987	60	43.03±3.99	2.95±0.32	110.0
	120	41.96±4.26	2.86±0.33	106.7

Table 4

Effect of light-technique irradiation on the yield-increase of red pepper

Type	Duration (s)	Mass/piece (g)	Yield/parcel (kg/m^2)	Yield in % of control
KM-622	0	36.0±4.01	0.685±0.071	100.0
	30	55.7±4.98	1.170±0.132	170.8
	70	56.5±5.11	1.300±0.153	189.7
	110	52.5±4.79	1.260±0.162	183.4
Kalocsai 50	0	28.8±3.04	0.576±0.061	100.0
	30	37.2±4.16	0.930±0.102	161.4
	70	30.8±3.53	0.800±0.092	138.8
	110	52.0±4.79	0.780±0.069	135.4

Table 5

Effect of light-technique irridiation on the yield-increase of cucumber (Nimbus)

Duration (s)	Yield (kg/m^2)	Yield in % of control
0	3.125±0.412	100.00
15	3.730±0.410	119.36
30	4.238±0.398	135.61
60	6.015±0.589	192.48
90	7.950±0.802	254.40

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Plant protection

EFFECTS OF ULTRASONIC IRRADIATION AND VACUUM INFILTRATION COMBINED WITH FUNGICIDES ON FUNGI-INFECTED SUNFLOWER SEEDS

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The authors have used the effects of ultrasonic irradiation (22 and 25 KHz frequency) and vacuum infiltration to increase the diffusion and permeability of fungicides to penetrate into the achenes of the sunflower hybrid NK-254, infected by fungi.

The germination of seeds was found to be 86% and the degrees of infection were as follows: *Alternaria* spp. 88%, *Botrytis cinerea* 14%. *Sclerotinia sclerotiorum* 17%.

The effectiveness of the four different fungicides was increased by both physical methods. In the case of Kelosild FW (Zn-Mn-8-oxyquinolate-dimethyl-dithiocarbamate) fungicide, the authors have achieved total freedom from infection and this product effected a long-lasting protection also in field experiments.

Both methods proved to be fungicide-saving and environmental; by their use the safety of sunflower production can be increased.

Keywords: dressing sunflower seeds, ultrasonic irradiation, vacuum infiltration

Introduction

As well known from the literature, sunflower has many dangerous fungal diseases. Their common features are that these can spread by infected seeds or by overwintering in growing areas.

For these reasons, the ways of protection against them are either breeding for resistance or effective seed dressing by fungicides. The authors wished to increase the effectiveness of seed dressing by the selection of suitable fungicides and by getting them within the pericarp of achenes with the help of ultrasonic irradiation and/or vacuum infiltration.

Our difficulties were increased by the fact that the fungal diseases of sunflower often result from mixed infections, as it happened also in our case. This increases the importance of the environment-friedly and fungicide-saving methods described in present paper. The mentioned physical methods yielded already favourable results in earlier studies (Nagy and Ratkos 1986, Ratkos and Nagy 1986, Nagy and Ratkos 1987).

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It is well known that, as a result of ultrasonic treatments the changes of specific ultrastructure within the living cells depend on the intensity and duration of the treatments; their effect on biological materials may be stimulative, restraining or destructive. They can thus limit the biological activity of treated organisms and may become even lethal. However, ultrasound doses that are lethal for fungal systems may also destroy the germinating capacity of treated sunflower achenes. On the other hand, if the irradiation is carried out in a solution of an appropriate fungicide, it is generally sufficient to apply the doses in the stimulative range (Heimann 1954, Jaenichen and Heimann 1955).

The effect of ultrasonic irradiation, increasing diffusion and permeability can be utilized in introducing certain fungicides into the seeds (Pohlmann 1951, 1969).

Earlier experiments were carried out generally with ultrasound of 800 KHz frequency and in irradiation tanks about 100 ml volume. In our experiments, ultrasounds of 22 and 25 KHz frequency and irradiation tanks of significantly larger volume (4–10 litre) were used.

Materials and methods

In our tests we used a 22 KHz constant frequency apparatus "KLN Ultraschall Universal Laborgerät' (made in Germany) and a 25 KHz constant frequency UC-002 BM Ultrasonic generator (made by Tesla Company, Bohemia).

The irradiations were carried out in an irradiation tank of 4 litre volume (within a range of 2.5 to 10 minutes), which doses exerted stimulative effects on the germination of sunflower seeds and on the growth of seedlings, as shown by the preliminary tests (Fig. 1).

We have carried out the ultrasonic treatments in fungicide solutions of different concentrations as indicated in Table 1 and with the above-mentioned ultrasonic irradiation parameters.

For comparison untreated seeds and ones soaked into the fungicide for about 30 minutes were used.

For vacuum infiltration an exsiccator jar was used, in which normal air pressure (about 1013 HPa) was maintained at the begin. The jar contained the 4 × 100 sunflower seeds in the appropriate fungicide solution. The air pressure could be reduced within 30 seconds to 40–50 HPa by means of a rotary air pump; after stopping this vacuum, the barometric pressure forced the dressing solution into the seeds through the micro-cracks of the pericarp into the achenes.

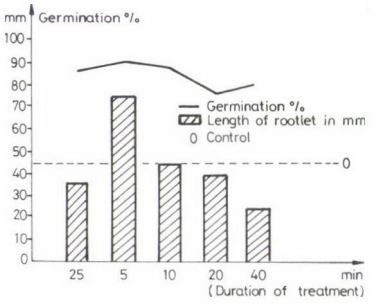

Fig. 1. Effect of ultrasonic treatment on the germination of sunflowers and the size of the rootlet depending on the duration of treatment

By the end of this procedure, a weight increase of approx. 30% of the dry net weight was observed, compared to the approx. 5% increase of the ultrasonic treatments and approx. 1% increase of simple soaking.

The sunflower hybrid NK-254 used in our experiments was infected mainly by Alternaria species and showed a germinating rate of 86%. The infection rates with other fungi are indicated in Table 2.

We have examined the effects of treatments mentioned above by germination tests in Petri dishes (4–8 × 100 seeds) as well as by field plot experiments (carried out with 50–50 seeds).

The names and concentrations of fungicides used are summarized in Table 1.

Table 1

Treating solution used in the experiments

Solution	Agents	Concentration used for seed treatments, %	Doses used for traditional dressing
Agrocit	50% Benomyl	0.15	100 g/100 kg
		0.40	
		0.70	
		1.00	
Dithane M-45	80% Mancoseb	0.20	100 g/100 kg
		0.50	
		0.80	
		1.00	
Kelokarb 80 WP	80% Zn-Mn-8-oxy-quinolate-di-methyl-dithiocarbamate	0.20	200 g/100 kg
		0.50	
		0.80	
		1.00	
Kelosild FW	20% Zn-Mn-8-oxy-quinolate-di-methyl-dithiocarbamate + 10% carbendazime	0.20	1:3 dilution rate
		0.50	
		0.80	
		1.00	

Table 2

The total fungal infection of NK 254 sunflower hybrid

Fungi	Degree of infection, %
Alternaria spp.	88.0%
Botrytis cinerea	14.0%
Sclerotinia sclerotiorum	17.0%
Aspergillus sp.	4.5%
Penicillium sp.	1.5%
Total degree of infection	125.0%
Germination percent	86.0%

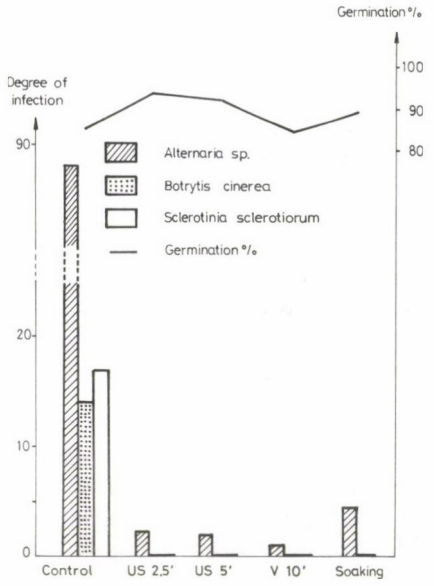

Fig. 2. Effect of Agrocit (1%) + Dithane M-45 (1%) fungicides combined with physical treatments on the degree of infection and germination of seeds

Results

First we carried out the ultrasonic and vacuum treatments with 0.2 to 1.0% concentrations of Agrocit (containing 50% benomyl) and Dithane M-45 (containing 80% mancoseb), as mostly recommended in literature.

According to our laboratory germination tests the 5 minutes ultrasonic irradiation (Us 5) and 10 minutes vacuum infiltration (V 10) reduced the *Alternaria* spp. infection only to 10% (even at fungicide concentrations of 1+1%), whereas the other fungi were completely destroyed (Fig. 2). Thus the fungicide combination was not sufficiently effective against Alternaria. Also Kelokarb 80 WP (80% Zn-Mn-8-oxyquinolate-dimethyl-dithiocarbamate) did not prove itself totally effective in lower concentrations (0.2–0,8%) in either treatment against Alternaria. By using an 1% concentration, however, both the ultrasonic irradiation (5 minutes) and vacuum infiltration treatments reduced the Alternaria infection to 2–2.5%, giving a substantially better result than the one obtainable by the traditional soaking method (Fig. 3).

Furthermore, as like it had been observed in our previous tests, the ultrasonic treatment increased also the germination rate.

In our further experiments Kelosild FW (20% Zn-Mn-8-oxyquinolate-dimethyl-dithiocarbamate + 10% carbendazime) fungicide was found the most effective, which has completely destroyed both *Botrytis cinerea* and *Sclerotinia sclerotiorum* and kept the rate of Alternaria spp. infection well below 10% in a 0.5% concentration (Fig. 4). At a 1% concentration both treatments resulted in seeds free of infection (Fig. 5).

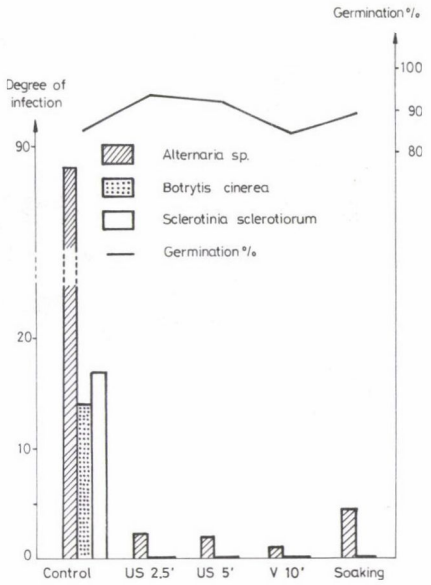

Fig. 3. Effect of Kelokarb 80 WP (1%) fungicide combined with physical treatments on the degree of infection and germination of seeds

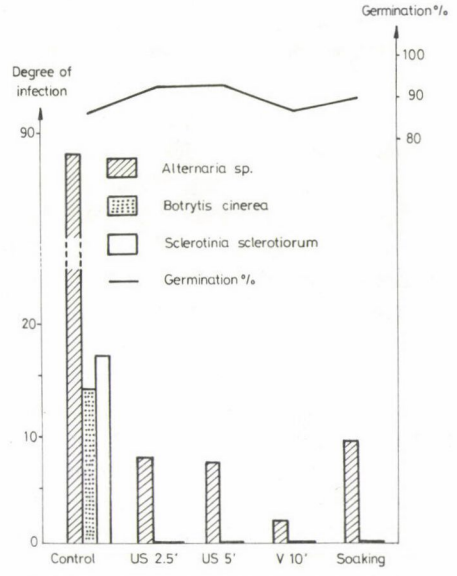

Fig. 4. Effect of Kelosild FW (0.5%) fungicide combined with physical treatments on the degree of infection and germination of seeds

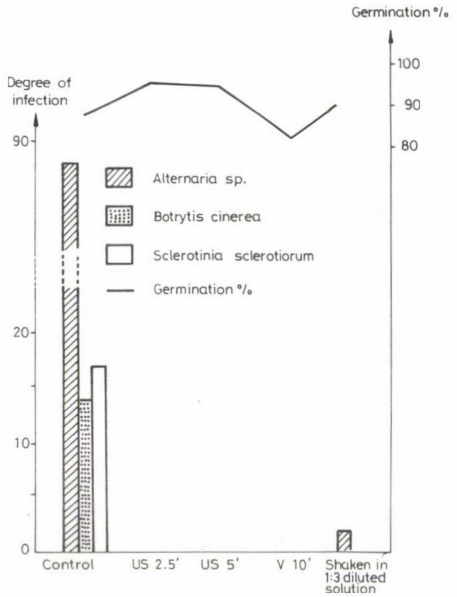

Fig. 5. Effect of Kelosild FW (1%) fungicide combined with physical treatments on the degree of infection and germination of seeds

In addition the ultrasonic irradiation increased while the vacuum infiltration decreased the germination rate by a few percents. By comparing the results with the ones obtained in the control "shaken in solution" dressing method we have found that the treatments with Kelokarb 80 WP (1% concentration) gave nearly the same result (percentage of fungal infection) as the 200 g/100 kg seed dose used experimentally and carried out in the traditional "wetted powder dressing" way. The method mentioned allows therefore a significant saving in fungicide amounts. The scale is nearly the same at vacuum infiltration of 0.8% concentration (Table 3).

More favourable results can be achieved as regards fungicide saving in case of Kelosild FW if we consider that for the same result we have to use 33% fungicide solution when applying the traditional "shaken in solution" treatment (30 minutes) (Tables 1, 3, 5). In our further experiments the Kelosild FW was not more effective by the combined ultrasonic (5 min) and vacuum infiltration (10 minutes) treatments (Table 4).

The ultrasonic treatment is, however, faster than the traditional one (5 minutes) and its stimulating effect on the germination can also be shown (Table 3).

On the other hand, vacuum infiltration can press fungicides through the microcracks of the pericarp which will not be lost from the achenes during drying and sowing. Because of these, both methods applied can be considered as environment-friendly.

We have completed the laboratory tests with field experiments as well, which have confirmed the advantages of both methods.

Table 3

The result of traditional wetted power dressing and fungicide treatments combined with physical methods

Treatments	Doses	Degree of infection %					Total degree of infection	Total degree of infection in the percentage of control	Germination	
		Alternaria sp.	Botrytis cinerea	Sclerotinia scl.	Penicillium sp.	Aspergillus sp.			%	in the % of control
1. Control		88	14	17	1.5	4.5	125	100	86	100
2. Traditional						3				
Agrocit+Dithane	100*+100*	14.5	–	1.5	–	2.5	19	15.2	90.5	105.2
M-45	200*	3	–	–	–	–	5.5	4.4	91	105.8
Kelokarb 30 WP	1:3 dil	0.5	–	–	–		0.5	0.4	92.5	107.5
Kelosild FW										
3. Ultrasonic										
Agrocit+Dithane	1% + 1%	9	–	2.5	1.5	–	13	10.4	92	106.9
M-45	1%	2.5	–	–	–	–	2.5	2	93	108.1
Kelokarb 80 WP	1%	–	–	–	–	–	–	–	95	110.4
Kelosild FW										
4. Vacuum										
Agrocit+Dithane	1% + 1%	9.5	–	–	–	–	9.5	7.6	84	97.6
M-45	1%	1	–	–	–	–	1	0.8	84	97.6
Kelokarb 80 WP	0.8%	–	–	–	–	–	–	–	85	98.8
Kelosild FW										

* = g/100 kg seeds

Table 4

The combined effect of Kelosild FW with ultrasonic (5 minutes) treatments and vacuum infiltration (10 minutes) on the germination per cent and the degree of infection of seeds

No.	Concentration	Degree of inf.%		Total degree of infection, %	Total degree of infection in the precentage of control	Germination	
		Alternaria sp.	other			%	in the % of control
1.	Control	88	37	125	100	86	100
2.	0.5%	1.5	–	1.5	1.2	87.5	101.7
3.	0.8%	–	–	–	–	84	97.6
4.	1%	–	–	–	–	81.5	94.7

Seeds treated with ultrasonic irradiation in 0.2% Kelosild FW for 5 and 10 minutes showed a higher emergence ratio, the plants earlier flowering and the yield showed higher oil content (48 and 49.3%) as compared to the 41.8% the control (Table 5).

Most probably due to the higher fungicide absorption (30% weight increase) the vacuum infiltration treatment resulted in a later flowering and the yield contained on the average only 37.9% oil (Table 5).

On plants that had developed from seeds treated with ultrasound and vacuum no fungal infection could be detected until 13 August in our field experiments, compared to the 20% infection of control plants at the same time. Sunflower plants that had received ultrasonic irradiation or vacuum infiltration as seeds have shown also in later stages a minimal degree of infection (Table 6).

Discussion

The seed treatment methods discussed above are both more effective and economical than the traditional ones.

According to our experiments, the stimulative effect of ultrasound resulted in a more favourable germination rate, more intensive growing in the seedling stage, healthier plants and in an increase of oil content.

It can be shown that both methods have fungicide-saving and environmentally safe features. It can be stated in general that:

1. according to our laboratory experiments Kelosild FW fungicide can be used with more success against Alternaria species with ultrasonic irradiation or vacuum infiltration than with the traditional methods;

2. the ultrasonic treatment was more efficient than the traditional one in each case because:

a) it increases the percentage of germination,

b) it increases the oil content,

c) the saving in fungicide amounts is significant in case of Kelokarb 80 WP and even more with Kelosild FW.

Table 5

The growing dynamics of plants developed of treated seeds with Kelosild FW (0.2%) solution combined with physical methods

Treatments	No. of leaves		Height of plants (cm)			Flowering %					Oil %
	20 May	29 May	max.	min.	average	14 July	15 July	17 July	18 July	21 July	
1. Soaked for 30 minutes	2–4	6	180	80	122	30.30	48.48	63.63	66.66	75.75	41.8
2. Ultrasonic (5 min.)	2–4	4–6	165	80	124	14.81	23.62	37.03	48.14	70.37	48.0
3. Ultrasonic (10 min.)	2–4	6	215	34	132	26.66	35.71	56.66	63.33	86.66	49.3
4. Vacuum (10 min.)	2–4	2–4	131	62	105	21.42	35.71	50.00	53.57	60.71	37.9

Table 6

The percentage appearance of infection on plants developed of treated seeds (field experiments)

Treatments	17 July		13 August				22 August			
	foot infection	leaf inf.	foot inf.	leaf inf.	crown inf.	total inf.	foot inf.	leaf inf.	crown inf.	total inf.
1. Soaked for 30 minutes	0	0	20.00	0	0	20.00	26.67	0	13.33	40.00
2. Ultrasonic (5 min.)	0	0	3.57	0	3.57	7.14	7.14	7.15	17.85	32.14
3. Ultrasonic (10 min.)	0	0	0	0	0	0	3.70	0	7.47	11.11
4. Vacuum (10 min.)	0	0	0	0	0	0	6.67	0	13.33	20.00

3. Both methods are fungicide-saving and environment-protecting, because the active materials do not pollute the surrounding micro-environment but remain inside the achene shells.

4. More plants developed that resisted to the infection; by vacuum-infiltration:

a) fungicide amounts equal to 30% of the original weight of seeds can forced through the microcracks of the pericarps of achenes, where they then stay,

b) the effectivity of fungicides is increased,

c) the disadvantages are moderate reduction of germination and decrease of oil content.

Finally, we may observe that no comparison is available at the moment with the currently used seed dressing methods (seed dressing machines). It has been mentioned, however, that in case of Kelosild FW the same result has been achieved than with "shaken in solution" dressing and with a 33% seed dressing solution (Tables 1, 3). These represent far more fungicide wasting than a 1% fungicide combined with an ultrasonic treatment. The more, the latter method is faster and can be done continuously.

We are sure that these treatments can be carried out under field conditions as well. For industrial purposes (cleaning) ultrasonic tanks of large capacity are used. The vacuum infiltration can also be done in large volume, pressure resistant tanks.

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Plant genetics and breeding

WHEAT QUALITY BREEDING AND THEORETIC STUDIES IN CHINA

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Wheat (*Triticum aestivum* L.) is one of the main crops in China and the yearly wheat planting area and production are about 30 million hectares and 9.5 million metric tons, respectively. Wheat breeding in China commenced in the 1920s and the quality breeding of wheat began just ten years ago. But multiple research has been done and many achievements have been made on the collection, screening and utilization of wheat genotype resources for good quality breeding and theoretic studies. General information on wheat breeding and some research results achieved in theoretic studies on wheat quality in China are presented in this article.

Keywords: wheat, quality breeding, protein content, gluten content, baking quality

Introduction

Wheat is cultivated all over China. The total planting area is about 30 million hectares yearly, 84% of which is winter wheat and 16% spring wheat. The two largest wheat planting areas are in Henan Province and Shandong Province. There are 5.3 million hectares in Henan Province alone. The national wheat planting area, average yield and total production in recent years are shown in Table 1 (FAO, 1991). But since the weather, soil, farming conditions and variety type vary in different regions, the wheat yields in different regions also vary.

Table 1

Wheat planting area, yield and production in China

	1978–81	1989	1990	1991
Total area (1000 ha)	28930	29842	30754	30151
Yield (kg/ha)	2047	3043	3194	3151
Total production (1000 MT)	59196	90810	98232	95003

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Wheat growing in China is divided into three main regions and ten sub-regions, based on wheat variety type and weather conditions. The three main regions are:

- Northern Winter Wheat Region
- Southern Winter Wheat Region
- Spring Wheat Region

The ten sub-regions and their characteristics are presented in Table 2.

Wheat breeding research has been carried out since the 1920s and great achievements have been made in recent years. The wheat varieties used before the 1950s were local varieties (such as Youzimai, Changzhouhong, etc.) and in the 1950s were local varieties (such as Mazhamai, Quality, etc.), with some imported varieties (such as Mentana, CI12203, Ardito, etc.) and a few selected varieties (such as Bima 1, Nanda 2419, Bima 4, Shannu 205, Nuda 36, etc.); in the 1960s there was a larger proportion of imported varieties (such as Abbondanza, Funo, St1472/506, etc.) and created varieties (such as Beijing 8, Jinan 2, Neixiang 5, etc.). Since the 1970s almost all the varieties (such as Taisan 1, Fengchan 3, Beijing 10, Bainu 3217, Manyang 11, Jimai 5418, etc.) have been created by wheat breeders.

Table 2

*Ten wheat planting sub-regions and their characteristics**

Sub-region	Variety type	Day light sensitivity	Sowing time	Date of maturity	Vegetation period (day)	Main disease and pest
North-eastern SWR	S	sensitive	Apr. 15	July 20	90	rusts, scab
Northern SWR	S	sensitive	Mar. 20–Apr. 15.	July10–Aug. 20	90–120	leaf rust
North-western SWR	S	sensitive	Mar. 10	July15–Aug. 10	120–130	leaf rust
Xinjiang SWWR	S	sensitive	Apr. 10	Aug. 10	120	
	SW	sensitive	Sep. 15	July25–Aug. 5		
Quingzhang SWWR	S	sensitive	Mar. 20–Apr.10	Aug. 25–Sep. 15	140–170	
	SW	sensitive	Sep. 20	Aug. 25–Sep. 15	330	
Northern WWR	SW	sensitive	Sep. 10	June 20	260	leaf rust, powdery mildew
Huang-Huai WWR	WS	medium	Nov. 10.	May 25–June. 10	230	leaf rust
	WW	medium	Oct. 10	May 25–June. 10	230	powdery mildew
WWR in middle and Lower Reaches of	WS	non-sens.	Oct. 20–Nov. 10	May 20	200	scab
Yangtze River	WW	non-sens.	Oct. 20–Nov. 10	May 20	200	powdery mildew
South-western WWR	WS	non-sens.	Oct. 20–Nov. 10	May 20	180–200	scab,
	WW	non-sens.	Oct. 20–Oct. 20	May 20	180–200	powdery mildew
Southern SWR	S	non-sens.	Nov. 25	Mar. 25–Apr. 10	120	scab, powdery mildew

* SWR: spring wheat region; S: spring type; SWWR: spring-winter wheat region; SW: strong winter hardy; WWR: winter wheat region; WS: weak spring; WW: weak winter hardiness

At present, the aim of wheat breeding in China is, in general, to achieve high yield potential and stability and high quality for a variety. But there are different standards in different regions. In Henan Province, for example, a new variety should yield 10% more than the standard varieties, should possess leaf rust and powdery mildew resistance and drought-heat-wind tolerance, and should mature early if it is to be used.

Wheat quality breeding research was commenced later in China than in the developed countries. It was in the late 1970s that some wheat breeding researchers began to introduce the results and techniques of wheat quality breeding from abroad and proposed some important ideas about how to carry out wheat quality breeding in China (Wu Z. S., 1976; Wu R. S., 1979; Zhao W. G., 1979; Li Z. Z., 1982; Wang J., 1983). Official research on wheat quality breeding began in 1982. Much research has been done and many achievements have been made in the past ten years on the following aspects: the screening and utilization of wheat genotype resources for high quality; the inheritance of wheat quality traits; the relationships between quality traits and agronomic characters; the methods and techniques of wheat quality breeding; the relationship between wheat quality and environmental conditions, etc.

The quality of wheat varieties in China

Over the past ten years wheat breeders in China have collected and screened millions of genotypes which possess high protein content, high lysine content and good processing quality, and have created some varieties with both good agronomic characteristics and high processing quality.

The qualitative criteria of wheat with good bread making quality are suggested in Table 3 (Wan F. S. et al., 1989). But, at present, there are no official quantitative criteria of wheat with different end uses in China.

The Crop Research Institute of the Chinese Agricultural Academy of Science tested 1070 wheat varieties which were widely cultivated in various parts of China in 1983 and 1984.

The average protein content of these varieties was 12.57%. The Variety Resource Research Institute of the Chinese Agricultural Academy of Science tested 572 wheat varieties which were included in the "Wheat Variety Records in China" in 1982. The average protein content of the varieties was 12.76% (8.07–20.42%), with an average protein content of 13.5% for spring wheat varieties and 12.39% for winter wheat varieties. The results indicated that the protein contents of most varieties were not too low but the quality of the gluten was very poor. Most of the varieties were not suitable for baking good bread (Mei J. F. et al., 1991). In 1988 Wan F. S. et al. collected and tested 79 good varieties which occupied the main wheat planting area of sixteen provinces. The average protein content was 13.7% (10.09–16.64%); the average wet gluten content was 29.6% (17.0–39.1%); the average dough development was 3 (1–11.3) minutes; the average valorimeter value was 49.1 (31.3–82.0); the average bread volume 586.4 (433.3–867.5) cm^3; the average bread score 62.6 (27–97). These results revealed that most of the wheat quality criteria of some varieties tested had almost reached the level

Table 3
Suggested qualitative criteria of wheat with good bread making quality in China

Item	Value
Test weight (kg/hl)	>79
Thousand kernel weight (g)	35–40
Grinding resistance (sec.)	20–30
Crude protein (%)	13–15
Wet gluten (%)	>30
Zeleny value (ml)	>40
Farinograph test	
– water absorption (%)	55–60
– dough development (min.)	>4
– stability (min.)	>8
– breakdown time (min.)	>12
– degree of softening (B.U.)	20–50
– valorimeter value	50–70
Bread baking test	
– bread volume (cm^3)	>650
– specific volume (cm^3/g)	>4.5
– score	>80

of similar varieties abroad. But most of the varieties had disadvantages, especially as regards processing. The gluten elasticity of most varieties was medium or poor. The processing quality of the wheat variety should therefore be emphasized in future wheat breeding research in China (Wan F. S. et al., 1989; Ling Z. J., 1989).

Testing techniques and genetic studies on wheat quality traits have been carried out extensively in China. The results of theoretical studies have guided the parent selection, mating and progeny treatment in the wheat breeding programme.

Progress of theoretic studies on grain protein content

The inheritance of protein content

Protein content is an inherited trait and is influenced by many factors (Li Z. Z., 1986; Liu G. T., 1985): The results of studies on the inheritance of protein content were different, depending on the materials tested, analytical methods, environmental conditons, etc. (Li Z. Z., 1986).

Wheat protein content is a quantitative character dominated by multiple genes (Zhu M. Y. et al., 1984; Wang Z. D., 1987; Li Z. Z., 1986; Shuen B. F., 1986) distributed on all the chromosomes (Li Z. Z., 1986). Some researchers reported that the protein content was controlled by a few major genes and many minor genes (Mei G. F. et

al., 1983): Low protein content was governed by a number of dominant genes (Zhou M. Y. et al., 1983; Zhou G. B., 1988) and high protein content by recessive genes (Zhou M. Y. et al., 1983).

Most studies revealed that wheat protein content was commonly controlled by both additive and non-additive genes, but the former were more important (Zhou M. Y. et a., 1984; Li Z. Z., 1985; Wang Z. D., 1987; Zhou G. G., 1989). The dominant gene effect had much more influence on protein content than the additive effect did (Zhou G. J., 1990; Wang Z. D., 1987). Some researchers suggested that protein content was affected significantly by additive, dominant, dominant–dominant, additive–additive and additive–dominant effects (Jie J. Z., 1986).

Few studies have been made on the gene location of protein content. High protein content and high lysine content are related mainly with the chromosomes of group A, partly with group B and least with group D (Li Z. Z., 1986). Some researchers have suggested that the genes which affect protein content are located on all the chromosomes in wheat (Wu Z. S., 1976; Shan B. S., 1985).

Heterosis of protein content in progeny

The heterosis of protein content in the F_1 generation varied greatly, depending on different combinations. Wang (1987) analysed 21 hybrid combinations in the F_1 generation and found that 9.5% plants had higher protein content, 23.8% had intermediate protein contents and 66.7% had lower protein contents compared with those of their parents. But compared with the protein yields of the parents, 91% had higher protein yields, 9% had intermediate values and none had lower protein yields. Wang (Wang Z. Y. et al., 1991) studied 30 hybrid combinations in the F_1 generation and found that 3.3% plants had higher protein contents, 60% had intermediate values and 36.67% had lower protein contents compared with those of their parents. But again, when comparing protein yields, 66.67% had higher values than their parents, 30% were intermediate and 3.33% had lower values (Wang Z. Y. et al, 1991; Wu Y. T. et. al., 1988). Based on the results above, it would appear to be more difficult to obtain heterosis for protein content in the F_1 generation than for protein yield.

In the F_2 generation the frequency of protein content followed a normal distribution. The average protein content of the progeny was between that of their parents but transgressive segregation sometimes occurred. The average protein contents in the F_3 and F_4 populations were close to those of their parents; about 44% plants in the F_3 generation had values above the average protein content of their parents and 30% in the F_4 generation (Wu Z. S., 1976).

Correlations existed between the wheat protein contents in different generations. This correlation was significantly positive between the F_1 generation and its parents (Zhan K. H., 1991) and between the F_1 and F_2 generations (Tian S. M., 1988). The protein content of the progeny could therefore be predicted from the average protein content of the parents (Mei G. F. et al., 1991).

Estimates of the heritability of protein content

The heritability values estimated for protein content differed, depending on the experimental design, the relationship between the materials tested, the estimation method and the environmental influence, etc. Most studies suggested that the range of heritability in the broad and narrow senses for protein content was 0.11–0.82 and 0.055–0.70, respectively (Zhan K. H., 1991; Chai Q. F., 1990; Zhao B., 1989), while that for protein yield 0.25–0.56 and 0.14–0.25, respectively (Wan Z. Y. et al., 1991; Zhao B., 1989). The results above revealed that the heritability of protein content was higher than that of protein yield. That is to say, the protein yield was more strongly influenced by environmental conditions (Zhan K. H., 1991; Chai Q. F., 1990; Zhao B., 1989).

Correlations between protein content and other traits

Grain protein content, grain yield and yield components

The correlation betweeen grain protein content and the grain yield and yield components was either negative or positive, and the degree of correlation was also different. There was a significant negative correlation between grain protein content and grain yield (Fan L. et al., 1983; Shuen Z. L., 1988; Ling Z. J. et al., 1989; Gue R. L. et al., 1989; Li H. Z., 1983; Wang S. J., 1989; Zhang Z., 1989), and a significant (Zhou Q. G. et al., 1988; Li H. Z., 1983; Ling Z. J. et al., 1989; Zhang Z., 1989) or non-significant negative (Shuen Z. L., 1988; Yang C. L., 1988; Gue R. L. et al., 1989; Xie R. S., 1990; Li Z. Z., 1988) correlation between protein content and 1000-kernel-weight. Similar relationships were found for spikes per plant and grains per spike (Gue R. L. et al., 1989; Li H. Z., 1983; Yang C. L. et al., 1988; Li Z. Z., 1988).

The results above indicated that high protein content was negatively correlated with the majority of desirable agronomic characteristics. But these relationships were not absolute and it was possible to create varieties with high quality and high grain yield potential (Liu G. T., 1986; Ding S. K., 1985; Zhang B. B., 1988).

Grain protein content and other characteristics

A positive correlation between grain protein content and plant height existed significantly (Zhou D. Y. et al., 1983; Shuen Z. L., 1988; Gue R. L. et al., 1989) or non-significantly (Zhang Z., 1989). But it was not absolute. Some semidwarf varieties had high protein contents (Wei Y. M., 1989).

The correlation between grain protein content and grain yield was significantly positive (Gue R. L. et al., 1989).

Grain protein content and other quality traits were influenced greatly by environmental conditions. A single wheat variety demonstrated different protein contents in different years, locations and farm management systems and even in different plants or tillers. The protein content of a variety varied from 8% to 18% depending on the

environmental conditions. Grain protein content and quality could, to some extent, be improved by certain cultivation techniques, especially by irrigation and fertilizer utilization in the later stages of development (Liu G. T., 1985).

Relationship between protein components and nutritional and processing qualities

The relationship between protein content and protein components

Different wheat varieties performed differently as regards quality, depending on variations in their protein contents and protein components. Positive correlations between grain protein content and gliadin and glutenin contents were most significant, having correlation coefficients of 0.8992 and 0.8257, respectively (Wang S. J., 1989).

Positive correlations existed between gliadin content and glutenin content, and between albumin content and globulin content. A negative correlation existed between gliadin or glutenin content and albumin or globulin content (Wang S. J., 1985; Huo Q. T., 1992).

Grain protein components and processing quality

The processing quality of a wheat variety was due mainly to the quantity and quality of the gluten, which was in turn governed by the gliadin and glutenin contents. The quantity and quality of gliadin governed dough elasticity and those of glutenin controlled dough plasticity. A variety had good processing quality when it had a proper proportion of gliadin and glutenin (Jang S. H., 1991; Zhao Y. M. et al., 1990). A larger amount of albumin and globulin would raise the nutritional quality of a variety, but too much of these would spoil the processing quality (Wang Z. D., 1987).

Inheritance of grain protein components

An extensive study on the inheritance of protein components would be helpful for the improvement of grain quality because the protein content and the proportion of protein components has a direct influence on nutritional and processing quality (Liu G. T. et al., 1988). Research results revealed that high values of absolute gliadin content, relative glutein content and relative globulin content were governed by recessive alleles and low values by dominant alleles (Huo Q. T. 1992).

The high-molecular-weight (HMW) subunits of glutenin, which are related to bread baking quality, showed great differences between varieties but were similar within a variety. They were inherited through dominance or maternal effects in the F_1 generation (Liu G. T. et al., 1988) and they were controlled by genes on chromosomes 1B and 1D, and probably on 4B (Liu G. T., 1990). α-gliadins, β-gliadins and r-gliadins were governed by genes located on the short arms of the sixth group of homologous chromosomes and the ω-gliadins on the short arms of the first group (Ding H., 1988).

Relationship between baking quality and other traits

Baking quality and gluten

Baking quality is a multiple trait and was the result of the co-action of gluten quantity and quality and the chemical characters of starch (starch quality and amylase activity) (Ding L. P., 1982; Wang L. Q. 1989).

A significant positive correlation was found between wet gluten content and protein content (Ling Z. J. et al., 1989; Xie R. S., 1990; Huo Q. T., 1992; Li Z. Z. et al., 1990). The correlations between gluten content and the sedimentation value and loaf volume were significant (Xie R. S., 1990), while the correlations between gluten content and some agronomic characteristics were negative (Xie R. S., 1990; Ling Z. J. et al., 1989).

The gluten quality of a wheat variety influenced its baking quality significantly. The glutenin in gluten mainly controlled dough mixing time, while the gliadin mainly governed loaf volume and crumb structure (Ding L. P., 1982; Li Z. Z., 1986; Wang L. Q. 1989). The inheritance of gluten quality in the offspring exhibited medium or partly positive transgression compared with that of its parents (Wang L. Q., 1989).

Baking quality and sedimentation value

Sedimentation value was an important criterion for evaluating gluten quality and it had a significant positive relationship with protein content and baking quality (Li Z. Z. et al., 1990; Xie R. S., 1990; Ling Z. J. et al., 1989; Huo Q. T., 1992). It was a trait with highly dominant inheritance. The heritability range for sedimentation value was 0.54-0.87 in the F_2 generation and 0.47-0.89 in the F_3 generation. The correlations between the plant lines in the F_2 generation and the plant systems in the F_3 generation were positive, as were those between the plant systems in the F_3 generation and the F_4 generation (Wang L. Q., 1989). Therefore, it was worth selecting sedimentation value beginning from the F_2 generation.

Baking quality and Pelshenke Test Number

The Pelshenke test number of the wheat grain, a measure of the quality of the wheat flour, had significant relationship with loaf volume and dough mixing time (Li Z. Z. et al., 1990). The heritability of the Pelshenke test number, which was governed by multigenes with additive effects, was high (Li Z. Z. et al., 1990; Wang L. Q., 1989).

Baking quality and starch characters

The quality of the starch is inherited through multiple genes. The activity of the amylases, especially α-amylase, could be measured by the falling number and using a viscosimeter. The falling number of the wheat grain is an indirect criterion indicating the

α-amylase content in the grain. Amylase activity is an inherited trait of a wheat variety (Ding L. P., 1982; Wang L. Q., 1989). It is not clear, however, how the amylases influence baking quality (Wang L. Q., 1989).

Conclusion

Even though wheat quality breeding aimed at improving bread baking quality was not begun untill the 1980s, many genotypes have been collected and some wheat cultivars with good baking quality have been put into production. Theoretic studies on some aspects of wheat quality breeding have served breeding practice directly and indirectly. The breeding methods and testing techniques for quality traits are gradually being completed and standardised. Some wheat breeders have been to advanced countries to learn quality trait testing techniques and the relevant apparatus for testing quality traits has been imported and put into use. Foreign genotypes with different good quality characteristics are imported to China every year but most of them are not adopted to Chinese conditions and possess late maturity, tall stature and susceptibility to diseases.

Crossing programs involving multiple crosses and back crosses, and offspring selection using the pedigree method are mostly used in China. The screening of offsprings for good quality traits is begun in the F_2 generation using easy testing methods requiring small amounts of samples, such as the SDS sedimentation test, the Kjeldahl method, etc. The gluten content, rheological and baking properties of the offspring are tested after F_4 generation.

Since the Chinese diet is different from that of other countries, wheat quality breeding for other end uses, such as steamed bread, noodles, dumplings and cakes, etc., is also being carried out and the quality criteria, testing methods and apparatus required for these end products are being studied.

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Animal husbandry and genetics

THE ROLE OF GROUP SIZE IN DEVELOPING HOUSING MANAGEMENT OF DAIRY COWS I. EFFECTS ON MILK PRODUCTION

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Two experiments with two replications in each were carried out to study the effect of group size on milk yield. In Expt. 1 groups consisting of 30, 40, 50 and 80 lactating Holstein-Friesian cows, respectively, were housing in sheds with deep litter and concrete paved, uncovered feeding areas. In Expt. 2 15, 25, 30, 40, 55 and 71 Hungaro-Friesian cows were kept in confinement with free stalls and inside feeding alley. All cows were milked in herringbone milking parlours (2 raws and 8 milking units). Experimental periods lasted for a fortnight in all cases. Milk yield was recorded individually at each milking, milk samples were taken for chemical analysis at start and end of periods. Results reveal that group size may not exert any significant influence neither on milk yield per milking nor on milk composition. Thus, forming large groups are allowed in creating milk production units up to levels as it was investigated under the limitations of this study.

Keywords: dairy cow, loose housing, group size, milk yield, milk composition

Introduction

Present state of the question

To improve the profitability of milk production, it seems imperative to increase its efficiency and develop more rational technologies. In dairy farms there are two possibilities to achieve this goal: either by biological methods, genetic and selection work, or through physiological interventions on the one hand, and with technical methods, by improving the ecological factors, on the other hand. The question is, considering the necessary harmony between an animal and its environment, which method or combination of methods would result in optimum production. Becze et al. (1976) emphasize that the environmental factors act through their interrelations directly and quantitatively on the animal, and as such they are mutually inseparable. The regulatory function ensuring the homeostasis of the animal organism postulates tolerance. Individuals lacking adequate tolerance, unable to adapt themselves to the modern efficient technologies, fall out of production. Thus, the role of a state of balance between an animal and its environment cannot be left out of consideration. In keeping milk-type cows, the source of the adaptation problems is the lack of a good general condition of the animals shown by their behaviour, and the inadequate harmony between

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their needs and their environment. The task is two-way: everything must be made to adjust the technical environment to the needs of the animals on the one hand, and the animals' adaptability to the changed technical environment must be improved, on the other (Czakó, 1977, 1981). Czakó (1977) calls the change-over to the modern animal production a kind of second "domestication", since the animals are exposed to changes of nearly the same importance as in the early period of domestication.

No doubt, the biological demands of the animals cannot be fully satisfied, because the development of keeping technologies always needs compromises. Yet, improving the harmony is a task of basic importance, since the degree of efficiency of the energy transformation depends upon this. Namely, adaptation is an energy-consuming process (Kovács, 1980; Czakó and Sántha, 1984). Thus, genotypes with which an optimum production level can be attained under up-to-date conditions (Czakó, 1976), as well as technological solutions promoting the fulfilment of the animals' needs, must be found.

Under farm conditions, the cattle are forced (1) to comply with their group mates and with the group effects, and (2) to accommodate themselves to the technical solutions (Czakó, 1981). It is known that the cattle is kept in group, not against their natural bent, but because they have a definite need to have contact with their group mates. The explanation of this phenomenon is that animals belonging to the same group that graze and rest together want to accommodate themselves to the environment as a group rather than individually (Sambraus, 1975). Group forming is thus a natural phenomenon, and the role played by age is more decisive than that by body weight (Czakó and Sántha, 1977). Moreover, socializing is a definite specific demand of the cattle (Reinhardt, 1980). Consequently, the behaviour of not one animal but of the whole stock must be examined from the stock- and environment diagnostical standpoint (Kovács, 1980).

According to Arave and Albright (1981) the role of the group number and the number of groups to be formed has not been properly studied in the case of cattle. The cow stocks of experiment stations are relatively small in number, many divergent experiments are carried out with them, and the facilities available are not sufficient for investigations of this character either, although it would be an urgent task. On the basis of experiences gained in practice, it is a generally accepted opinion that the group number should not exceed 50–60, as this makes an optimum arrangement possible and reduces waiting by the milking house to minimum. The authors cited are of the opinion that the upper limit of the size of group is when the members of the group still can recognize each other. However, the recognizable number remains to be determined, and perhaps it cannot even be determined owing to the changing group mates and environmental background – they say. The group of non-domesticated cattle is strong up to a herd of 50, and this is the limit where cattle in loose housing still recognize their group mates (Kolb, 1981).

At the same time, in the question of group number – with the artificial environment, the preconditions of up-to-date keeping taken into consideration – the opinions vary according to the way the problem is examined.

Dohy (1977) emphasizes the interaction between genotype and group size. Although the cattle breeds do not vary concerning their accommodation to the modern technologies, it is still necessary to develop genotypes with which an optimum production

level can be attained. In the case of cattle, those populations are preferable in which the genetic variance of certain qualitative properties is high, while that of the responses given to the environment is low (Czakó, 1977). The technological tolerance as an aspect of selection becomes more and more dominant (Czakó and Sántha, 1984).

In their work on the modern feeding systems, Coppock, Bath and Harris (1981) say that in the United States the improvement in the output of milk production is due first of all to an increase in the genetic trend. Of the variance of the milk production of cows some 25% can be attributed to genetic effects. Besides, dairy farms strong in numbers become more and more frequent. With an increase in the farm accommodations the technology is focussed on the cow groups, neglecting individuality. Earlier, in 1955, a mere 6% of the cows were milked in milking parlours, but this proportion grew by 1980 to 60%. Group feeding has come into prominence, where in the case of *ad libitum* feeding the dominance relations – if the cows have become accustomed to the technological system – play hardly – if any – role. The technological research has not paid sufficient attention to the role of the group number. There are hardly any data on the number of cows per group that would offer sufficient information for the optimum utilization of fodder. Parallel with an increase in the population number, technological and feeding aspects are mostly taken into consideration when grouping the cows. Following regrouping, the social structure in groups small in number is formed anew in 24 hours, and though agressivity redoubles during this period, it does not affect the milk production. According to Coppock (1977) out of the social hierarchy and the effects of feeding, the latter are much more spectacular than those resulting from the breaking up of the social organization. In dairy farms strong in numbers, placing of animals in sub-groups has – according to the author cited above – the advantage that the feed rations of full value need less modification, if there are more than one – preferably a minimum of 3 groups. In the case of smaller groups the population undoubtedly can be better kept in hand, but the labour costs of feeding will grow, and an increase in the investment costs of the farm cannot be left out of consideration either (Sainsbury, 1967).

To summarize, with the various elements and components of the technological systems of up-to-date milk production taken into consideration, it seemed necessary to study the role of the group number, with special regard to the trend of milk production. All the more so, because in this subject many questions are uncleared, the answers to which are indispensable for developing the technological systems concerned.

Our experiment were planned, carried out and evaluated on the above considerations with the aim of answering to the following concrete questions:

– Is the number of cows kept together an important factor from the standpoint of the amount of milk per milking?

– Is the daily variation of milk production in correlation with the group number, and is there any interaction between the two factors?

– Does accommodation within the cow shed in the case of the same number of animals cause any considerable difference in milk production per milking?

Materials and methods

In order to settle the above questions, we carried out two experiments.

In the course of the first experiment two groups of 80 Holstein-Frisian cows each, in the second half of their first lactation period, were kept loose in deep-litter house connected with a concrete covered exercise with roofed feedbox. The resting space was 5.9 m^2, the exercise yard 5.4 m^2, the length of feeding box 0.9 m per animal, irrespective of the group number. The experiment consisted of three phases, 14 days each, with A and B parallel replications. In the first phase of replication A, the 80 cows were kept in one group; in the second phase, they were divided at random in two groups of 40 animals each, one of which was placed at the edge, the other in the middle of the house; in the third phase, the animals were divided in a smaller and a larger group of 30 and 50, respectively and similarly at random. In the first, second and third phases of replication B, the group numbers were: 30 and 50; 80; and 40-40, respectively.

Replications	Experimental phases		
	I	II	III
A	80	$40_e + 40_m$	30+50
B	30+50	80	$40_e + 40_m$

e = groups placed at the edge of the cow shed
m = groups placed in the middle of the cow shed

The animals were allowed to consume the complete feed rations of full value portioned several times daily *ad libitum* during the 24 hours of the day. The cows were milked twice a day: from 8 to 9 p.m. and from 10 to 12 a.m. in a herringbone milking parlour with 2 × 8 milking units, recording for each milking the order and time of entering the parlour and the amount of the milk produced, as well as the side of the parlour the animal went to. To determine the fat and protein content of milk, samples were taken from each cow on the occasion of evening milking on the 2nd and 11th day and at morning milking on the 3rd and 12th day of each experimental phase. The fat content of the preserved samples was determined by a turbidimetric Milkotester apparatus, and the protein content by Pro-milk equipment in the milk laboratory of the Cattle Breeding Dept. of the Research Institute for Animal Husbandry.

In the second experiment two groups, each of 70 Hungaro-Frisian milking cows of various lactation were kept in confinement with resting boxes. The walking and feeding area was 2.3 m^2/cow, the resting box 1.70 × 1.25 m, the length of feeding box 0.38 m/cow. In the same way as in the first experiment, in both – A and B – replications three experimental phases, each of 14 days were determined.

The experimental phases started in each case on Monday at 12^{00} a.m., and ended two weeks later at 12^{00} a.m. again. In the first phase of replication A, the cows were divided at random in two groups, one with 15 and one with 55 animals; in the second phase all the 70 cows were kept in one group; while in the third phase groups of 30 and 40 were formed. In the first phase of replication B, the 70 cows were together; then in the second experimental phase, groups of 30 and 40; while in the third phase, groups of 25 and 45 individuals were formed.

Replications	Experimental phases		
	I	II	III
A	15+55	70	30+40
B	70	30+40	25+45

In the feeding area formed within the cow-house the cows were allowed to consume the complete mixture portioned several times a day *ad libitum*. Milking took place in a herringbone milking parlour with 2 × 8 boxes between 8 and 9 p.m. and 8 and 9 a.m. In the same way as in the first experiment, the milk production, order and time of entering the parlour as well as the side on which the cow entered the parlour were recorded for each milking of each animal. Milk samples were taken from each cow separately on the occasion of the evening milking on the 2nd and 11th day, and at the morning milking on the 3rd and 12th day of each experimental phase; the fat- and protein content of the samples was determined by the same methods as in the first experiment.

When composing the groups, we took the stage of lactation, the milk production and the condition of the animals into consideration in both experiments.

The milk production data of both experiments were evaluated ANOVA after Weber (1972).

Results

First experiment

The trend of the amount of milk obtained per milking was evaluated by analysis of variance, where the aim was to separate the following effects: (1) replications (A and B), production levels ($x<16.2$ kg daily production, $16.2 \leq x \leq 19.5$ kg and $x>19.5$ kg daily milk), (3) milking time (evening and morning), (4) group number (80, 50, 30, 40_e and 40_m, where *e* and *m* indicate groups with the same number of animals placed at the edge, and in the middle of the cow shed, respectively).

The additive effects exerted, according to examination, on the amount and composition of the milk produced are summed up in Table 1. Since between the averages of the two parallel replications significant differences were not found, the data of the two experiments concerning groups with the same number of cows were combined for evaluation.

Table 1

Effect of production level, milking time and group number on the amount of milk produced per milking and on the composition of milk (First experiment)

Designation	Amount of milk (kg)	Composition of milk (%) butterfat	protein
1. *Production levels* (daily amount of milk)			
I. $x < 16,2$	7.39	–	–
II. $16.2 \leq x \leq 19.5$	8.91	–	–
III. $x > 19.5$	10.65	–	–
Significance	xxx	–	–
2. *Milking time - evening*	7.19	2.84	3.57
morning	10.73	2.63	3.54
Significance	xxx	xxx	xx
3. *Group number*			
80	8.95	2.83	3.56
50	8.86	2.66	3.59
30	9.10	2.77	3.40
40_e	8.88	2.69	3.57
40_m	8.99	2.66	3.62
Significance	N.S.	N.S.	xxx

e = groups placed at the edges of the cow-shed
m = groups placed in the middle of the cow-shed
N. S. = $P > 0.05$
xx = $P < 0.01$
xxx = $P < 0.001$

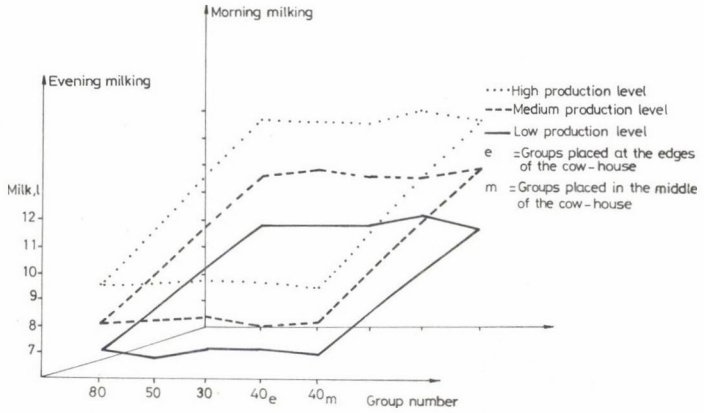

Fig. 1. Effect of group size on milk yield of dairy covos.
(Expt. 1. Combined means of replications A and B)

The differences between the mean values of the production levels are statistically significant at high level of probability. By forming classes our primary aim was to reduce the disturbing effect possibly originating from the large volume of milk production per milking. Within the different group size and identical group, the effect of number or arranging the animals in the cow-shed could not be pointed out, so we think that its role is insignificant in milk production. The effect of the milking time is highly significant; the ratio of evening to morning milk production is 40:60%, and the explanation is the different milking interval.

The interaction between group number and milking time is shown in Fig. 1. Its low measure, as explained by the figure, is that while within the group number the effect of the time of milking (evening and morning) is regular, with the averages of the amount of milk produced in the evening and in the morning, respectively, the mean values of the group number differ not in the same place. The data of butterfat content determined from the milk samples taken per milking at the beginning and end of the experimental phases were again evaluated by analysis of variance.

It is remarkable that the butterfat content is generally low compared to what can be expected from the breed, which can be explained by the fact that the cows were in the middle of their lactation period. On the other hand, they consumed a complete mixture based mostly on green forage, poor in crude fibre, with relatively high concentrate ratio, and as a result of these factors they were not able to fully realize their genetic capacities in butterfat production. As to our own aspects of examination, we consider the differences between the two replications, and between the mean values of the evening- and morning milking to be important. The causes are supposedly the different feed supply on the one hand, and the shifting of the milking intervals, on the other. The differences in mean values according to the group number are with a few exceptions non-significant and non-consequent. The difference pointed out can be traced back to the outstanding average of the group of 80. In the case of the protein content, although

among the additive effects the differences between replications A and B and between the evening and morning milk production are significant, still they do not appear to be important. In the course of evaluating according to the group number, we could not establish a definite systematical order for the averages of the protein content, in spite of the significant differences occurring.

Second experiment

Since in this experiment groups of 70, 55, 40, 30 and 15 animals in replication A, and of 70, 45, 40, 30 and 25 cows in replication B were formed, there was no possibility to point out the effects of the replications. Therefore, a combined evaluation was carried out. The following effects and interactions were analyzed: (1) production level ($x<12.1$ kg daily production of milk, $12.1 \leq x \leq 14.4$ kg daily amount of milk, or $x > 14.4$ kg milk production), (2) milking time (evening and morning) and (3) group number. The results are contained in Table 2.

Table 2

Effect of production level, milking time and group number on the amount of milk produced per milking and on the composition of milk (Second experiment)

Designation	Amount of milk (kg)	Composition of milk (%) butterfat	protein
1. *Production levels* (daily amount of milk)			
I. $x < 12.1$	5.41	–	–
II. $12.1 \leq x \leq 14.4$	6.69	–	–
III. $x > 14.4$	8.07	–	–
Significance	xxx	–	–
2. *Milking time*			
evening	6.53	4.93	4.09
morning	6.83	5.09	4.05
Significance	xxx	xx	N.S.
3. *Group number*			
70	6.70	5.08	4.04
55	7.21	4.62	4.00
45	6.50	4.96	4.09
40	6.56	5.17	4.13
30	6.54	5.06	4.11
25	6.43	5.08	4.12
15	6.93	4.83	4.01
Significance	xxx	xx	xxx

N. S. = $P > 0.05$
X = $P < 0.05$
xx = $P < 0.01$
xxx = $P < 0.001$

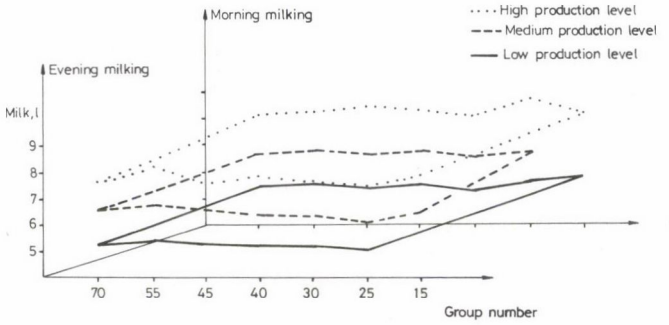

Fig. 2. Effect of group size on milk yield of dairy cows
(Expt. 2. Combined means of replications A and B)

Between the mean values of milk production per milking as broken down to production levels, highly significant differences were found. Although, between the averages of evening and morning milk production, the difference is not – in our opinion – great, their ratio being about 49:51, still the differences are significant. The differences between the averages of the group numbers – while mostly significant – are inconsequent. No linear scale can be set up between the mean values. Special mention is worth being made here of the 6.70 kg milk production average per milking in the cow group of 70, the 7.21 kg average in the group of 55 and the 6,43 average production in the group of 25.

We tried to give a visual illustration of the possible interaction between milking time (evening or morning) and group number (Fig. 2). According to this figure, the role of the milking time is unequivocal, namely, the average amount of milk produced in the morning generally is more than that produced in the evening. At the same time, the differences between the group averages separated by milking time are inconsequent. The figure clearly shows the moderate role of these interactions.

The results of the butterfat analyses of milk samples taken per cow and per milking were evaluated by combining the two replications. The variance sources separated in the variance analysis are: (1) effect of group number and (2) of milking time. The additive effect of the milking time was found to be significant, with lower butterfat values for the evening milk and higher ones for the morning milk, unlike the earlier experiences. The significant difference in the group number is due to the fact that, in the case of the group of 55, the mean value of the butterfat content is very low.

According to the results of analyses of milk protein contents, the additive significant differences of mean values according to group number probably originate from the interaction of group number and milking time. Namely, within the group number the milk protein contents of evening and morning milk differ considerably only in the groups of 45, 40 and 30, while in a comparison of the two milking times by group number, significant differences only appear among the mean values of the evening milking.

Discussion

From the results of our examinations, we can conclude that, in the trend of the amount of milk produced per milking, no important role is played by the size of the group – at least up to a number approaching 100 –, taking the frames of the present investigation into consideration. In this trend the effect of the shift in the milking interval is unambiguous; the amount of milk produced following the longer milking interval increases proportionately to time, consequently the amount of milk produced after the shorter interval will be smaller. The concentration of milk changes accordingly: decreases or increases, respectively.

The publications in which the effect of group number on milk production is analyzed are very few. On this subject, research work has but recently started.

In the experiment carried out by Nagy (1983) with Holstein-Friesian F_1 cows, milking cows in groups of 50, though they produced more milk than those in groups of 100, the difference compared to the milk production of the group of 20 still was not significant. In our earlier experiments (Szűcs et al. 1984) we also found that the amount of milk produced in the evening and in the morning was independent from the group number; and, in the case of identical number from the arrangement of cows within the shed, it only varied with the milking interval. Czakó (1981) reported a 6–8% increase in milk production when the cows were kept in smaller groups. Czakó and Sántha (1984) see the advantage of keeping cows in small groups in a 8–12% increase in milk output. In our earlier investigations (Szűcs et al., 1977) young fattening bulls responded with decreased performance to increased housing density and group number, though the individual reactions of the animals were highly varied. According to data by Eckstein and Seidemann (1972) under the given condition (5, 10, 20 and 40 individuals, respectively, in one group) keeping in a large group did not cause decrease in performance when raising young heifers. The milk production and health condition of cows kept in groups of 65–70 in a house with slatted floor did not change, compared to the earlier used groups of 30 – reported by Kaiser (1971). In his opinion larger cow groups can be fit in without the depression of milk production in the technological processes. With dairy farms of 1000 taken into consideration, Admin and Zjukina (1977) think it expedient to form groups of 100, although they reckon with the possibility of forming sub-groups, e.g. of freshly milked cows, depending on the technological demands. In a block-type dairy farm with accommodation for 3200 Andrejev (1980) composed groups of 24, 48, 64, 80 or even more, and found that in groups of 10 to 100 the competition for feed had an unfavourable effect on feed intake, milkability and milk production.

Evaluating the literary data and summarizing the results of our own investigations, we think it reasonable to draw the following conclusions:

– Under the conditions described, the role of the group number is not important in regard to the amount of milk produced per milking. This means that cows can be kept in larger groups without any disadvantage to milk production.

– The effect of milking intervals is unambiguous, and the amount of milk produced following a longer interval is larger than it is after a shorter interval.

– We think that the parameters of milk concentration are not primarily influenced by the systematic effects we studied, but the role of other effects, e.g. the effect of feeding and of the stage of lactation, may be much more decisive. The identification of the effects mentioned may form the subject of further experiments. The role of the milking interval in the trend of the butterfat content could be observed in this experiment, too.

– Insofar as the farm technology is not otherwise a limiting factor, then from the standpoint of milk production there is supposedly no objection to forming milk-cow groups of an even larger number. Moreover, we think it possible to compose groups somewhat more populous than the largest group in our experiment; that is, to extrapolate the results without any disadvantage to milk production.

– The effect of the arrangement of the cows within the cow-house concerning the factors examined was not observed.

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THE ROLE OF GROUP SIZE IN DEVELOPING HOUSING MANAGEMENT OF DAIRY COWS II. ETHOLOGICAL ASPECT

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In a 2 × 2 factorial experiment using lactating Holstein-Friesian and Hungaro-Friesian cows the effect of group size (15–80 head) was investigated on behaviour parameters of animals. Expt. 1 was conducted in cow sheds with deep litter serving as resting area which was connected to a conrete paved feeding area without cover. Expt. 2 was carried out on animals housed in confinement with free stalls. In the first and last 48 hours of experimental periods lasting for a fortnight behaviour pattern of animals were recorded individually and/or for each group at 10 minute intervals. Time spent resting, standing, eating and ruminating was recorded and calculated for 24 hours and circadian rhythm of behaviour traits was investigated, as well. It was concluded that cows kept in various size exhibit species specific behaviour pattern independent of the number of individuals. By adapting to the artificial environment supplied by the housing system cows utilise the time and space staying at their disposal. The daily rhythm of lying is biphased, that of eating is also biphased or occasionally triphased. Rumination was restricted to resting periods.

Keywords: dairy cow, loose housing, group size, behaviour, resting, eating, ruminating, circadian rhythm

Introduction

In cattle keeping, the animals are confined to fully artificial environmental conditions in order to increase the efficiency of production, while their keepers, with highly diversified motives, control many biological functions (Becze et al., 1976). According to Czakó and Sántha (1976) the reason for the 10–20% decline in production observed with a change-over from stanchion barn system to loose housing – often attributed to the technological tolerance – is due to other factors. They emphasize that the role of the examined factors of technological tolerance in production is not quite clear so far. On the other hand, 80% of the causes of culling is – mainly in the case of high producing cows – of technological origin. The deleterious effects of forced adaptation can be lessened in groups of 40–50 (Czakó and Sántha, 1984). The wrong group size may cause social tension (Gere, 1977). In large populations the social hierarchy cannot be observed, as it is usually present only in small groups where the members live together over a long period (Fraser, 1974). Other authors are also of the opinion that in larger groups definite social hierarchy can hardly develop, because the cows are not able to remember all their mates (Arave et al., 1974; Whittlestone et al., 1971). According to Kovalčikova (1981) the order of rank cannot be precisely established even in cow groups with more than 30 individuals, since only cows first in rank can be spotted. In the case of small groups the order of hierarchy is linear, while the same cannot be said for larger

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groups. In the opinion held by Syme (1974) the competitive order as a means of measuring the social dominance is not always one-dimensional, it is rather more a case of multidimensional structure. According to Sambraus (1975) the possible number of y rank relations in a population of x number is:

$$y = \frac{x(x-1)}{2}$$

Our calculations showed that in a group of 20 the possible rank relations were 190, in the case of 50 cows 1225, while with 100 individuals 4950 in number. However, the number of clear combinations varies between 50 and 100%, compared to the possible relations even in the case of a smaller population (Sambraus, 1975). A further problem in clarifying the dominance relations is raised by the necessity of a constant regrouping owing to technological considerations, although Coppock, Bath and Harris (1981) state that the cows can become accustomed to regrouping. The negative effect of repeated regrouping then gradually decreases. The social hierarchy, at the same time, exercises its function in competitive situation only (Arnold and Dudzinski, 1978; Brackel and Leis, 1976; Czakó, 1977, 1984; Friend and Polan, 1974; Gabr, 1973; Kongaard, 1981; Sambraus, 1973). This is supposed to explain that correlation is seldom found between social dominance and milk production (Albright, 1971; Czakó, 1978).

So, from the point of view of ethology it is highly important:

(1) to find out what behaviour response a group of animals gives to stressors arising from artificially developed keeping technology systems, highly differing from the natural biotops, and

(2) to determine the limits within which a genotype is able to adapt itself to the changing ecological conditions (Arave and Albright, 1981). Although according to Kovalčik and Kovalčikova (1978) the technological adaptability of cattle ranges between relatively wide limits, the effect of group number on behaviour has not been clarified at all (Porzig, 1980).

Starting from the above outlined facts, we thought it reasonable to look into the following questions:

– Do the fundamental behaviour functions – resting, feed intake, rumination – follow specific patterns in dairy-cow groups of different size?

– Does the comfort behaviour of cow groups of different size show any disturbance in choosing voluntarily the place of staying, depending on the technological system?

– How does the periodicity of resting, eating and rumination change with the group number?

Analysing the role of the group number we planned and carried on our examination series with the above objectives taken into consideration.

Materials and methods

Concerning the technologies applied and studied, and the arrangement of the experiments, we only refer here to the section "Materials and methods" in our first publication. As to the behaviour of the animals, we equally made ethological observations in the A and B replications of the first experiment. The observations were made at

the beginning of the experimental phases from 12 a.m. on the first day to 12 a.m. on the third day, and at the end of the experimental phases from 12 a.m. on the 10th to 12 a.m. on the 12th day, over 48 hours on each occasion, recording every 10 minutes the individual cows eating, and counting in each group the cows lying in the shed and in the walking area, and those standing and moving and ruminating. The data were summarized per hour and for the first and second 24 hours.

In the second experiment the behaviour studies were carried out with the same method as in the first experiment with the difference that they only covered the groups set in replication A.

On evaluating the behaviour studies, the eating time was subjected to analysis of variance after the method of Weber (1972). The average values of the other characteristics were analysed by simple comparison. The circadian rhythm of the behaviour patterns was drawn up on the basis of hourly summarized frequency with the aid of a plotter, using computer. The changes of lying, eating and rumination during the day in each group of A and B replications are represented with the first and second day of observations at the beginning and end of the experimental phase separately indicated.

Results

Resting, standing, eating, rumination

First experiment. Out of the results of the behaviour studies the combined average values of the daily time spent by cows kept in groups of different size in lying, standing, eating and rumination are shown in Table 1. Within this, the time spent by the animals in standing in the exercise yard and in the resting area, respectively, was also recorded. The cows spent 50–55% of the day in standing and moving. The ratio of staying within this in the resting area and in the exercise yard combined with feeding alley, respectively, ranged between 15:85 and 11:89% in the cow groups of different number. In the case of the same number of cows (n = 40) there was no essential difference according to either

Table 1

Daily averages of behaviour patterns
(First experiment, A and B replications)

Designation	Group number				
	80	50	40_e	40_m	30
Standing/moving total	755	766	733	726	792
exercise yard	650	672	650	646	677
resting area	105	94	83	80	115
Lying/resting total	684	674	707	715	648
exercise yard	102	89	99	110	117
resting area	582	585	609	605	531
Eating total	299	304	303	311	326
Rumination total	379	396	373	394	398
lying	290	305	281	306	300
standing	89	91	92	88	98

e = groups placed at the edges of the cow-shed
m = groups placed in the middle of the cow-shed

the average time of standing or place of staying. However, it is remarkable that in groups of 30 the time of standing and moving slightly increases (55%), and the proportion of the time of standing in the resting area rises. Nevertheless the phenomenon remains within the limit values of biological behaviour. The time spent by the cows in resting is 45–50% of 24 hours. On warm summer nights, the cows liked to lie down even in the concrete covered exercise yard; the ratio of lying in the exercise yard and in the deep litter resting area ranged between 13:87 and 18:82.

In the first experiment the cows spent an average 20–22% of the daily time available for them in consuming their feed rations. Since in the course of the behaviour studies the eating time of the animals was individually fixed, there was possibility for a statistical analysis. According to the analysis, with the reduction of the group number the daily time of eating significantly increased, even if only in a small measure. The primary cause of the difference is that the mean value obtained with the group of 30 is higher than in all the other groups.

The fact that the mean values obtained at the beginning and end of the experimental phases and on the first and second day show no considerable differences confirms the adaptability of cows after regrouping.

The daily time of rumination was found to be 26–28%. The ratio of ruminating, lying to standing, ranged between 75:25% and 78:22%. Signs suggesting systematic effects by group number were not found.

In the first experiment, the role of the group number in the trend of the basic behaviour data could hardly be decisive. The differences in average values – according to whether the latter originated from the beginning or the end of the experimental phases, or from the first or the second day of observation –varied between the biological limit values, and signs suggesting abnormal changes were not found in any of the behaviour parameters.

Second experiment. Ethological studies were possible in the A replication of the second experiment. The cows were kept in a shed with resting boxes. The average values of the basic parameters of behaviour – broken down as described in the first experiment – are contained in Table 2. The standing and moving of the animals was 49–52%, irrespective of the group size. Within this the proportion of the time spent in standing in the walking areas and the feeding area was 72–84%, the ratio of the time spent in standing in the resting boxes to the total time of standing was 18–28%. In the resting boxes 48–51% of the 24 hours was spent by the cows in lying. It seems that variations in the group number did not influence the average values of the daily time of lying. In the walking areas the animals never lay down.

An analysis of the mean values of daily eating times in the different treatments shows that the time spent in eating was about 24%, and as to its tendency the cows took longer to eat when the group number was reduced; the effect was significant.

The mean values of the daily total time of rumination did not considerably change with the reduction of the group number, their proportion in the 24 hours of the day is 30–40%. The ratio of rumination lying, and standing in the walking area and resting boxes ranged between 71–29% and 82–18%, unambiguous tendencies suggesting the effect of the group number were not found.

Table 2

Daily averages of behaviour patterns
(Second experiment, A replication)

Designation	Group number				
	70	55	40	30	15
Standing/moving total	715	748	710	724	745
exercise yard	568	582	561	524	625
resting area	147	166	149	200	135
Lying/resting total	725	692	730	716	695
Eating total	337	332	374	361	399
Rumination total	465	469	426	458	495
lying	370	331	350	351	359
standing	95	138	76	107	136

Since abnormal averages deviating from the natural limits of value were not found, it seems that the role played by the group number in the basic patterns of behaviour was not considerable under the conditions of the present examination series. Keeping in groups of different siez and regrouping were tolerated by the cows, due to their adaptability.

Circadian rhythm of the behaviour patterns

First experiment. It is known that cattle in a natural keeping system follow a concerted, allelomimetic activity in accordance with their specific behaviour patterns. Starting from the mentioned phenomenon, we thought it proper to examine what the trend of the daily continuity – circadian rhythm – of the basic behaviour patterns of milk-type cows kept in groups of different size was. On the basis of the frequency of occurrence we drew the daily changes of lying, eating and rumination for each group of replication A and B, by means of a computer. The drawings show the first and second-day results side by side, the results recorded at the beginning and end of the experimental phases separately in a coordinate system. Through the outlined method a visual analysis of occasional differences coming from the group number, the adaptation and the differences of the days of observation became possible.

We have no possibility to publish all figures within the present paper, so we confine ourselves to show the circadian rhythm of the groups of cows consisting of 30 and 80 individuals, respectively, from replication A concerning lying/resting, eating and rumination, in Figs. 1 and 2.

The figures show at once that the natural daily circadian rhythm of resting, eating and rumination is determined – irrespective of the size of group – first of all by the technological operations, and among them by milking above all. In fact, the daily twice of milking form the limit values in the daily continuity of the behaviour patterns.

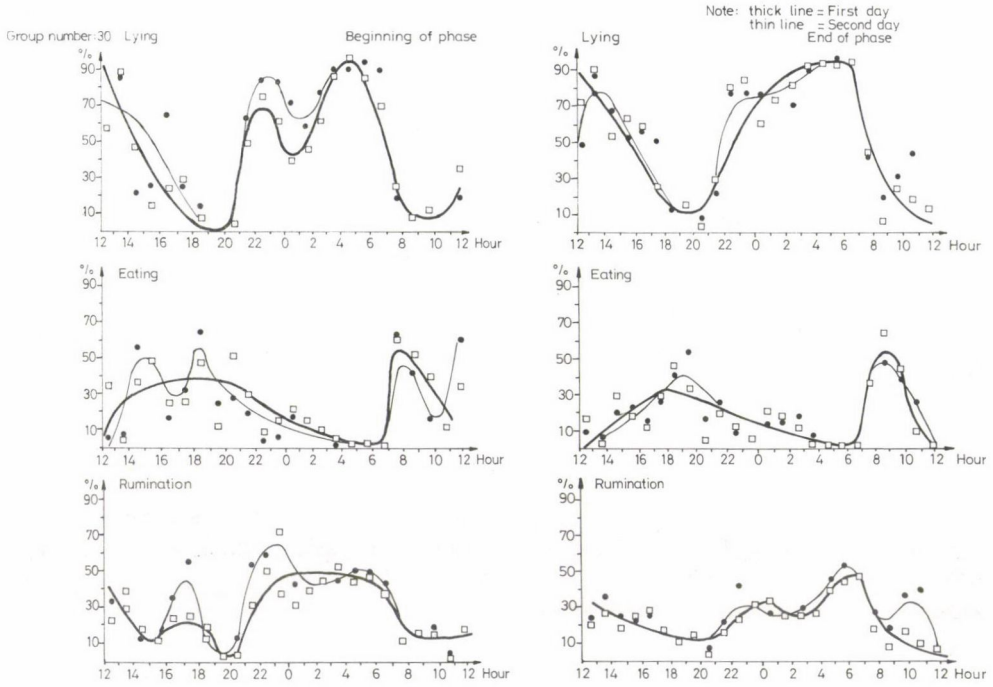

Fig. 1. Effect of group size on circadian rhythm of behaviour pattern of dairy cows in sheds with deep litter (Expt. 1, replication A)

As for lying, it consists of two phases: a shorter daily and a longer night phase. In the longer night phase, following the evening milking, the numerical proportion of cows lying down suddenly increases, to reach at a slowing rate maximum at dawn. After sunrise, at the time of the first feed distribution the cows stand up and go to the feeding troughs in large numbers. The shorter daily resting period following the morning milking shows a more varied picture; having reached the peak it ceases at a decreasing rate. From time to time it happens that the continuity of the phases is temporarily broken by a short period of activity due to highly diversified causes, e.g. littering, storm and so on.

Eating shows a more varied pattern. It must be emphasized that on the farm complete rations were distributed several times during the day, and the cows consumed it *ad libitum*. Feed was regularly before the animals long after midnight. In fact, two, occasionally three, main eating periods can be determined; the curves are, however, less peaky, and the phases gradually flatten. The eating periods are occasionally broken by milking late in the morning. After the appearance of the feed-distributor cart the cows go to the feeding troughs and eat in large numbers, irrespective of the size of group.

The daily continuity of rumination also can be divided into two periods, which follow the periodicity of lying, a shorter daily and a longer night period. However, in consequence of the shorter daily total time of rumination, its curve is placed lower in the system of coordinates, compared to lying.

The results are more characteristic of the keeping system, and this did not cause abnormal behaviour patterns. The role of the size of group was not found to be decisive. Minor differences in the circadian rhythm of the behaviour patterns between the two days of observation and between the beginning and the end of the experimental phases can partly be explained by daily changes in the technological operation. At the same time, the similarity of the curves characteristic of the beginning and end of the experimental phases suggests that regrouping hardly causes disturbance in the circadian rhythm of the behaviour of cows kept in groups of different size. Moreover, owing to the adaptability of the animals, there is no depression in their behaviour.

Second experiment. In the second experiment (Figs 3 and 4) a longer night and a shorter daily lying period can be distinguished, separated first of all by the milking times. The role of the group number is not felt in the circadian rhythm of resting. The cows ate every hour of the day, and cows consuming feed could almost always be found by the feeding troughs. The eating curves usually are two-peaked. The fact that eating still was protracted can be traced back to the 50% feeding space of the keeping system. The daily continuity of rumination – though at a lower level – follows the rhythm of lying, the curves usually are two-peaked. Yet, similarly to the curve of eating, they are flatter than in the first experiment. The peaks naturally can be clearly seen.

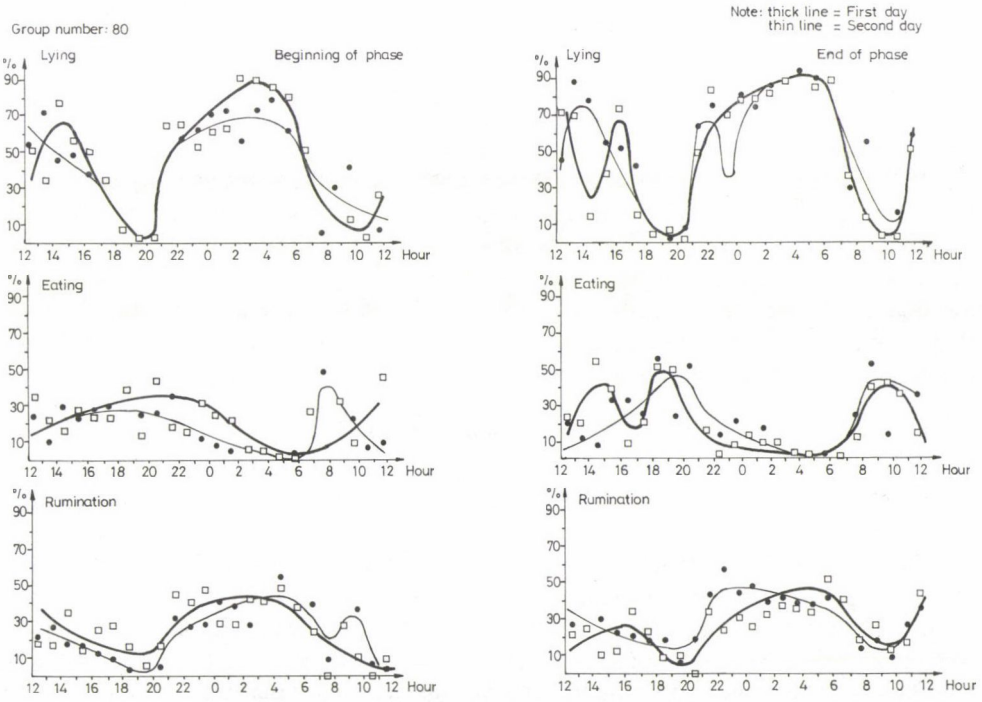

Fig. 2. Effect of group size on circadian rhythm of behaviour pattern of dairy cows in sheds with deep litter (Expt. 1, replication A)

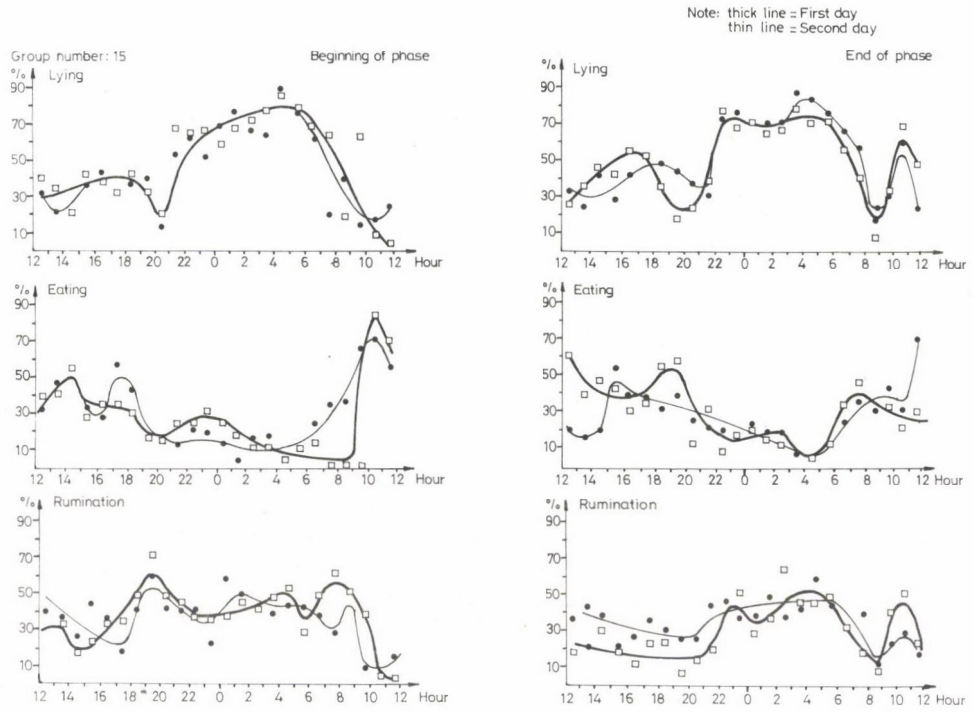

Fig. 3. Effect of group size on circadian rhythm of behaviour of dairy cows in sheds with free stalls(Expt. 2, replication A)

The effect of group number was not observed in the circadian rhythm of the behaviour patterns. They did not even show considerable differences on the first and second day of observation, nor at the beginning and end of the experimental phases. At the beginning of the experimental phases following regrouping, no signs suggesting disturbance in the circadian rhythm of the cows' behaviour patterns were observed. Regrouping was readily tolerated by the cows, they almost at once accommodated themselves to the new conditions.

Discussion

Average values of basic behaviour parameters, and their distribution

In his lecture discussing keeping systems that satisfy the demands of animals, Rist (1981) proposed an evaluation according to pathological, physiological and ethological parameters. He mentioned that a keeping system is suitable when the course, length of time and frequency of the different behaviour patterns do not much deviate from the specific ones. The aim of the ethological investigations is peculiarly specific, depending on the animal population used and the technology applied – writes Kovalčik (1981). The animals must have sufficient time to rest and to consume their feed, and such a situation

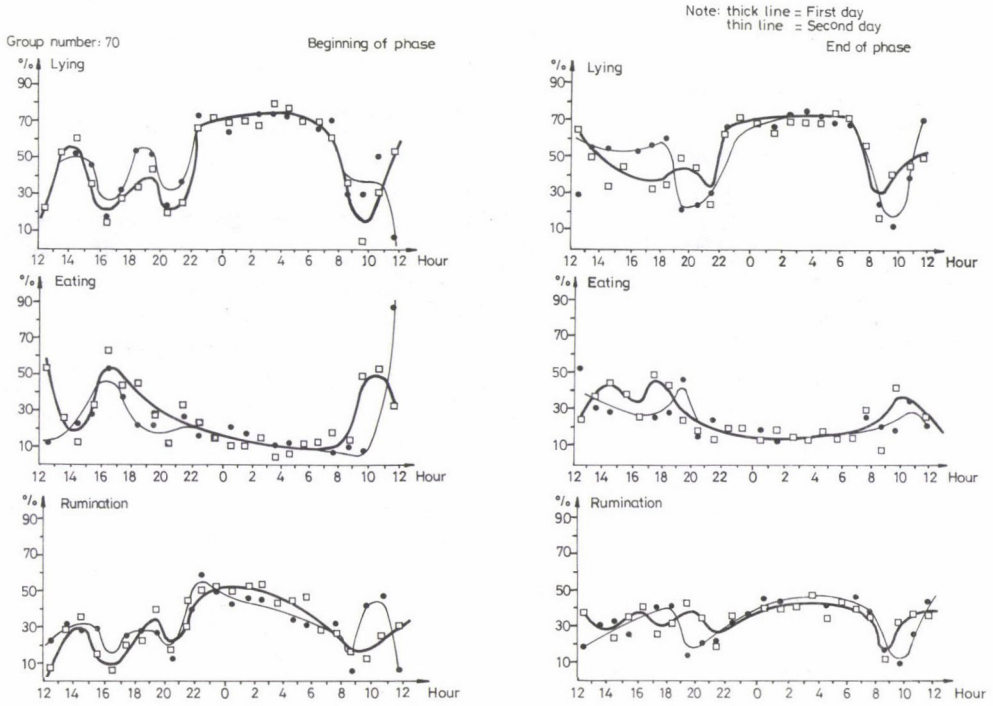

Fig. 4. Effect of group size on circadian rhythm of behaviour of dairy cows in sheds with free stalls(Expt. 2, replication A)

should possibly be created in which conflicts between the animals seldom occur. In regard to social tension, the number of cows within a group may play a special role. On the basis of experiences gained in practice, the generally accepted opinion is – according to Arave and Albright (1981) – that the group size possibly should not exceed 50–60 individuals, since this makes a classification according to production possible, whereby the time of waiting before milking is reduced to minimum. At the same time, establishing a stable social structure is a reasonable aim, and its precondition is that the animals know each other. However, the dominant hierarchy is only functional when some resource of vital importance is absent. According to Coppock et al. (1981) out of the effects of disorders of feeding and social structure the former plays the primary role. The behaviour as a signalizing system, as an integrated phenomenon of life will show whether the environment ensured for the animal satisfies its demands (Czakó, 1977).

The primary indicators of the comfort behaviour, in the case of different group number as well, are the daily periods of the basic behaviour patterns, such as standing and moving, lying and resting, feed intake and rumination. The periods of resting, eating and rumination can be regarded as normal when they range between the limit values of 33–59%, 8–40% and 17–40% of the 24 hours, respectively. In our preliminary-, first and second experiment these values were within the intervallums indicated (lying: 45–55 and 48–51%, eating: 20–22 and 23–28%, rumination: 26–28 and 30–34%). Nagy (1980) found similar times, 23% for eating and 31% for rumination, while Czakó et al., (1981)

pointed out 40–55% for lying and 21% for eating in a cow group of 60–80 kept in a loose housing system. They did not find signs suggesting aggressiveness.

In the first and second experiment the cows spent 21–33%, 22-25% and 18–27% of the total time of rumination, respectively, ruminating standing. The proportion of ruminating while lying was in the same order: 67–79%, 75–78% and 73–82%. Cattle are known to ruminate mostly lying (Bárczy and Czakó, 1962; Dávid, 1983; Fraser, 1974; Czakó, 1978; Ruckebusch, 1970; Porzig, Tembrock, Engelmann, Signoret and Czakó, 1969; Molnár et al., 1977; Szabó and Béri, 1984; Okomoto, 1976; Gordon and McAllister, 1970). The fact that in the above investigations the milk-cows spent about 75% of the total period of rumination while lying, irrespective of the size of group, suggests that changes in the number of animals in a group had no disturbing effect.

From the basic behaviour data obtained in our own experiments and published by the relevant literature, respectively, we can draw the conclusion that, in the case of a smooth technology of keeping, feeding and milking, the size of group has hardly any decisive role within the examined limit values from the standpoint of the daily period of resting, eating and rumination. Namely, the cattle are able to accommodate themselves to the technology within rather wide limits (Kovalčik and Kovalčikova, 1978).

The place of the cows' staying is a function of the technology applied. In our first experiment the cow-house had a deep-littered resting area connected with a exercise yard with outdoor feeding alley. The groups of 40-80 spent 52% of the daily 24 hours in the exercise yard and 48% in the resting area. Only the groups of 30 stayed somewhat longer (55%) in the exercise yard, which partly can be explained with a slight increase in the period of eating. The lying period also decreased, though to a small extent. However, in a characteristic way the cows in the group of 30 used the exercise yard for a longer time. In the course of investigations made by Czakó et al. (1981) from the daily 44% lying time the cows rested 23–25% in the cow-shed and 18–19% in the exercise yard. In the experiment conducted by Bárczy and Czakó (1962) in an open cow-shed the cows spent the larger part of the day in the shed not only in the rainy and colder November days but even in July. In a keeping system with resting box, indoor feeding space and outdoor exercise yard Ádám et al. (1977) found that in summer the cows liked to lie down in the exercise yard, while owing to the feed uptake in the house they mostly stood. Of the 24 hours of the day in summer they spent 43% in the corral, 50% in the house. In winter the time spent in the corral fell to 5%. In warm weather the cows do not like to stay in the shadeless corral. On windy, snowy days they stayed in the cow-house. In the course of earlier investigations (Szűcs and Molnár, 1975) we found that in a deep-littered cow-shed including a feeding space and combined with concrete covered exercise yard the cows spent in summer 38.2% of the day in the exercise yard, 53.9% in the littered resting place and 7.9% in the waiting place and milking shed. The proportions did not considerably change in winter, they were in the above order: 42.2, 50.8 and 7.0%. In the exercise yard the cows did not lie down, while of the time spent in the resting place they lied 80–85%. In the experiment of Metz, Mekking and van Diepen (1981) the extension of the exercise yard increased the time spent there, but it did not influence either the eating or the ruminating time or even the milk production. The time of lying in the boxes decreased.

In the second experiment cow groups of different size were kept in confinement with resting boxes. The cows spent 49–52% of the day in standing and moving and 48–51% in lying. The animals lied exclusively in the boxes. The proportion of the time spent standing in the boxes was 18–28% of the total time of standing. Walker-Love and Laird (1964) found that the Ayrshire and Friesian cows spent 48,4% of the day lying, 7.2% standing in the resting boxes, and the proportion of the day spent in the resting space outside the boxes was 4.95%. The grey Sakar cows kept in herds of 100–200 by Tomova and Gorinov (1981) spent 44 and 47%, respectively, of 24 hours in the exercise yard instead of the covered shed even at temperatures of +2 – –6°C. They think that the primitive cattle breeds do not demand special buildings. According to Wander and Fricke (1969) the cows visit the cow-shed with a regular daily periodicity irrespective of the season:

Part of the day	Use of the cow-shed (time %)	
0 – 6 a.m. night rest (2nd part of the night)	high	80 – 100%
6 – 10 a.m. feeding (morning)	low	0 – 20%
10 a.m. – 2 p.m. day-time rest (afternoon)	medium	40 – 50%
2 – 6 p.m. feeding (afternoon)	low	0 – 20%
6 – 12 p.m. night rest (1st part of the night)	increasing	50 – 70%

In the course of our investigations the cows made use of the available space and time by accommodating themselves to the artificial environment provided by the housing system, the technology did not restrict their specific demands to such an extent that in their interaction with the varying group number they would have become a limiting factor concerning the comfort behaviour.

Daily periodicity of the behaviour patterns

Discussing the role of allelomimetics in the large-scale housing system of farm animals Altmann (1981) says that, in the case of a change-over from a small-group to a large-group housing system, the individual is affected not only by the change in the physical environment but also by the increased size of the group, as social interactions make their effects felt. Among social animals, copying the behaviour is a typical phenomenon, variously known as group effect, transmission of mood, collective action or allelomimetics. It is an innate mechanism, and can be harmful (e.g. panic), but may even be useful, when through the simultaneity of the behaviour, it makes living together more favourable for the group. From the point of view of the group, it is favourable if the behaviour is coordinated in space and time. It may play an important role in a production-oriented, purposeful farm animal keeping, because the disturbance of the rhythms of resting and activity may cause reduction of performance. From the point of view of the individual and its performance, the copying of behaviour lies in the synchronization of behaviour within the group. In the life of milk type cows, group activity plays an important role. In cattle the circadian basic rhythm of the behaviour patterns has been described by many authors (Metz, 1975; Czakó et al., 1981; Tschirch

and Sommer, 1970; Szűcs et al., 1979; Sambraus, 1975; Barker et al., 1973; Bogart et al., 1977; Nagy, 1980; Pearce, 1965; Gere, 1977; Walker-Love and Laird, 1965; Okomoto, 1976; Molnár et al., 1977; Szabó and Béri 1984; Dregus et al., 1979; Webb et al., 1963; Legosin et al., 1976; Schmisseur et al., 1966; Porzig et al., 1969; Ruckebusch, 1970; Czakó, 1978; Szűcs and Molnár, 1975; Ádám et al., 1977; Campling and Morgan, 1981; Arnold and Dudzinski, 1978; Dávid, 1983; Bárczy and Czakó, 1962; Fraser, 1974; Kolb, 1981; Stricklin et al., 1976). The animal systematically divides the 24 hours of its day into resting and activity periods – writes Koch (1963).

Normal is the behaviour – states Porzig (1980), if most of the individuals of a species or breed of animal show the same activity under certain environmental conditions. His definition for the concept of behaviour disorder is: "Spontaneous changes in behaviour in consequence of unfavourable environmental conditions which in some individuals of a given group lead to temporary or lasting deviation from the normal patterns of behaviour, weaken the resistance of the organism to pathogens." He emphasizes that the relation of stocking density and size of group to possible behaviour disorders has not been cleared up at all. Although the technological system employed may modify the behaviour, the natural basic rhythm of the latter remains unchanged (Gordon and McAllister, 1970). Under natural conditions the illumination, in artificial biotope the technological operations, play a role in its regulation (Kovalčik and Kovalčikova, 1978; Gordon and McAllister, 1970; Metz, 1975; Sambraus, 1971; Bárczy and Czakó, 1962). It is therefore indispensable when developing a rational housing system to take into consideration the behaviour of the animal and satisfy its specific demands (Nagy, 1979).

In the first and second experiment we examined closely how in two loose-housing systems the daily periodicity of the behaviour patterns – lying, eating, rumination – changed, and whether disorders in their circadian rhythms could be observed under the influence of the varying group number. Porzig (1980) pointed out disorders of this character in the daily rhythm of eating depending e.g. on the feeding technique. In combibox the eating curve was two-peaked, in the case of silage self-feeding, it was unnaturally even throughout the 24 hours of the day, while in self-binding combibox it was definitely two-peaked. The author traces back the disorder to a frustrated condition that acts as a stressor. In the circadian rhythm of rumination Hughes and Reid, as well as Rakes (cited by Gordon and McAllister, 1970) also pointed out similar disorders.

Under natural conditions, in the pasture the main resting period of the primitive cattle breeds falls to the night hours (Arnold and Dudzinski, 1978). Dávid (1983) found that, of the daily 8.5–9-hour resting time, the proportion of the night period was 75–85%. According to Porzig et al. (1969) the main lying period in the pasture is between 10 p.m. and 5 a.m. In the case of milk-type cows the main lying period also falls to the night hours (Legosin et al., 1975). Gere (1977) found that the periodicity of lying was determined by the working order, half of he daily lying period fell between 8 p.m. and 3 a.m. According to our earlier examinations 55% of the lying period was between 9 p.m. and 6 a.m. (Molnár and Szűcs, 1977). In another examination series (Dregus et al., 1979) the distribution of the two main lying periods – between 6.30 p.m and 8.30 a.m. at night and between 8.30 a.m. and 6.30. p.m. in daytime – was 65–87 and 35–13% respectively.

Our own examination results reconfirm the earlier observations that in the loose housing system of cows the circadian rhythm of lying has two phases, and its division is primarily determined by the technological operations. The night period of lying usually is longer, the day-time one shorter. In our experiments the periodicity of lying was independent from the size of group, and corresponded to the specific behaviour characteristics of cattle.

The circadian rhythm of feed intake is most strikingly described by Kolb (1981). In summer grazing, it can be divided in 5 phases: (1) from 4 to 6 a.m (2) from 8 to 10 a.m., (3) from 11.30 a.m. to 12.30 p.m., (4) from 3 p.m. to 4.30 p.m. and (5) from 6.30 p.m. to 8.30 p.m.; in October the number of the periods is reduced to four. The Hungarian authors Szabó and Béri (1984) found that the Hungarian Grey cows graze in daily three, the Hereford cows in daily five, phases. Porzig et al., (1969) distinguish absolute grazing times. Cattle also graze at night (Dávid, 1983; Szabó and Béri, 1984; Stricklin et al. 1976). Arnold and Dudzinski (1978) call the night grazing a „secondary" eating period. In warm weather a grazing cow may consume more at night than in daytime (Fraser, 1974). The main periods are: shortly before sunrise, in the morning, early in the afternoon and at evening twilight. The intervals are filled in with lounging, resting and rumination. Arnold and Dudzinski (1978) note, however, that the rhythm of the feed intake activity of cow during grazing can be influenced by such factors as the temperature and humidity of air, the geographic situation, the genotype, the weather conditions (clouds, wind, rain), the available grass, and the individuality of the animal. The periodicity of the cows' grazing can be excellently calculated – Stricklin et al. (1976) emphasize.

According to Campling and Morgan (1981) in the case of housed milk-cows the daily rhythm of silage comsumption varied. In the case of self-feeding the largest number of cows were usually observed by the silage stack immediately before and after the afternoon milking, and the fewest between midnight and the morning milking. The number of the eating periods was 6 on the average, though it varied with the quality of the silage: in the case of a higher acid content silage the animals visited the feeding place more frequently than when the acid content of the silage was lower. With fodder supplied from automatic feeder we earlier found (Szűcs et al., 1981) that the daily fodder comsumption was approximately even; yet, the circadian rhythm in which active and less active phases (4 periods each) alternated could be observed. In the active periods the average fodder consumption per hour decreased, while in the less active periods it increased. Periodicity may have been in connection with the milking times and the time of portioning the mass fodder. Webb, Colenbrander, Blosser and Waldern (1963) reported that 74% of the total eating time fell between morning and 6 p.m., while the eating period between 3 and 6 p.m. was 27%. The phenomenon that in open and closed shed the cows eat at night was observed by Bárczy and Czakó (1962) too. With feeding *ad libitum* Nagy (1980) distinguished two main eating periods: one between 6 and 12 a.m., the other from noon to 6 p.m. In the cow-house with a resting box, Ádám et al. (1977) pointed out three peak periods of eating: between 5 and 6 p.m., and between 6 and 7 and 8 and 9 a.m. In the present study with complete rations given *ad libitum,* the periodicity of eating was also observed. We found two, occasionally three eating periods

in the circadian rhythm. However, this may be influenced by the available feeding space. In the course of our observations, we found that the cows ate even in the late evening hours, and though the number of cows staying by the feedbunks gradually decreased, eating was protracted far after midnight. The circadian course of eating is independent of the group size.

The rumination of cows is controlled by quite a series of endogenous and exogenous factors (Porzig et al., 1969). Rumination is called by Arnold and Dudzinski (1978) the second period of feed consumption. Cattle are able to exert digestive activity even while resting, far from pasture, under a lean-to roof, and even in bad weather. They usually lie down while ruminating (Fraser, 1974; Dávid, 1983; Kolb, 1981; Czakó and Béri, 1984; Arnold and Dudzinski, 1978; Bárczy and Czakó, 1962; Gere, 1977; Ruckebusch, 1970). Investigations in Hungary showed two main periods of rumination: 6-12 a.m. and 6–12 p.m. (Nagy, 1980), 9 a.m.–3 p.m. and 6 p.m.–6 a.m. (Molnár et al., 1977). With feeding once a day (9 a.m.) in the experiment carried out by Pearce (1965) with sheep, the circadian rhythm of rumination was characterized by inactivity after eating, then rumination of increasing intensity followed, reaching a maximum in the early morning hours. After that, the ruminating activity decreased until the next feeding. Feeding *ad libitum,* on the other hand, resulted in the break-up of the circadian rhythm of rumination, which went on throughout the day, apparently without regularity. Theories concerning the role of rumination are reported by Ruckebusch (1970): (1) for wild animals the quick uptake of food is necessary; later they are able to chew it peacefully, (2) partial supply of the ruminal content with oxygen and saliva to ensure optimum conditions for fermentation and (3) breaking the feed into small pieces so that it can pass from the rumen to further sections of the digestive canal. Factors playing a role in controlling the rumination: the crude fibre content of the feed, characteristics of the species, feed composition and digestibility of the portion, its physical form and quantity of the portions. The feed intake is limited by the ruminal content, and rumination is the only mechanism that accelerates the decomposition of cellulose and enables the feed to pass from the rumen to the stomach.

For cows – especially on a high level of production – peaceful conditions must be created for an undisturbed rumination. In our experiments, the group size did not influence the periodicity of rumination. We observed two – a shorter daytime and a longer night – periods, and found no signs suggesting the disorder of the rhythm of rumination.

The activity of the cows showed the following order: eating, pause, rumination, pause, sleeping.

The basic question studied by us: the different size of group did not prove a factor causing disorders in the daily rhythm of the behaviour patterns. Its continuity and course are a function of the technological operations, milking, and feeding, which do not mean gross interference in regard to periodicity shown in natural keeping. The slight differences after regrouping and between the days of examination prove the adaptability and technological tolerance of cows.

From the results of our experiments and the data of the relevant literature, the following conclusions can be drawn:

– As regards the basic behaviour patterns, cows in groups of different size follow the model characteristic of the species. Thus, in their duration, course and frequency, an increase in the group number hardly causes any disorder. At the same time, the milking cow is widely able to accommodate itself to various elements of the production technology, to the systems of feeding, milking and housing, without any harmful effect on its general condition and milk production.

– The comfort behaviour of cows is characterized by their voluntary choice of resting places. Irrespective of the group size, the animals make use of the available space and time by adapting themselves to the artificial environment provided by the housing system. Thus, the technologies employed in the present examination series, in their interactions with the varying group size did not restrict the specific demands of the animals to such an extent that they could become important factors.

– The daily rhythm of lying has two phases: it is divided in a longer night and a shorter daytime period. The periodicity of eating also has two, occasionally three phases, depending on the system of feeding in the different treatments. Rumination is confined to the resting periods. Its periodicity coincides with lying-resting, being divided in a shorter day-time and a longer night period.

– In the circadian rhythm of behaviour the basic question examined: the varying group size did not prove a disturbing factor concerning the allelomimetic behaviour of the milk-cows. The continuity and course of the examined behaviour patterns in the given technologies were in accordance with the specific characteristics of cattle on the one hand, and reflected the technological operations, feeding, milking, on the other. Disorders of adaptation did not even occur in groups of larger size.

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Lectures

LONGEVITY OF SEXUAL ORGANS OF EUROPEAN AND JAPANESE PLUM VARIETIES

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Sexual organ longevity of 4 European prune (*Prunus domestica* L.) varieties (Besztercei Bt. 2, Bluefre, President, Stanley) and 6 Japanese plum (*Prunus salinica* Lindl.) varieties (Burbank, Duarte, Elephant Heart, Methley, Santa Rosa, Shiro) were tested in 1984 and 1985.

The end of stigma longevity, start of anther's dehiscence following the opening of flowers and end of dehiscence were determined. Results showed that temperature had a significant effect on both stigma longeity and anther's dehiscence. The end of stigma longevity – after the anthesis – was long in both European (3–5 days) and Japanese (2–4 days) plums, when the average daily temperature was below 10 °C. When temperature was above 13 °C, stigma longevity was short. Both European and Japanese plums can be characterized by protogyny.

Anther dehiscence proved to be more sensitive to changing temperature than was stigma longevity. Anthers of Japanese plum varieties are extremely sensitive to increasing temperature. Anther's dehiscence was continuous, culminating after 12:00 in Japanese plums, and after 14.00 in European plums.

Keywords: secretum, pollen germination, viability generative organs, effective pollination period

Introduction

The pollination season is very short for the plum. The highest rate of fruitset may be expected, when flowers are pollinated in the early stage, or in the peak of anthesis.

When secretion of the stigma starts, which is to help pollen grains adhere and grow tubes (Stösser, 1985), it signifies sexual maturity.

Before the anthesis there was no secretum observed on the stigma of Stanley variety (Cresti et al., 1985). The changing colour of stigma shows its aging through green-yellow-and finally brown colours. Pollen grains grew their tubes *in vivo* even if the stigma was brown and papilles shrivelled. In this case fruitset failed due to degeneration of tubes (Stösser, 1985).

Radhava and Nair (1960) found the stigma susceptible a day before the starting of anthesis, and it lasted for 3–4 days. Stigma viability was determined by Dorsey (1919), Bellini and Bini (1978) to last 4–6 days. According to Levickaja and Kotoman (1980) stigma viability lasted for 7 days, but petals fell in the 3–4 days. When the stigma was pollinated, it started browning 3–5 days after the anthesis.

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Anther dehiscence and pollen spread is also effected by ecological conditions.

Anther dehiscence of all of the plum varieties tested by Nyéki et al. (1985) started within a day after opening of flowers and lasted for 51–93 hours. Dehiscence of anthers of the same flower may last only for one day, when the weather is favourable, but may last for 4–5 days when it is rainy (Bellini and Bini, 1978).

Nyéki et al. (1985) observed protogyny, which means that the stigma matures before the anthers open.

Materials and methods

Studies were carried out in the Variety-Test Orchard of Siófok State Farm. Trees were planted on myrobalan seedlings rootstocks in 1978. Observations were based on the method by Nyéki (1974). The change of stigma colour and anther dehiscence were observed between 8–18 o'clock on 10 numbered flowers of each variety.

This paper is about the study the most important period of stigma viability in respect of natural pollination, what lasts from the early stage of the anthesis till stigma turns brown.

The end of anther dehiscence refers to the period between the opening up of the first and last anthers.

Results

Longevity of sexual organs

Functioning periods of stigmas of European prune varieties have different lengths. In 1985, it lasted for 3–59 hours in the case of President variety. In 1985, in a flower of Bluefre variety, the stigma started browning after 100 hours. Anther dehiscence started 0–31 hours after opening of flowers, and lasted for 64–122 hours. The overlap in functioning of sexual organs was 0–56 hours.

Table 1

Longevity of sexual organs of European plum varieties
(Siófok, 1984–1985)

Variety	Stigma longevity (hours)			Anther dehiscence following anthesis (hours)				Percentage of opened anthers when the stigma turned brown		
				start	duration					
	1984	1985		1984	1985		1984	1984	1985	
		a	b		a	b			a	b
President	32.6	26.6	47.7	2.6	0.7	7.8	117.0	60.3	83.3	82.9
Bluefre	26.7	29.0	55.0	10.8	1.9	34.1	99.2	42.6	73.1	37.2
Stanley	30.4	22.5		18.0	2.8		104.0	46.6	83.6	
Besztercei Bt.2	35.6	26.4		11.4	2.4		95.2	77.5	79.4	
L.S.D. 5%	10.9	3.5	11.8	9.1	1.6	18.6	11.5	15.1	9.4	17.3

Duration of examination: 1984 –27.04 – 03.05.
1985 a –23.04 – 24.04.
b –25.04 – 01.05

Table 2

Longevity of sexual organs of Japanese plum varieties
(Siófok, 1984–1985)

Variety	Stigma longevity (hours)		Anther dehiscence following anthesis (hours) Start		duration	Percentage of opened anthers when the stigma turned brown	
	1984	1985	1984	1985	1984	1984	1985
Methley	34.7		2.9		47.8	96.0	
Santa Rosa	42.0		12.0		46.0	90.7	
Burbank	48.7	28.7	2.8	0	51.4	98.5	96.8
Shiro	43.2		5.4		54.0	94.7	
Elephant heart	51.1		12.1		49.8	98.2	
Duarte	50.6	25.0	9.1	0	53.0	98.4	100
L.S.D.$_{5\%}$	7.4	8.5	5.5		12.5	4.7	3.5

Duration of examination: 1984 –18.04 – 22.04.
1985 –23.04 – 24.04.

In the case of Japanese plums, there were 24–74 hours recorded from flower opening till the browning of the stigma. This period of certain varieties has very different lengths, as in the case of European prunes. Anther dehiscence started 0–29 hours after the flowers opened and lasted for 23–73 hours. The overlap in functioning of sexual organs was 6–71 hours.

The average per variety is presented in Tables 1 and 2. In 1984, when temperature was high Japanese plums kept their viability for a longer period than did European prunes. However, the period of anther dehiscence of European prunes was twice as long as that of Japanese plums.

In 1985, under similar conditions the viability of the stigma was close in the two groups.

The browning of the stigma started on the first and second day in the case of European plums and on the second and third day in the case of Japanese plums. Almost all of the stigmas tested lost their viability on the 3rd day.

When daily average temperature was under 10 °C stigma longevity was long in both group (3-5 or 2-4 days), and short when average temperature exceeded 13 °C.

In spite of the big variability of characteristics, there is a significant difference between the varieties. Stigma longevity of Stanley variety was similar to that of other European prunes in 1984, but it was significantly shorter in 1985. However, in both years its anther dehiscence was most retarded.

Stigma longevity of President showed no significant difference to the average, but anther dehiscence was early and intensive. It seems to be antagonistic to the fact that the longevity of anther dehiscence lasted significantly long (117 hours in 1984). Most of the anthers opened up early, but some (7.9% of them) failed until the 5th day of observation. This may be due to abnormal anthers.

In the group of Japanese plums, Methley bloomed at the earliest, and its stigmas had the shorted longevity (34.7 hours). Anther dehiscence started soon after the anthesis (within 2.9 hours).

Daily process of sexual organs

The variability of stigma viability and anther dehiscence is presented in Figs 1 and 2.

Anther dehiscence is continuous, but it has low intensity till 11 o'clock. It is very intensive from 12 o'clock in the case of Japanese plums and from 14 o'clock in the case of European prunes till 17 o'clock.

In 1984 the most anthers opened up on the first day in the case of Japanese plums and on the third day in the case of European prunes. Between the 19th and 22nd of April anther dehiscence of Japanese plums was intensive due to the warm weather.

Between April 28th and May 1st it was slow in the European prunes due to the cool weather, and there was no considerable daily difference, as occurred in the case of European prunes.

In 1985 the start of anthesis was closely followed by anther dehiscence due to the high temperature, and all tested flowers of Japanese plums had opened anthers at the beginning of the anthesis. After the analysis of data for the two years, the results are as follows, regarding intensity of anther dehiscence.

	Low	Medium	High
European prunes	under 14 °C	14–20 °C	over 20 °C
Japanese plums	under 15 °C	15–18 °C	over 18 °C

Between the above temperature grades the rater of opened anthers/hour are as follows:

	Low	Medium	High
European prunes	1 – 5%	2 – 10%	4 –16%
Japanese plums	1 – 2%	2 – 10%	6 – 24%

Results show that intensity of anther dehiscence depends more on temperature than does stigma viability.

Longevity of stigmas became short due to the warm weather, but anthers opened up a higher rate, than they had done in the previous year.

Anthers of Japanese plums are more sensitive to the temperature than those of European prunes, because:

– anther dehiscence closely follows the beginning of anthesis
– they reach maximum intensity of dehiscence earlier in the day (11–13 o'clock)
– the period is short between the opening up of the first and last anthers.

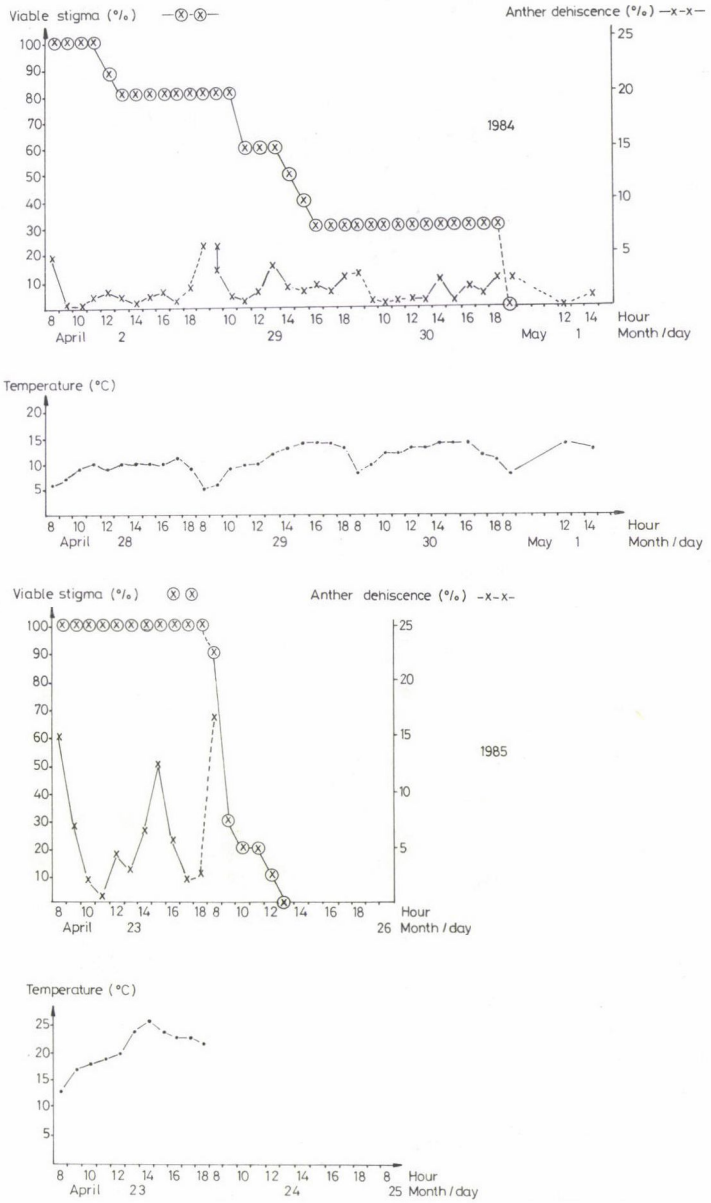

Fig. 1. Function ability os sexual organs in President flowers

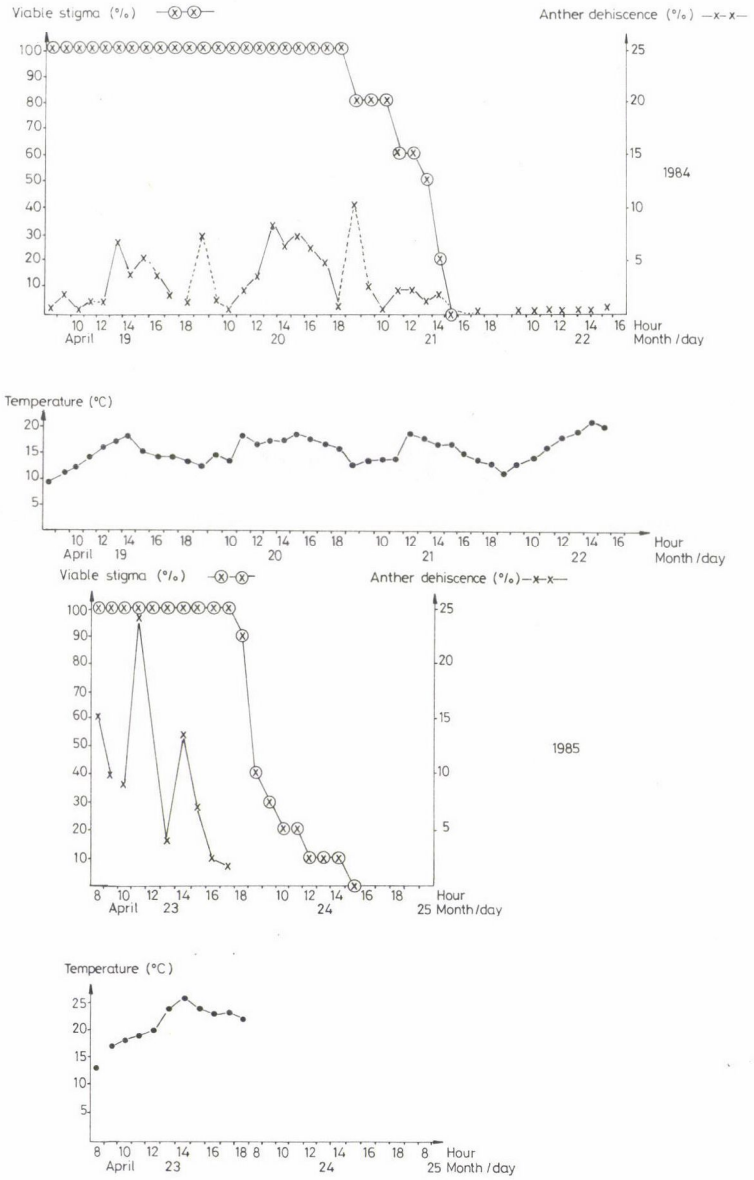

Fig. 2. Function ability of sexual organs in Burbank flowers

Discussion

Cross pollination in balloon stage showed, that the stigma was susceptible 1–2 days before the anthesis. This is in accordance with Randhawa and Nair (1960). Longevity of stigmas is reported in the literature to last for 3 (Randhawa and Nair, 1960) or 7 days (Levickaja and Kotoman, 1980). In our study, stigmas lost their viability in 1–2 days, when temperature was over 13 °C, and within 3–4 days when it was under 10 °C.

Anther dehiscence in one flower may last only 1 day in warm weather, and 4–6 days in cool weather.

When the rate of opened anthers is lower than 10% (2–3 per flower) the pollen supply is adequate only on the second day. Considering that on the first day of flowering there are only a few opened flowers, we may say that in the case of cool weather the pollenizer variety has an adequate pollen supply only from the third day of flowering. In the case of warm weather and intensive flowering, the pollen supply may be adequate even on the first day.

A continuous pollen supply for the mother variety can be obtained if one pollenizer starts flowering 2 days earlier, the other starts 2 days later.

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FLOWERING CHARACTERISTICS OF EUROPEAN (*PRUNUS DOMESTICA* L.) AND JAPANESE (*PRUNUS SALICINA* LINDL.) PLUMS AT THE CULTIVAR AND INTRA-INDIVIDUAL LEVEL*

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In Hungary, at the present stage of production, the inclusion of pollinating cultivars is a precondition of high yields. For the selection of most suitable pollenizer the knowledge of the date, duration and dynamics of flowering is important. This can be achieved by the Index of Flowering (Máthé, 1977) elaborated and successfully used with numerous horticultural, medicinal and fruit crops.

It could be established that the flowering characteristics of both European and Oriental plums can be very well characterized by the *Index-V.* In the experiments both European, as well as Chinese and Japanese plum cultivars, tree individuals and populations have been analysed also in view of the annual flowering variations. The ultimate aim of the analysis of flowering was to determine the simultaneously flowering cultivars to be used as pollinators.

It was also concluded that the data provided by *Index-V* be included in the data-banks of plant collections, gene banks. They can also facilitate agricultural plant protection activity taking place at the time of flowering.

Keywords: *Prunus domestica* L., European-plums, *Prunus salicina* Lindl., Oriental plums, flowering, Index of Flowering, *Index-V,* course and dynamics of flowering, cultivars, intra-individual variability

Introduction

In Hungary the choice of both European and Japanese plum cultivars is relatively modest. Besides the main cultivar 'Besztercei' (*P. domestica* convar. hungarica), attempts are going on to introduce late flowering, Yugoslavian, Rumanian and also possibly Japanese cultivars (*P. salicina* Lindl. Syn.: *Prunus triflora* Roxbgh.) (Szabó, 1989).

According to Szabó (1989) the most popular Hungarian plum cultivar 'Besztercei' being susceptible to Plum Pox virus, the introduction of oriental plums seems to be reasonable in Hungary, although owing to the relatively high heat and light demand of these plum species, frost damages are relatively frequent. All cultivars studied in Hungary have proved to be self-sterile; therefore, the application of pollinating cultivars is necessary. It is also stated by him that the Oriental plums are more precocious than the European plums.

Although the main goal is to select self-fertile cultivars, at the present stage of production a relatively large choice of pollinating cultivars is a precondition of high yields. Consequently, the flowering phenological characterization of cultivars is still of

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great importance. This fact is also underlined by the results of a self-compatibility (fertility) test including 120 cultivars grown in Hungary, between 1950–1969. It was established that 1/4 of the cultivars is capable of producing satisfactory yields, whereas 2/3 of the cultivars produced yields though not in an economically feasible extent. Therefore, pollinators are likely to be still needed in the long run. For the selection of most suitable pollinators, the knowledge of the date and duration of flowering is important (Tóth, 1980). No matter how compatible the plums are in the generative or physiological way, should the flowering period of cultivars or species be not overlapping (if only partially, e.g. two-three days), no sufficient pollination can take place.

According to Keulemans (1993), pollination and fruit set problems are causes for low yields and irregular bearing under Belgian climatic conditions.

In a most comprehensive long range study of plum cultivars, lasting over 22 years, Surányi (1993) established that besides virus infection, changes in the flower structure caused by both tree-age and annual variations of the ecological factors could be reasons for fertility problems in plums.

The high rate of deformed flowers (up to 87.5%) in the observations on the anatomical–morphological deformations of plum flowers reported by Misic (1993) also verify the above conclusion.

Investigations into the longevity of sexual organs of plum cultivars (Nyéki and Szabó, 1993) indicating a 2–4 day's stigma longevity after anthesis, have also indicated importance of appropriate pollinating cultivars.

Most recently two comprehensive analyses have been published on the cultivar evaluation of both European and Japanese plums grown in Hungary (Nyéki, 1989) and Szabó (1992). Both authors emphasized the necessity to elaborate an objective method for the comparison of cultivars. In the present work, this was attempted by adapting the flowering index for the numerical expression of flowering in plums. Thus, the aim of present investigations was twofold: firstly, to verify the applicability of the numerical approach in expressing the process of flowering; secondly, to characterize the flowering properties of both European and Japanese plums at the cultivar and – where it was possible – at the intra-individual level.

Materials and methods

I. European plums (Prunus domestica L.)

The experiments with the European plum cultivars (*P. domestica* L.) were carried out in the "Siófok" State Farm, at Siófok, in 1982–85. The intra-individual variations were studied at Csány, between 1984–86.

The flowering phenological observations involved the following cultivars: 'Besztercei Bt. 2', 'Bluefre', 'Cacanska Najbolja', 'Cambridge Gage', 'Czar', 'Debreceni Muskotály', 'Early Besztercei', 'Early Italian', 'Early Laxton', 'Italian prune', 'Krikon', 'Ontario', 'Pozegaca', 'President', 'Prune d'Agen', 'Reine Claude de Bavay', 'Stanley', 'Richards Early Italian', 'Ruth Gerstetter', 'Tuleu Timpuriu', 'Victoria'.

II. Japanese plums (Prunus salicina Lindl.)

The experiments with the Japanese plum cultivars (*P. salicina* Lindl.) were carried out in the "Siófok" State Farm, at Siófok, in 1982–85. The individual variations were studied between 1984 through 1986.

The flowering phenological observations involved the following cultivars: 'Burbank', 'Duarte', 'Elephant Heart', 'Methley', 'Santa Rosa', 'Shiro'.

III. Evaluation and comparison of the flowering of cultivars

The flowering dynamics, and flowering sequence of cultivars were characterized by the Index of flowering (*Index-V*) elaborated by Máthé (1977).

The flowering index was calculated on the basis of some 400–1000 observations/cultivar.

The following phenological stages were distinguished: closed buds (flowers prior to anthesis), anthesis (fully open flowers) and flowers in post-anthesis (beyond the flowering stage, with withered petals). The observations were carried out daily, between 4–5 p.m.

Results and discussion

I. Basic characteristics of flowering

1. European plums

On the basis of flowering phenological observations of 21 European plum cultivars, it could be established that flowering in plum (*Prunus domestica* L.) can be characterized by the same equation (regularities) as described for the fruit crops apple (*Malus domestica* L.) (Máthé et al., 1993), pear (*Pyrus communis* L.) (Máthé et al., 1993), cherry (*Prunus avium* L.) (Máthé et al., 1993), sour cherry (*Prunus cerasus* L.) (Máthé et al., 1993), apricot (*Prunus armeniaca* L.) (Máthé et al., 1993), and peach (*Prunus persica* L.) (Máthé et al., 1993).

Figures 1/a–b illustrate this from the examples of cvs. Ontario, Bluefre, President, and Richard's Early Italian. The characteristic feature of this type of distribution, i.e. that the frequency of flowers in anthesis culminates at a date when the decreasing rate of unopened buds and the increasing rate of flowers in the stage post-anthesis is at a quasi-equilibrium, holds also true for European plums.

The index-values, as calculated from above values, vary between –1 and +1 and can be fitted to a Sigmoid curve (Figure 1/b), characteristic of the growth processes of living organisms. The time range falling between the index-values –0.25 and +0.25 corresponds to the phenophase of 'mass flowering' with more than 50% of the flowers in the phenophase of anthesis, a period most crucial in regard to fertilization.

2. Japanese plums

Similarly to the European plums, the flowering phenological observations of 6 Chinese and Japanese plum cultivars have demonstrated that the course of flowering in oriental plums (*Prunus salicina* Lindl.) shows the same regularities.

Figure 2/a illustrates the frequency distribution of flowers of selected cultivars in the three subsequent phenophases (i.e.: bud opening, anthesis, post-anthesis).

The index-values calculated from above values (Figure 2/b) varying between –1 and +1 and can also be fitted to a Sigmoid curve.

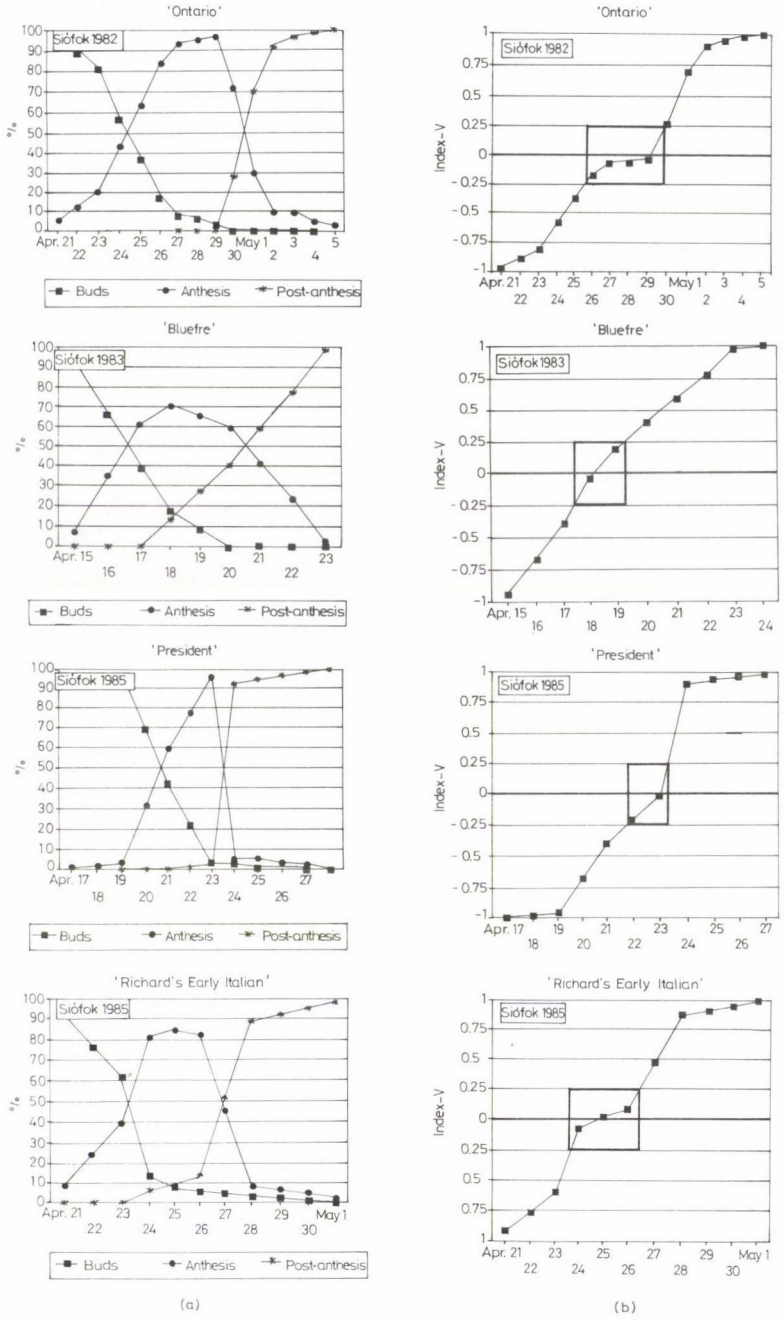


Fig. 1. Basic characteristics of the frequency distribution of generative organs of selected plum cultivars

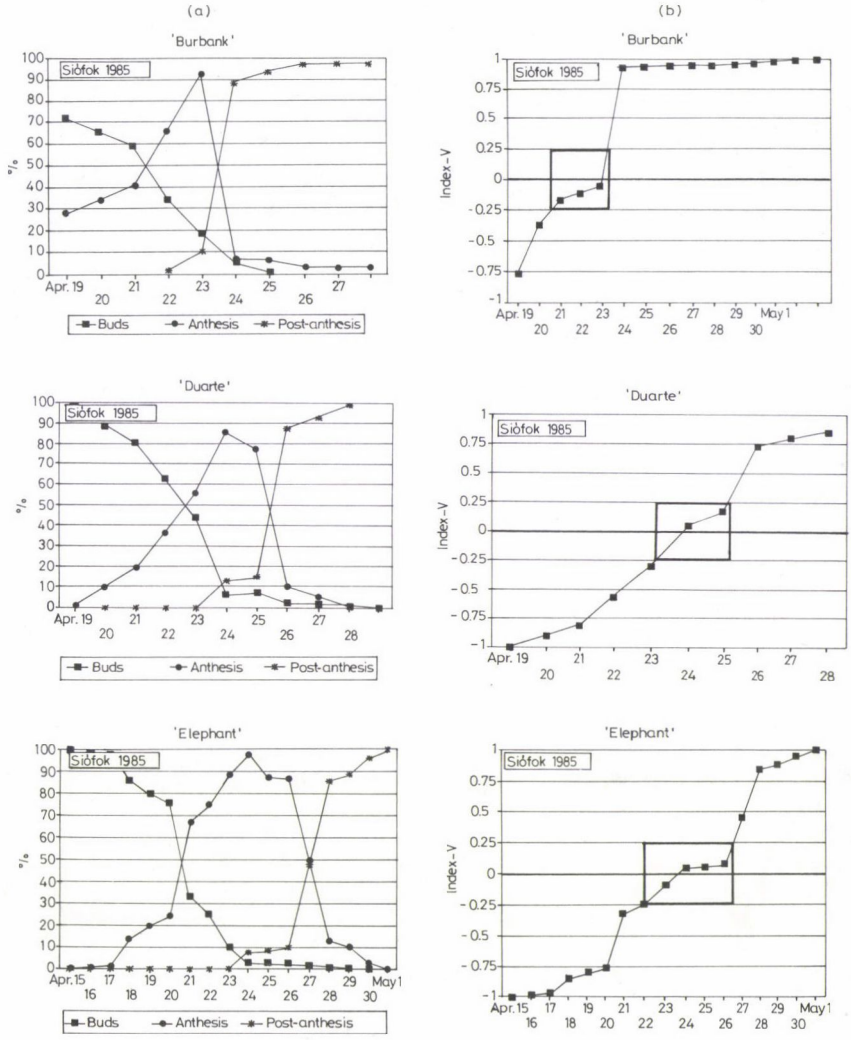
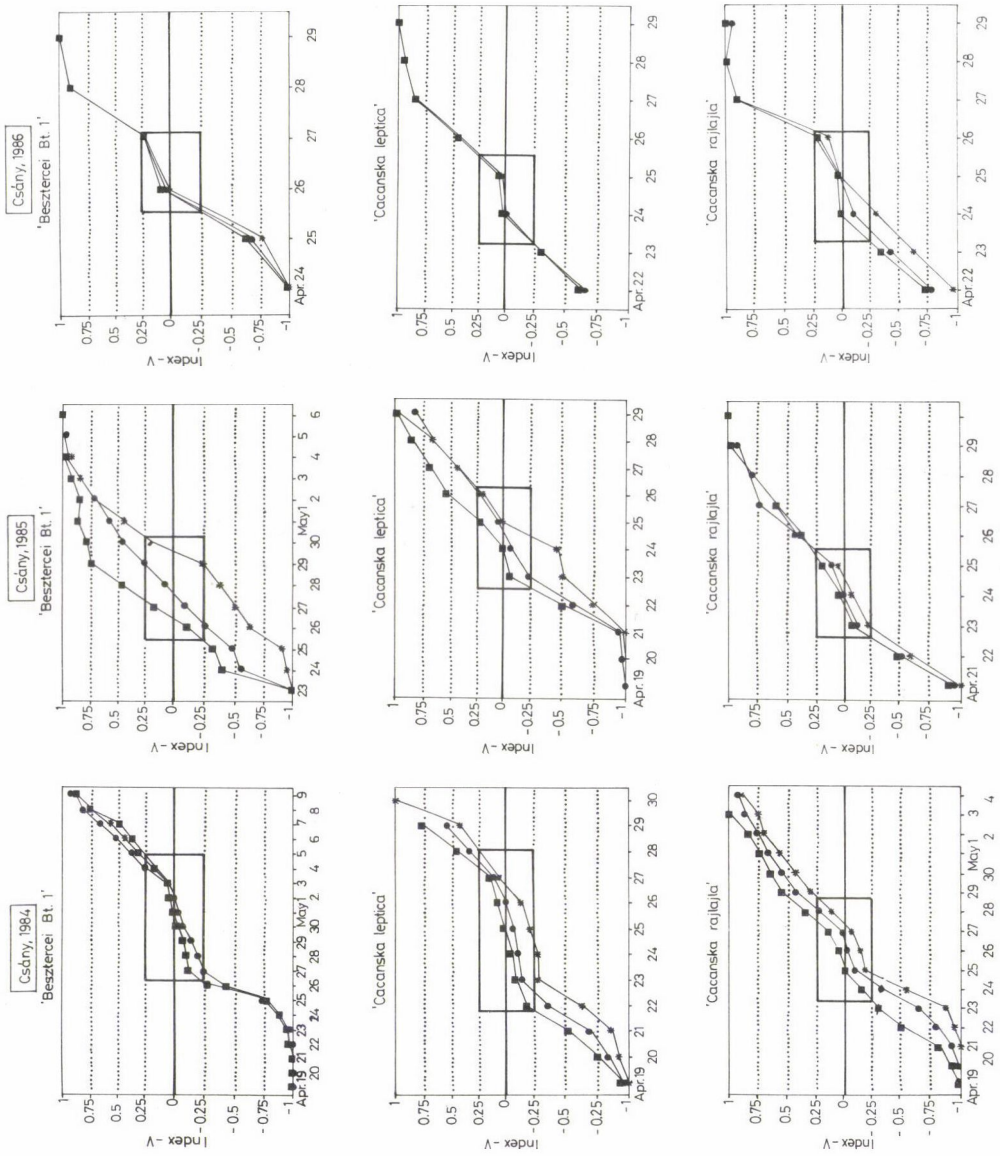


Fig. 2. Frequency distribution of flowering phenophases and the corresponding *Index-V* values

II. Dynamics of flowering at the cultivar and inter-individual level

1. European plums

In our comprehensive investigation, flowering characteristics of plums were observed at two locations: at Csány (I) and Siófok (II). The observations carried out for 3 years allow for some conclusions on the flowering properties at both the cultivar and individual levels.


Csány, 1986
'Besztercei Bt. 1'
'Cacanska leptica'
'Cacanska rajlajla'
Csány, 1985
'Besztercei Bt. 1'
'Cacanska leptica'
'Cacanska rajlajla'
Csány, 1984
'Besztercei Bt. 1'
'Cacanska leptica'
'Cacanska rajlajla'
Index - V
Apr. 24
Apr. 22
Apr. 19
Apr. 21
May 1

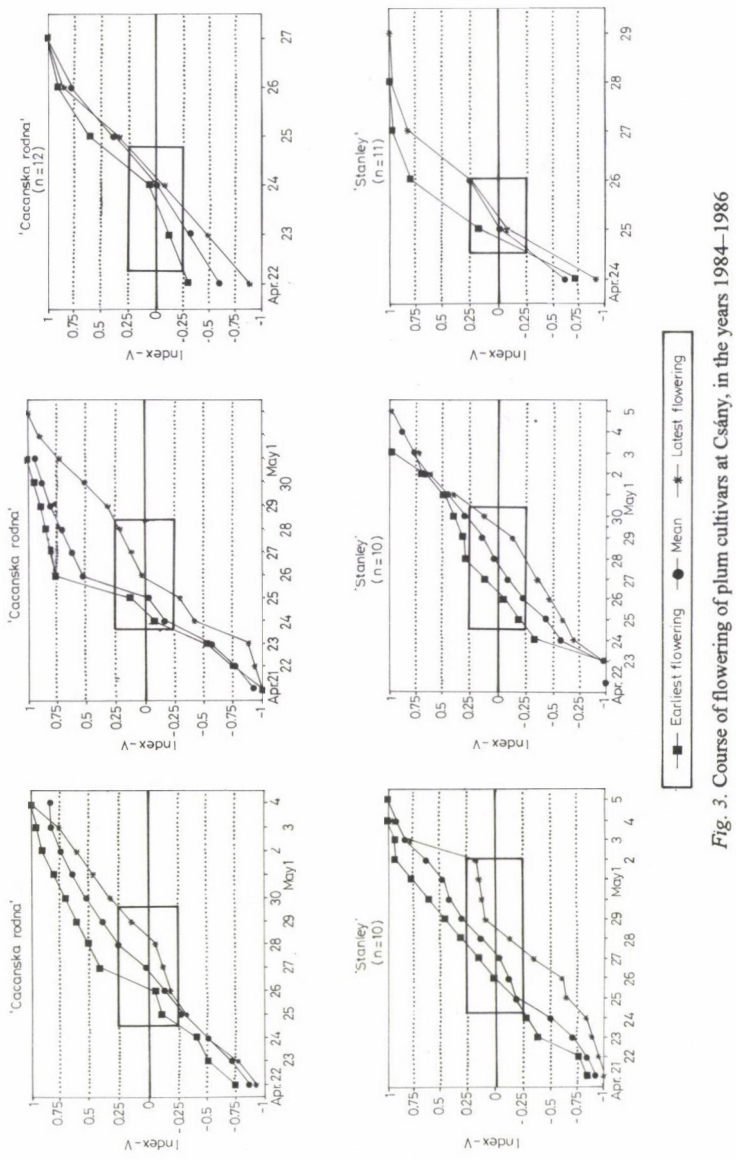


Fig. 3. Course of flowering of plum cultivars at Csány, in the years 1984–1986

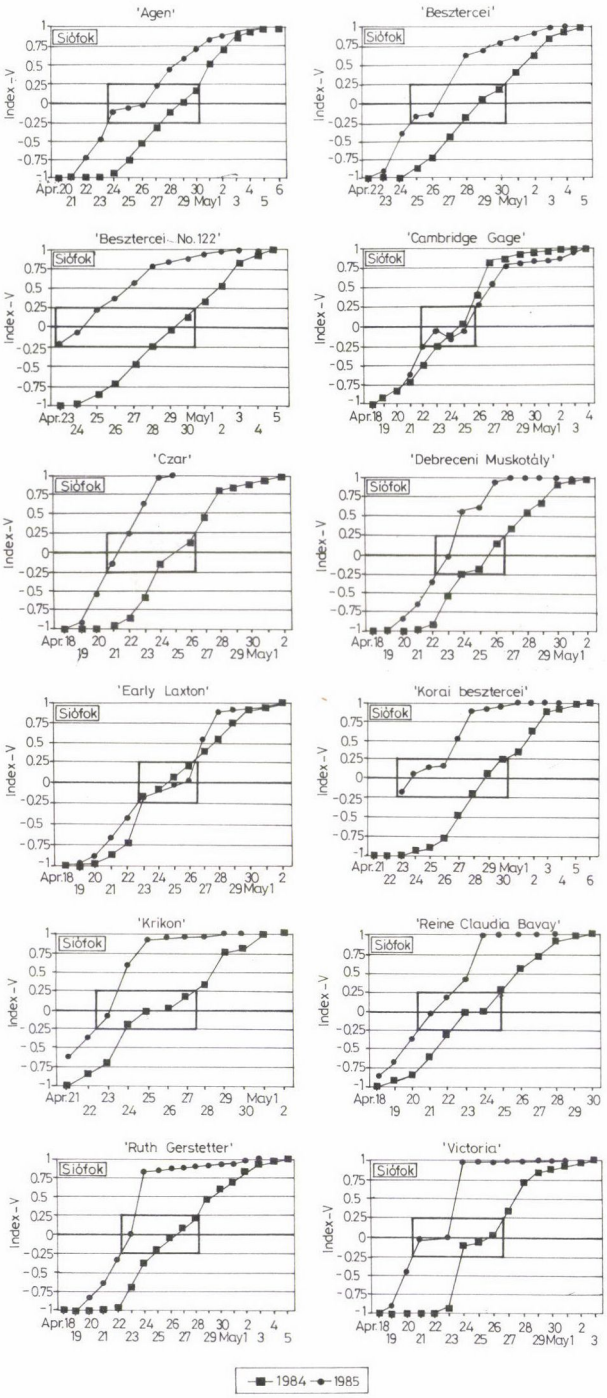


Fig. 4. Course of flowering of the early, mid-late and late-ripening trees of plum cultivars (Csány, 1985)

(a) Observations at Csány

The results of a three years' (1984–1986) experiment at Csány are summarized in Fig. 3. The observations involved 5 cultivars. The course of flowering of the earliest/latest flowering trees was plotted against the mean of 9 to 19 individual trees. The figures based on 3 years' data clearly indicate a narrow discrepancy in the starting date of mass flowering of cultivars not exceeding the narrow time range of one or two days, nearly irrespective of the weather conditions. This dynamic flowering process calls attention to the possible difficulties in choosing the right pollinating cultivar.

Similarly, in all three years of the experiment, the start of the flowering period of most cultivars fell within a narrow range of three days (April 22 and 25). The only exception was cv. 'Besztercei Bt. 1', which cultivar, in 1984, was the latest to start mass flowering (April 26).

These findings seem to refer to the peculiar role of genetic control of flowering in plum.

As to the individual variability in flowering dynamics (expressed as the duration of mass flowering) of trees, in the case of most cultivars, only less pronounced differences could be detected. It is, however, contradictory that both the slowest and the highest rate of flowering-dynamics was observed in the case of the same two cultivars, 'Besztercei Bt.1' and 'Stanley' in the years 1984 and 1986, respectively. In 1984, the flowering of both cultivars seemed to be rather prolonged (mass flowering lasted ca. 8 days); whereas in 1986, it was extremely rapid, lasting ca. 1.5 days, which fact seems to refer to a greater dependence of flowering-dynamics of the impact of ecological factors.

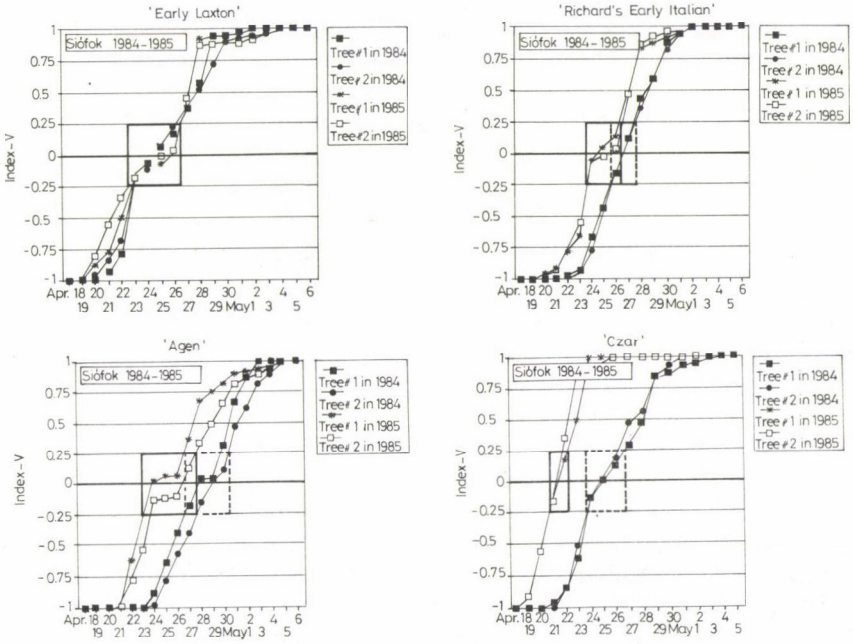


Fig. 5. Course of flowering of selected European plum tree individuals (Siófok, 1984–1985)

(b) Observations at Siófok

At an other location, at Siófok, in the years 1984–1985, the observations were made on 12 cultivars. Remarkably, in both years and in the case of all cultivars, mass flowering started within a time-range of 4 days (April 20–24). In 1984, with the exception of cv 'Early Laxton', almost all cultivars were more precocious, mass flowering started 1 to 7 days earlier, e.g. cv. 'Cambridge Gage' and 'Besztercei No. 122', respectively. In flowering-dynamics, however, no similar regularities could be detected (Fig. 4).

In a similar experiment with individual trees of 18 cultivars, in 1985, the onset of mass flowering occurred within the time range of 3 days' (April 21–24), whereas its dynamics seemed to be more varied (duration of flowering between 1 to 5 days, cb. 'Early Italian' and cv. 'Cacanska rajlajla', respectively. Based on this experiment, however, no significant individual variability could be established.

The possibly less expressed individual variability among plum cultivars could be verified by the observations summarized in Fig. 5, where the course of flowering of 2–2 trees of 4 cultivars was studied, in 1984 and 1985. Also in this case, the only major differences can be established in terms of annual variation.

(c) Geographic variability (Csány vs. Siófok)

By way of the complex analysis of cultural ecosystems at two locations, i.e. orchards at two locations (Csány vs. Siófok) further results could be obtained as to the ecological flexibility of cultivars.

Although, the cultivars studied were different, it could be established that, in comparison to 1985, in 1984 both at Csány and Siófok, mass flowering started 1–5 days earlier. Remarkably, the first day of mass flowering was also the same (April 22), at both locations. These results will have to be interpreted, also in view of the meso-climatic influences, which – seemingly – have less impact on flowering than do the genetic factors.

(d) Annual variability of flowering

Although the flowering time of cultivars is in most cases a genetically fixed trait, the start and duration of flowering can vary under the influence of ecological, physiological, agrotechnical, etc. factors.

It seems to be a remarkable feature of plums that – as illustrated by data of the earliest and latest flowering tree individuals of 5 cultivars, studied in the years 1984 through 1985, at Csány that the start of the mass flowering phenophase fell within a period of 2–3 days (April 22–26). As this phenomenon holds true at both cultivar and intra individual level of each cultivar, it seems to justify the results on the relatively strong genetic fixedness of this character. Similar tendencies could be observed with farther 12 cultivars, studied in 1984 and 1985, at Siófok, where in all instances, mass flowering in 1984 was more precocious than in 1985.

Flowering dynamics, however, as expressed by the length of the mass-flowering phenophase, seems to be more varied, implying the higher susceptibility to the weather conditions, at the time of flowering. As extreme examples, in 1985 the cv. 'Cacanska najbolja' had a short mass flowering period of 2–3 days, in 1985, whereas the cv. 'Stanley' blossomed for nearly 6 days, in the same year. Apparently, the flowering dynamics of the latter cultivar reflects most flexibly the weather conditons prevailing at the time of flowering, i.e. in 1984, flowering dynamics during the period of mass flowering was rather low (flowering lasted nearly 9 days), whereas in 1986, it was very rapid (1.5 days).

(e) Flowering sequence of cultivars

The establishment of the flowering sequence of cultivars renders possible the selection of pollinating cultivars. This can be made on the basis of index values. Table 1 summarizes the observations made at Siófok, on 9 cultivars, between 1982–1985. Remarkably, the cultivar 'Ontario' was the most precocious in all four years of the experiment, whereas the other cultivars showed a differing rate of variability that we expressed by the Coefficient of Variation.

The rather low values (between 0 and 29%) reveal a peculiar feature of plums, i.e. that the flowering date of cultivars is relatively stable. (This conclusion can be made also in comparison with other fruit crops.) Thus, the cultivars 'Ontario', 'Besztercei Bt. 2', 'President' and 'Italian Blue' retain a high degree of flowering stability, whereas some other cultivars, e.g.: cv. 'Bluefre' change their position more flexibly.

Table 1

Flowering sequence of European plum (Prunus domestica L.) cultivars at Siófok, in the years 1982 through 1985

Cultivar	Year				4 years'	Final	C. V.
	1982	1983	1984	1985	average	sequence	(%)
Ontario	1	1	1	1	1	1	0
Grower's Late Victoria	4	2	3	2	3	2	30.15
President	3	3	2	3	3	2	15.75
Bluefre	2	4	5	4	4	3	29.06
Early Italian	7	7	4	5	6	4	22.59
Richard's Early Italian	5	5	7	6	6	4	14.42
Italian Blue	6	6	6	7	6	4	6.92
Stanley	8	8	9	8	8	5	5.25
Besztercei Bt.2	9	9	8	9	9	6	4.95

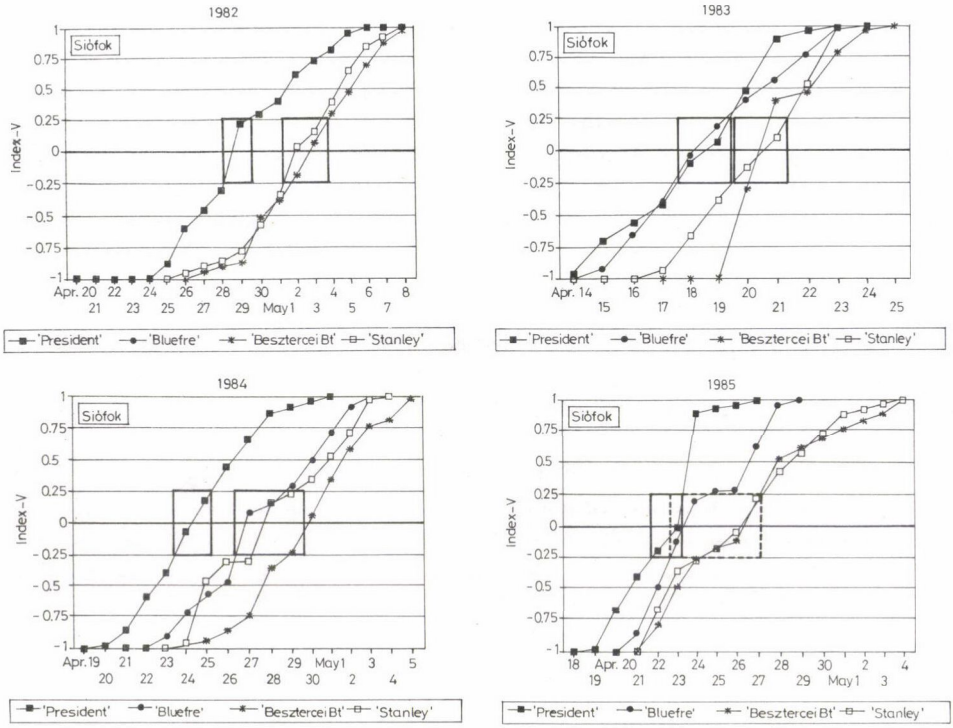


Fig. 6. Course of flowering of plum cultivars in 1984 and 1985 (Siófok)

(f) Simultaneous flowering of cultivars (selection of pollinizer cultivars)

In view of the fact that the position in the flowering sequence, even over many years of experimentation, does not necessarily express the simultaneous blossoming of various cultivars, the selection of possible appropriate pollinizers necessitates a more thorough analysis. This can be achieved in the easiest way by plotting pollinizer and self-sterile cultivars in the same figure. Thus, e.g. in Fig. 6, pollination conditions of 4 cultivars are analysed, in 4 vegetation periods (1982–1985). As, in two vegetation periods, i.e. in 1982 and 1984, the mass flowering of the self-sterile cultivar 'President' precedes that of the possible pollinators cv. 'Bluefre', 'Besztercei Bt. 1' and 'Stanley', it can be concluded that in certain years these cultivars are inappropriate pollinizers for cv. 'President'. Therefore, on the basis of our observations, preference should be given to the cv. 'Bluefre' and 'Stanley', that both in 1983 and 1985, blossomed simultaneously with cv. 'President'. Similar and long term analyses can facilitate the selection of pollinizer cultivars and contribute to increasing the reliability of high yields.

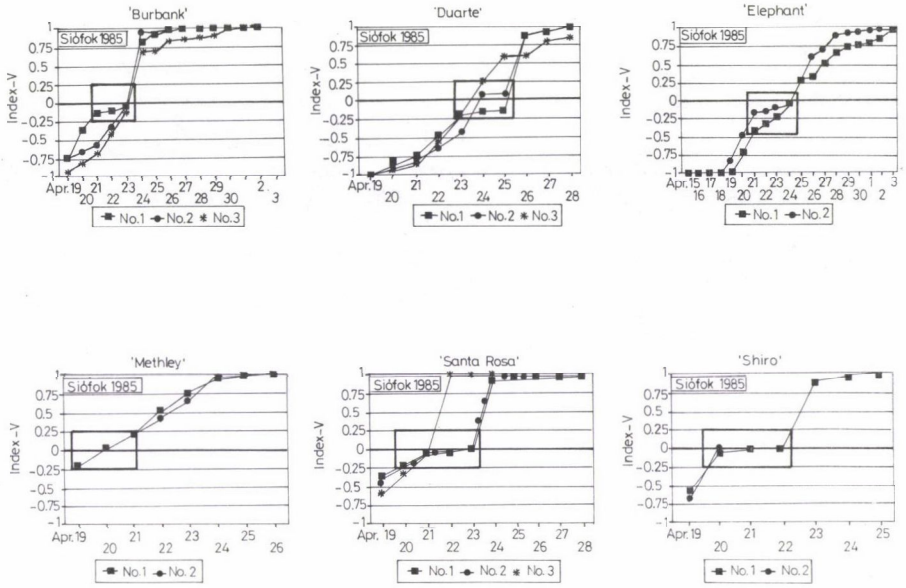


Fig. 7. Simultaneous flowering of the self-sterile cv. 'President' vs. possible pollinating cultivars (Siófok, 1982–1985)

2. Japanese plums

Based on the index values, we have tried to compare the flowering characteristics of individual plum trees (intra-individual variation). Figure 7 depicts the course of flowering of six cultivars, in 1985. Although there are minor variations among the cultivars in terms of the onset of flowering (cv. Methley is the most precocious, whereas cv. Duarte the latest flowering), within the cultivars, however, both the start and the dynamics of flowering seems to be less varied.

(a) Annual variability of flowering

Annual variations in the start of mass flowering could be detected in the case of Japanese plum cultivars. In 1983 all cultivars seemed to come to flower 4–7 days earlier. Among them the cv. Methley was equally precocious both in 1982 and 1983. It is a remarkable characteristics that, in certain cultivars, the annual variations were so low that full flowering (*Index-V* = 0) occurred nearly on the same day. For example cv. Duarte (Apr. 23, 1984 and Apr. 24, 1985), cv. Methley (Apr. 9, 1983 and Apr. 10, 1982).

The study of inter-cultivar variations on the annual basis reveals the tendency that cv. Methley was most precocious in the years 1983 through 1985, whereas cv. Duarte was among the latest flowering cultivars in all four years of the experiment.

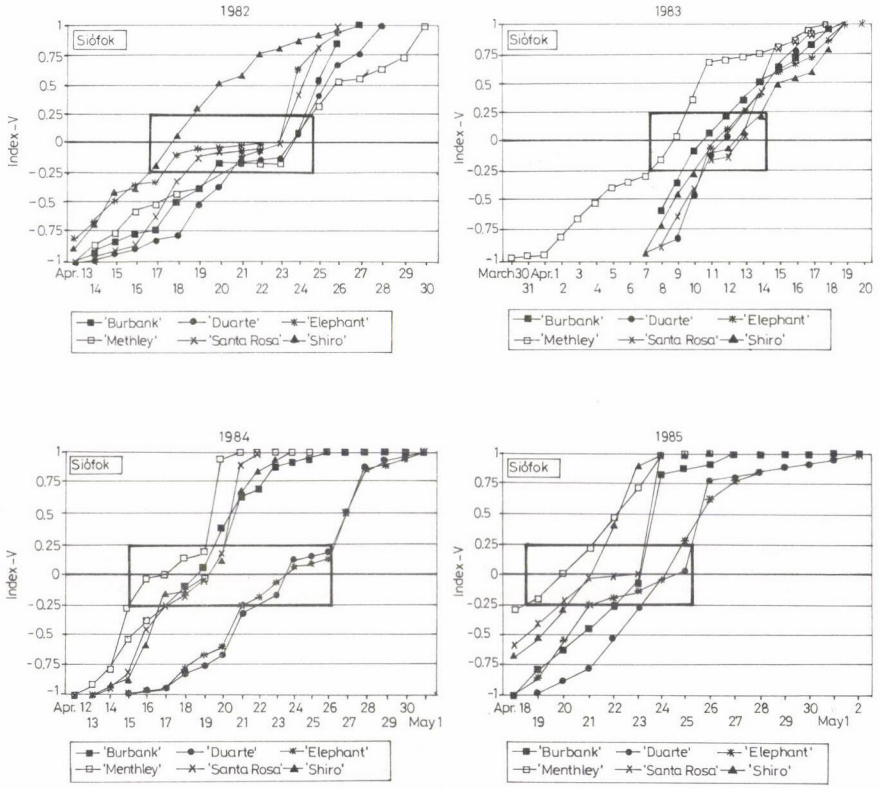


Fig. 8. Flowering dynamics of individuals trees of Japanese plums

(b) Flowering sequence of cultivars

Figure 8, prepared on the basis of *Index-V* values, allows also for the assessment o simultaneous flowering of cultivars. For example, it can be postulated that for th pollination of cv. 'Duarte' the years 1982 and 1983 were extremely favorable, as mos cultivars blossomed within nearly the same time range (Apr. 18–25, 1982 and Apr. 9–14 1983), whereas in 1984 and 1985 only the cv. 'Elephant Heart' could be considered as a reliable pollinizer for the cv. Duarte.

The flowering sequence of cultivars, as calculated on the basis of the *Ind-V* value for mass flowering (V = 0) is tabulated in Table 1. As a unique feature of the *Index-V,* w have elaborated a novel method for establishing the flowering sequence of cultivars Based on the date of mass flowering (V = 0) the sequence of cultivars is established o an annual basis. The coefficient of variance calculated from the data allows for th assessment of reliability of position in the flowering sequence.

The detailed analysis of 6 cultivars has revealed that the cv. Methley was the mos precocious in 3 out of 4 years. Owing to the 5th position in 1982, the reliability of thi value is, however, not too high, which is expressed also by the coefficient of varianc

(CV = 86.6%). In this respect, the cultivar taking in the 6th position is most stable (CV = 15.8%).

Our investigations into the flowering sequence of 6 Japanese plum cultivars (Table 1) have established that the cv. Methley is inclined to precocious flowering. In 3 out of 4 years of the study, it was this cultivar that blossomed at the earliest. The high value of the coefficient of variance (86.6%) seems to indicate that this precociousness is far from being a stable trait, especially as compared with the other cultivars, among which the late flowering character of cultivar Duarte seems to be genetically more fixed (CV = 15.8%).

On the basis of these observations, it is to be emphasised that, in contrast to the special literature, the "fixed flowering sequence" of cultivars needs further analysis, which can be carried out by the *Index-V* fairly easily and probably with fine precision.

Conclusions

I. European plums (Prunus domestica L.)

In our experiments, the flowering dynamics was studied at two locations, at Csány and Siófok. The 3 years of measurements indicate a relatively narrow discrepancy in the onset of mass flowering. On the other hand, all cultivars studied at Csány displayed a rapid flowering process. In both 1984 and 1985, all cultivars started mass flowering within a time range of 5 days. Flowering dynamics (expressed as the duration of mass flowering) did not show cultivar specific changes. Remarkably, however, both the highest and the lowest rate of flowering dynamics was recorded in the case of the same cultivars, i.e. 'Besztercei Bt. 1' and 'Stanley' in 1984 and 1986, respectively.

At another location, at Siófok, in the years 1984–85, mass flowering started within a time-range of 4 days. Some of the cultivars (e.g. 'Prune d'Agen', 'Early Laxton', 'Besztercei No. 122') displayed characteristic low-flowering dynamics, whereas the majority of cultivars seemed to be most varied in this respect. As a general feature it can be stated that in comparison with 1985, in 1984 the flowering process was more rapid, e.g.: in the case of cultivars 'Debreceni muskotály' 'Krikon'1, etc.

In agreement with the special literature (Tóth, 1957; Szabó, 1989; Nyéki, 1989), on the basis of the comparative analysis of 9 cultivars over a 4 years' period, it could be stated that the flowering sequence of plum cultivars is a relatively fixed trait (Fig. 9). In this respect, an especially striking example is the cv. Ontario, which was the first to enter the period of mass flowering, in all 4 years of the experiment. The 0 value of the coefficient of variance also refers to the stability of this trait. The latest flowering date was recorded with the cv. Besztercei Bt. 2. Apart from the two extremes, several cultivars are identically placed in the flowering sequence, which circumstance seems to indicate that they are likely to blossom simultaneously, or at least rather closely, in the average of 4 years.

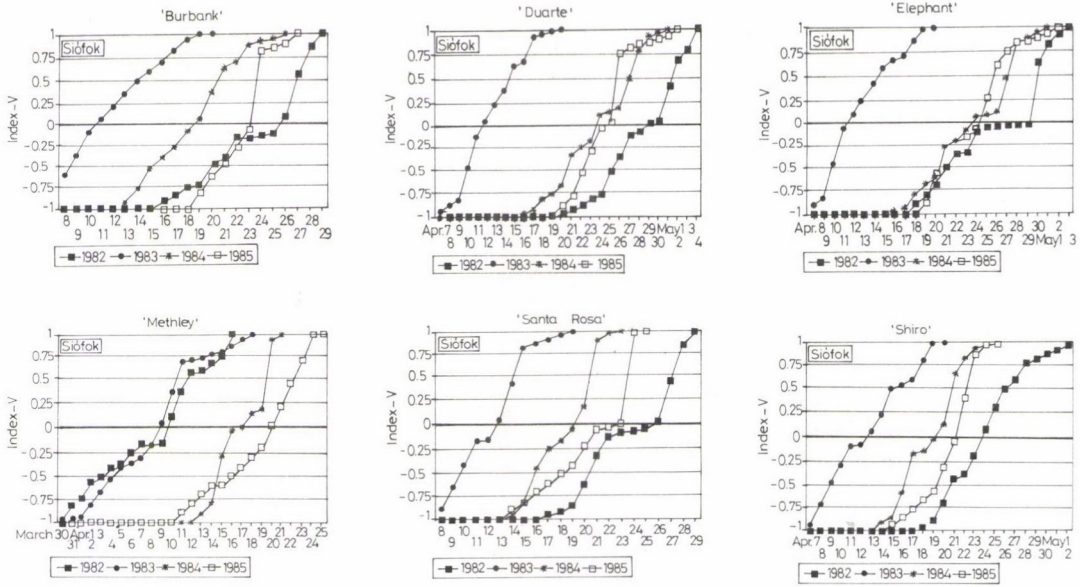


Fig. 9. Annual variability of flowering in selected Japanese plum cultivars

Table 2

Flowering sequence of Oriental plums (Prunus salicina Lindl.), at Siófok, in 1982–1985

Cultivar	Years				4 years' average	CV
	1982	1983	1984	1985		(%)
1. Methley	5	1	1	1	2.00	86.6
2. Shiro	1	3	2	3	2.25	36.2
3. Santa Rosa	3	4	2	2	2.75	30.2
4. Burbank	4	2	2	5	3.25	40.0
5. Elephant	2	5	3	4	3.50	31.9
6. Duarte	6	5	4	6	5.25	15.8

II. Japanese plums (Prunus salicina Lindl.)

The analysis of 6 cultivars over a 4 years' period indicates a rather fixed flowering dynamics of Japanese plums (Table 2). In 1982, mass flowering was rather prolonged lasting for an average of 7–8 days. 1983 was characterized by a rather rapid flowering process of an average of 4 days. Despite these annual variations, the flowering dynamics of individual cultivars was, however, less varied, which is indicated by the more-or-less parallel slanting of the individual curves of flowering.

In harmony with data by Szabó (1989), the start of mass flowering shows annual variations. Similarly to the European plums, the year 1983 could be characterized by the most precocious flowering (start of mass flowering Apr. 7). As a contrast, in 1982, 1984 and 1985, flowering started within a narrow range of 2 days (Apr. 15–17).

In comparison with the significant annual variations of European plums, this finding seems to indicate a higher genetic control of this trait in Japanese plums.

III. General applications of the Index of Flowering (Index-V)

In the course of our investigations, it could be established that the flowering characteristics of both European and Oriental plums can be very well characterized by the application of the Index of Flowering (*Index-V*). Consequently, on the basis of our experiences, the following main applications can be recommended:

1. Characterization of the course of flowering of individual cultivars,
2. Comparison of cultivars, populations, annual flowering variations with the aim of
   – selecting the simultaneously flowering cultivars to be used as pollenizers
   – facilitating agricultural plant protection activity at the time of flowering, etc.
   – including the data thus obtained in the data-banks of plant collections, gene banks, etc.

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Reviews

INTEGRATION OF TRADITIONAL AND NEW METHODS IN ANIMAL BREEDING*

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"If today we see farther,
it is because we can look
round from the yesterday's
altitude of our elders."
(Newton)

I began to make acquaintance in 1955, as a member of the scientific student circle at Professor Arthur Horn's department, with the secrets of the research work that serves the science of animal breeding. In a couple of years I was convinced that a continuous improvement and increasing efficiency of breeding demands the synthetization, integration of traditional and new, up-to-date principles and methods according to the requirements and possibilities of the practice of breeding. For nearly 40 years I have been trying to serve, with my scientific activity the professional training, the sphere of applied research and the practice of breeding alike. This is illustrated by my publications and citations listed in the "References".

My first work – even resulting in international interest and application – was the elaboration and testing of the *"fault-point of persistency" of milk production* (Dohy, 1957) (Table 1), which called attention to the importance and influencing factors of persistency. In these days this selection trait is coming to the forefront of interest (Dohy, 1989).

In 1960 I worked out and published a simple method (6) to *express the fertility of cows* drawing attention to the great importance of this trait and to the necessity of its objective examination (Table 2). (This work of mine was cited by Professor Sándor Cseh in his university text-book.)

In collaboration with Antal Dunay I worked out an index called *"index of relative milk production"* (Dunay and Dohy, 1961) (Table 3), which we applied as an indirect indicator of feed conversion, a selective means of a more efficient production with several of my co-workers and pupils (Dohy, 1963; 1971; 1976; Dunay and Dohy, 1963; Dohy and Guba, 1976; Dohy and Kelemeri 1967; Dohy and Kiss, 1970; Dohy and Ludrovszky, 1966). Our publications have been made use of by foreign research workers, too (mainly in Germany and Czechoslovakia) in their experimental investigations.

In 1961 I published a complex, still practical index called *"index of relative lifetimeproduction of cow"* (Dohy, 1961; 1963) (Table 4), which again aroused the specialists' interest (e.g. Professor Szajkó improved and employed this method), and was even discussed at international conferences (Dohy, 1975a; 1975b; 1976).

* On the basis of the academic inaugural lectuire delivered on 20 October, 1993

0238–0161/94–95/$ 5.00

Table 1

„Fault-point of persistency"
(Dohy, 1957)

$P = \frac{100 \times (Sd1 + Sd2)}{M}$
„P" = fault-point of persistency „Sd1" = the amount of the differences in the average daily milk kilograms of the various lactation months from the highest average value <from M> „Sd2" = the amount of the differences of the daily average value of the one after following months „M" = the daily average value of the highest production month
„P" value smaller than 100 = excellent persistency between 101 and 160 = good " between 161 and 220 = middle " between 221 and 280 = inferior " above 280 = bad "

In the 1960s the *modernization of breeding planning* more and more became a timely problem. Motive and possibility to it was given by the international integration blossoming out in animal breeding too, and by the wide adaptability of deep-frozen sperma in cattle breeding. On the basis of foreign experiences (e.g. Fewson's work) I published a *model* in 1970 concerning breeding planning (Dohy, 1970; 1972) (Fig. 1), which has been included in university text-books and international handbooks (similarly to my works mentioned above), contributing to the modernization of the national breeding strategies.

In common with Sándor Bozó, Antal Dunay and Károly Rada, we elaborated a comprehensive plan for the *evaluation of the Holstein-Friesian breed and organization of its breeding in Hungary* (Bozó et al., 1975) (Fig. 2). This work – as a publication of the Research Institute of Animal Breeding – was utilized in the national breeding planning and organization mainly by the state farms and by co-operative farms voluntarily undertaking a pioneer role. This synthetizing work that modified the way of looking at things also aroused the specialists' interest in the neighbouring countries, as proved e.g. by the publications of the International Agricultural Review.

Table 2

Fertility index of the cow
(Dohy, 1960)

$T = 100 - (K + 2i)$
T = fertility index K = age of cow at 1st calving (months) i = av. calving interval (months)
"T" value over 47 = good fertility between 41 – 47 = middle fertility under 41 = bad fertility

Table 3

Index of relative milk production
(Dunay and Dohy, 1961)

$$I_r = \frac{F.C.M(kg) \times 100}{(chest\ girth, dm)^2}$$

I_r = index of relative milk production
F.C.M = fat corrected milk (= 0,4 × milk kg + 15 × fat kg) (lactation or yearly milk yield)
dm = 10 cm

I_r	over 1500			= excellent relative milk production
	between	1301	and 1500	= very good relative milk production
	between	1001	and 1300	= good relative milk production
	between	801	and 1000	= medium relative milk production
	between	601	and 800	= poor relative milk production
	under	600		= bad relative milk production

Table 4

Index of relative lifetime production of the dairy cow

$$I_{re} = I_h \times \frac{I_r}{100}$$

$I_{ré}$ = Index of relative lifetime production

$$I_h = \frac{\text{Productive life – span of the cow (year)} \times 100}{\text{Total life – span of the cow (year)}}$$

I_h	over	80	= excellent relative productive life-span
	between	76–80	= very good relative productive life-span
	between	71–75	= good relative productive life-span
	between	51–70	= medium relative productive life-span
	under	51	= bad relative productive life-span

I_r = Index of relative milk production (see Table3)

I_{re}	over	1000	= excellent relative lifetime production
	between	701–1000	= good relative lifetime production
	between	401–700	= medium relative lifetime production
	under	401	= bad relative lifetime production

Table 5

„Optimum milk protein production index"
(Dohy, Boda and Kovách, 1980)

$$\text{Optimum milk protein production index} = \frac{\text{Milk protein kg}}{150} \times \text{milk kg}$$

Milk protein kg = protein yield in 1st lactation (305 days)
milk kg = 1st lactation yield (305 days)

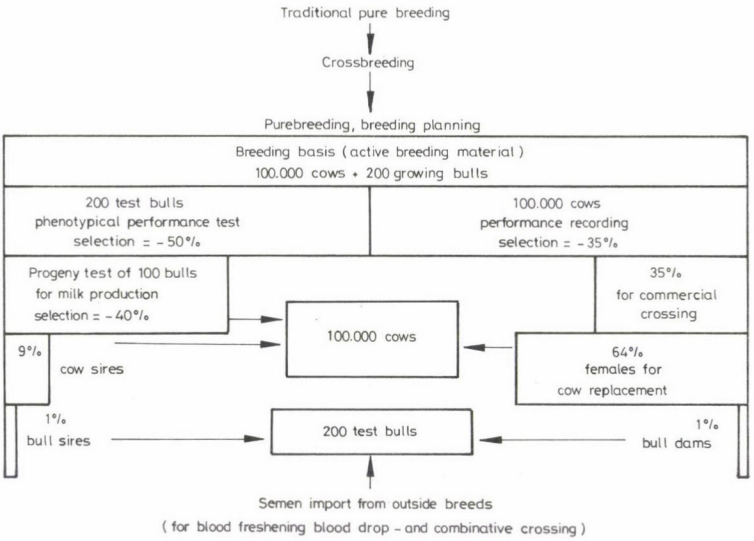

Fig. 1. Model for planning of cattle breeding (Dohy, 1970, after Fewson, modified)

In the second half of the seventies, we began to deal with the questions of applying dependent and independent selection limits, at the Agricultural College of Kaposvár. As a result we elaborated a practical selection index called the *optimum milk protein production index* (Dohy, 1992) (Table 5), and by using a wide data basis demonstrated its

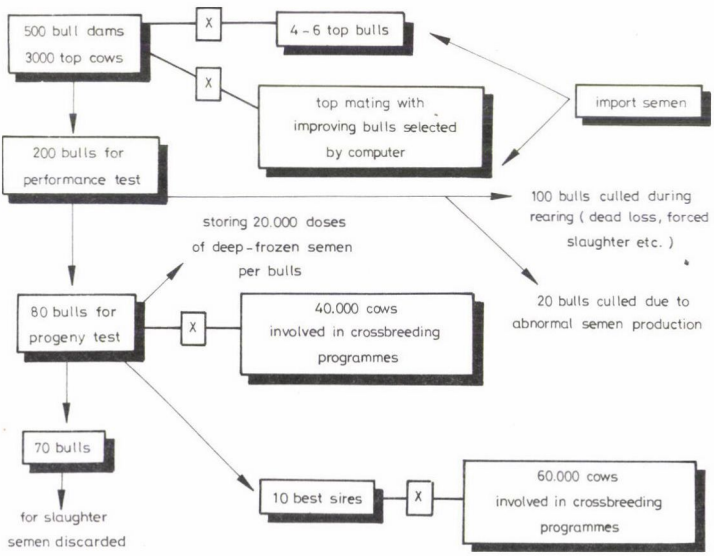

Fig. 2. Breeding plan for utilization of the Holstein - Friesian breed in Hungary(Bozó et al., 1975)

Table 6

Superiority of the best 10% of the population (expressed in %) and its deviation from the results of the entire population, depending on the selection index applied in the breed studied
(Boda, et al., 1989)

Production trait	Breed	Hungarian index	German index	Norwegian index	Dutch index
	Holstein	13.3	11.4	12.3	7.4
Milk, kg	Mountain Fleckv.	9.7	8.8	9.7	4.0
	Jersey	11.9	11.2	11.8	3.1
	Holstein	–2.5	3.7	2.0	7.0
Milk fat %	Mountain Fleckv.	–0.5	0.5	0.5	3.0
	Jersey	–1.0	0.6	0	4.3
	Holstein	0.3	1.8	1.2	3.7
Protein %	Mountain Fleckv.	0.9	1.8	1.2	4.2
	Jersey	–0.7	–0.2	0.5	3.7
	Holstein	10.0	15.2	14.3	14.8
Milk fat kg	Mountain Fleckv.	9.4	9.4	9.9	6.4
	Jersey	11.0	11.9	11.6	7.4
	Holstein	13.4	12.8	12.8	10.5
Protein kg	Mountain Fleckv.	10.4	10.4	10.4	6.9
	Jersey	17.6	17.1	17.1	5.6

German index = 0.09 × milk fat kg – 2,43 × fat % + 0.06 × protein kg + 3.88 × protein % + 4.5 (after Panicke)
Norwegian index = 0.22 × milk kg + 7 × fat kg + 15 × protein kg (after Skjervold)
Dutch index = 0.316 × milk kg + 260 × fat % + 500 × protein % (after Dommerholt)

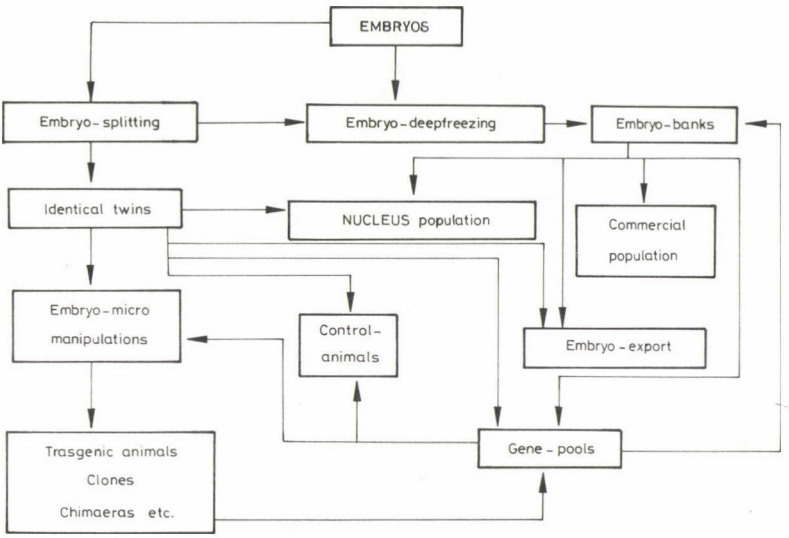

Fig. 3. Some possibilities for utilization of embryos for future cattle production systems(Dohy, 1986)

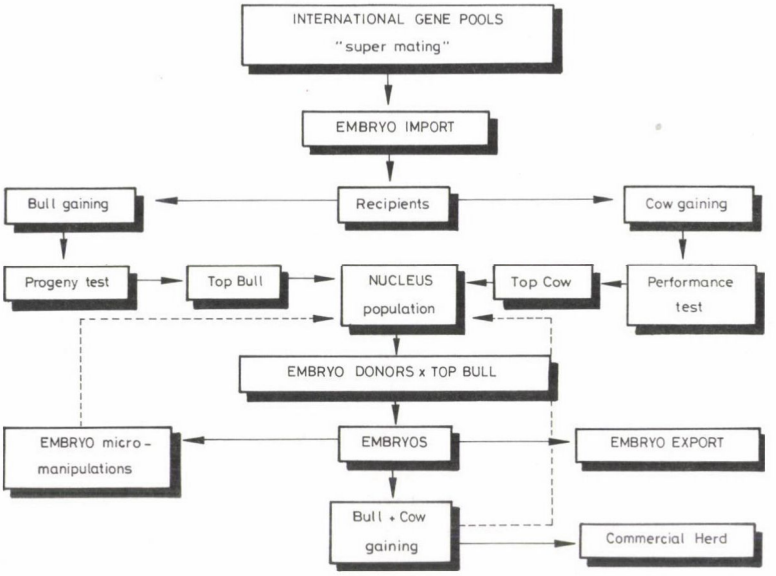

Fig. 4. Systematic utilization of ET in breeding plan (Dohy, 1986)

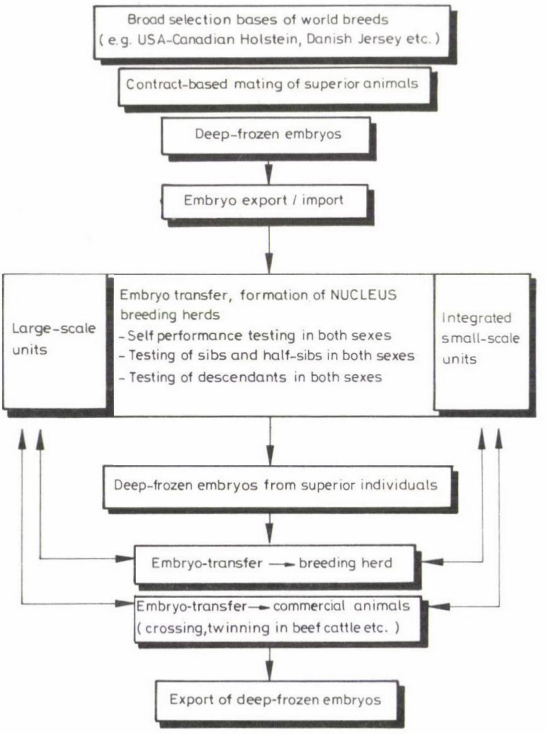

Fig. 5. Diagram of the new nucleus breeding strategy (Dohy, 1987; 1988)

efficiency calling attention to the necessity and possibility of increasing economically – through genetics and breeding – the production of milk protein (Boda et al., 1989; Dohy 1983a; 1989b; 1992) (Table 6).

In 1986, at a conference organized jointly by the Section of Agricultural Sciences, Section of Biological Sciences and Section of Medical Sciences of the Hungarian Academy of Sciences, I pointed out the *genetic and breeding possibilities based on embryo transplantation and embryo micro-manipulations,* incorporating them into a system and inserting them in our breeding strategy, a participant of the international integration (Dohy 1989a; 1989b; 1991a; 1991c) (Figs 3, 4). With this synthetizing work I also called attention to the possibility that with the help of the *embryo banks* we can prepare an efficient defense *against gene erosion,* which is becoming an ever more important and constant task.

In the second half of the eighties, the elaboration of a *comprehensive breeding strategy on the basis of the results of new biotechnical and biotechnological investigations* became necessary and increasingly important (Dohy, 1987; 1989a; 1989b; 1991a; 1991c) (Fig. 5). In this the MOET (multiple ovulation and embryo transfer) and the nucleus herds are in the focus of the breeding work. This breeding system – shown and published on international fora too – fits well in the frames of the "global breeding strategy" acting as catalyzer in the interest of a co-ordinated utilization of the gene bases of world breeds and their highly reproducible, mutually advantageous exploitation.

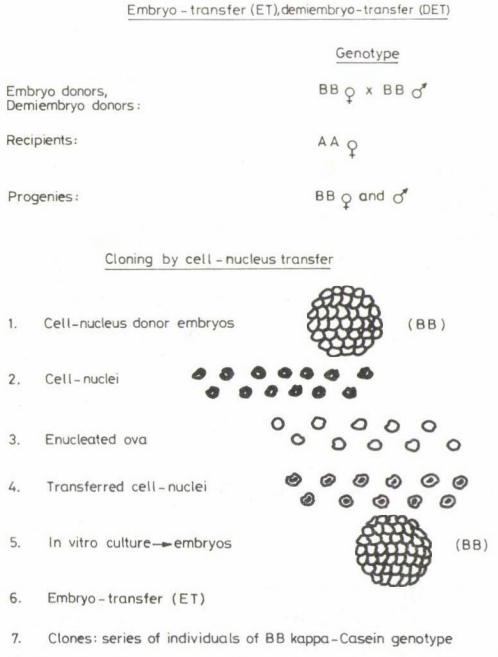

Fig. 6. Utilization of ET and DET and cloning for producing cattles of BB kappa Casein genotype (Dohy, 1993)

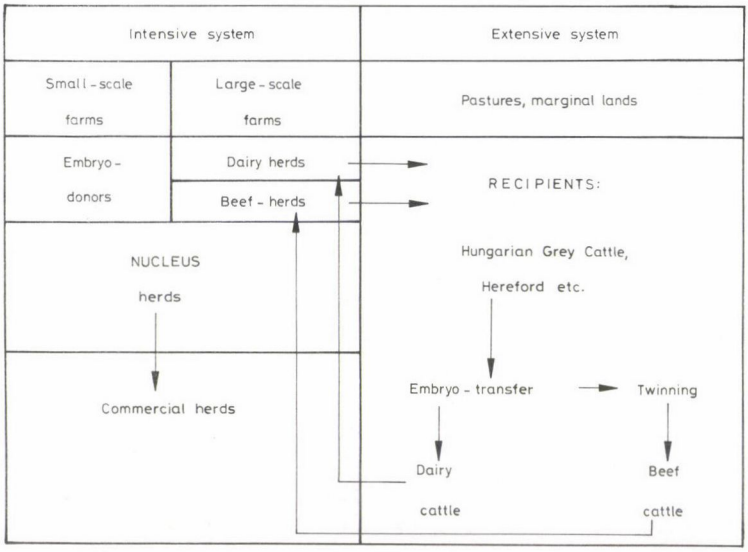

Fig. 7. Integration of intensive and extensive cattle breeding and production systems (Dohy, 1993)

On the basis of the results and experiences of the previous decades, the 1990s lay emphasis on quality in animal production, programmed "mass production" of high genetic value breeding animals (by means of cloning as well), and – with the view of "sustainable development" – environment protecting natural animal keeping, through a rational realization of extensive ("low input") and intensive ("high input") animal breeding systems working in manifold interactions. This work that means a complex task is urged by the transformation of the property relations, by the radical renewal of the Hungarian agriculture and of animal breeding within it. In this spirit I worked out a *breeding system for bringing into existence a population producing milk* (obtained from BB kappa casein genotype animals) *more suitable for cheese production.* In this selection and mating system *embryo transfer and use of demiembryos as well as cloning by cell-nucleus transplantation* are equally components showing a direction for further investigations (Bögre and Dohy, 1992; Bősze and Dohy, 1993; Dohy, 1991b; 1991c; Pethő et al. 1992) (Fig. 6).

For the integrated operation of the *intensive animal breeding system* (the sphere of nucleus herds) and the *extensive animal keeping system* (making use of grasslands and marginal areas cheaply, in the manner of a "nature-lover") I also worked out a *model,* which I demonstrated at an international conference organized in 1993 at the Gödöllő University of Agricultural Sciences ("New strategies for sustainable rural development"). This field of research – which includes the mentioned biotechnical and biotechnological possibilities – gives us new and perspectivic tasks: to the Institute of Animal Breeding of the Gödöllő University of Agricultural Sciences and to our co-operating partners (Fig. 7).

I continue to make every effort with my co-workers to amalgamate the traditional valuable methods, experiences, research results with the latest achievements of the modern science and technics with the firm intention of helping to raise the Hungarian animal breeding to a higher level and find a new path of development for it.

For my *ars poetica* I have chosen the following three words:

Cure,
Build
Improve!

The breeder serves this triple aim with his vocational activity never forgetting Mihály Váci's dramatic warning:

"The first man has stepped on the Moon.
The last two thousand million wait with
agony: – when to reach
at least the nourishing Earth."

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AUXIN AND CYTOKININ BIOASSAYS: A SHORT OVERVIEW

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Introduction

Plant Growth Regulators (PGRs), especially the auxins and cytokinins, play a key role in plant development (Kamalay and Goldberg, 1980; Goldberg, 1988; Gray and Purohit, 1991). By means of bioassays, a compound can be tested to determine whether it exerts auxin or cytokinin activity. In this paper, auxin and citokinin bioassays are summarized and briefly discussed from Darwin's (1896) assumption (Table 1) to recent achievements.

In general, there are five groups of classical PGRs: basic cytokinins, natural ethylene, acidic auxins, abscisic acid, and gibberellins (Bonner 1933; Letham, 1963, 1971; Staden and Davey, 1979). There are also several groups of compounds with growth regulating activity: (1) synthetic hormone analogues (Mornet et al., 1979; Horgan 1987; Horgan and Scott, 1987), (2) hormone antagonists (Leopold and Klein, 1952; Christou and Barton, 1989; Nagy and Tabi, 1982), (3) hormone inhibitors (Varga and Köves, 1959; Köves and Sirokman, 1973; Lürsen and Reiser, 1987), (4) hormone herbicides (Corbett, 1974), (5) antidotes or safeners (Kőmíves and Dutka, 1989; Halmann 1990),

Table 1

The history of auxins and cytokinins
(Doby 1965; Moore 1979; MacMillan 1980)

	Auxins	References	Cytokinins
1	"Downward moving sap"	Du Hamel (1758)*	"Upward moving sap"
2	"Organ-forming substances"	Sachs (1880)	"Organ-forming substances"
3	"Lateral light-inductive factor"	Darwin (1896)	
4		Wiesner (1892)	Cell division factor
5	Synthetic IAA	Ellinger (1904)*	
6	"Transportable factor"	Paál (1919)	
7		Haberlandt (1921)	"Wound hormone"
8	Growth promoting substances	Went (1928)	
9	a-, b- Auxins**	Kögl et al. (1934a)	
10	Heteroauxin	Kögl et al. (1934b)	
11	3-Indoleaceticacid	Haagen-Smit et al. (1946)	
12		Miller et al. (1956)*	Kinin, phytokinin
13		Skoog et al. (1965)	Cytokinin

* in Moore (1979), ** cyclopentene derivatives, they were proved to be artifacts.

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Table 2

The eight different types of auxins and auxin metabolites

Natural auxins			Synthetic auxins					
Phenyl derivaties		Indole auxins	Naphtalene acids	Chlorophenoxy acids	Benzoicacids	Pyridines	Benzo triazoles	Dithio carbamates
Amino acid precursors							Benzoisothia-(oxa) zoles	
PHE	THYR	TRY						
PAA	PHAA	IAA	NAA	2,4-D	2,5-DBA	3,5,6-TPA	BTR	CDDC
t-CA		ClIAA	NOXA	2,4-DP	2,3,6-TBA		BIA	
		N_3IAA	NOAC	2,4,5-T	3,6-DMBA		BOA	
		IBA		2,4,5-TP				
		IPRA		4-CPA				
Auxin – gluco/ribosides/tides								
Auxin – amino acid conjugates								

BIA=1,2-benzoisothiazole-3-acetic acid, BTR=benzotriazole, BOA=1,2-benzisoxazole-3-acetic acid, t-CA=trans-cinnamic acid, ClIAA=4-chloro-indoleacetic acid, CDDC=carboxydimethyl-dithiocarbamate, 4-CPA=4-chlorophenoxy-acetic acid 2,4-D=2,4-dichlorophenoxy-acetic acid, 2,5-DBA=3-amino-2,5-dichlorobenzoic acid, (CHLORAMBEN), 3,6-DMBA=3,6-dichloro-2-methoxybenzoic acid (DICAMBA), 2,4-DP=2-(2,4-dichlorophenoxy)-propionic acid (DICHLORPROP), IAA=-indole-3-acetic acid, IBA=indole-3-butyric acid, IPRA=indole-3-propionic acid, NAA=1-napthacetic acid, N_3IAA=azidoindoleacetic acid, NOAC=methyl-2-naphthoxy-acetate, NOXA=2-naphthoxyacetic acid, PAA=phenylacetic acid, PHAA=para-hydroxy-phenylacetic acid, PHE=-phenylalanine, 2,4,5-T=2,4,5-trichlorophenoxyacetic acid, 2,3,6-TBA=2,3,5-trichorobenzoic acid, 2,4,5-TP=2-(2,4,5-trichlorphenoxy)-propionic acid (FENOPROP), 3,5,6-TPA=4 amino-3,5,6,-trichloropicolinic acid (PICLORAM) TRY=tryptophan, TYR=tyrosine.

Table 3

The three different types of cytokinins and cytokinin metabolites

	Free bases		Metabolites	
PURINE CYTOKININS	ISOPENTENYL	2iP, Z	3-,7-,9-N-GLUCO	0-GLUCO
	AROMATIC	BA	and	and
	HETEROCYCLIC	KIN	RIBOSIDES/TIDES	RIBOSIDES/TIDES
UREA CYTOKININS	PU, DPU, CPPU, TDZ	–	–	–
BENZIMIDAZOLE DERIVATIVES	BACN	–	DBR	–

BA=6-benzylaminopurine, BACN=benzymidazole acetonitrile, CPPU=N-[2-chloro-4-pyridyl]-N" phenylurea, DBR=5,6-dichlorobenzimidazole-1-b-D-riboside, DPU=1,3-diphenylurea, 2iP=N-6-[2-isopentenyl] adenine, KIN=6-furfurylaminopurine, TDZ=thidiazuron, N-phenyl-N'-1,2,3-thiadiazol-5-yl urea, Z=6-[4-hydroxy-3-methyl-but-2-enylamino]purine, PU=phenylurea

and (6) plant alkaloids with hormone type activity (Buta and Kalinski, 1988). In addition (7) a number of miscellaneous types of non-classical PGRs are to be found: brassinosteroids, elicitors, oligosaccharides, glycoproteins and poliamines (Yopp et al., 1981; Corbett, 1974; Bruinsma, 1980). Besides, (8) hormone metabolites (Laloue and Pethe, 1982) also have biological activity. The loose connections between the molecular structure and hormone activities underline the importance of bioassays in classifying and determining the new bioactive compounds (Bearder, 1980; Zhao, et al. 1992; Kanbe et al., 1993).

Intensive research has been focused on finding new growth-regulating compounds, such as modified auxins like azido-auxins (Lee et al., 1984), modified cytokinins like thidiazuron (Thomas and Katterman, 1986; Visser et al., 1992), CPPU (Halmann, 1990) and new types of PGRs like S-(carboxymethyl)-dimethyldithicarbamate for agronomical purposes (Kerk et al., 1957), benzimidazole (Person et al., 1957), picloram (Beyl and Sharma, 1983), dicamba (Conger et al., 1983), benzothiazole (BIA) and benzizoxazole (Branca et al., 1990). No general theory exists which enables us to predict the hormone activity from the molecular structure. While the molecular structure requirements for PGR-activity are evident in the cases of abscisic acid, gibberelins and ethylene, in the case of auxins, except for certain types (Porter and Thimann, 1965), and in the case of cytokinins, it is still an open question, because of their various molecular structures (Tables 2; 3). Auxins comprise over ten types of compounds with different molecular structures, but with similar biological activity (Table 2). Cytokinins include three main groups of compounds (Table 3).

The discovery of the correlation between the electronic and steric properties of PGR molecules led to the formulation of QSAR (Quantitative Structure-Activity Relationship). In this way, the new PGR molecules can be designed by computer programmes (Halmann, 1990). Obviously, bioassays are necessary to verify bioactivity.

Bioassays

Different plants of monocots and dicots are used for bioassays (Tables 4, 5). The value of any bioassay ultimately depends on the specificity and selectivity of the observed biological responses on test objects (Nissen, 1985; 1988).

When the responses are compared, bioassays for auxins and cytokinins can be classified as: (1) Cell elongation, (2) cell division, (3) cell function, and (4) cell differentiation (Tables 4, 5).

From the observation of Darwin (1896) on *Phalaris* coleoptile, several assays were developed on the coleoptile of different monocot plant species, especially of *Avena* (Boysen-Jensen, 1913; Paál, 1919; Stark, 1921). In *Avena* coleoptile tests, both curvature and straight growth were used (Bentley, 1950; Kefford, et al., 1962). This technique was extended to other plant species, e.g. *Ageratum* (Bottelier, 1954), cucumber (Katsumi et al., 1965) and sunflower (Brauner, 1966).

Several other effects of auxins (Table 4) were also used for bioassays, e.g. initiation of tomato parthenocarpy (Zimmermann and Hitchcock, 1940), *Lotus* hairy root test (Shen et al., 1988) and in the *de novo* root initiation assay *in vitro* (Gyulai et al., 1992; 1993).

Table 4

Bioassyas for auxins

I. Cell elongation assays

Auxin induced coleoptile curvature assays

1. *Avena* Coleoptile Section Test (Boysen-Jensen 1913; Paál 1919; Stark 1921)
2. *Avena* Coleoptile Curvature Test (Went 1928; Went and Thimann 1937)
3. *Avena* Deseeded Coleoptile Test (Skoog 1937)
4. *Avena* Geocurvature Test (Kaldewey et al. 1969)
5. *Avena* Coleoptile Split Test (Rayle 1973)
6. *Phalaris* Etiolated Coleoptile Phototropic Test (Darwin 1896)

Auxin induced coleoptile straight growth assays

7. *Avena* Coleoptile Straight Growth Test (Bonner 1933, 1949; Schneider 1938; Bentley 1950; Sirois 1966)
8. *Avena* Peeled Coleoptile Test (Bonner 1934; Nitsch and Nitsch 1956)
9. *Avena* Micro Straight Growth Test (Rietsema 1949)
10. *Corn* Coleoptile Test (Bridges and Wilkins 1973)
11. *Rice* Coleoptile Test (Yamada 1954)
12. *Sorghum* Coleptile Test (Ramana et al., 1971)
13. *Wheat* Coleoptile Test (Nitsch and Nitsch 1956; Hancock et al. 1964)

Auxin induced different curvature assays

14. *Ageratum* Petiole Curving Test (Bottelier 1954)
15. *Bean* Internode Curving Tests (Meudt and Bennett 1978; Strand and Kaminek 1985)
16. *Citrus* Petal Curving Test (Goldschmidt and Monselise 1966; Goldschmidt 1968)
17. *Pea* Stem Split Curving Test (Went 1934; vanOverbeek and Went 1937)
18. *Pea* Quartered Stem Split Curving Test (Thimann and Schneider 1939; Ockerse and Galston 1967)
19. *Tomato* Petiole Curving Test (Kazemi and Kefford 1974)

Auxin induced different elongation assays

20. *Avena* First Internode Test (Nitsch and Nitsch 1956; Crosby et al. 1961)
21. *Cabbage* Hypocotyl Elongation Test (Andersen and Muir 1966)
22. *Cucumber* Hypocotyl Elongation Test (Katsumi et al., 1965; Sakurai et al. 1974)
23. *Mung Bean* Hypocotyl Elongation Test (Goldberg 1980)
24. *Pine* Hypocotyl Elongation Test (Wodzicki and Wodzicki 1973)
25. *Pea* Epicotyl Elongation Test (Christiansen and Thimann 1950)
26. *Sunflower* Hypocotyl Elongation Test (Brauner 1966)

II. Cell division assays

27. *Soybean* Cotyledon Callus Test (Miller 1963)
28. *Tobacco* Callus Test (Murashige and Skoog 1962)

III. Cell function assays

29. *Bean* Leaf Inhibition Test (Brown and Weintraub 1950)
30. *Cichorium* Root Disk Water Uptake Test (Rutherford et al. 1966)
31. *Coleus* Leaf Abscission test (Luckwill 1956)
32. *Tomato* Parthenocarpy Induction Test (Zimmermann an Hitchcock 1940)

IV. Cell differentiation assays

Auxin controlled rooting assays

33. *Avena* Root Inhibition Test (Bonner and Koepfli 1939)
34. *Bean* Root Test (Luckwill 1956)
35. *Corn* Root Test (Swanson 1946)
36. *Cucumber* Cotyledon Rooting Assay (Zhao et al. 1992)
37. *Cress* Root Section Test (Moewus 1949)
38. *Lettuce* Root Test (Ready and Grant 1948)
39. *Lotus* Hairy Roots Test (Shen et al., 1988)
40. *Pea* Root Test (Aberg 1950; Audus and Thresh 1953)

Auxin induced morphogenesis assays

41. *Tobacco* Root Regeneration Test (Skoog and Miller 1956; Murashige and Skoog 1962)
42. *Tobacco* Test With Auxin Selectivity (Gyulai et al. 1992, 1995).

Table 5

Bioassays for cytokinins

I. Cell elongation assays

Cytokinin induced coleoptile elongation assays

1. *Avena* Coleoptile Elongation Test (Shrank 1957; 1958)
2. *Wheat* Coleoptile Elongation Test (Rothwell and Wright 1967)

Cytokinin induced expansion assays

3. *Bean* Leaf Disc Expansion Test (Miller 1956)
4. *Cucumber* Cotyledon Expansion Test (Green and Muir 1978; Zhao et al. 1992)
5. *Lemna* Frond Growth Test (Hillman 1957; Loeffler and VanOverbeek 1964)
6. *Lettuce* Cotyledon Expansion Test (Ikuma and Thimann 1963)
7. *Radish* Cotyledon Enlargement Test (Kuraishi 1959)
8. *Radish* Cotyledon Expansion Test (Letham 1971)
9. *Spirodel* Frond Growth Test (Letham 1967a)
10. *Xanthium* Cotyledon Expansion Test (Esashi and Leopold 1969)

II. Cell division assays

Cytokinin induced callus proliferation assays

11. *Bean* Pod Parenchyma Test (Wehnelt 1927; English and Bonner 1937)
12. *Carrot* Callus Test (Caplin and Steward 1949; Steward and Shantz 1955)
13. *Carrot* Root Callus Test (Letham 1963, 1967a)
14. *Soybean* Cotyledon Callus Test (Miller 1960, 1963)
15. *Soybean* Hypocotyl Section Test (Manos and Goldthwaite 1976; Newton et al. 1980)
16. *Tobacco* Stem Slab Test (Skoog and Miller 1956)
17. *Tobacco* Pith Callus Test (Murashige and Skoog 1962)

III. Cell function assays

Cytokinin induced pigment formation assays

18. *Amaranthus* retroflexus Betacyanin Test (Bamberger and Mayer 1960, Köhler and Conrad 1966)
19. *Amaranthus* caudatus Betacyanin Test (Bigot 1968; Biddington and Thomas 1973; Reda and Rasmussen 1975)
20. *Bean* Etiolated Leaf-Disc Test (Millewr 1963)
21. *Cucumber* Chlorophyll Formation Test (Fletcher and McCullagh 1971; Fletcher et al. 1982)
22. *Soybean* Deoxysoflavone Test (Miller 1969)

Cytokinin included leaf senescence delaying assays

23. *Avena* Leaf Chlorophyll Retention Test (Thimann and Sach 1966; Varga and Bruinsma 1973)
24. *Barley* Leaf Chlorophyll Retention Test (Kende 1964, 1965; Letham 1967b; Engelbrecht 1971)
25. *Bean* Leaf Chlorophyll Retention Test (Goldthwaite and Laetsch 1967)
26. *Radish* Leaf Chlrophyll Retention Test (Kefford et al. 1968)
27. *Rumex* Leaf Chlorophyll Retention Test (Goldthwaite 1972; Manos and Goldthwaite 1975)
28. *Xanthium* Leaf Chlorophyll Retention Test (Richmond and Lang 1957; Osborne and McCalla 1961)
29. *Wheat* Leaf Chlorophyll Retention Test (Person et al. 1957)

Cytokinin stimulated transpiration assays

30. Avena Leaf Transpiration Test (Luke and Freeman 1967; Tetley and Thimann 1974)

IV. Cell differentiation assays

Cytokinin induced morphogenesis assays

31. *Barley* Root Inhibiton Test (VanOnckelen and Verbeek 1972)
32. *Impatiens* Lateral Shoot Initiation Test (Bozsik 1983)
33. *Funaria* Moss Bud Formation Test (Szweykowska 1962; Hahn and Bopp 1968; Brandes and Kende 1968)
34. *Lettuce* Seed Germination Test (Skinner and Shive 1956; Skinner et al. 1957).
35. *Pea* Etiolated Stem Inhibiton Test (Sommer 1961)
36. *Pea* Lateral Bud Induction Test (Thimann and Sachs 1966)
37. *Pohlia* Moss Bud Formation Test (Mitra and Allsopp 1959)
38. *Tobacco* Shoot Regeneration Test (Skoog and Miller 1956; Murashige and Skoog 1962)
39. *Tobacco* Test With Cytokinin Selectivity (Gyulai et al. 1992, 1995)
40. *Tortella* Moss Bud Formation Test (Gorton et al. 1957)

The first cytokinin-dependent cell division tests were based on tobacco pith (Skoog and Tsui, 1951; Patau et al., 1957; Skoog and Miller, 1956; Das et al., 1958; Murashige and Skoog, 1962). The system was widened to carrot (Steward and Shantz, 1955; Letham, 1963; 1966), and to soybean (Miller, 1963; Manos and Goldthwaite, 1976; Newton et al., 1980). The cytokinin-initiated leaf expansion-effect was used first in a bean leaf test (Miller 1956); later, other dicot plants were found to be responsive, e.g. radish cotyledon (Kuraishi 1959; Letham 1971), *Spirodela* frond (Letham, 1967b) and cucumber cotyledon (Greend and Muir, 1978).

A special part of the cytokinin bioassays were based on the initiation of pigment formations like betacyanin in *Amaranthus* (Bamberger and Mayer, 1960; Elliot, 1979), chlorophyll in cucumber (Fletcher and McCullagh, 1971) and deoxyisoflavone in soybean (Miller, 1969).

From the observation of the cytokinin-inducable chlorophyll retention effect (Richmond and Lang, 1975; Person et al., 1957; Reilly, 1986), several assays were developed to barley (Kende, 1964, 1965), *Avena* (Thimann and Sachs, 1966) and radish (Kefford et al., 1968). As a result of the key role of cytokinins on plant morphogenesis, different morphoregulatory assays were developed for moss bud formation (Skinner and Shieve, 1956; Gorton et al., 1957; Hahn and Bopp, 1968), for tobacco plant regeneration (Skoog and Miller, 1956) and for selective tobacco shoot initiation (Gyulai et al., 1992, 1995).

Limitations

Different bioassays have various limitations and disadvantages. The seed germination assays (Skinner and Shive, 1956; Skinner et al., 1957) can be influenced not only by cytokinins but by many other factors such as red light, gibberellins and thiourea (Miller, 1963).

The kinetin-retarded senescence assays are responsive not only to cytokinins but also to benzimidazole or large amount of sugar, as was observed in the tests of *Xanthium* (Osborn and McCalla, 1961) and *Rumex* (Goldthwrite 1972). Therefore, their uses are very limited (Varga and Bruinsma, 1973).

The leaf expansion cytokinin tests (Miller, 1956; Kuraishi, 1959) are limited by the side effect of cobalt ions, red light, and the cross effect of gibberellins (Miller, 1963). In the cytokinin tests of tobacco and carrot, cross effects of IAA and myo-inositol were found (Miller, 1963; Letham, 1967a) and there was a tendency of soybean cotyledons to become independent of cytokinins (Newton et al., 1980).

Assays based on callus or suspension cultures involved limitations of habituation (Gautheret, 1934; 1938; 1960; Köves and Szabó, 1987; Christou, 1988).

Coleoptile test were considered to be highly specific for auxins, until the observation of Shrank (1957, 1958) and Rothwell and Wright (1967) demonstrated that there was not only auxin but cytokinin activity in coleoptile tests (Table 5).

Leaf expansion and pigment production assays for cytokinins showed difficulties of standardization and hormone specificity (Letham, 1971; Ladó et al., 1975).

Betacyanin accumulation in *Amaranthus* assays (Table 5) exhibited cross activity, being enhanced not only by cytokinins but also by fusicoccin, KCl, $NaNO_3$, KNO_3 and phosphate ions (Elliot, 1979).

In bioassays based on leaf senescence retardation, the protein synthesis inhibitors could imitate the cytokinin-effect (Tetley and Thimann, 1974).

Facilities of tissue culture techniques made it feasible to increase hormone specificity (Linsmayer and Skoog, 1965; Gamborg et al., 1968, Malone and Dix, 1986; Schenk and Hildebrandt, 1972), eliminating the synerging effects of PGRs (Bhattacharaya et al., 1978; Hasenstein and Evans, 1986). In *Xanthium* cotyledon tests the gibberellin cross activity was eliminated successfully by the application of mannitol in the test media (Esashi and Leopold, 1969).

A new selective bioassay for auxins and cytokinins was reported recently (Gyulai et al., 1993, 1995). It was based on the ability of cytokinins to induce adventitious shoots and the auxins to induce adventitious roots applied alone on tobacco leaf discs cultures, giving a so-called "all-or-none" (Hahn and Bopp, 1968) reaction. The new bioassay eliminated the synerging effects of auxins and cytokinins. Abscisic acids and gibberellins did not show activity. The method was patented in Hungary (Gyulai et al., 1992).

The new physicochemical (PHLC, GC-MS etc.) and immuno assays (ELISA, RIA, etc.) opened a new dimension in PGR science (Letham, 1966; Ernst et al., 1983; Sutter and Cohen, 1992). Nevertheless, bioassay tests are always needed to prove bioactivity.

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Book reviews

Tierzüchtungslehre. Ed.: Prof. DR. HORST KRÄUSSLICH, Ulmer, Stuttgart, 1994. p. 464.

The international literature of the profession of animal breeding was enriched by an extremely valuable systematizing and synthetizing book in the middle of 1994. This 4th edition of the work *Tierzüchtungslehre (Science of animal breeding)* resulted from a complete revision of the earlier editions. The compilation was done by Prof. Dr. Horst Kräusslich, professor at the Animal Breeding Institute of the Ludwig-Maximilians Universität, Munich, who invited another thirty German, Austrian and Swiss experts of international fame to write the individual chapters. The book appeared in the series Tierzuchtbücherei (Animal Breeding Library) edited by the Ulmer Publishing House, Stuttgart, in German language. (Further volumes of the series contain detailed information on the science of farm animal breeding.) The book runs to 464 pages, made complete by 137 pictures and 131 tables. Orientation in the extremely concise text is facilitated by a detailed subject index.

The book is divided into ten main chapters and many sub-chapters.

(1) Introduction (H. Kräusslich)

(2) Importance and strategies of animal production (H J. Langholz)

"The development of animal breeding cannot be understood without a knowledge of the natural, economic and political conditions."

(3) History of animal breeding (M. Röhrs, B. Mayr)

"The 12000-year history of animal breeding has offered many – often surprising – experiences, concerning among others the effects of domestication and the biological productivity of the animal species."

(4) Basics of genetic

Molecular genetics (H. Geldermann)

Cytogenetics (G. Stranzinger)

Mendelian genetics (F. Pirchner)

Genetics of quantitative properties: population genetics (P. Glodek)

"Acceleration of the development of breeding in the past decades is largely the consequence of applying Mendelian genetics. Today the special branches of genetics offer indispensable bases for breeding."

(5) Property complexes of farm animals

Colour and design (W. Schlote)

Type (N. Künzi)

Reproduction (D. Schmidt)

Adaptation and disease resistance (B. Senft)

Growth and meat production (G,. Schönmuth, G. Seeland. W. Neumann)

Milk production (H. O. Gravert)

Wool production (R. Wassmuth)

Egg production (J. Petersen)

Work performance, rideability, race performance (H.-J. Schwark)

Functional interactions (F. Ellendorff)

"In the course of breeding, efforts are made to improve definite characteristics. It is based on disclosing the genetic diversity of the characteristics and the correlations between them."

(6) Measuring and evaluation of properties as preconditions of selection and mating.

Performance tests (H.-J. Langholz, F. Schmitten, H.-J. Schwark)

Evaluation of data (A. Essl)

Estimation of breeding value (L. Demple)

"The knowledge of correlations between genetic variation and properties is only able to serve the purposes of breeding if the properties can be measured and evaluated at acceptable costs. That is why the systems of performance test were developed, which in principle, and partly in practice, are becoming internationally more and more uniform. The performance tests form the basis of breeding value estimation, and indirectly of selection. The breeding value estimation is based on the theories and models of population genetics. The increasing masses of data are processed continuously in high capacity computer centres. To solve the task, a great bulk of special, not explicitly animal breeding knowledge is required."

(7) Biotechnics

Structure and function of sexual organs: artificial insemination (W. Leidl)

Embryo transfer (W. Holz)

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Genome analysis (M. Förster)
Gene transfer (G. Brem)
„The development of genetics, biotechnics and computer technics in the second half of this century has radically changed the science and practice of animal breeding. The results of biotechnics are utilized first of all in reproduction and in the field of gene technics. The artificial insemination is a reproduction method of world-wide dominance in some animal species and lines of utilization. With its application, the number of paternal progeny groups has increased by leaps. In the female sex, the same is promoted by the embryo transfer. Intensive breeding programmes are no longer imaginable without this (e.g. MOET).
The genome analysis is aimed at acquiring a knowledge of the genetic constitution of breeding animals so as to make the selection decisions more reliable. Primarily through the gene diagnostics, the hereditary defects are to be eliminated, and the so-called major genes identified. The gene mapping renders it possible to make use of the genetic markers in selection. With the help of successful gene transfers, breeding lines possessing revolutionarily new properties can be brought into existence."

(8) Applied breeding
Breeding methods (D. Simon)
Breeding planning (D. Fewson)
Integrated breeding and production programmes (E. Kalm)
Breeding strategies in the tropics (P. Horst)
The steps of the breeding process are: determination of the goal of breeding, performance examination, estimation of breeding value, selection, mating, turning of breeding progress into the field of production. When planning the process of breeding, the economic factors besides the genetic parameters must also be taken into consideration. Then the breeding of farm animals can remain competitive for a long time. In the breeding programmes, the importance of the individual properties changes from time to time as a function of this too.

(9) Organization and institutions of animal breeding (H. Kräusslich)
"The breeding programmes generally cover large populations. This requires efficient breeders' organizations. At the same time the actual work of breeding starts from intensively managed nucleus stock-farms. An efficient cooperation between nucleus stock-farms and commercial farms can be realized only by a high level organization."

(10) Summary of the past and outlook for the future (H. Kräusslich).

The book *Tierzüchtungslehre (Science of animal breeding)* is recommended to all those who demand a general knowledge of animal breeding in the course of their theoretical or practical activities, and who wish to continue working in accordance with the requirements of our days.

Z. SÜPEK

Radioecology and environmental protection by ANDRÁS S. SZABÓ, Ellis Horwood Ltd., pp. 258, 1993.

The author published the first version of the book – written in Hungarian in 1985 –, of which a review was also written by me in the periodical "Agrokémia és Talajtan."

The book agrees in many respects with the Hungarian version as regards its structure; the author naturally completed the material with the scientific experiences of the past 8 years. This was – unfortunately – made obligatory by the Chernobil disaster that occurred not very long after the appearance of the first book. I emphasize that these data – first of all in Hungarian relations – gain publicity in a summarized way in this place.

In his book the author remained faithful to his earlier good qualities: the book is readable, professionally correct and easy to understand. Perhaps– as it was not marked for a text-book – it is unnecessarily pedantic.

The author tries to lay great emphasis on the biological, physiological, agricultural correlations of radiations. The value of the book as a source is considerably increased by the large number – nearly double compared to the first book – of tables and figures which contain abundant information.

Some critical remarks:

a) In the chain of nutrition most authors refer to the crust of the Earth, since all nutritive elements originate from there.

b) The concept of rheology is used in a much wider sense than it is in the book.

c) The Hungarian edition contained some 800 literary references, but in this book they are only 500 in number, which in itself would not be a serious deficiency. However, as every book can also be regarded as a source, a more detailed bibliography would have been welcomed. And a great fault is the absence from the list of the names of many authors referred to, even in tables.

To sum it all up, Szabó's book is a valuable work. And well translated for its English edition.

I. PAIS

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Zinovev, L. S., Matalova, T. S. (1976): Protaviteli, bezopasnie dlya klubenykovykh bakterii. *Zashchita Rastenii*, **5,** 29–31.

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Plant Physiology and Biochemistry

PIGMENT ACCUMULATION STUDIES IN TABLE BEET (*BETA VULGARIS* L., *SUBSP. ESCULENTA GURKE VAR. RUBRA* L.) DURING THE GROWING PERIOD

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VEGETABLE CROPS RESEARCH INSTITUTE, KECSKEMÉT, HUNGARY

(Received: 13 November 1992; accepted: 8 December, 1993)

The pigment accumulation trials aimed at finding out in which developmental phase red pigment quantities were the highest.

In table beet varieties sown in July, the betacyanin and vulgaxanthin contents were the highest on the 75th day of root development, followed later by considerable decrease.

In the August trial, the pigment accumulation maximum was observed in an earlier phase (50th day of development) which could be explained by the decreasing sunshine hours in autumn. At the 75th day of growth, a considerable decrease in the betanin content was observed. Thus, as indicated by trials, sowing in August cannot be recommended.

The pigment content evolution in table beet root is influenced considerably by numerous environmental factors (light, temperature, soil moisture content, nutrients, etc.). Consequently, the continuation of trials is well motivated.

Key words: *Beta vulgaris* L., *subsp. esculenta Gurke var. rubra* L., betanin, vulgaxanthin, growing period, pigment content

Introduction

Almost all of the beet roots produced in Hungary are processed in factories mostly for pickles and, in a very small amount, for dried products (beet root powder).

The colour of the root is due to two main pigment groups found in cell vacuoles – the yellow betaxanthins and the red betacyanins (Nilsson, 1970).

Piattelli et al. (1965) identified two yellow pigments – vulgaxanthin-I and vulgaxanthin-II – in the table beet. Betanin, the main betacyanin component in beets, comprises 75–95% of the total red pigment content (Pash et al., 1975); the remaining part consists of isobetanin, isobetanidin, prebetanin and isoprebetanin (Elbe et al., 1972).

The structure formula of the most important beet root pigments can be seen in Fig. 1.

Mabry et al. (1962, in Nilsson, 1970) found a similarity in the structure of betanidin (an aglycone of betanin) to that of alkaloids and adapted the name of chromoalkaloids to describe natural substances of such a type.

Nilsson (1970) improved the spectrophotometric method to study the pigments in question. λ_{max} 535–540 nm absorption maximum was found for betanin and λ_{max} 476–478 nm for betaxanthin (Aronoff and Aronoff, 1948). The pigment content of the root can be characterized by its principal pigments (betanin and vulgaxanthin-I) or by its total red and yellow pigment quantity (betacyanin, betaxanthin) expressed in mg/100 g fresh weight (Elbe et al., 1972). (Both variations can be found in scientific articles.)

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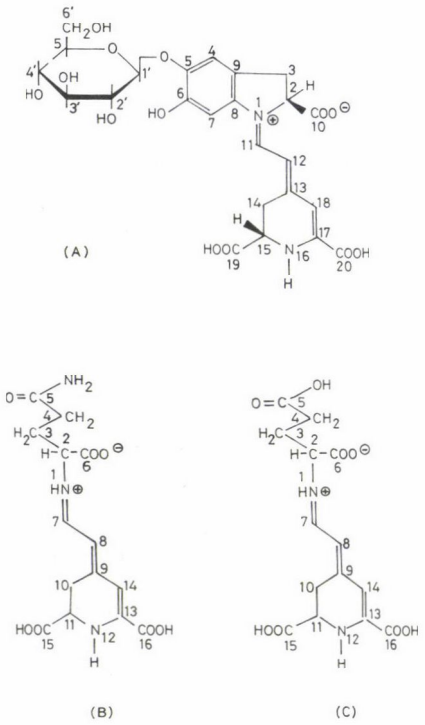

Fig. 1. A betanin, B vulgaxanthine I, C vulgaxanthine II

During processing the yellow pigments will decompose readily, so the colour of the product is determined mostly by the quantity of red pigments (Kramer and Smith, 1946). Kaack (1977) found nearly 60% loss in the pigment content of products during processing, compared with the raw material, followed by a further 10% decomposition in 6 month storage at 5 °C.

Thus, it is important to study pigment accumulation during the growing period in order to obtain useful information as to optimal harvest time, when root size (yield) and pigment content favour processing.

Scientists abroad, however, differ in their opinions as to changes in the red pigment content.

Lorenz (1947) found that air temperature and the length of the growing period influenced the pigment content of the root considerably. He observed senescence of the roots and a considerable decrease in pigments in autumn, among beets sown in early spring (February, March).

Shannon (1972) stated that the betacyanin content of beet roots sown at the beginning of June reached its maximum on the 109th day of the growing period, followed later by a gradual decrease. He explained the phenomenon by the increase in root diameter. Kaack (1977) made similar observations in his trials.

Nilsson (1973) measured in August the highest betacyanin amount in beet roots sown in May and explained it by the ripening of roots. In a second crop beet roots sown in June the highest betacyanin quantity was found in autumn, while betaxanthin increased gradually during the growing period.

He suggested that the pigment content of the root depended on the age of the root and on the temperature conditions of the growing period.

Watson and Gabelman (1982) suggested the influence of genotype and that of the sowing date. They stated that low temperatures promoted the increase of pigment concentration, thus confirming the observations of Sistrunk and Bradley (1970).

Wolyn and Gabelman (1986), however, supposed the genetic potential of the variety to be responsible for the amount of pigment accumulation. In their trial, the beet was sown in June and July and samplings were taken on the 50th, 75th and 100th days of the growing period. They found that in roots sown in July, with moderate or high betacyanin-betaxanthin ratio, the red pigment concentration was three times more than in varieties sown in June. In varieties of low pigment ratio, the pigment content did not increase considerably.

They found that the cool autumn air temperature favourably affected the phenotypic appearance of the genetic background determining pigment concentration.

The intensity of the violet-red pigmentation of the root can also be affected by environmental factors (Banga, 1962). High light intensity increases (Weichman, 1987) while high mineral fertilizer and manure doses decrease (Patzelt-Dollnick, 1986) the pigment content of the roots.

Materials and methods

Trials were carried on in the Vegetable Crops Research Institute in Kecskemét in a random block design of 4 replications with two different sowing dates: 5 July 1991 and 18 August 1991.

The Hungarian and foreign varieties tested in the trial are listed in Table 1. The variety Bordó, well known in Hungarian production, served for control. The plot size was 2.5 m^2 with 20–25 plants/m^2 population density.

Table 1

List of varieties studied in the pigment accumulation trial

Variety	Trial date	Origin	Growing period	Root shape
'Bordó'	July and August 1991	home variety, released 1981	long	globe
'Detroit'	July 1991	home variety, released 1951	short	globe
'Rubin'	July and August 1991	home variety, released 1992	midlong	globe
'Mobile'	July and August 1991	the Netherlands	midlong	globe
'Norton'	July and August 1991	the Netherlands	midlong	cylindric
'Egipsky Crosby'	July 1991	the Netherlands	short	globe
'Tardel'	July 1991	the Netherlands	midlong	globe
'Rubia'	July 1991	Denmark	midlong	globe
'Little Ball'	July 1991	the Netherlands	short	globe
'Pronto'	July 1991	the Netherlands	midlong	globe
'Forono'	July and August 1991	Denmark	long	cylindric

To study the accumulation rate of pigments, samples were taken on the 50th and 75th days in varieties of short growing period sown in August; and in varieties sown in July samples were also collected on the 100th and 135th days. The content of red and yellow pigments (mg%) were determined. Due to lack of labor capacity, measurements were made in one replication only using mean samples of total 20 roots; 5 roots of each replication. Nilsson's (1970) method was used to determine pigments quantitatively.

The absorption maximum of betanin and vulgaxanthin-I (478 and 537 nm) were measured spectrophotometrically to determine pigment concentration. Results are given in mg betanin and mg betaxanthin-I/100 g fresh weight, respectively.

The root diameter was also determined. Measurements were evaluated by correlation tests.

Results

To study the rate of pigment accumulation in table beet roots, varieties were sown at two different dates: 5 July 1991 and 18 August 1991. Results of the first sowing date (5 July) are presented in Table 2. In samples taken on the 50th day of the growing period the highest betanin content was found in the varieties Norton, Egipsky Crosby and Pronto (146.7, 144.9 and 145.6 mg%). At the second sampling date this value decreased in Norton, Egipsky Crosby and Rubia, falling to 58.5 mg% in Norton. The variety candidate Rubin excelled in uniform red pigment accumulation (91.8 and 116.1 mg%, respectively) associated with good root diameter (3.02 cm). In the other varieties tested in the trial, 22–54 mg% pigment surplus was found as related to previous trial results. In Pronto this value was nearly identical on the 50th and 75th days (145.6 and 151.2 mg%, respectively).

Table 2

Pigment accumulation trends in table beet varieties sown in July

Variety	50th day			75th day			100th day			135th day		
	pigment content mg%		root	pigment content mg%		root	pigment content mg%		root	pigment content mg%		root
	betanin	vulga-xanthine I.	diameter cm	betanin	vulga-xanthine I.	diameter cm	betanin	vulga-xanthine I.	diameter cm	betanin	vulga-xanthine I.	diameter cm
'Bordó'	80.1	50.4	1.92	107.1	92.7	3.08	83.3	43.0	4.71	60.3	34.1	7.10
'Rubin' fj. (10)	91.8	57.6	1.94	116.1	105.3	3.02	77.0	33.5	5.90	56.0	37.2	7.50
'Detroit'	68.4	53.1	2.03	99.9	81.0	3.17	51.3	25.4	6.47	46.0	18.7	8.00
'Mobile'	101.7	61.2	2.42	123.3	100.8	2.81	101.3	45.2	4.92	76.2	38.1	6.54
'Norton'	146.7	92.7	1.79	88.2	89.1	3.11	58.6	31.2	4.40	66.7	37.5	6.32
'Egipsky Cr.'	144.9	94.5	1.85	135.9	109.8	3.12	88.3	36.0	5.33	64.3	39.2	7.30
'Tardel'	85.5	52.2	1.57	139.5	106.2	2.87	83.7	33.6	4.80	71.5	35.2	7.10
'Rubia'	98.1	63.9	1.81	85.5	81.0	3.44	84.6	34.2	5.49	65.3	31.6	7.75
'Little Ball'	68.4	44.1	1.98	108.9	93.6	3.79	67.5	35.0	5.40	63.2	32.0	7.70
'Pronto'	145.6	44.1	1.74	151.2	127.8	3.54	83.0	50.3	4.62	69.5	40.2	6.36
'Forono'	88.2	55.8	1.20	129.6	101.7	2.63	73.1	31.0	4.52	71.1	37.0	5.38
			1.84			3.12			5.14			7.00

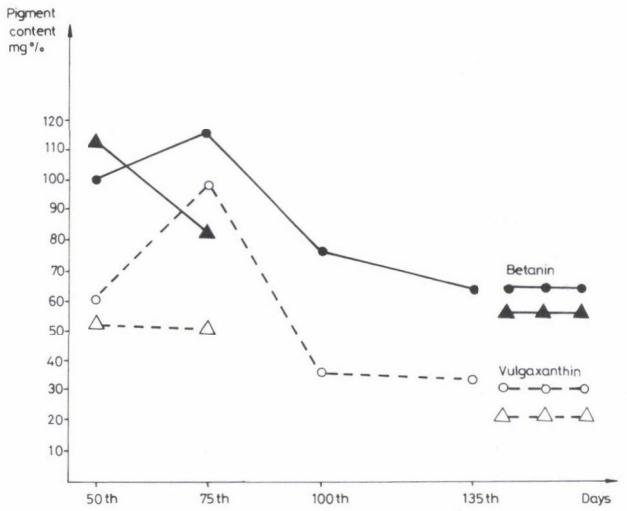

Fig. 2. Pigment accumulation trends in table beet varieties sown in July and August

At the third sampling date (100th day) pigment content decreased considerably in every variety, but especially so in Detroit, where it approached 50%. Figure 2 illustrates well the trend of change in betanin content. On the 135th day of the growing period, results showed a further decrease, confirmed also by the strong negative correlation between the number of days and the betanin content ($r = -0.608$) (Table 3).

During the growing period, the vulgaxanthin content of varieties reached its maximum on the 75th day, excepting Norton and Egipsky Crosby, which had the highest values on the 50th day (92.7 and 94.5 mg%). The yellow pigment content

Table 3

Multicorrelation analysis in table beet lines

Studied characters	Number of days	Betanin content	Vulgaxanthine I. content	Root diameter
Varieties sown in July:				
Number of days	1.000	–0.608	–0.559	0.962
Betanin content		1.000	0.832	–0.649
Vulgaxanthine I. content			1.000	–0.609
Root diameter				1.000
$Chi^2 = 192.54$				
FG = 6				
Varieties sown in August:				
Number of days	1.000	–0.710	–0.054	0.916
Betanin content		1.000	0.176	–0.481
Vulgaxanthine I. content			1.000	0.117
$Chi^2 = 31.84$				1.000
FG = 6				

increased suddenly in the mean of varieties, as also represented in Fig. 2. At the third sampling date (100th day) these values decreased in Rubin, Detroit, Tardel and Forono to one third. Gradual decreases or persistence could be observed on the 135th day as well.

In our trials, relationships among the number of days, red and yellow pigment contents and the root diameter were evaluated by correlation analysis (Table 3). Results indicated inverse relation of the number of days to yellow pigment accumulation, as proved by the moderate correlation of r = –0.559.

There was a close correlation between the accumulation of betanin and vulgaxanthin (r = 0.832) suggesting identical trends in the accumulation of the two pigments.

The trials indicated a negative correlation between the diameter of the growing roots and the betanin and vulgaxanthin contents (r = –0.609 and r = –0.649, respectively) as also proved by Watson and Gabelman (1982).

Results of the two trials – July and August sowing – are compared in Table 4. In beet root varieties sown at the end of summer, the betanin content had maximum values on the 50th day of the growing period. By the 75th day, however, a decreasing tendency could be observed (Fig. 2) as also confirmed by the correlation trend (r= –0.710) between the number of days and betanin content. In trials sown in July, the tendency was increasing at the time (75th day).

The lowest value in vulgaxanthin content was measured in the variety Forono on the 50th day (37.2 mg%), while in the other varieties the value varied between 46.3 and 58.7 mg%. Similar to the red pigment content, a decrease could be observed on the 75th day in contrast to the varieties sown in July where the pigment content increased intensively.

By the advancing growing period the yellow pigment content decreased slightly.

Correlation between root diameter and pigment quantity had a tendency character in vulgaxanthin (r = 0.117) and it is loose, though inverse, in the betanin (r = –0.481).

Table 4

Pigment accumulation comparison in table beet varieties sown in July and August

aa Variety	50 th days						75th days					
	5 july			18 August			5 July			18 August		
	pigment content mg%		root diameter cm	pigment content mg%		root diameter cm	pigment content mg%		root diameter cm	pigment content mg%		root diameter cm
	betanin	vulga-xanthine I.		betanin	vulga-xanthine I.		betanin	vulga-xanthine I.		betanin	vulga-xanthine I.	
'Bordó'	80.1	50.4	1.92	118.3	58.7	2.31	107.1	92.7	3.08	88.0	45.7	4.23
'Rubin' fj.	91.8	44.8	1.94	102.0	46.3	2.54	116.1	81.9	3.02	92.1	46.0	4.45
'Norton'	146.7	72.1	1.79	95.4	55.9	2.05	88.2	69.3	3.11	65.7	63.2	3.96
'Forono'	88.2	43.4	1.20	100.0	37.2	1.36	129.6	79.1	2.63	68.1	44.2	3.30
'Mobile'	101.7	61.2	2.42	142.3	58.0	2.46	123.3	100.8	2.81	93.9	52.8	4.25
			1.85			2.14			2.93			4.04

Conclusion

In our trials, accumulation of the red pigment content in most varieties reached its maximum on the 75th day of the growing period (18th September) due, probably, to the higher light intensity of the summer months. In samples taken on the 100th and 135th day, pigment contents decreased considerably in roots of increased volume, as also proved by the negative correlation (r =–□0.608) between the number of days and red pigment content. Our results found confirmation by Watson and Gabelman (1982).

In our conditions, the vulgaxanthin content showed a similar tendency (r = –0.599) in contrast to Nilsson (1973) who found a gradual increase in vulgaxanthin till the end of the growing period in beet root varieties sown at different dates.

In varieties sown in August, the trend of pigment accumulation was contrary to those sown in July, probably due to the decreasing sunshine hours. Our trials did not confirm results of Sistrunk and Bradley (1970) who stated that cool temperature affected pigment accumulation favourably. In our trials, betanin decreased considerably by the 75th day while vulgaxanthin stagnated or decreased but slightly, as also proved by the inverse correlation between root diameter and red pigment content (r = –0.481). In our trials, this sowing date did not affect the red pigment accumulation favourably in beet roots.

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PHYSIOMORPHOLOGICAL CHANGES IN TRITICALE IMPROVED BY PYRIDOXINE APPLIED THROUGH GRAIN SOAKING

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The seedlings grown from the grains soaked in 0.001 (T_2), 0.01 (T_3) and 0.1 (T_4) % aqueous solution of pyridoxine hydrochloride (Vitamin B_6) showed better growth as exhibited by their shoot length, leaf area, fresh and dry weight at 10, 15, 20, 25 and 30 day-stages of growth, than water soaked control (T_1). These seedlings were also characterised with larger reserves of pyridoxine, chlorophyll, nitrogen, phosphorus, potassium, soluble and insoluble carbohydrate and protein and higher activity of nitrate reductase (NR). Most of the characteristics gave better response to T_3 which interacted best with cv. Tigre "S" of triticale.

Key words: triticale, pyridoxine, nitrate reductase, chlorophyll, carbohydrate, protein, growth

Introduction

Although there is a wide gap in assigning a definite role to the vitamins of B-group in the growth of higher plants, pyridoxine is still known to regulate a number of processes. Pyridoxine (Vitamin B_6) is involved as a co-factor in the synthesis of amino acids (Lehninger 1982), α-amino-laevulinic acid synthetase, a key enzyme in porphyrin synthesis (Kikuchi et al., 1958) and auxins (Moore and Shaner, 1967): Pyridoxal phosphate effectively stimulates photosynthetic phosphorylation (Black and Sanpietro, 1968), total chlorophyll content and photosynthetic activity in plants (Kodandaramaiah and Rao, 1984, Samiullah et al., 1988). Therefore, the response of two varieties of triticale to pre-sowing grain treatment with pyridoxine was studied and is discussed below.

Materials and methods

The healthy, previous year, grains of two cultivars of triticale, namely: Tigre "S" and Muskox "S" received from CIMMYT (Mexico), after being surface sterilised with 0.01% mercuric chloride, were soaked in water (T_1), 0.001 (T_2), 0.01 (T_3) and 0.1 (T_4) % aqueous solutions of pyridoxine hydrochloride for 8 h at 20 2 °C. Six pre-soaked grains representing a treatment were sown in each earthen pot (6" size) lined with polythene sleeve and filled with acid washed sand and placed in a glass house on a raised plateform at 20 ± 2 °C with a light duration of about 10 hours. In all, 160 pots were used to raise the seedlings of each variety thus, included 40 pots per treatments. 100 ml of the full nutrient solution (Hewitt, 1966) was provided to all the pots every day in the morning. Thinning was done on the emergence of the seedlings, leaving only 3 healthy seedlings per pot. Ten pots from each treatment were taken out randomly from day 10 to 30 at an interval of 5 days. All the 30 plants, representing each treatment, were divided randomly into three groups of 10 plants each, to represent the number of replicates. The shoot of each plant was assessed for its length, leaf area and fresh and dry weight.

The shoot was also subjected to chemical analysis. The activity of nitrate reductase was studied following the method of Jaworski (1971). Chlorophyll was determined by the method of Mackinney (1941). The contents of soluble and insoluble carbohydrate and protein were estimated according to Yih and Clark (1965) and Lowry et al. (1951), respectively. The methodologies adopted by Hochberg et al. (1944) and Lindner (1944), respectively, were used for determining pyridoxine and nitrogen contents.

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Table 1

Effect of pre-sowing seed treatment with pyridoxine on shoot length per plant (cm) in two varieties of triticale
(Mean of three replicates)

Treatments (% aqueous solution of pyridoxine)	Sampling time (days after sowing)														
	10			15			20			25			30		
	Varieties														
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1 (Water)	15.20	15.70	15.45	20.30	21.57	20.94	23.97	23.57	23.77	27.57	26.47	27.02	29.07	29.87	29.47
T_2 (0.001%)	16.40	15.80	16.10	22.37	20.47	21.42	24.60	23.70	24.15	26.23	26.30	26.27	30.47	32.50	31.49
T_3 (0.01%)	19.53	16.73	18.13	27.93	23.03	25.48	31.10	27.17	29.14	33.80	30.17	31.99	38.57	35.43	37.00
T_4 (0.1%)	15.80	20.37	28.09	21.27	25.23	23.25	23.40	31.27	27.34	26.23	34.20	30.22	29.20	38.20	33.70
Mean	16.73	17.15		22.97	22.58		25.77	26.43		28.46	29.29		31.83	34.00	
Critical difference at 5%															
Treatment			0.39			0.72			0.77			0.73			0.88
Variety			0.27			N.S.			0.54			0.51			0.62
Treatment × variety			0.55			1.02			1.08			1.03			1.24

V_1 – Tigre "S"; V_2 – Muskox "S"; N.S. – Non-significant

Results and discussion

The soaking treatment of the grain in aqueous solution of pyridoxine hydrochloride not only favoured the germination process of the grain (Haque et al., 1988) but the resulting seedlings had roots which were longer and more numerous (Haque, 1989). It will naturally lead to significantly better meristematic activity together with consequent cell enlargement in the shoot too. The resulting seedlings were, therefore, longer (Table 1) and produced the leaves with larger surface area (Table 2), at most of the stages of growth. It favoured an increase in the fresh and dry weight (Tables 3 and 4) of the shoot, as compared with the control. Favourable effects of pyridoxine, irrespective of its mode of application, on shoot of other plants at advanced stages of growth have also been reported by Kozhin and Kravatsov (1973) and Samiullah et al. (1988).

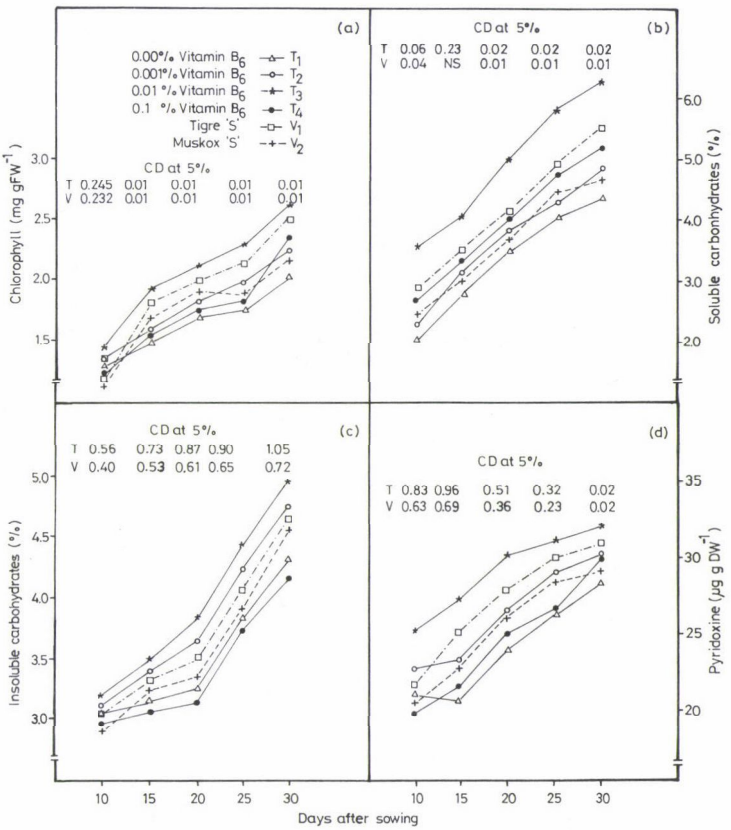

Fig. 1. Effect of pre-treatment of grains with pyridoxine on chlorophyll content (a), soluble carbohydrate (b), insoluble carbohydrate (c) and pyridoxine content (d) in the shoot of two varieties of triticale, at early stages of growth. (CD – Critical difference, NS – Non-significant, T – Treatment, V – Variety)

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Table 2

Effect of pre-sowing seed treatment with pyridoxine on leaf area per plant (cm^2 in two varieties of triticale
(Mean of three replicates)

Treatments (% aqueous solution of pyridoxine)	Sampling time (days after sowing)														
	10			15			20			25			30		
	Varieties														
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1 (Water)	5.71	5.18	5.45	12.98	9.30	11.14	20.50	12.26	16.38	26.30	17.51	21.91	30.24	22.20	26.22
T_2 (0.001%)	6.13	5.36	5.74	1.378	10.66	12.22	22.32	13.67	18.00	31.32	19.11	25.22	35.50	24.24	29.87
T_3 (0.01%)	6.97	5.75	6.36	15.40	10.82	13.11	29.44	15.30	22.37	33.52	21.22	27.37	37.22	25.87	31.55
T_4 (0.1%)	5.50	5.13	5.32	12.65	8.66	10.66	20.12	12.02	16.07	26.21	17.36	21.79	30.89	22.99	26.94
Mean	6.08	5.36		13.70	9.86		23.10	13.31		29.34	18.80		33.46	23.82	
Critical difference at 5%															
Treatment	0.01					0.18			0.21			N.S.			1.19
Variety	0.01					0.13			0.15			N.S.			0.84
Treatment × variety	0.02					0.26			0.30			N.S.			1.68

V_1 – Tigre "S"; V_2 – Muskox "S"; N.S. – Non-significant

Table 3

Effect of pre-sowing seed treatment with pyridoxine on fresh weight (g) per plant in two varieties of triticale
(Mean of three replicates)

Treatments (% aqueous solution of pyridoxine)	Sampling time (days after sowing)														
	10			15			20			25			30		
	Varieties														
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1 (Water)	0.44	0.44	0.44	1.19	1.11	1.15	1.50	1.44	1.47	1.92	1.91	1.92	2.34	2.28	2.31
T_2 (0.001%)	0.56	0.44	0.50	1.48	1.13	1.31	1.99	1.50	1.74	2.54	1.93	2.24	3.11	2.52	2.82
T_3 (0.01%)	0.61	0.47	0.54	1.58	1.17	1.38	2.16	1.68	1.92	2.68	2.63	2.65	3.37	2.89	3.13
T_4 (0.1%)	0.49	0.43	0.46	1.51	1.05	1.28	1.92	1.44	1.68	2.51	1.92	2.21	3.18	2.44	2.81
Mean	0.52	0.45		1.44	1.11		1.89	1.51		2.41	2.09		3.00	2.53	
Critical difference at 5%															
Treatment	0.01					0.02			0.02			0.03			0.02
Variety	0.01					0.01			0.01			0.02			0.16
Treatment x variety	0.02					0-02			0.03			0.04			0.32

V_1 – Tigre "S"; V_2 – Muskox "S"

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Table 4

Effect of pre-sowing seed treatment with pyridoxine on dry weight of shoot (g) per plant in two varieties of triticale
(Mean of three replicates)

Treatments (% aqueous solution of pyridoxine)	Sampling time (days after sowing)														
	10			15			20			25			30		
	Varieties														
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1 (Water)	0.027	0.024	0.026	0.188	0.175	0.182	0.268	0.255	0.262	0.341	0.330	0.336	0.415	0.404	0.410
T_2 (0.001%)	0.033	0.026	0.030	0.235	0.181	0.208	0.348	0.267	0.308	0.451	0.343	0.397	0.561	0.442	0.502
T_3 (0.01%)	0.037	0.029	0.033	0.255	0.184	0.220	0.370	0.300	0.335	0.477	0.404	0.441	0.598	0.513	0.556
T_4 (0.1%)	0.025	0.022	0.024	0.231	0.166	0.199	0.342	0.256	0.299	0.445	0.337	0.391	0.560	0.431	0.496
Mean	0.030	0.025		0.227	0.177		0.332	0.270		0.429	0.354		0.534	0.448	
Critical difference at 5%															
Treatment	0.001					0.007			0.009			0.006			0.008
Variety	0.001					0.005			0.006			0.004			0.005
Treatment × variety	0.002					0.010			0.012			0.008			0.011

V_1 – Tigre "S"; V_2 – Muskox "S"

Table 5

Correlation between various growth and chemical parameters with shoot dry weight

Parameters	Correlation coefficient (r)				
	Days after sowing				
	10	15	20	25	30
Growth characteristics					
Shoot length plant^{-1}	N.S.	N. S.	N. S.	N. S.	N. S.
Leaf area plant^{-1}	0.91**	0.91**	0.86**	0.84**	0.81**
Shoot fresh weight plant^{-1}	0.99**	0.99**	1.00**	0.94**	1.00**
Chemical characteristics					
Nitrate reductase activity	0.92**	0.74*	0.75**	0.85**	0.81**
Soluble protein content	0.71**	0.64**	0.76**	0.72**	0.70**
Insoluble protein content	0.83**	0.87**	0.85**	0.60**	0.87**
Soluble carbohydrate content	0.60*	0.50*	0.74**	0.75**	0.80**
Insoluble carbohydrate content	0.93**	0.68**	0.63*	N. S.	0.50**
Chlorophyll content	0.89**	0.87**	0.88**	0.86**	0.87**
Nitrogen content	0.92**	0.88**	0.88**	0.95**	0.94**
Phosphorus content	0.86**	0.68**	0.68**	0.72**	0.75**
Potassium content	0.80**	0.83**	0.81**	0.72**	0.66**

* Significant at 5%; ** significant at 1%; N.S.-Non-significant

The chemical analysis of the shoot of these seedlings, at regular stages of growth, will possibly help us in tracing the mechanism by which pyridoxine might be regulating higher rates or growth. Shoot pyridoxine reserves (Fig. 1d) increased as the growth progressed but it was more prominent in those raised from pyridoxine treated grains. Although not much information is available to explain these observations but presumably pyridoxine treatment acted on some unknown physiological process in the seed and/or shoot to enhance its own level in the leaves.

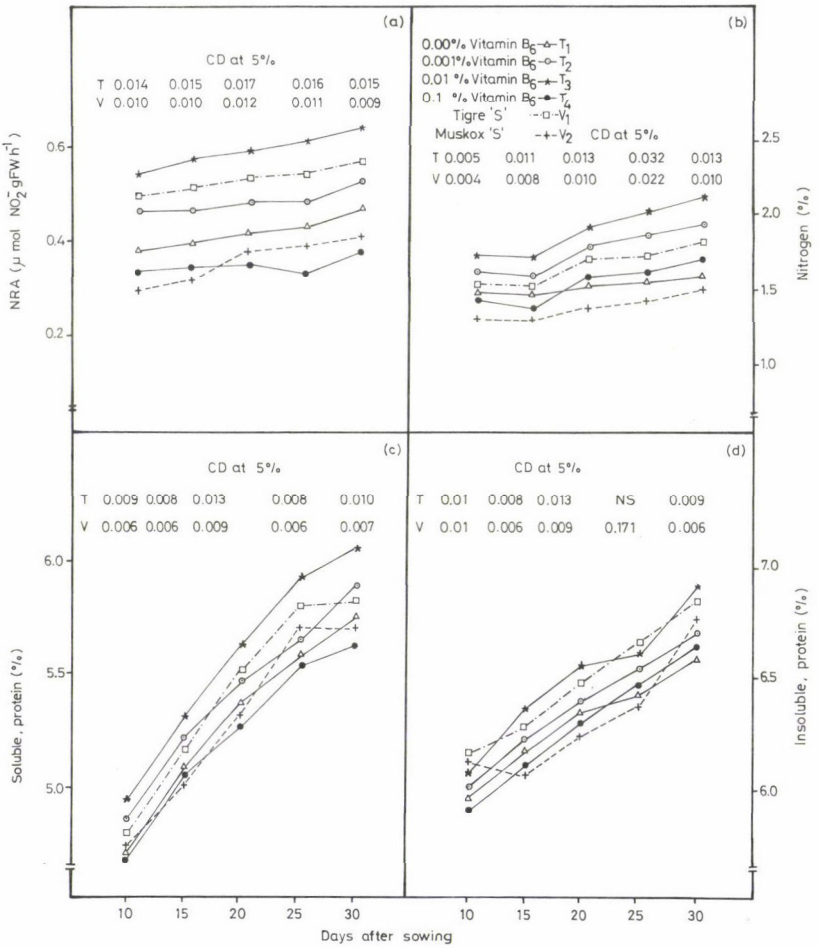

Fig. 2. Effect of pre-treatment of grains with pyridoxine on (NRA) nitrate reductase activity (a), nitrogen content (b), soluble protein (c) and insoluble protein content (d) in the shoot of two varieties of triticale, at early stages of growth. (CD – Critical difference, NS – Non-significant, T – Treatment, V – Variety)

Better root growth (Haque, 1989) increased the soil exploring capacity of the seedlings raised from pyridoxine treated grains, therefore they removed larger quantities of the nutrients (Figs 2b, 3a and b). Increased availability of nutrients, particularly that of nitrate, must have led to the observed increase in the activity of nitrate reductase (Fig. 2a) because of its induced synthesis by the substrate (Candella et al., 1957). Cumulative effects of the enhanced level of nitrate and NRA increased total nitrogen content of the shoot (Fig. 2b). Samiullah et al. (1988) also reported similar observations in other plants, at advanced stages of growth. Further assimilation of nitrogen by the vitamin B_6 in the seedlings raised from pyridoxine treated grains must have been favoured because of its involvement in the synthesis of amino acid (Lehninger, 1982). These seedlings, therefore, possessed larger quantities of soluble and insoluble proteins (Figs 2c and d) and exhibited a pattern similar to that observed for NRA (Fig. 2a). Kodandaramiah (1983), while reporting similar observations, suggested that vitamin B_6 is probably acting as a co-factor in protein synthesis and has been supported by Rao et al. (1987).

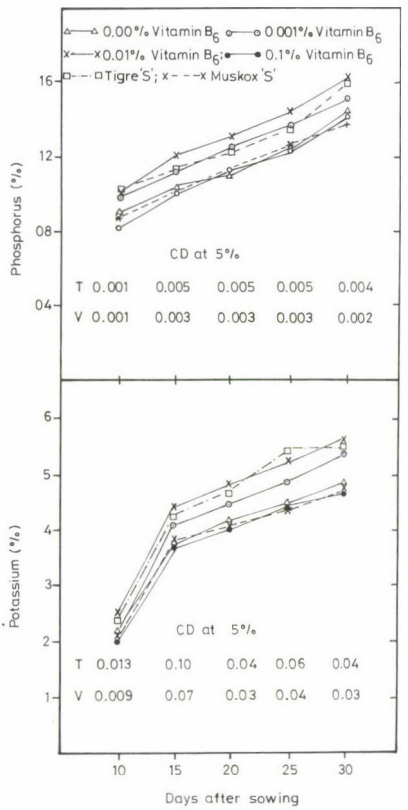

Fig. 3. Effect of pre-treatment of grains with pyridoxine on phosphorus (a) and potassium percentage (b) in the shoot of two varieties of triticale, at early stages of growth. (CD – Critical difference, T – Treatment, V– Variety)

The increased availability of porphyrin in whose synthesis pyridoxal phosphate, the active form of pyridoxine, is involved as a co-factor of α-amino-laevulinic acid synthetase (Kikuchi et al., 1958) must have elevated the level of chlorophyll (Fig. 1a). This, in association with increased leaf area (Table 2), might have exerted a marked effect on the photosynthetic capabilities of the plants, thus possessing more carbohydrates (Figs 1b and c). In addition to this, shoot dry weight was significantly correlated with all growth (except shoot length) and chemical characteristics (Table 5).

In general, Tigre "S" (V_1) was more responsive to pyridoxine treatment than Muskox "S" (V_2), probably due to the difference in grain vitamin reserves (1.53 and 1.64 μg g DW^{-1}, respectively). The grains of V_1 soaked in 0.01% (T_3) solution of pyridoxine hydrochloride produced the plants which exhibited maximum values for most of the characteristics. A higher concentration of this vitamin might have proved to be supra-optimal for these early stages of growth.

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COMPARISON OF OPTIMUM MOISTURE ENVIRONMENTS AGAINST STRESS ENVIRONMENTS FOR DEVELOPING DROUGHT-RESISTANT TEF (*ERAGROSTIS TEF* /ZUCC./ TROTTER) VARIETIES*

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In order to sketch appropriate breeding strategy for developing varieties for low moisture areas using the more than 2000 germplasm accessions found at the Debre Zeit Agricultural Research Center, an experiment was conducted using a random selection of 21 pure lines having different panicle forms and similar maturity. The genotypes were tested in contrasting environments (optimum and low moisture) for two seasons in a randomized complete block design of three replications.

Analyses of variance for each environment showed that there was variation among the test genotypes, indicating the possibility of developing varieties for different moisture regimes. Correlation coefficients between drought susceptibility index and grain yield were highly significant ($P \leq 0.01$), and positive and negative in low and high moisture environments, respectively, suggesting that different traits are desired in the two contrasting environments. Moreover, comparison of the grain yield of top and bottom 5 lines in contrasting environments revealed that poor yielders in optimum environments gave reasonable yield in low moisture environments and *vice versa*. Similarly, good yielders in optimum environments gave low yield in moisture stress environments and *vice versa*. It was, therefore, concluded that direct selection be practiced in the target environment.

Key words: tef, *Eragrostis tef* (Zucc.) Trotter, moisture stress, drought-resistant

Introduction

Tef is considered to be the most widely adapted of all major cereal crops (maize, sorghum and wheat) in Ethiopia because of its drought tolerance and adaptation to a wide range of soil conditions. As a result, it is grown in many adverse environments. Though the breeding work in Ethiopia generated several lines adapted to mid- and high-altitude areas, attempts to do the same for lowland areas are of limited success. In fact, testing experimental strains screened at mid-altitude areas at high-, mid- and low-altitude areas was the strategy adopted. However, the slow progress in developing varieties for moisture stress areas makes it necessary to devise appropriate breeding strategy based on experimental evidence to efficiently exploit the more than 2000 germplasm accessions collected from Ethiopia, which is the center of origin and diversity for tef.

Breeding for moisture-limited environments raises two main issues (Ceccarelli et al., 1987). The first is whether breeding for stress environments should rely on selection under favourable conditions and subsequent testing of selected materials in stress environments, or on direct selection under stress conditions. Though selecting

* A research conducted at Debre Zeit Agricultural Research Center, P. O. Box 32, Debre Zeit, Ethiopia

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in favourable conditions has been supported by some data (Frey, 1964; Mederski and Jeffers, 1973; Richards, 1982) where certain genotypes that do well under stress conditions also were obtained, the supremacy of the second strategy has been emphasized by many researchers (Bidinger, 1980; Boyer and McPherson, 1975; Ceccarelli et al., 1987; Johnson, 1980; Srivastava et al., 1983) working on wheat, barley and some perennial herbaceous weedy plants. However, information of this kind is lacking in tef where a great deal of its production area is affected by drought. Therefore, the objectives of the present study are:

1. to see the correlation among grain yield, total biomass, harvest index (HI) and drought susceptible index (DSI) in wet and dry environments.
2. to see the effect of selection for grain yield in wet environments on grain yield, biological yield, HI and DSI in dry environments, and *vice versa.*
3. to suggest a breeding strategy for low moisture areas.

Materials and methods

The experiment was conducted at Debre Zeit Agricultural Research Center in 1990/91 main cropping season under rainfed conditions and 1991/92 off-season using irrigation. During the main season, two sowing dates (normal and late planting) were used under fertilized and unfertilized conditons each, and similarly in the off-season, two fertility conditions (fertilized and unfertilized) were subjected to two moisture regimes each (stressed and non-stressed). This gave a total of eight environments representing various moisture levels, productivity and seasons (Table 1).

Twenty-one pure lines of tef having nearly similar maturity representing different panicle forms and randomly selected from tef germplasm pool have been evaluated under the eight environments mentioned earlier in a randomized complete block design of three replications. Recommended management practices have been uniformly applied to all trials.

Data were collected on DSI (Fischer and Maurer, 1978), grain yield, biological yield and HI. Analysis of variance, and linear correlation were performed for each environment and *t*-test was performed to see the reponse of high and low yielding genotypes selected under optimum moisture regimes in moisture stress environments, and *vice versa.*

Table 1

Characteristics of the test environments

Environment	Season	Fertility condition	Moisture (mm) rainfall/irrigation
E_1	Main season (normal planting)	Fertilized	320
E_2	Main season (late planting)	Fertilized	205
E_3	Main season (normal planting)	Unfertilized	320
E_4	Main season (late planting)	Unfertilized	205
E_5	Off-season (optimum irrigation)	Fertilized	390
E_6	Off-season (stressed)	Fertilized	220
E_7	Off-season (optimum irrigation)	Unfertilized	390
E_8	Off-season (stressed)	Unfertilized	220

Table 2

Mean square (MS) and coefficients of variation (CV) for grain yield, biological yield, harvest index and drought susceptibility index in eight environments

Environment	Grain yield		Biological yield		Harvest index		Drought susceptibility index	
	MS (kg/ha)	CV (%)	MS (kg/ha)	CV (%)	MS	CV (%)	MS	CV (%)
E_1	69970**	11	6996500**	17	0.02**	12		
E_2	2250**	21	118750**	13	0.01**	26	0.11	9
E_3	27000**	14	189500**	6	0.04**	12		
E_4	2750**	24	92250**	13	0.02**	27	0.11	7
E_5	57630**	15	614420**	14	0.02**	14		
E_6	1970**	22	109270**	18	0.01**	27	0.20	11
E_7	21700**	17	170610**	13	0.03**	13		
E_8	1850**	23	86210**	16	0.01**	25	0.23	10

** Significant at 1% probability level

Results and discussion

Combined analyses of variance for all environments showed that there were significant genotype X environment interaction. Hence, a table was presented for each environment. As can be seen from Table 2, there were highly significant differences ($P \leq 0.01$) among genotypes for all characters measured in all environments indicating variation among the experimental strains and possibility of developing varieties for different moisture regime using direct selection from the tef germplasm collection. As expected, coefficients of variation were higher for low moisture environments (E_2, E_4, E_6 and E_8) than high moisture environments (E_1, E_3, E_5 and E_7). Otherwise, they all were within in acceptable range based on experience at Debre Zeit.

Table 3

Correlation coefficients among drought susceptibility index and grain yield, biological yield, and harvest index in eight environments

Environment	Grain yield	Biological yield	Harvest index
High moisture			
E_1	0.46**	0.42**	0.40**
E_3	0.41**	0.23**	0.38**
E_5	0.26**	0.51**	0.50**
E_7	0.52**	0.42**	0.42**
Low moisture			
E_2	–0.74**	–0.53**	–0.29*
E_4	–0.82**	–0.62**	–0.42**
E_6	–0.80**	–0.57**	–0.43**
E_8	–0.84**	–0.62**	–0.45**

*,** Significant at 5 and 1% levels of probability, respectively

Table 4

Average grain yield (100 kg/ha), biological yield (100 kg/ha), harvest index and drought susceptibility index in contrasting environments of tef genotpyes selected for grain yield under low (E_2) and high (E_1) moisture conditions (Main season and fertilized)

Character	Low moisture (E_2)			High moisture (E_1)		
	Top 5	Bottom 5	Difference	Top 5	Bottom 5	Difference
Grain yield (E_1)	17.58	6.58	11.00**	35.18	45.38	–10.20**
Grain yield (E_2)	9.18	14.48	–5.30*	49.43	25.88	23.55**
Biological yield (E_1)	108.63	106.43	2.20	158.83	159.03	–0.20
Biological yield (E_2)	100.63	129.53	–28.90*	151.18	107.00	44.18**
Harvest index (E_1)	0.18	0.18	0.10**	0.27	0.29	–0.02
Harvest index (E_2)	0.15	0.19	–0.04*	0.34	0.19	0.15
Drought susceptibility index	0.46	0.89	0.43**	0.89	0.61	0.28*

*,** Significant at 5 and 1%, respectively using *t*-test

As can be seen from Table 3, highly significant ($P \leq 0.01$) negative correlation coefficients were found between the drought susceptibility index (DSI), and grain yield, biological yield and harvest index (HI) under low moisture environments, indicating that large yields are associated with higher levels of drought resistance, whereas in high moisture environments positive and significant ($P \leq 0.01$) correlation coefficients were found. Positive and significant correlation coefficients between DSI and grain yield in wet environments have been reported by Fischer and Maurer (1978) and interpreted as indication of the existence of traits which are desirable under drought and undesirable under favourable conditions or *vice versa*. In other words, lines selected under favourable conditions will be different from those selected in unfavourable conditions or *vice versa*.

This was further tested by comparing grain yield of the top and bottom 5 lines in contrasting environments (Tables 4, 5, 6 and 7). The lines with the highest and lowest grain yield under low moisture environment (E_2, E_4, E_6 and E_8) were found to differ significantly ($P \leq 0.05$) in high moisture environments, the bottom 5 lines producing

Table 5

Average grain yield (kg/ha) biological yield (100 kg/ha), harvest index and drought susceptibility index 100 in contrasting environments of tef genotypes selected for grain yield under low (E_4) and high (E_3) moisture conditions (Main season and unfertilized)

Character	Low moisture (E_2)			High moisture (E_1)		
	Top 5	Bottom 5	Difference	Top 5	Bottom 5	Difference
Grain yield (E_3)	12.18	3.40	8.78	30.88	34.05	–3.97*
Grain yield (E_4)	4.73	8.85	–4.12*	36.86	13.13	23.73**
Biological yield (E_3)	67.13	65.43	1.70	98.75	102.28	–3.53
Biological yield (E_4)	41.15	58.08	–16.93**	110.75	65.23	45.52**
Harvest index (E_3)	0.22	0.11	0.11**	0.28	0.22	0.06
Harvest index (E_4)	0.12	0.15	0.03	0.40	0.22	0.18**
Drought susceptibility	0.59	0.79	–0.20	0.91	0.69	0.22*

*,** Significant at 5 and 1%, respectively using *t*-test

Table 6

Average grain yield (100 kg/ha), biological yield (100 kg/ha), harvest index and drought susceptibility index in contrasting environments of tef genotypes selected for grain yield under low (E_8 and high E_7) moisture conditions (off-season and unfertilized)

Character	Low moisture (E_8)			High moisture (E_7)		
	Top 5	Bottom 5	Difference	Top 5	Bottom 5	Difference
Grain yield (E_7)	12.04	3.52	8.52 *	31.02	33.96	–2.94*
Grain yield (E_8)	4.86	8.76	–3.90 *	35.13	12.97	22.16*
Biological yield (E_7)	64.28	63.91	0.37	91.57	92.20	–0.63
Biological yield (E_8)	40.03	57.62	–7.59	100.19	66.04	34.15*
Harvest index (E_7)	0.21	0.11	0.10*	0.26	0.21	0.05
Harvest index (E_8)	0.13	0.17	0.04	0.41	0.20	0.21**
Drought susceptibility index	0.58	0.80	0.22*	0.90	0.69	0.21*

*,** Significant at 5 and 1%, respectively using *t*-test

larger yield than the top 5 lines. Similarly, the bottom 5 lines under high moisture environments (E_1, E_3, E_5 and E_7) produced larger yield than the top 5 lines when grown in low moisture environments. The lines selected for higher and lower grain yield under low moisture were found to affect harvest index, whereas those selected under high moisture affected both harvest index and biological yield. This is additional evidence for the existence of traits which are of advantage under drought and disadvantageous under optimum environments or *vice versa.* Moreover, lines selected for large grain yield under low moisture were more drought resistant than those selected for low grain yield, whereas lines selected for large grain yield under high moisture are more drought susceptible than those selected for low grain yield as judged by drought susceptibility index.

Both positive and negative correlation coefficients were found between DSI and grain yield, biological yield and HI in high and low moisture environments, respectively, indicating that different traits are operating under the two contrasting environments

Table 7

Average grain yield (100 kg/ha), biological yield (100 kg/ha) harvest index and drought susceptibility index in contrasting environments of tef genotypes selected for grain yield under low (E_6) and high (E_5) moisture conditions (off-season and fertilized)

Character	Low moisture (E_6)			High moisture (E_5)		
	Top 5	Bottom 5	Difference	Top 5	Bottom 5	Difference
Grain yield (E_5)	15.30	6.01	9.29*	34.01	43.42	–3.41*
Grain yield (E_6)	8.13	12.56	–4.43*	46.22	23.92	22.30*
Biological yield (E_5)	927.21	918.72	8.49	132.81	133.02	–0.21
Biological yield (E_6)	912.20	956.41	–44.21	130.15	101.61	28.54*
Harvest index (E_5)	0.15	0.04	0.11**	0.26	0.28	–0.02
Harvest index (E_6)	0.12	0.17	–0.05	0.31	0.16	0.15*
Drought susceptibility	0.41	0.88	–0.47*	0.85	0.57	0.28*

*,** Significant at 5 and 1%, respectively using *t*-test

(Ceccarelli et al., 1987; Fischer and Maurer, 1978). Moreover, comparison of the top and bottom 5 lines in contrasting environments (Tables 4, 5, 6 and 7) indicated that lines selected under favourable conditions were different from those selected under unfavourable conditions. This finding agreed with the findings of researchers emphasizing the supremacy of direct selection under stress conditions in breeding for moisture limited environments. It is, therefore, concluded that breeding for low moisture area in tef should be based on direct selection in the target environments.

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EFFECTS OF SEEDING RATE AND FILLER RATIOS ON GRAIN YIELD AND STRAW YIELD OF TEF (*ERAGROSTIS TEF* (ZUCC.) TROTTER)

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This study was conducted 1. to examine responses of tef grain yield and straw yield to varying seeding rates and filler ratios, and 2. to investigate the possibility of using lower seeding rates in combination with filler. A factorial combination of four seeding rates (15, 25, 40 and 55 kg/ha) and three filler ratios (0:1, 2:1 and 4:1 sand to tef in phase I and 0:1, 10:1 and 20:1 sand to tef in phase II) were tested in a randomized complete block design of four replications at Debre Zeit (Vertisol) Debre Zeit (Inseptisol) and Akaki (Vertisol).

The results indicated that grain yield and straw yield were not affected by filler ratios and the later by seeding rates as well. In general, better grain yield was obtained with a seeding rate of 25 kg/ha. Therefore, this rate can be used either alone or in combination with filler (e.g. soil).

Key words: tef, *Eragrostis tef* (Zucc.) trotter, seeding rate, filler

Introduction

Tef is one of the most important cereals in Ethiopia. Its grain is used as human food and as straw, mainly for reinforcing mud for plastering walls of buildings, and as livestock feed (Tadesse, 1969). Furthermore, the total production and amount consumed excels any other cereal crop in Ethiopia (Ethiopian Nutrition Survey, 1959).

Effects of seeding rates on yield and other plant attributes are well documented in other cereal crops. Baker (1982) reported that grain yield and straw yield of spring wheat cultivars changed with seeding rates under certain environmental conditions, but remained unchanged under others. A study conducted by the institute of agricultural research (1986) at Ghinchi on wheat and at Holetta on food barley, both locations in the central highlands of Ethiopia, revealed no significant effects of seeding rates tested in the range of 75–150 kg/ha on grain yield. Moreover, Singh and Rammohan (1983) got the same result on wheat. Nevertheless, Thorat, Bhosale and Patil et al. (1983) on wheat found increased yield with higher seeding rates than lower rates. In addition, Adjeie-Twum et al. (1986) working on maize (non-tillering variety) reported that the highest dry matter yield and grain yield were obtained at a respective population density of 53,333 and 67,666 plants/ha when a population density ranging from 38,095 to 88,888 plants/ha was tested.

Concerning tef, several experiments were conducted to study the effects of seeding rates on grain yield in the past (Asrat Felleke, 1965 and Debre Zeit Agricultural Research Center, 1983). In most of these studies, seeding rates tested within 15 and 50 kg/ha showed a non-significant difference in grain yield, probably due to compensation through tillering and other yield components. However, it is impractical

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to uniformly distribute lower seeding rates over a hectare due to the smallness of tef seeds (100 seed weight = 35 mg). Partly for this reason, 25 kg/ha was recommended in the past. Nevertheless, there were complaints by some farmers and extension agents that the recommended rate was not enough. Moreover, straw yield was neglected in the above studies despite its importance. Therefore, the objectives of this study were 1. to examine responses of tef grain yield and straw yield to varying seeding rates and filler ratios and 2. to investigate the possibilities of using lower seeding rates in combination with filler.

Materials and methods

The study was conducted at Debre Zeit on two soil types (Vertisol and Inseptisol) and Akaki (Vertisol) using a widely adapted, high yielding and late maturing variety named DZ-01-354 from 1984 to 1988, the years 1984 and 1985 being phase I and 1988 being phase II. Four seeding rates (15, 25, 40 and 55 kg/ha) and three filler ratios were evaluated in factorial combination using a randomized complete block design with four replications. The filler was prepared in such a way that sand particles that passed through 1.00 mm mesh but retained on a 0.75 mm mesh were used. Similarly, tef seeds retained on a 0.75 mm mesh were used. The sand particles and tef seeds then obtained were combined on the basis of number to formulate the various proportions of sand and tef. In phase I, filler ratios were 0:1, 2:1 and 4:1 of sand to tef. In phase II, the filler ratios were changed to 0:1, 10:1 and 20:1. Therefore, results were presented for each phase.

Data on grain yield were collected for all years and all plots, whereas only two replications and two years (1986 and 1988) data were collected concerning straw yield.

Table 1

Effect of seeding rates and filler ratios on grain yield of tef grown at Debre Zeit, Phase I.

Treatment	Location	
	Debre Zeit (Vertisol)*	Debre Zeit (Inseptisol)*
Seeding rate kg/ha		
15	34.01a	29.55a
25	33.32a	28.94a
40	33.42a	29.41a
55	32.83a	27.86a
Filler ratios		
(sand to tef)		
0:1	33.24a	28.51a
2:1	33.06a	28.35a
4:1	33.88a	29.95a

Means followed by a common letter are not significantly different at 0.05 level of probability.

*Mean of 1984 and 1985 experiments.

Results and discussion

Results of the analysis of variance indicated that there were significance ($P \leq 0.05$) differences in the interaction between treatment (seeding rates and filler ratios) and locations, hence, results were analysed and presented on phase and location basis.

Phase I

Seeding rates and filler ratios showed non-significant differences (Table 1) in grain yield on both Vertisol and Inseptisol. Similarly, seeding rate-filler ratio interaction was not significant.

Phase II

Seeding rates and filler ratios revealed non-significant differences in grain yield at Debre Zeit on Inseptisol (Table 2) and this is also true with all other interactions (seeding rate X filler ratio seeding rate X year, filler ratio X year, seeding rate X filler ratio X year). Effects of seeding rates were significant ($P \leq 0.05$) for grain yield at Akaki and at Debre Zeit on the black soil (Table 2). At Akaki, the seeding rate of 25 kg/ha with a mean grain yield of 2340 kg/ha differed significantly from the other

Table 2

Effect of seeding rates and filler ratios on grain yield (100 kg/ha) of tef at three location, Phase II.

Treatment	Location		
	Akaki*	Debre Zeit (Inseptisol)**	Debre Zeit (Vertisol)***
Seeding rate (kg/ha)			
15	21.20b	26.96a	21.27a
25	23.40a	29.62a	24.32b
40	21.87b	30.06a	25.40b
55	20.91a	31.20a	26.17b
Filler ratios			
(sand to tef)			
0:1	22.02a	29.17a	28.51a
10:1	21.28a	29.94a	28.35a
20:1	22.24â	29.27a	29.95a

Means followed by a common letter are not significantly different at 0.05 level of probability.

* Mean of 1989 and 1988 experiments.
** Mean of 1986 and 1987 experiments.
*** Mean of 1986, 1987 and 1988 experiments

Table 3

Effect of seeding rates and filler ratios on straw yield (100 kg/ha) of tef at three locations, Phase II.

Treatment	Location		
	Akaki	Debre Zeit (Vertisol)	Debre Zeit (Inseptisol)
Seeding rate (kg/ha)			
15	94.74a	99.60a	99.80a
25	98.22a	100.01a	100.00b
40	98.28a	87.00a	98.27b
55	100.09a	100.67a	101.02b
Filler ratios			
(sand to tef)			
0:1	100.23a	99.99a	98.05a
10:1	100.07a	101.74a	101.74a
20:1	100.01a	200.23a	100.23a

Mean followed by a common letter are not significantly different at 0.05 level of probability.

rates. On the Vertisol of Debre Zeit, however, 25, 40 and 55 kg/ha out-yielded the lowest seeding rate (15 kg/ha) significantly but the differences among them were not significant. Similar to Inseptisol at Debre Zeit, filler ratios gave non-significant grain yield differences on Vertisol at Debre Zeit and Akaki.

Concerning straw yield, seeding rates, filler ratios and all other interactions (seeding rate X filler ratio, seeding rate X year, filler ratio X year, seeding rate X filler ratio X year) showed non-significant differences. Straw yield of seeding rates and filler ratios was given in Table 3 as per location.

Unlike Vertisols at Debre Zeit and Akaki where 25 kg/ha seeding rate was the best, seeding rates showed no response in grain yield on Inseptisol at Debre Zeit (Tables 1 and 2). However, straw yield showed no response to changing seeding rates at any one of the locations (Table 3). Similar results were obtained by Baker (1982) working with spring wheat cultivates where grain yield and straw yield of spring wheat cultivars changed with seeding rate under some environmental condition and remained unchanged under others.

On the other hand, filler ratios showed a non-significant effect on both grain yield and straw yield at all locations. Therefore, it is better to use the earlier recommendation of 25 kg/ha. Indeed there were complaints among farmers around Debre Zeit that 25 kg/ha was insufficient to physically cover a hectare. However, the use of filler (other than sand), as suggested by the present study, in combination with 25 kg/ha seeding rate seems to avoid unnecessary wastage of seeds by using seeding rates over 25 kg/ha, as is currently practiced.

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PHYSIOLOGICAL STUDIES OF SOME HEAVY METALS ON *HELIANTHUS ANNUUS, VIGNA SINENSIS* AND *TRITICUM VULGARE* I. GROWTH CRITERIA, PIGMENTATION, PHOTOSYNTHESIS AND RESPIRATION

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A water culture technique was used to study the effect of high concentrations of some essential elements (Co, Cu, Zn), on the respiration rate, pigmentation, photosynthesis and respiration in sunflower, pea and wheat plants.

Transpiration rates were more affected by the three heavy metals used and decreased as the metal increased in the culture media and the degree of inhibition was arranged as Cu > Co > Zn.

The photosynthesis was reduced, while the respiration increased at the low level (2 mM and 4mM) of the three metals.

Key words: copper, cobalt, zinc, *Helianthus annuus, Vigna sinensis, Triticum vulgare*

Introduction

Heavy metals can be divided into two categories: essential (viz. Cu, Ni, Mn, Zn,Co, Fe) which are required in trace amounts for different metabolic processes and non-essential (those toxic even at low concentrations, e.g Hg, Ag, Pb, Cd, Sn, etc.). However, essential heavy metals are also reported to be toxic at elevated concentrations (Woolhouse, 1983).

The phytotoxicity of heavy metals on growth and dry matter production and pigmentation of a number of cultivated plants has been reported by some investigators (Agarwala and Kumar, 1962; Schroeder and Vinton, 1962; Deckok, 1965; Perry and Erlanger, 1971; Shaddad et al., 1988).

Materials and methods

Sunflower (*Helianthus annuus*), pea (*Vigna sinensis*) and wheat (*Triticum vulgaris*) were used in this investigation. Five day-old seedlings were selected for uniformity and transferred for two weeks to freshly prepared 1/2 strength Pfeffer's nutrient solution at concentrations similar to those used by Arnon and Hogland (1940). The pH value of the nutrient solution was 5.5±2. Two week-old plants were treated with different concentrations of the applied heavy metals. This was carried out by exposing the plant roots for 7 days to a nutrient solution containing ($CoCl_2$, $CuSo_2$ and $ZnSO_4$) at concentrations of (2 mM, 4 mM, 6 mM and 8mM). All solutions were renewed every three days. Each treatment was carried out in three replicates. Transpiration rates were measured during the period of treatment as described by Bozcuk (1975). The pigment fractions (Chl.a, Chl.b and carotenoids) were determined according to Metzner et al. (1965) using the spectrophotometric method. Oxygen evolution was determined using the manometric technique developed by Warburg (Otto). At the end of the experimental period (10-days) the freshly harvested organs, roots and shoots (stems and leaves) were weighed, then dried in an oven at 85 °C.

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Results

Visual symptoms of cobalt and copper appeared as yellowish spots, spread over leaves of sunflower and pea; but, in the case of wheat, non appeared. Curled leaves appeared at the higher concentrations of zinc treatment (8mM).

The dry matter of sunflower, pea and wheat generally decreased due to the effect of different concentrations of Co, Cu and Zn, except in the case of wheat roots, which showed some increase in dry weight at the low levels of used elements (Table 1).

The tested three heavy metals induced a significant reduction in the rate of transpiration. This reduction was increased along with the increasing of heavy metals level (Table 2). The degree of metal inhibition of transpiration rate in the three plants was arranged as follows:

$$Cu > Co > Zn$$

The oxygen evolution (photosynthesis) was reduced as the metal concentration increased, and the degree of inhibition arranged in the order: Cu > Co > Zn in the three tested plants (Table 3); but the oxygen uptake (respiration) increased at the lower concentration (2mM and 4 mM) in the case of sunflower and pea leaves. There above the O_2 uptake was retarded as the concentration of metal increased.

Table 1

Effect of various concentrations of cobalt, copper and zinc on dry matter (g/plant) of sunflower, pea and wheat plants

Treatment		Dry matter (g/plant)					
		Sunflower		Pea		Wheat	
		Root	Shoot	Root	Shoot	Root	Shoot
	0	2.88	2.62	2.17	1.87	1.17	1.87
Cobalt	2 mM	1.91*	2.19	1.71*	2.08	1.48	1.75*
	4 mM	1.48**	2.20	1.68*	2.23	1.75**	1.86
	6 mM	1.39**	2.19	1.68*	2.44*	1.77**	1.81
	8 mM	1.30**	1.90	1.13*	1.46	1.84**	1.75
	$L.S.D._{5\%}$	0.68	0.82	0.21	0.52	0.36	0.12
	$L.S.D._{1\%}$	0.98	1.58	0.59	0.74	0.52	0.17
	2 mM	1.95*	2.18	1.82	2.56*	1.75**	1.83
Copper	4 mM	1.81*	2.08	1.74	2.36	1.72**	1.80
	6 mM	1.83*	2.05	1.89	2.32	1.69**	1.73**
	8 mM	1.53**	1.86	1.16	2.25	1.55**	1.65**
	$L.S.D._{5\%}$	0.79	1.01	0.12	0.52	0.17	0.13
	$L.S.D._{1\%}$	1.13	1.52	0.59	0.75	0.20	0.15
	2 mM	2.52	2.32	1.74	2.01	1.80**	1.50
Zinc	4 mM	1.85*	2.10*	1.53	1.72	1.65**	1.18**
	6 mM	1.76*	2.83	1.15	2.12	1.13	1.10**
	8 mM	1.60*	1.77**	1.11	1.13**	1.19	1.11**
	$L.S.D._{5\%}$	0.79	0.41	0.14	0.41	0.14	0.07
	$L.S.D._{1\%}$	1.73	0.58	0.63	0.52	0.19	0.10

* Significant (P = 0.05) and ** highly significant (P = 0.01) differences as compared with control

Table 2

Changes in transpiration rate (g H_2O dm^{-2} leaf $area^{-1}$ day^{-1}) of sunflower, pea and wheat plants treated for 7 days period with different concentrations of cobalt, copper and zinc

Treatment		Rate of transpiration		
		Sunflower	Pea	Wheat
	Cont	20.93	13.49	11.37
Cobalt	2 mM	16.63**	8.65**	11.13
Cobalt	4 mM	14.32**	4.32**	7.75**
Cobalt	6 mM	05.39**	3.59**	4.90**
Cobalt	8 mM	04.28**	0.38**	3.72**
Cobalt	$L.S.D._{5\%}$	1.29	1.31	1.18
Cobalt	$L.S.D._{1\%}$	1.84	1.65	2.55
Copper	2 mM	6.83**	9.81**	9.72**
Copper	4 mM	5.24**	6.44**	7.95**
Copper	6 mM	4.05**	5.60**	6.18**
Copper	8 mM	2.75**	3.17**	3.34**
Copper	$L.S.D._{5\%}$	1.64	1.61	0.96
Copper	$L.S.D._{1\%}$	2.30	2.29	1.39
Zinc	2 mM	15.12**	10.10**	9.95
Zinc	4 mM	09.89**	8.06**	7.72**
Zinc	6 mM	07.18**	6.25**	6.88**
Zinc	8 mM	04.93**	1.56**	4.12**
Zinc	$L.S.D._{5\%}$	2.12	1.32	1.44
Zinc	$L.S.D._{1\%}$	3.02	1.88	2.06

* Significant (P = 0.05) and ** highly significant (p = 0.01) differences as compared with control.

The pigments content of the tested plants was generally decreased at all concentrations of the three heavy metals (Co, Cu, Zn). This reduction was more pronounced at the high concentrations of the used elements. It can be noticed that the dry matter yield and photosynthesis processes were decreased as the concentration of metal increased.

The inhibition occurred in this order: Co > Cu > Zn in the case of sunflower plants and in this order: Cu > Co > Zn in the case of pea plants (Table 4).

Discussion

The inhibitory effect of the three metals on growth rate of the roots and shoots of the tested plants add more support to the results obtained by other investigators (Page et al., 1972; Haghiri, 1973; Collins, 1981; Barceleo et al., 1986 and Shaddad et al., 1988). It has been assumed that the growth reduction by heavy metals may be in particular the result of inhibited cell division (Hasset et al., 1976; Reese and Robert, 1984).

The inhibitory effects on pigment biosynthesis in the tested plants, at the different levels of the three heavy metals used, concurs with the results obtained by some other investigators, using various plants with different metals (Paivoke, 1985; Stobart et al., 1985 and Parekh et al., 1990). It can be generalized that one of the commonly reported

Table 3

Effect of various concentrations of cobalt, copper and zinc on photosynthetic and respiratory oxigen (μ mols O_2/mg Chl^{-1}/h) of sunflower, pea and wheat

Treatment		μ mols O_2/mg $Chl^{-1}h^{-1}$					
		Sunflower		Pea		Wheat	
		Photosynthetic oxigene	Respiraty oxygen	Photosynthetic oxigene	Respiraty oxygen	Photosynthetic oxigene	Respiraty oxygen
	Cont	34.50	10.59	25.02	12.32	23.90	15.31
Cobalt	2 mM	25.37**	18.90**	20.35**	13.32	14.30**	11.18**
Cobalt	4 mM	15.41**	16.79*	18.75**	14.85*	13.20**	10.16**
Cobalt	6 mM	13.90**	13.90	15.20**	07.35**	04.46**	04.80**
Cobalt	8 mM	11.22**	10.05	10.35	07.00**	03.51**	02.20**
Cobalt	$L.S.D._{5\%}$	3.44	5.53	1.7	2.30	0.65	0.68
Cobalt	$L.S.D._{1\%}$	4.91	7.89	2.35	3.65	0.80	0.97
Copper	2 mM	15.18**	13.80*	17.30**	15.52**	14.90**	13.00**
Copper	4 mM	13.68**	14.50*	15.05**	16.37**	14.60**	14.00**
Copper	6 mM	12.20**	12.20	10.30**	11.02	4.50**	01.50**
Copper	8 mM	08.81**	11.00	9.85**	08.03**	2.80**	00.80**
Copper	$L.S.D._{5\%}$	2.51	2.97	2.05	1.45	0.58	0.11
Copper	$L.S.D._{1\%}$	8.96	4.24	5.62	2.15	0.81	0.16
Zinc	2 mM	25.67	24.29**	27.37	13.75	13.31**	7.75**
Zinc	4 mM	37.88	23.12**	25.22	12.00	12.14**	4.65**
Zinc	6 mM	23.08**	15.60	20.35	10.77	11.43**	3.16**
Zinc	8 mM	18.06**	5.32	15.00**	9.95	07.30**	2.14**
Zinc	$L.S.D._{5\%}$	3.45	5.32	5.27	2.95	0.54	0.11
Zinc	$L.S.D._{1\%}$	4.93	7.60	6.95	3.88	0.77	0.15

* Significant (P = 0.05) and ** highly significant (P = 0.01) differences as compared with control

symptoms of heavy metal toxicity was the decrease in chlorophyll content of leaves (Haghiri, 1973; Baszynski et al., 1980 and Shaddad et al., 1988). The decrease in the photosynthetic activity as O_2 evolution may be due to the destructive effect of heavy metals on the ultrastructure of chloroplasts (Barceleo' et al., 1988). In addition (Van Assche and Clijstevs, 1986) recorded that Zn, at concentrations of 200 ppm, inhibits the photosynthesis in *Phaseolus vulgaris* and interferes with photochemical chloroplast reactions.

The data reveal that the O_2 uptake (respiration) was slightly increased, especially at the low doses of metals, but at high levels of metals, the O_2 uptake decreased sharply. This is in agreement with Lee et al. (1976), who found that different metals at low concentrations (0.4 and 0.5 mM) increased the rate of respiration and concluded that heavy metals increased the activity of hydrolytic enzymes responsible for senescence. In this respect Barceleo' et al. (1988) found that cadmium increased the ethylene production; on the other hand, Bittel et al. (1974) found that heavy metals inhibit the respiration due to their interference with the electron transport which inhibits the phosphorylation mechanisms, and also due to their interference with the sulfhydryl group of respiratory enzymes (Miller et al., 1973).

It was found that the different concentrations of heavy metals (Cu, Co and Zn) used had reductive effects on transpiration rates of the tested plants. The results of this study and those obtained by others suggest that heavy metals inhibited transpiration

Table 4

Pigments contens (mg/g f. wt) of sunflower, pea and wheat leaves, after treated for 7-days period with different concentrations of cobalt copper and zinc

Treatment		Chlorophyll pigments content (mg/g fresh wt)								
		Sunflower			Pea			Wheat		
		Chl.a	Ch.b	Carot	Chl.a	Chl.b	Carot.	Chl.a	Chl.b	Charot.
Cobalt	0	0.63	0.44	0.12	4.27	2.21	1.02	0.76	0.49	0.35
	2 mM	0.75	0.39	0.11	2.15**	1.78*	1.03	0.56**	0.38**	0.31**
	4 mM	0.71	0.38	0.10*	0.89**	1.06**	1.00	0.63**	0.35**	0.22**
	6 mM	0.47*	0.32*	0.09**	0.89**	0.79**	1.03	0.57**	0.41**	0.24**
	8 mM	0.33**	0.27**	0.08**	0.60**	0.52**	0.51**	0.42**	0.28**	0.16**
	$L.S.D._{5\%}$	0.13	0.12	0.02	0.31	0.29	0.13	0.06	0.04	0.008
	$L.S.D._{1\%}$	0.19	0.17	0.03	0.45	0.41	0.19	0.09	0.05	0.01
Copper	2 mM	0.81**	0.45	0.11	0.24**	0.42**	0.18**	0.67**	0.47	0.21**
	4 mM	0.61	0.38*	0.08*	0.19**	0.27**	0.15**	0.50**	0.37**	0.27**
	6 mM	0.63	0.24**	0.10	0.16**	0.18**	0.06**	0.48**	0.34**	0.15**
	8 mM	0.57	0.17	0.06*	0.15**	0.18**	0.05**	0.44**	0.24**	0.04**
	$L.S.D._{5\%}$	0.11	0.19	0.03	0.08	0.15	0.02	0.04	0.04	0.02
	$L.S.D._{1\%}$	0.13	0.27	0.07	0.27	0.07	0.03	0.08	0.05	0.03
Zinc	2 mM	0.61	0.40	0.12	0.29**	1.58**	0.53**	0.56**	0.36**	0.17**
	4 mM	0.56	0.36	0.11	0.25**	0.26**	0.05**	0.53**	0.31**	0.18**
	6 mM	0.41**	0.34	0.11	0.14**	0.25**	0.04**	0.49**	0.29**	0.17**
	8 mM	0.42**	0.23	0.09*	0.12**	0.11**	0.04**	0.06**	0.26**	0.15**
	$L.S.D._{5\%}$	0.12	0.16	0.03	0.22	0.02	0.01	0.06	0.04	0.01
	$L.S.D._{1\%}$	0.17	0.23	0.05	0.32	0.03	0.02	0.09	0.06	0.02

* Significant (P=0.05) and ** highly significant (P=0.01) differences as compared with control

by interference with stomatal function and/or by retarding water uptake by the test plants (Bazz et al., 1974; Robert and Williams, 1978 and Carlson et al., 1975). A possible mode of action of heavy metals may be due to their interference with diffusive resistances of leaves to CO_2 and water vapour transport (Robert and Williams, 1978). In this respect, the heavy metal cadmium, which is known to affect a host of enzyme systems (Vallee and Ulmer, 1972), may alter stomatal function, indirectly, by affecting the potassium ion pump controlling the movement of potassium between guard cells and adjacent subsidiary cells (Zelitch, 1971).

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HEAVY METAL CONTENT OF FLUE-CURED TOBACCO LEAF IN DIFFERENT GROWING REGIONS OF HUNGARY

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The objective of the present study was to provide information on the heavy metal content of flue-cured tobaccos produced in different growing regions of Hungary, and to examine relationships between certain environmental factors and the metal content in the leaves.

A two-year investigation was conducted including 192 farms randomly selected in the seven main growing regions across the country.

Cured leaf samples from the mid-stalk position were taken by farms. Determination of Cd, Ni, Pb, Cr, Sr, Co, Zn, Mn and Fe was made by the ICP technique using wet ashing with cc. HNO_3 and cc. H_2O_2 method.

The available element content in corresponding soil composite samples was determined using the ammonium-acetate + EDTA method. Analysis of variance and correlation analysis were used to determine the relationship between the environmental factors and the characteristics studied.

Soil acidity appeared to be the most important factor to influence the concentration of Cd, Ni, Sr, Co, Zn and Mn in the leaves, with highly significant negative correlation between the pH value in soil and leaf metal content. Higher levels of available metals in the soil did not result in increased metal concentration in the leaf. Leaf Pb and Cr were positively correlated with rainfall.

Leaf concentrations of Cd, Ni, Co and Zn differed significantly among the growing regions, according to differences in soil pH. The lowest values (as mg/kg) for the regional means of leaf Cd (0.50), Ni (0.33), Pb (0.38), Co (0.02), Zn (19.7) and Mn (82) were obtained in the central part of Hungary, where the reaction of tobacco soils is neutral or slightly alkaline. Extreme high leaf metal concentrations were found in the North-East (Cd 1.89, Ni 5.40, Pb 1.17, Co 0.52, Zn 57.6, Mn 471) where the mean soil pH value is 4.50.

Key words: tobacco, heavy metals, soil pH, cadmium, nickel, lead, chromium, cobalt, strontium, zinc, iron, manganese

Introduction

Although heavy metals have long been recognized as toxic to humans, it is only recently that their occurrence in the environment has been intensively studied. Tobacco smoking has been established as one of the origins of heavy metal in men. A number of investigations have been carried out worldwide on the natural occurrence of heavy metals in tobacco leaf (Adamu et al., 1989; Frank et al., 1977; 1991; Murty et al., 1986). Bell et al. (1988) showed also the residual effects of land applied municipal sludge on tobacco heavy metal composition.

Generally speaking, cadmium is of particular concern, as tobacco *(N. tabacum)* is known to easily accumulate this element in the leaves (Clark and Brennan, 1983; Frank et al., 1977; Wagner et al., 1988). Part of the heavy metals in the tobacco leaf is transferred to the smoke (Cogbill and Hobbs, 1957; Westcott and Spincer, 1974). The level of metals taken up by the plants is the function of a number of factors, including

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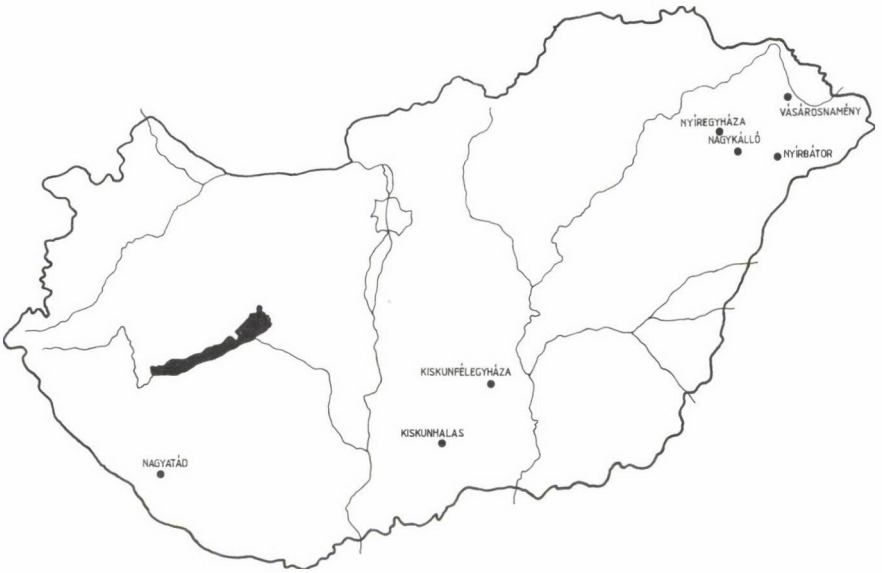

Fig. 1. Tobacco growing regions selected for the study across Hungary, with the tobacco marketing stations indicated

soil properties (Adamu et al., 1989; Bell et al., 1988; Kádár, 1992) and genotype (Kádár, 1992; Wagner et al., 1988).

No comprehensive information is yet available on the heavy metal content of flue-cured tobacco produced in Hungary. The objective of the present study was (i) to survey the metal content of the tobacco leaf produced in different growing regions, and (ii) to determine the most important environmental factors influencing the metal concentration in the leaves.

Materials and methods

A two-year investigation was conducted from 1990 to 1991 including 192 farms, each of which planted to one of the nine flue-cured cultivars available for commercial production in that period. The farms were randomly selected with a distribution pattern proportional to the hectarage of flue-cured tobacco in each particular region. The soils typically exhibit coarse texture, with a clay content of less than 10%. The geographical lay-out of the seven growing regions selected for the study, with the tobacco marketing stations indicated, is shown in Fig. 1.

Cured leaf composite samples of the 2nd ('light B') grade were taken from the mid-stalk position by farms. Determination of Cd, Ni, Pb, Cr, Sr, Co, Zn, Mn and Fe was made by ICP technique, using cc. HNO_3 and cc. H_2O_2 for wet ashing. Total nitrogen, total alkaloids and reducing sugars were determined by Kjeldahl, UV spectrophotometric and colorimetric methods, respectively.

Soil composite samples consisting of 8–10 subsamples were taken from the upper 20 cm layer of each field in spring, prior to transplanting and fertilizer application. The available heavy metal content of the soil was determined by the ammonium-acetate + EDTA method (Lakanen and Erviö, 1971) using the ICP technique.

Rainfall data recorded by the National Meteorological Service were used in this study, including 34 of the reporting meteorological stations. Only rainfall totals for June and July were taken into account. It had been earlier demonstrated that climatic conditions of these two months are of particular importance for yield, quality and chemical composition of flue-cured tobacco in Hungary (Gondola, 1990). The data processing included correlation and variance analysis to determine the relationship between the environmental characteristics and tobacco parameters studied.

Results and discussion

Data presented in Table 1 indicate that the variability of heavy metals in the cured leaf, expressed by the CV %, is higher than that of total alkaloids, total N and reducing sugars, over the seven growing regions and the two years. Among the heavy metals studied, Cr has the highest and Cd has the slightest variation. The levels of Cd, Ni, Pb and Cr (the most commonly studied toxic metals in tobacco) are lower than data published from other studies on cured leaf (Adamu et al., 1989; Bell et al., 1988; Frank et al., 1977; Westcott and Spincer, 1974) and on cigarette blends (Bell and Mulchi, 1990). The level of the essential elements Mn, Zn, Fe is consistent with other foreign published data on flue-cured tobacco (Tso, 1990).

Correlations for leaf components and environmental factors are shown in Table 2. Leaf Cd, Ni, Sr, Co, Zn and Mn content related negatively to soil pH. This finding generally agrees with results reported by other investigators (Adamu et al., 1989; Bell et al., 1988; Murty et al., 1986). There was no significant correlation between the pH value of the soil and leaf Pb and Cr content. These two elements were, however, positively associated with rainfall.

Table 1

Means and variations of tobacco leaf components, soil pH and total rainfall over the growing regions and years (n=192)

Variables	Mean	Minimum	Maximum	CV %
Total alkaloids %	1.91	0.40	6.65	58
Total N %	2.36	1.34	3.74	22
Reducing sugars %	13.76	1.51	30.37	48
Cobalt mg/kg	0.24	0.01	5.95	208
Lead mg/kg	0.66	0.01	9.02	176
Chromium mg/kg	0.77	0.01	21.00	222
Cadmium mg/kg	1.15	0.22	4.53	63
Nickel mg/kg	2.40	0.01	15.90	109
Zinc mg/kg	34.7	8.4	209.0	74
Iron mg/kg	224.1	60.0	1704.0	75
Manganese mg/kg	247.6	30.3	3072.0	136
Strontium mg/kg	285.4	26.6	1373.0	89
Humus %	1.24	0.28	6.12	62
Soil pH (KCl)	5.69	3.52	8.10	26
June + July total rainfall mm	129.0	55	226	36

Table 2

Correlation matrix of leaf chemical components, leaf metal contents, soil properties and rainfall
(Tobacco growing regions of Hungary, 1990–1991, n=192)

Leaf/envir. properties	Total alk.	Red sugars	Total N	Cd	Ni	Pb	Cr	Sr	Co	Zn	Mn	Fe	pH	Clay	O.M.	Rain-fall
				Heavy metals												
Total alk.	–	–xxx	xxx	NS	NS	NS	–x	xx	NS	NS	NS	NS	NS	NS	NS	–xxx
Red. sugars		–	–xxx	NS	NS	x	NS	–xxx	NS	NS	NS	NS	NS	–x	NS	xxx
Total N			–	x	xx	NS	NS	xxx	xx	x	xxx	NS	–xxx	NS	NS	–xxx
Cadmium				–	xxx	NS	NS	xxx	xxx	xxx	xxx	NS	–xxx	xx	NS	NS
Nickel					–	NS	xxx	xxx	xxx	xxx	xxx	xx	–xxx	NS	NS	NS
Lead						NS	NS	NS	xx	xxx	NS	xx	NS	NS	NS	x
Chromium							–	NS	NS	NS	NS	NS	NS	NS	NS	xxx
Strontium								–	xxx	xx	xxx	x	–xx	NS	NS	–xxx
Cobalt									–	xxx	xxx	NS	–xx	NS	NS	NS
Zinc										–	xxx	NS	–xxx	NS	NS	NS
Manganese											–	NS	–xxx	–x	NS	NS
Iron												–	NS	NS	NS	NS
Soil pH													–	NS	x	–
Soil clay														–	xxx	–

NS – not significant
x – significant at the 0.05 level
xx – significant at the 0.01 level
xxx – significant at the 0.001 level

Table 3

Means for soil pH, rainfall, leaf metal contents and for some selected chemical components
(Tobacco growing regions of Hungary, 1990 and 1991, n= 192)[X]

The seven growing regions	Soil pH	Rainfall	Total alk.	Red sugars	Total N	Co	Cr	Cd	Pb	Ni	Zn	Fe	Sr	Mn
										/Heavy metals/				
Vásárosnamény	4.50	161	1.60	18.0	2.42	0.52	1.65	1.89	1.17	5.40	58	244	395	471
Nyírbátor	4.55	131	1.85	14.8	2.47	0.22	0.38	1.25	0.49	2.83	48	178	174	264
Nagykálló	4.73	122	1.28	17.3	2.18	0.39	0.99	1.24	0.98	3.70	29	246	182	291
Nagyatád	4.98	153	2.22	11.3	2.58	0.10	0.66	1.25	0.43	1.35	31	208	319	228
Nyíregyháza	5.51	118	1.66	11.9	2.33	0.42	0.83	1.11	1.08	2.77	36	229	457	277
Mean	4.85	137	1.72	14.7	2.40	0.33	0.90	1.35	0.83	3.21	40	221	305	306
Kiskunhalas	7.55	129	1.41	14.8	2.00	0.02	0.64	0.50	0.39	0.50	27	183	203	89
Kiskunfélegyháza	7.78	116	2.13	13.7	1.94	0.02	0.45	0.54	0.38	0.33	20	312	181	82
Mean	7.66	122	1.77	14.2	1.97	0.02	0.54	0.52	0.38	0.42	24	248	192	86
$L.S.D._{5\%}$ (for the particular regions)	0.59	NS	NS	NS	NS	0.43	NS	0.50	NS	3.16	32	NS	NS	NS

[X] Rainfall = June + July total in mm
Total alkaloids, nitrogen and reducing sugars in %
Heavy metal contents in mg/kg d.m.

Table 4

Summary of means for soil pH, rainfall, leaf metal contents and for some selected chemical components (Tobacco growing regions of Hungary, 1990 and 1991, n=192)[X]

Measured parameters	1990	1991	L.S.D. 5%
Total alkaloids	2.34	1.12	0.44
Total nitrogen	2.48	2.07	0.35
Reducing sugars	11.3	17.8	5.1
Cobalt	0.28	0.20	NS
Lead	0.38	1.03	0.56
Chromium	0.57	1.03	NS
Cadmium	1.07	1.15	NS
Nickel	2.73	2.09	NS
Zinc	34	36	NS
Iron	264	193	NS
Manganese	314	172	NS
Strontium	387	159	165
Soil (KCl)	5.61	5.69	NS
Rainfall	99	166	34

[X] Rainfall = June + July total in mm
Total alkaloids, nitrogen and reducing sugars in %
Heavy metal contents in mg/kg dry matter

Of the metals studied, only Cd gave significant positive correlation with the clay content of soils. None of the metals were influenced by the organic matter levels in soil. Leaf Fe content showed no significant correlation with any of the environmental parameters studied. Highly significant, positive correlations were found between and among leaf Cd, Ni, Sr, Co, Zn and Mo levels. Cr vs. other metals were not correlated, except for the relationship between Cr and Ni. Highly significant positive correlations were observed, however, for rainfall vs. reducing sugars and negative correlations for rainfall vs. total alkaloids and total N.

The differences in leaf metal content among the growing regions are presented in Table 3. L.S.D. values were computed for the means of the seven regions using years as replications. Significant differences existed among the regions for Cd, Ni, Co and Zn, as well as for soil pH. Lower leaf metal concentrations were obtained in regions where soil pH values were higher. A 3.8-fold range was found for mean concentrations of Cd among the regions.The same values for the ranges of Ni and Co amounted to 16.4 and 32,3 respectively.

Although the variations for Pb, Cr and Mn were not significant, their concentrations in the leaf also tended to be higher in regions with lower pH value. These findings are in accordance with the general knowledge on the influence of soil acidity on the plant available level of most microelements. Tobacco soils are highly acidic in North-Eastern Hungary, which is why tobaccos produced in these regions accumulate higher amounts of heavy metals than do tobacco plants in the central part of the country, where soils are neutral or slightly alkaline.

Decreasing soil acidity by liming has been reported to lower the plant uptake of Cd and of some other heavy metals (Bell et al., 1988; McLean, 1976). Lime application is therefore highly recommended to most of the tobacco soils in the North-East of the

country. Heavy metal content of tobacco leaf from the South-West region (Nagyatád) are, in general, between that of the North-East and of the central regions, according to differences in soil pH.

Total rainfall for June and July differed significantly between the two years, with the 1990 season being drier (Table 4). Of the metals studied only Pb and Sr changed drastically between the years, with the lower value of Pb and the higher value of Sr obtained in the drier 1990. An earlier study showed that in wet years the soil phosphorus together with Sr is more available for the crops, while Pb will be partly washed down from the leaves (Kádár, 1992). The change of total alkaloids, total N and of reducing sugars from one year to the other followed the pattern typical for these components (van Bavel, 1953; Gondola, 1989).

Finally, the relationship for leaf metals vs. available metal content in the soil was examined in 1991 (Table 5). Only Ni exhibited a significant positive correlation between soil and plant values. The relationship for the other elements was either not significant or there was a significant negative correlation (Sr, Mn). It can be concluded that, apart from Ni, higher levels of available metal content in the soil do not result in increased metal uptake in the leaves. The heavy metal limit values for soil testing and related recommendation work should be established taking into consideration other soil properties (e.g. pH, clay and humus content).

Table 5

Coefficients of correlation between plant-available heavy metals in the soil and heavy metal contents in the cured leaf (Tobacco growing regions of Hungary, 1991, n=80)

Metals	Coefficients of correlation (r)	
Cd	–0.207	NS
Ni	0.251	x
Pb	–0.152	NS
Cr	–0.059	NS
Sr	–0.475	–xxx
Co	0.002	NS
Zn	–0.010	NS
Mn	–0.290	–xx
Fe	0.082	NS

NS – not significant
x – significant at the 0.05 level
xx – significant at the 0.01 level
xxx – significant at the 0.001 level

Conclusions

Soil pH appeared to be the most important factor influencing the concentration of Cd, Ni, Sr, Co, Zn and Mn in the leaves of flue-cured tobacco. Higher levels of soil-available metals did not result in increased metal concentrations in the leaf.

Mean leaf content of Cd, Ni, Co and Zn differed significantly among the seven growing regions studied, according to differences in soil pH. Higher values of leaf metal concentrations were obtained in the North-Eastern regions, where soils are highly acidic. Liming of acid soils in North-East Hungary is recommended to lower the leaf heavy metal uptake.

Because of the general discrepancy between soil-available heavy metal content and leaf concentrations (plant uptake), the heavy metal limit values for soil testing and related recommendation work shall be established, taking into consideration other soil properties such as pH, clay and humus content.

The mean level of the essential elements, Mn, Zn, Fe was consistent with other foreign published data on flue-cured tobacco, while the most commonly studied toxic metals of Cd, Ni, Pb and Cr were found lower than other international studies showed. As it might be expected, significant positive correlations were observed between total rainfall and reducing sugar content and negative correlations for rainfall vs. total alkaloids and total N.

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EFFECT OF HYDROTHERMIC TREATMENT ON THE ILEAL AND FAECAL DIGESTIBILITY OF HYBRID MAIZE NUTRIENTS BY YOUNG PIGS

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The authors studied the effect of hydrothermic treatment (Bocchi technology) on the absorption of nutrients of normal and waxy hybrid maizes in young female F1 pigs of large white × Dutch lowland supplied with small intestine T-cannula, with 4 animals used per treatment, and determined the ileal and faecal digestion coefficients of the nutrients (protein, amino acid, starch etc.). The use of chrome oxide as indicator obviated the quantitative collection.

According to the analyses, the ileal digestion coefficients of the untreated normal and waxy hybrids show no significant differences except for cystine and methionine, while the analysis of the faeces indicated a significantly better digestion of crude protein and amino acids – with the exception of tyrosine and lysine – in the case of the waxy hybrid. The digestion coefficients of starch content show no considerable differences, neither between the breeds nor as a result of the treatment.

In response to the Bocchi technology all the nutrients examined, except threonine, methionine, leucine, tyrosine and starch, were significantly better digested in the normal hybrid according to both the ileal and the faecal analyses. As to the ileal digestion coefficients of the waxy hybrid, those for threonine, cystine and lysine are not significantly better; while, according to the faecal analysis, none of the amino acids – except cystine – give statistically proved better results.

Key words: hybrid maizes, nutrient content, amino acid composition, pig small intestine T-cannula, ileal and faecal digestibility

Introduction

A considerable proportion (about 80%) of fodder mixtures for pigs consists of cereals, in Hungary mostly of maize. Maize is primarily a source of energy for pigs; but, owing to the high rate of its conversion, it also plays an important role in the protein supply of the animals. Today hybrid maizes are almost exclusively used in feeding, with a part of which (opaque) the aim has been to increase their lysine content, while in another part of them changes have been brought about in the composition of starch, the source of energy. The starch content, the main energy source of the hybrid maize with normal endosperm, generally consists of 25% amylose and 75% amylopectin; while the starch of the waxy hybrid is made up to 100% of amylopectin (Rosa et al., 1977a; Perez and Aumaitre, 1979).

Feed production has widely introduced thermic treatment technologies capable of increasing the digestibility of nutrients. A procedure of this kind is flake, in the course of which hydrothermic and mechanical treatments cause changes in the structure of the feed's nutrients (Bocchi, 1985). The favourable effect of these changes can be checked by *in vivo* digestion tests.

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Although a separate feeding of maize hybrids has not become a general practice in pig feeding yet, it is reasonable to carry out digestion biological examinations and determine the rate of conversion of the different nutrients, so as to call the plant breeders' attention to the dietetical differences of maize hybrids and through, this, to the aspects of animal feeding.

In the different parts of the pig's digestive system the absorption of the nutrients varies, and the digestion coefficients determined by the traditional faecal analysis do not sufficiently explain concerning the absorption of the proteins, amino acids and starch originating from the feed. By means of a T-cannula built surgically in the last section of the pig's small intestine it is possible to determine the precaecal, hereinafter ileal digestibility of the nutrients, studying thereby the absorption of proteins, amino acids and starch.

It was thus reasonable that in the framework of a comparative study in young pigs, supplied with small intestine T-cannula, we determined the digestibility of the nutrients of two different maize hybrids (normal and waxy), and the influence of treatments with the Bocchi technology on the absorption of the nutrients.

Materials and methods

Experimental feeds

The experimental fodder mixture contained 96% normal and waxy maize, and 4% premix. Four groups were formed; two of them consumed untreated maize, while the other two were given maize treated with Bocchi technology (Table 1). The waxy hybrid originated from Kunszentmiklós (Egyetértés Cooperative Farm), while the maize with normal endosperm came from the Herceghalom Farm. Both maize hybrids had been treated with Bocchi technology: the maize grains were flocculated through "steam+pressure+shearing".

Experimental animals

In the ileum of young female large white × Dutch lowland F_1 pigs (30.0±2.0 kg on the average), at a distance of about 15 cm from the caecum, the T-cannula was surgically implanted. The pre- and postoperative tending of the animals took place with a method developed at our Institute (Kubovics et al., 1989). After the operation the animals were placed separately in pig-pens, where drinking water was available according to need. The experiment was started following recovery after the operation. In the experiment four pigs were given the same treatment. Each animal was weighed before the experimental feeding and when the collecting period was completed. The average body weight of pigs in the experimental period ranged between 35.6 and 44.7 kg.

Chymus and faeces collecting

Feeding, chymus and faeces collecting of animals supplied with an intestinal fistula took place as described in the paper by Szelényiné et al. (1991): chymus and faees collecting was carried out four times a day over 80 minutes on three days of the 5-day experimental phase, following 9 days of preliminary feeding. In the feed ration chrome oxide was mixed as an indicator in the form of CrNDF (Cr_2O_3 content 365 mg/kg), whereby quantitative collecting could be dispensed with.

The digestion coefficients were established on the basis of the difference between the nutrients taken up from the feed and those discharged in the chymus and faeces, respectively.

Table 1

Percentage composition of experimental feeds

Designation	Normal		Waxy	
	Hybrid maize			
	Untreated	Bocchi-treated	Untreated	Bocchi-treated
Maize	96.0	96.0	96.0	96.0
Pig premix[X]	4.0	4.0	4.0	4.0
Total	100.0	100.0	100.0	100.0
Average daily feed uptake by pigs g	1225±50	1265±47	1135±161	1200±82

[X] 1 kg premix contains: Ca 16.17%; P 4.35%; NaCl 9.70%; Na 3.80%; A vitamin 131250 NE; D_3 vit. 30625 NE; E vitamin 350 mg; K_3-vit. 21.87 mg; B_1-vit. 8.2 mg; B_2-vit. 28.87 mg; B_6-vit. 20.78 mg; B_{12}-vit. 0.23 mg; nicotinic acid 65.62 mg; BHT antioxydant 1032.5 mg; Zn 1381.25 mg; Fe 1381.25 mg; Cu 1062.50 mg; Mn 690.62 mg; I 20.72 mg; Se 2.12 mg

Laboratory analyses

The laboratory analyses were performed (dry matter, crude protein, crude fat, crude fibre etc.) from the feed, faeces and chymus samples according to MSZ (Hungarian standard) 6830. The amino acid composition was determined by analysis based on the Moore–Stein principle, after hydrochloric acid hydrolysis (Amino-chrom II type analyser). For the reliable determination of methionine and cystine, performic acid oxidation was used, from which the oxidized products: Cys (O_3H) and Met (0) were determined (Takarmánykódex 1990, Feed Codex 1990). The chrome indicator was determined after Fenton and Fenton (1979).

The dry matter, crude protein and amino acid contents were determined from fresh chymus and faeces samples, while the other determinations were made with samples dried at 60 °C.

The starch content of maize, and of chymus and faeces samples, were determined by Boehringer's "UV-test" after exposure with enzyme described in Seidler et al. (1988).

Statistical analysis

The experiment was evaluated by single-factor variance analysis (Sváb, 1973). Since the treatments showed significant differences for all factors examined, the differences were checked by pairs with t-test.

Results

Feed analyses

The analysis results of the experimental feeds are shown in Table 2. The crude protein content was about 20% more in the waxy than in the normal hybrid. The starch content, on the other hand, was 13% less in the waxy hybrid than in the normal one. In amino acid composition, there was hardly any difference between the two hybrids; in the table, the amino acids most important from the standpoint of pig breeding are only shown.

Table 2

Nutrient and starch content, and amino acid composition of the maize samples analysed
(On 1000 g dry matter basis)

Designation	Normal hybrid		Waxy hybrid	
	Maize			
	Untreated	Bocchi-treated	Untreated	Bocchi-treated
Nutrients (g)				
Organic matter content	954	960	959	955
Crude protein content	112	106	136	136
Crude fat content	43	45	40	48
Crude fibre content	28	23	27	24
Ash content	46	40	40	45
N-free extr.m. content	771	785	756	769
Starch content	700	649	608	611
Digestible energy content (MJ/kg)	18.68	18.11	18.43	19.00
Amino acid composition (% of dry matter)				
Threonine	0.30	0.28	0.33	0.35
Cystine	0.10	0.09	0.12	0.11
Valine	0.39	0.36	0.45	0.49
Methionine	0.18	0.17	0.17	0.17
Isoleucine	0.24	0.25	0.32	0.37
Leucine	1.07	1.01	1.22	1.34
Tyrosine	0.31	0.27	0.33	0.34
Lysine	0.26	0.29	0.27	0.31

Ileal and faecal absorption examinations

The nutrient and amino acid absorption results of the treated and untreated variations of the maizes examined are summarized in Figs 1, 2a and 2b.

In the case of the normal maize hybrid, the Bocchi technology significantly improved the digestibility of dry matter, organic matter and crude protein content, as proved by the chymus analysis (Fig. 1). The crude protein content of the waxy hybrid showed about 6% improvement in response to the treatment, while for the other nutrients only an improving tendency could be observed.

In response to the treatment, a slight improvement in the digestibility of starch was found in both varieties. On the basis of the ileal analysis, the digestibility of the starch content was an average of 97–98%, while the faeces analysis gave values exceeding 99%.

The changes taking place in the ileal and faecal digestion coefficients of the most important amino acids are shown in Figs 2a and 2b. In consequence of the treatment, there was a considerable difference between the two hybrids in the ileal digestibility of the amino acids; the digestibility of the lysine content in the normal hybrid improved from 58.5% to 72.7%, and – even if not to the same extent – improved the digestibility of cystine, valine, isoleucine and leucine too. Among the amino acids of

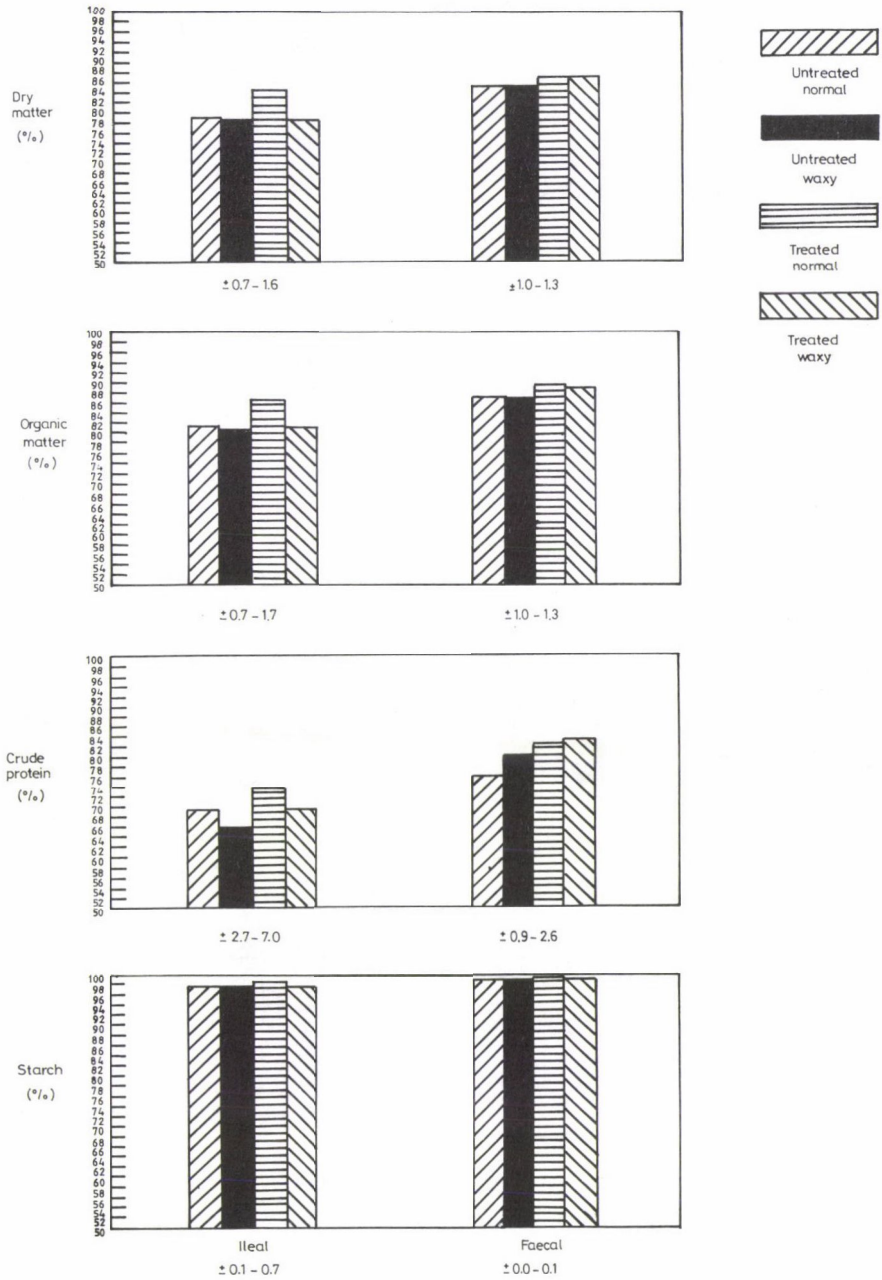

Fig. 1. Digestion coefficients of nutrients in normal and waxy maize hybrids

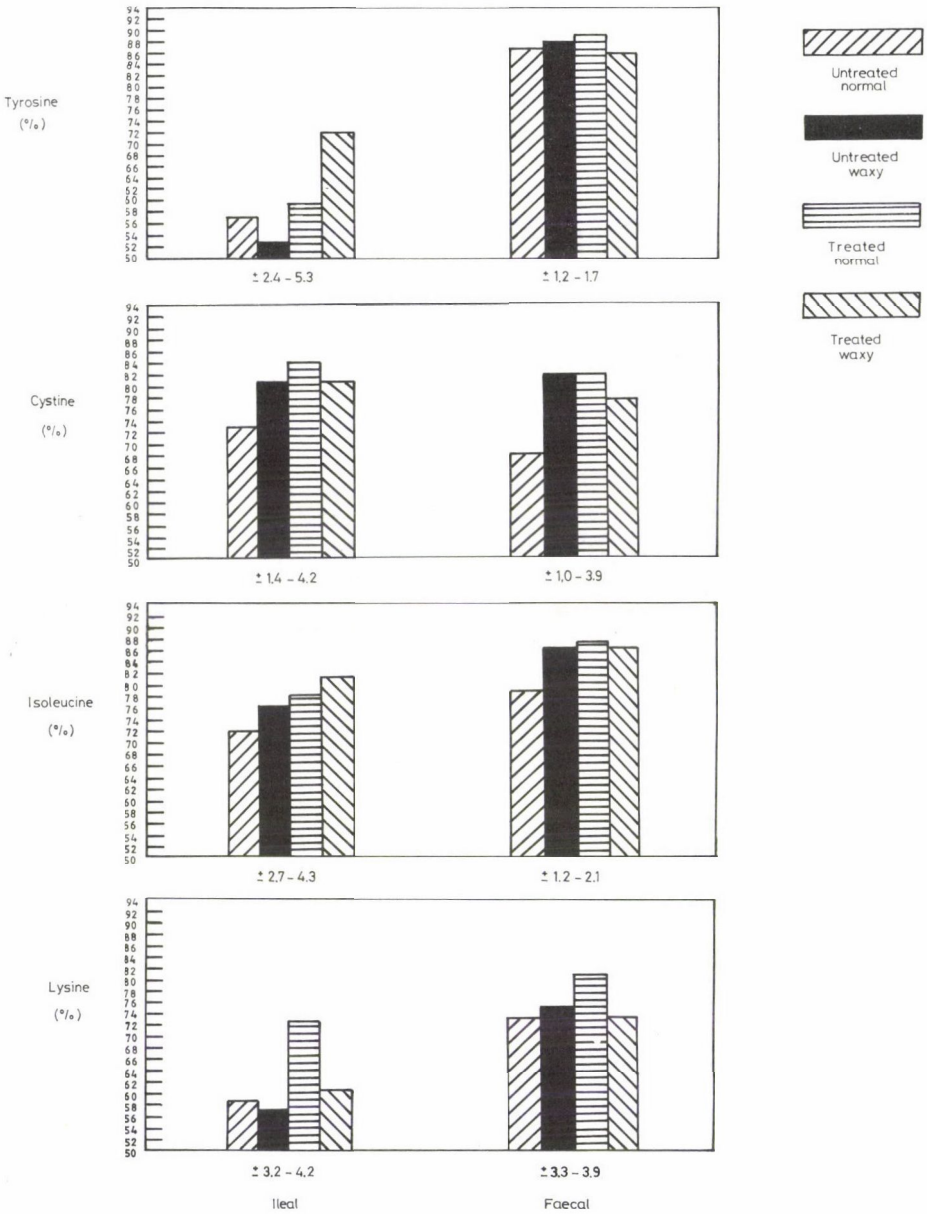

Fig. 2a. Digestion coefficients of the amino acid content of normal and waxy maize hybrids

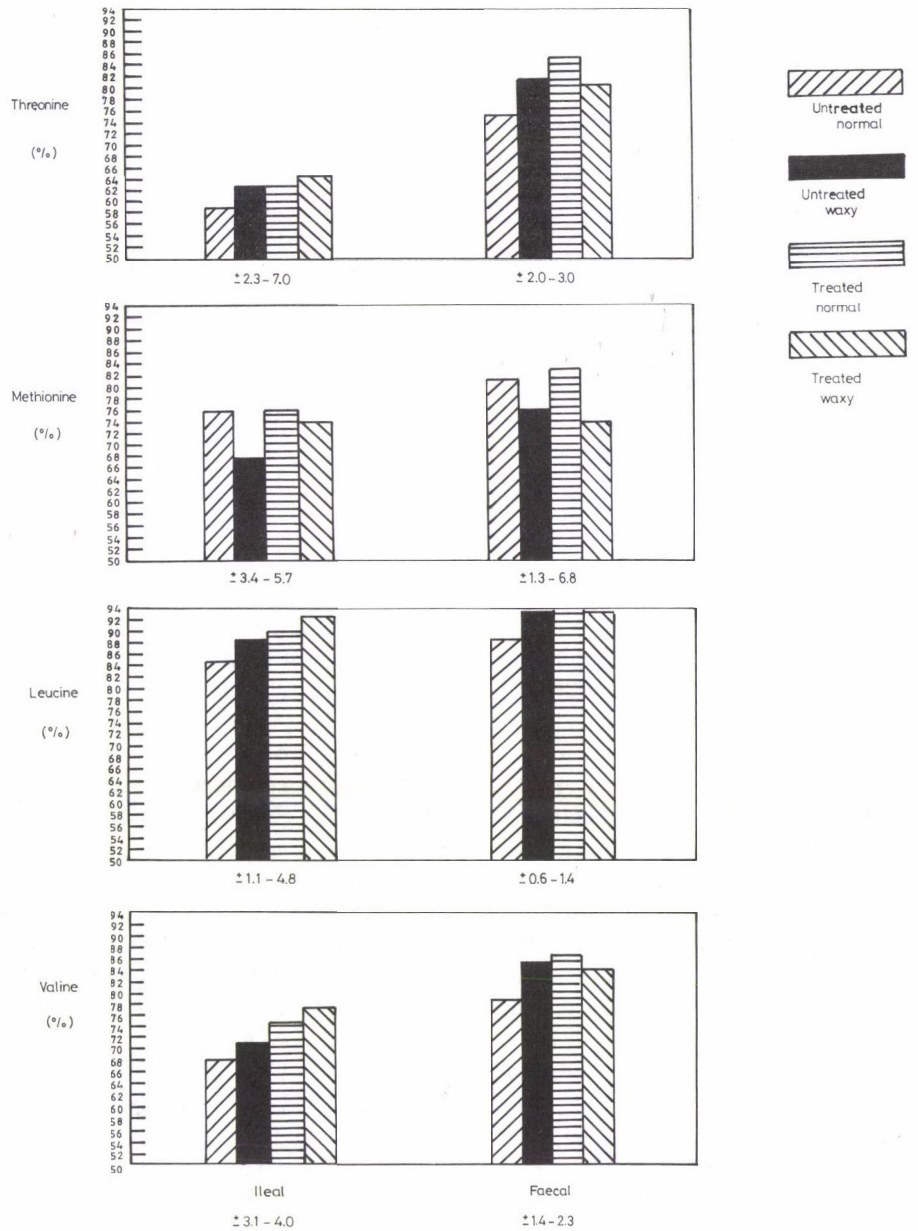

Fig. 2b. Digestion coefficients of the amino acid content of normal and waxy maize hybrids

Table 3

Differences between the percentage values of ileal and faecal digestion coefficients

Designation	Normal hybrid maize		Waxy hybrid maize	
	Untreated	Bocchi-treated	Untreated	Bocchi-treated
Dry matter	6.7	3.0	6.7	8.7
Organic matter	6.0	3.2	6.1	8.0
Crude protein	6.7	8.9	14.5	13.7
Crude fat	–18.7	–10.9	–25.0	–10.7
Crude fibre	–9.2	1.0	–6.6	8.8
N-free extr. matter	7.6	3.4	6.7	7.8
Crude ash	19.9	–4.2	20.4	25.1
Starch	1.8	0.9	1.4	1.7
Threonine	16.3	22.9	18.9	16.2
Cystine	–4.8	–2.4	–1.2	–3.2
Valine	10.9	12.4	14.7	7.2
Methionine	5.4	7.3	8.3	–0.2
Isoleucine	7.0	9.3	10.1	4.9
Leucine	4.0	4.0	5.1	0.9
Tyrosine	29.6	29.5	35.2	14.0
Lysine	14.7	8.4	18.4	12.9

the waxy hybrid the digestibility of lysine changed from 57.0% to 60.5%, while that of tyrosine from 52.8% to 72.0%; the valine, methionine, isoleucine and leucine showed about 5–7% improvement. The digestibility of threonine only slightly changed in either hybrid.

In the case of the normal hybrid, the faecal digestion coefficients of threonine, cystine, valine isoleucine and lysine substantially improved under the influence of the treatment. Out of the amino acids of the waxy maize, the digestion coefficients of methionine, tyrosine, threonine and lysine decreased in consequence of the treatment.

In Table 3 the differences of the digestion coefficients obtained by ileal and faecal analyses are summarized. Accordingly, it can be generally said that the differences between the praecaecal resp. postilelal digestibility values of the untreated normal and waxy hybrids are greater than when the same hybrids are treated with Bocchi technology. The greatest differences among the amino acids were shown by the tyrosine, threonine, lysine and valine. Sauer et al. (1977) only found a considerable difference in the case of threonine, though they indicated that the ileal amino acid digestion coefficients of cereals were lower than those obtained through faecal analysis. Hartog (1988) compared extruded to untreated maize making 60% of the fodder mixture of weaned piglets fitted with cannula. According to his results, the treatment caused some positive change in the ileal and faecal digestion coefficients of the nutrients and of the amino acids within. On the other hand, the differences between ileal and faecal digestion coefficients generally exceeded 10%. In the experiment carried out by Poel et al. (1990) the treatment of maize improved the *in vitro* digestibility to a considerable extent. Simultaneously, in their experiment with weaned piglets, with the exception of the organic matter and N-free extr. matter as well as of the amino acid cystine and proline, the ileal digestibility did not change.

Discussion

The crude protein content and amino acid composition of the normal hybrid maize shows the characteristics of a medium quality hybrid. Its starch content, on the other hand, is below the values published by Sauer et al. (1977) and Perez and Aumaitre (1979); but it comes close to the starch content given by Seidler et al. (1988). We note here that in our experiment we used the method of starch determination described by the latter authors. The crude protein content of the waxy hybrid in our experiment was higher than the value given in the papers mentioned above, while for the amino acid composition we found hardly any difference. However, the starch content was also found by the mentioned authors to be lower in the waxy hybrid than in the normal one. Besides the protein, the energy content of the feed – determined decisively by the carbohydrate – is one of the most important factors in the course of animal fattening. The carbohydrates form a heterogeneous group of compounds; their intestinal transformation varies both with the kind of reaction process and with the site of decomposition within the intestinal system. The readily soluble carbohydrates (starch and sugar) are decomposed by the enzymes produced by the organism itself, mostly in the small intestine, while the substance of the cell wall is decomposed through a microbial fermentation of energetically lower efficiency, which essentially takes place in the postileal intestine (Schulz, 1990, verbal information).

In similar experiments with pigs fitted with cannula, Sauer et al. (1977) and Freire et al. (1988) found that, in the case of an average maize hybrid, there was hardly any difference in ileal and faecal digestibility between lysine and methionine, while the digestibility of threonine and cystine as well as of dry matter and crude protein varied highly, and the starch was digested to an average of 98% by the end of the ileum. They also found that the different types of starch did not influence the fattening performance of the pigs. Rosa et al. *(1977a),* referring to other authors, point out that the starch of the waxy maize is more responsive to the enzyme hydrolysis, but this does not appear in the fattening performance of the animals.

Leeuwen et al. (1987), testing average maize samples with pigs fitted with cannula, found the ileal digestibility to be 70% for crude protein, 57% for lysine, 82% for methionine and 61% for threonine. Our analysis results obtained with either maize hybrids come close to these values. Also the digestion coefficients determined by faecal analysis in the experiment of the above authors showed a similar increasing tendency as in our experiment.

Evaluation of the results

In our experiment the treatment of the normal maize hybrid with Bocchi technology caused a significant change in the ileal digestion coefficients in the case of lysine, cystine, leucine as well as dry matter, organic matter and N-free extr. matter ($P<0.01$ and 0.1). The Bocchi technology likewise caused significant differences in the faecal digestion coefficients of all nutrients examined, except methionine ($P<0.01$; 0.1; 0.5).

As regards the ileal digestion coefficients of the waxy hybrid, the valine, methionine, isoleucine, leucine and tyrosine showed significant differences ($P<0.01$; 0.1; 0.5). As for the faecal digestion coefficients, the differences of cystine, further of dry matter, organic matter and crude protein were significant ($P<0.1$ and 0.5, respectively).

The difference between the ileal and the faecal digestion coefficients (Table 3) shows a higher value on the basis of the chymus analysis than the one obtained by faecal analysis only in the case of cystine. The difference in ileal and faecal digestion coefficient of crude protein between the untreated and the treated waxy hybrid is 14.5 and 13.7%, respectively; while, in the case of the normal hybrid, these values are 6.7 and 8.9%, respectively. Besides, out of the amino acids the faecal digestion coefficients for tyrosine, threonine, lysine and valine differ most from the values obtained in ileal analysis.

According to the chymus analysis, the apparent digestibility of amino acids both in the normal and the waxy hybrid significantly improved in response to the treatment, apart from a few exceptions; while, for the other nutrients significantly positive effects could be observed only in the normal hybrid.

When comparing the two maize hybrids for amino acid digestibility, we found that, except for methionine and tyrosine, the coefficients of the ileal amino acid digestion are better for the waxy hybrid; but, concerning the other nutrients, the values obtained by the chymus analysis were in all cases poorer.

On the basis of the faecal analysis the treatment resulted in a significant improvement in the digestion coefficients of amino acids and crude protein of the normal hybrid, while in the case of the waxy hybrid declining values were found. This result can be explained by the fact that the absorption of proteins and amino acids is completed by the end of the ileum, while in the postileal intestinal section the microbial protein synthesis takes place simultaneously with the protein decomposition. This protein shows a structure characteristic of the amino acid composition of the microbe proteins, and differs from the feed proteins. Thus, the quantities measured to the end of the small intestine are accepted as the possible quantity of amino acid absorption available in the intermediary metabolism for the synthesis of protein (Hermann, 1988; Schröder et al., 1989).

The digestion coefficients of the starch content do not show any considerable differences, neither between the two hybrids nor in response to the treatment. These results agree with those found by Rosa et al. (1977b) during their investigations; namely that, in the pig, the amylose and the amylopectin are totally digested. From this we can in turn draw the conclusion that the starch becomes converted in the digestive system of the young pig even without any treatment.

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HISTOLOGICAL CHANGES IN THE STEMS OF SOME *ROSA* SPECIES PROPAGATED BY LEAFY CUTTINGS AS AFFECTED BY IBA TREATMENTS. I*

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The effect of indole-3-butyric acid (IBA) on root development of *Rosa canina* and *Rosa rugosa* leafy cuttings was investigated. Four IBA concentrations (0, 500, 1000 and 2000 ppm) were used, and three propagation time (May 20th, June 8th and July 8th). Treatments with IBA resulted in a higher percentage of rooted cuttings of *Rosa canina* and were partially stimulating the rooting of *Rosa rugosa.* In *Rosa canina,* the best rooting (78.75%) was achieved with cuttings collected on June 8th and treated with 1000 ppm. of IBA Rooting was accompanied by histological changes that started with the proliferation of certain zones of phloem parenchyma and cambial zone. The adventitious roots were formed from the cambium tissue, and some of them were initiated at nodes, from parenchymatic bud and leaf gaps. In *Rosa rugosa,* some roots were initiated fromt he callus tissue outside of the cut surface.

Key words: *Rosa rugosa, Rosa canina,* propagation, cuttings, IBA treatment, histological studies

Introduction

The members of Rosa genus are important as ornamental plants in gardens and public parks. There are many species of roses. Some of them are grown for their beautiful and fragrant flowers; others, such as *Rosa canina* and *Rosa rugosa* are grown not only as rootstocks, but also as climbers or hedging plants. The traditional way of vegetative propagation for roses is budding. In recent years, propagation by cuttings is also used.

Large differences in rooting capacity of different roses are well known. Auxins can increase the rooting potential of a large number of plants.

Several researchers investigated the effect of IBA on the adventitious root formation of several rose species (Azimi and Bisgrove, 1976 on *Rosa multiflora;* Ivanicka, et al., 1977 on *Rosa pomifera;* Das et al., 1980 on *Rosa indica;* Khosh-Khui and Sink, 1982 on *Rosa hybrid* cv. Sonia and Mukhopadhyay and Bankar, 1987 on *Rosa hybrid* "Queen Elizabeth". However, there are still many difficult-to-root species which respond very little to auxin treatments.

Adventitious roots of some rose species were originated not only at the internodes from the non-differentiated secondary phloem and interfascicular cambium between the vascular bundles (Fahn, 1977), but also at the nodes from bud and leaf gaps (Jakson, 1986): The origin of adventitious roots from callus tissue and out-cut surface has been associated with difficult-to-root species (Mittempergher, 1964 on pear; Fouad, 1965, on pear; Girouard, 1967, on *Hedera helix;* Davies et al., 1982, on *Ficus pumila;* and Hicks, 1987, on apple).

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The aim of the present study was to investigate the effect of IBA on the rooting, and the histological changes that take place in the stem of *Rosa canina* and *Rosa rugosa* during the rooting, with or without IBA treatment; and to determine the best time of the year for making the cuttings.

Materials and methods

Three experiments were carried out at the Experimental Station and Laboratories of the Department of Floriculture and Dendrology, University of Horticulture and Food Industry, Budapest, Hungary, during the spring and summer of 1992.

Cuttings were taken from one-year-old shoots of both *Rosa canina* and *Rosa rugosa* collected from the University Garden and prepared from the basal or middle parts of the shoots in May 20th, June 8th and July 8th. The cuttings were quick-dipped for 5 sec in distilled water as control and in 500, 1000 and 2000 ppm alcoholic solutions of indolebutyric acid (IBA). All experiments were laid out in complete randomized split plot design, with four replications, 22 cuttings for each sub-plot. Two months after planting, the percentage of rooted cuttings, the percentage of callused cuttings, percentage of dead cuttings and the average length of roots (cm) were calculated and measured, and the average number of roots per rooted cuttings was counted. All data were statically analyzed according to the methods described by Sváb (1973).

For the anatomical studies, samples were taken from the base of cuttings 2 and 4 weeks after planting and at the end of the second experiment. The samples were immediately killed and fixed in FAA solution.* Later, they were washed in tap water before sectioning. Cross and longitudinal sections of 15–30 mm thick were prepared by using a seldge microtome. The sections were stained with light green-saffranin combination, cleared in oil cloves and mounted in canada balsam (Johansen, 1940) for microscopical examination and photography.

Results and discussion

A) *Effect of IBA on the rooting*

The results shown in Tables 1 and 2 indicate that the treatment with IBA induced the rooting process, but its effect depended on the concentration used, on the plant species, and on the time of propagation.

1. Rosa canina

Results in Table 1 indicate that IBA increased the percentage of rooting, number of rooted cuttings, number of roots per rooted cutting and length of rooted cuttings in all concentrations used, but had no significant effect on the percentage of callused cuttings in the first and third experiment in May and July. The medium concentration used (1000 ppm) was more effective in promoting root formation. The best rooting (78.75%) was achieved with cuttings collected on June 8th, and treated with IBA at 1000 ppm.

2. Rosa rugosa

Results in Table 2 indicate that this species is difficult to root, although, some success was obtained with IBA treatment. IBA in all concentrations used increased the percentage of rooting, percentage of callused cuttings, the number of rooted cuttings,

*FAA solution = 85 ml ethyl alcohol 70%: 10 ml Formalin: 5 ml glacial acetic acid.

Table 1

Effects of IBA and the time of propagation on rooting of Rosa canina L. leafy stem cuttings

(A) Propagation times	May					June					July				
	Characters					Characters					Characters				
(B) IBA Treatments ppm	Percent of rooting (%)	Percent of callused cuttings	Percent of dead cutting (%)	Average No. of roots per rooted cutting	Average length of roots rooted cutting	Percent of rooting (%)	Percent of callused cuttings	Percent of dead cutting (%)	Average No. of roots per rooted cutting (cm)	Average length of roots per rooted cutting (cm)	Percent of rooting (%)	Percent of callused	Percent of dead cutting (%)	Average No. of roots per rooted cutting	Average length of roots per rooted cutting (cm)
Control	33.8	42.5	66.3	2.44	4.13	43.8	70.0	56.3	1.70	2.93	33.8	43.8	66.3	1.76	1.54
500 ppm	46.3	50.0	53.8	7.67	6.09	63.8	72.5	36.3	6.40	5.95	41.3	46.3	58.8	3.43	4.18
1000 ppm	50.0	56.5	50.0	7.71	7.20	78.8	87.5	21.3	7.70	6.70	50.0	46.3	50.0	8.39	7.13
2000 ppm	36.3	46.3	63.8	6.63	6.38	62.5	70.0	37.5	5.43	5.60	40.0	47.5	60.0	6.66	5.18
I%	1.50	N.S.	11.5	3.80	3.41	19.0	3.1	19.0	1.59	1.56	2.69	N.S.	12.8	2.86	3.18
$L.S.D._{5\%}$	8.00	N.S.	8.02	2.64	2.37	13.2	2.14	13.2	1.10	1.08	1.87	N.S.	8.8	1.98	2.21

Table 2

Effects of IBA and the time of propagation on rooting of Rosa rugosa L. leafy stem cuttings

(A) Propagation times	May					June					July				
	Characters					Characters					Characters				
(B) IBA Treatments ppm	Percent of rooting (%)	Percent of callused cuttings	Percent of dead cutting (%)	Average No. of roots per rooted cutting	Average length of roots rooted cutting	Percent of rooting (%)	Percent of callused cuttings	Percent of dead cutting (%)	Average No. of roots per rooted cutting (cm)	Average length of roots per rooted cutting (cm)	Percent of rooting (%)	Percent of callused cuttings	Percent of dead cutting (%)	Average No. of roots per rooted cutting	Average length of roots per rooted cutting (cm)
Control	1.25	1.3	98.8	0.50	1.38	3.75	11.3	96.3	2.50	2.20	5.00	7.5	95.0	2.25	1.25
500 ppm	1.25	1.3	98.8	1.00	2.00	10.0	11.3	90.3	9.75	8.53	6.25	8.8	92,8	2.50	2.08
1000 ppm	3.75	3.8	96.3	1.25	2.10	12.5	21.3	87.5	14.0	8.63	7.50	15.0	92.5	6.38	3.50
2000 ppm	5.00	5.00	95.0	2.00	2.60	14.0	30.0	86.0	21.3	9.60	11.25	21.3	88.8	7.70	4.50
I%	N.S.	N.S.	N.S.	N.S.	N.S.	10.8	2.82	9.42	12.22	1.91	N.S.	2.76	N.S.	4.16	3.75
$L.S.D._{5\%}$	N.S.	N.S.	N.S.	N.S.	N.S.	7.52	1.96	6.56	8.49	1.33	N.S.	1.92	N.S.	2.89	2.48

number of roots per rooted cutting and the length of roots (cm) per rooted cutting, but the differences were not significant for all parameters in the first experiment and for the percentage of rooting in the third experiment. The best rooting (14%) was achieved with cuttings collected in June 8th and treated with IBA at 2000 ppm. Generally, *Rosa rugosa* stem cuttings showed a very poor rooting, in spite of treatments with IBA.

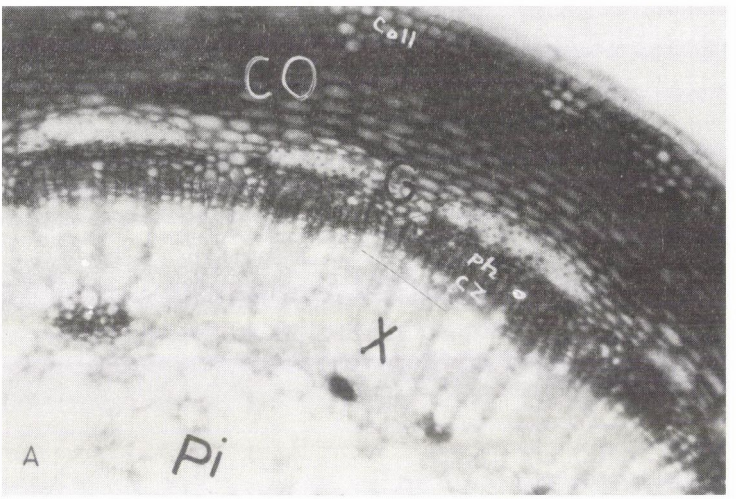

Fig. 1A. Cross section of stem cuttings of *Rosa canina* at the start of the experiment showing weak sclerenchymatic bands and large gaps in the sclerenchymatic ring (obj. 10x0c.4x). coll-chollenchyma, c – cortex, scb – sclerenchymatic bands, g–gaps in the sclerenchymatic bands, ph – phloem, cz – lambial zone, x–xylem, pi–pith

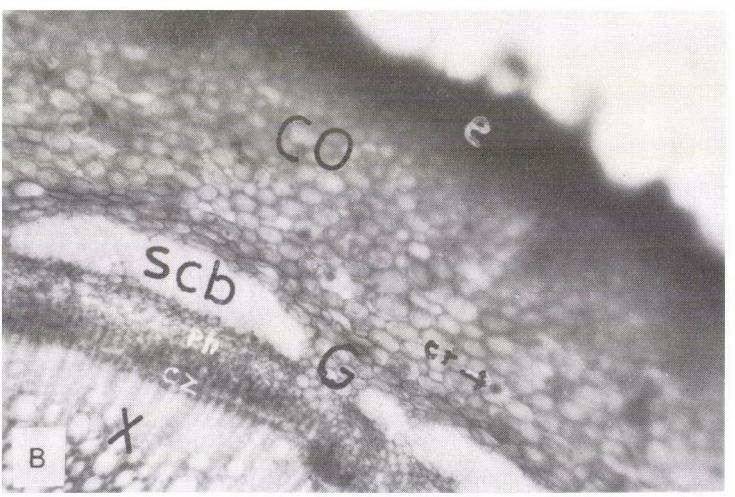

Fig. 1B. Cross section of stem cuttings of *Rosa rugosa* at the start of the experiment showing narrow gaps in the sclerenchymatic ring and thick phloem fiber (obj. 10xoc.4x). e – epidermis, cr – calcium oxalate crystal

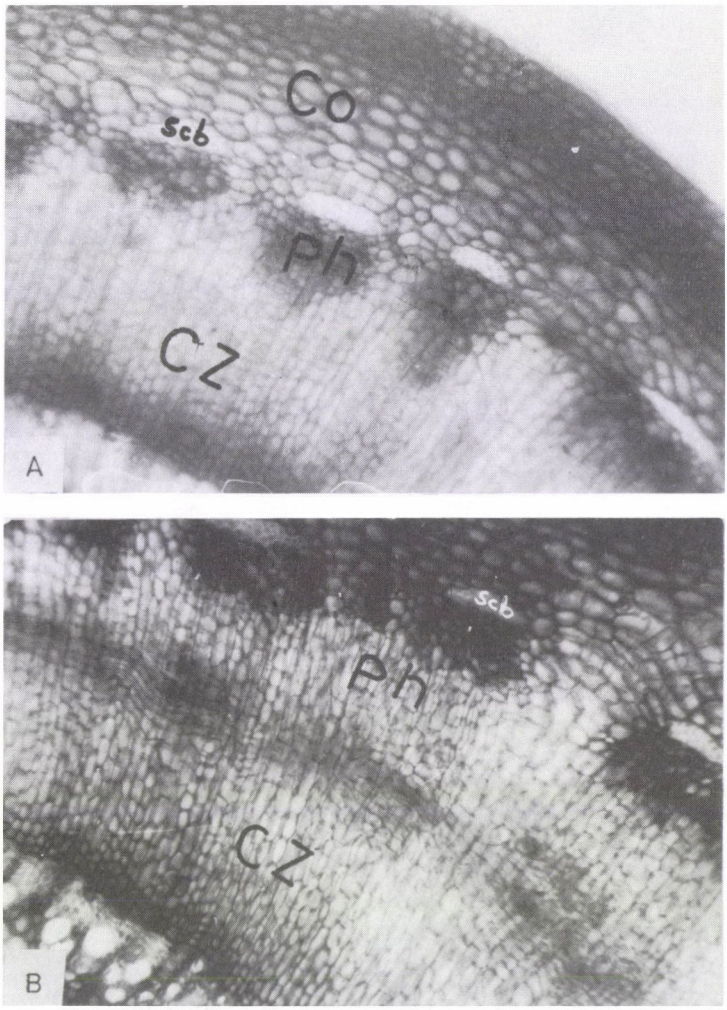

Fig. 2A–B. Cross sections through the base of *Rosa canina* stem cuttings 15 days after planting. (A) Control without treatment. (B) cutting treated with IBA (1000 ppm). Note the great proliferation int he cambial zone, phloem tissue and sclerenchymatic bands. (Obj. 10xoc.4x)

The stimulating effect of IBA on rooting was previously reported by many investigators on different rose species (Novikov, 1977; Ivanicka et al., 1977; Das et al., 1980 and Dubois and Vries, 1986).

The stimulation of the adventitious root formation noticed in our work may be attributed to the auxin, which causes cell elongation and swelling of tissues, cell division (callus formation) and formation of adventitious roots (Pierick, 1986). According to Thomas (1982), the effect of IBA is due largely to its accumulation at the base of cuttings, to a suitable level for initiation and development of roots.

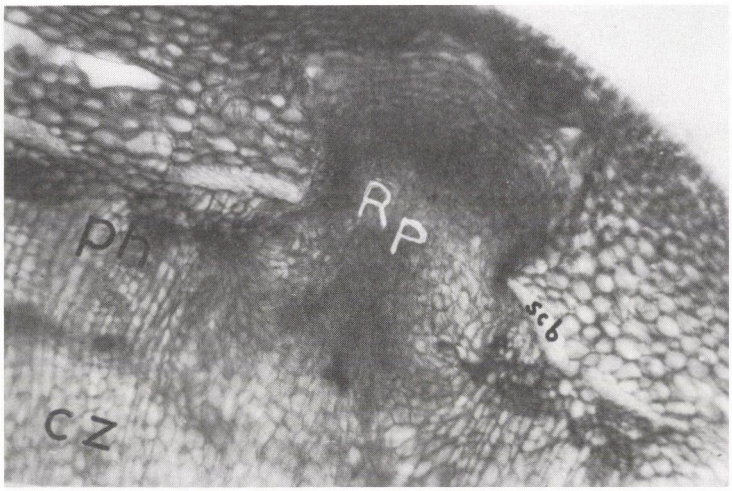

Fig. 3. Cross section through the base of cutting of *Rosa canina* as affected by IBA (1000 ppm) 15 days after planting. Note the root primordia initiated from cambial zone. It is composed of several meristematic cells with dense cytoplasme and is passing between the sclerenchymatic bands. (Obj. 10xoc.4x)

The best root formation on June 8th may be due to an increase in leaf area of mother plants (Vries and Dubois, 1988), which led to an increase of photosynthesis and carbohydrate content in the shoots, and increase the root promoting substances to suitable levels for rooting (Weiser, 1963). In addition, the reduction of rooting ability with age may be due to an increase of the fibers' development, and a decrease in the cambial activity (Davies, 1984). The ability for adventitious root formation generally declines with increasing age of parent stocks.

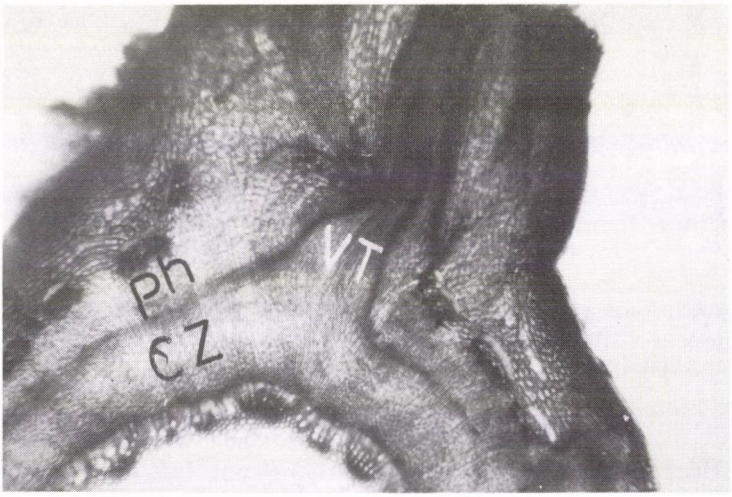

Fig. 4. Cross section of stem cutting of *Rose canina* 30 days after planting. Note the adventitious root emerging from a cutting and forming vascular connections between the main stem and the emerging root. (Obj. 3.2xoc.4x)

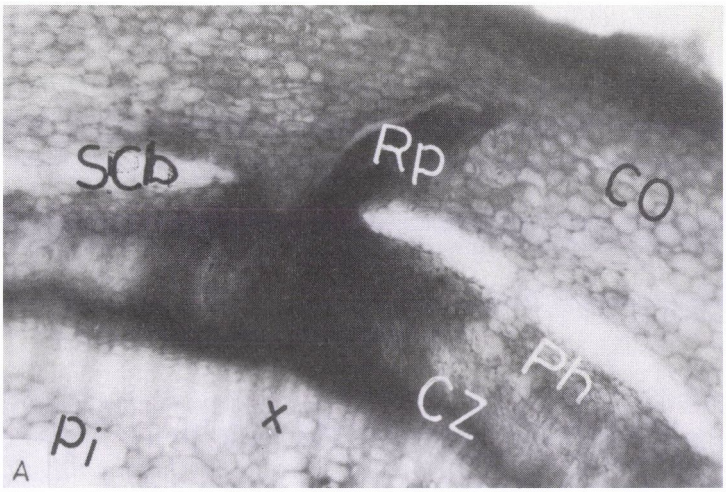

Fig. 5A. Cross section of stem cutting of *Rosa rugosa.* 30 days after planting, showing, the root priomorida initiated from cambial zone. It avoids the intense sclerechymatic bands. (Obj. 10xoc.4x)

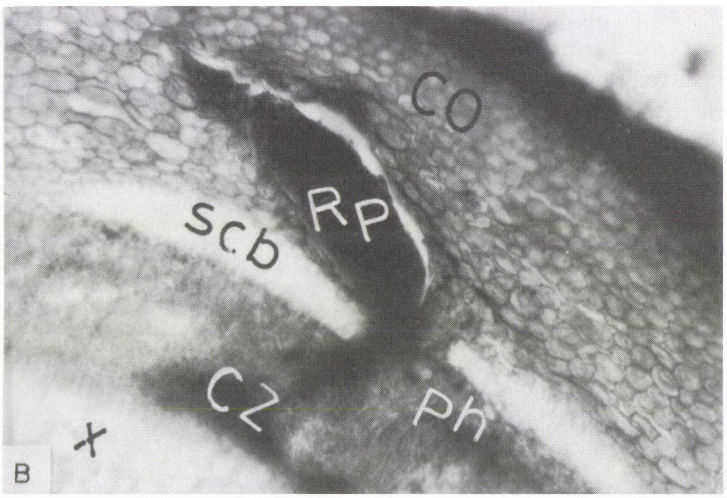

Fig. 5B. Cross section 30 days after planting showing an inclination of the root primordia to go downwards (obj. 10xoc.4x). co–cortex, Rp–root primordia, scb–sclerenchyma bands, ph–phloem, cz–cambial zone, x–xylem

B) *Anatomical changes in the stems during the rooting process*

Figures 1A and B show cross sections of *Rosa canina* and *Rosa rugosa* stem cuttings taken at the beginning of the experiment from one-year-old shoots. The stem of *Rosa canina* is characterized by a weaker cortex tissue, fewer primary pholem fibers, thicker phloem tissue, wider cambial zone, interfascicular cambium regions and large gaps in the sclerenchymatic ring, as compared with the stem of *Rosa rugosa.* A few scattered calcium oxalate crystals were found only in the cortex of *Rosa rugosa.*

Section taken from the basal part of *Rosa canina* cuttings, two weeks after planting, showed significant anatomical differences compared to those taken at the beginning of the experiment. Cuttings treated with IBA showed large changes, with a great proliferation and extension of the phloem parenchyma and cambial zone, especially in the regions of interfascicular cambium (Fig. 2). Along these modifications, some young root primordia were also initiated.The latter was composed of several meristematic cells with dense cytoplasmae and prominent nucleus (Fig. 3). The sclerenchymatic bands, which limit the vascular regions became narrowed and someof them were pushed into stem cortex tissue, and broken by the proliferating phloemparenchyma and cambial zone (Fig. 4). Almost the same anatomical changes were observed in *Rosa rugosa* four weeks after planting (Figs 5A and B)

It has been proposed that growth and passage of the root primordia include the enzymatic breakdown of tissues as well as a physical action on adjacent tissues (Bonnet, 1969; Blazich and Heuser, 1979 and White and Lovell, 1984).

In our experiment, some primordia showed an inclination to go downwards, which was probably due to the strong cortex cells, containing calcium oxalate crystals. However, some root primordia avoid the intense sclerenchymatic bands during their growth, and break through the cortex. These evidences are shown in Figs 5A and B.

At the end of the experiment with *Rosa rugosa,* longitudinal sections taken from the basal end of cutting showed that the adventitious roots were originated from the callus tissue on the basal cut surface, and some of them were originated at the cut surface from the cambial zone. Similar observations were made by Mittempergher (1964); White and Lovel (1984); and Hicks (1987).

The adventitious roots of both of them were developed not only at the internodes but also at the nodes. The cambial zone was the main tissue which developed root

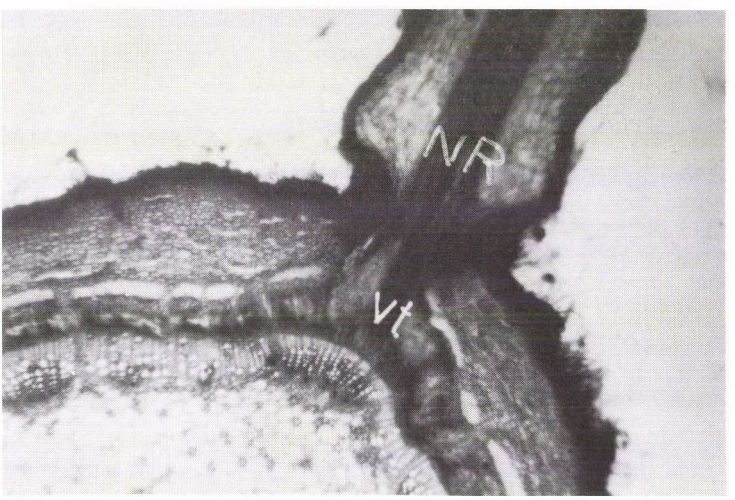

Fig. 6. Cross section of stem cutting of *Rosa rugosa* 30 days after planting. Note the adventious root emerging from a cutting and forming the vascular connection between the main stem and the emerging root. (Obj. 3.2xoc.4x)

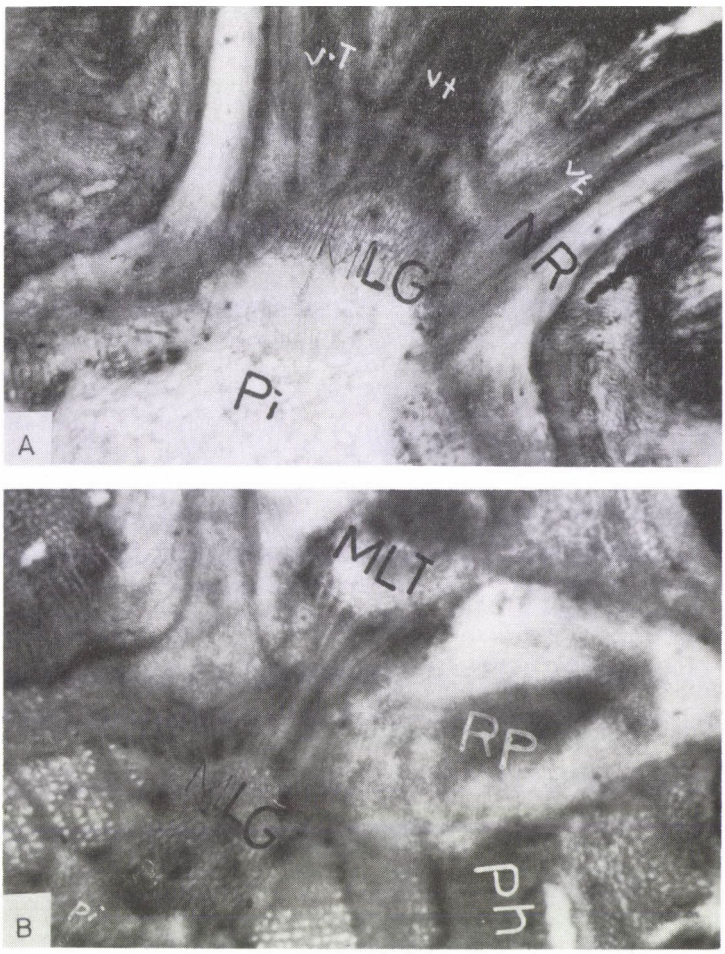

Fig. 7. Cross section at nodes of stem cuttings of (A) *Rosa canina* (B) *Rosa rugosa* at the end of experiment. Note the adventitious roots initiated from median and lateral leaf gaps. (Obj. 3.2xoc.4x)

primordia in both *Rosa canina* and *Rosa rugosa* (Figs 4 and 6). The nodal roots were associated with the parenchymatic bud and leaf gaps (Figs 7A and B). In this context, Doud and Carlson (1977); and Jakson (1986) reported similar results.

According to Bruck and Paolillo (1984), the nodes often have sites where xylem differentiation is initiated, and also may have regions of locally high auxin concentration. White and Lovell (1984) reported that the most important regions of cambial activity are always associated with traces from young leaves, which are probably exporters of auxins.

Vascular transition tissues were observed in the growing primordia which ensured the translocation of nutrients and hormonal factors to the growing roots (Figs 4 and 6). Similar results have been observed by other authors in several species (Hicks, 1987 on apple; Shawky et al., 1988 on pear; and Rodriguez et al., 1988 on hazel).

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CHANGES IN THE NUTRIENT CONTENTS OF ASPARAGUS (*ASPARAGUS OFFICINALIS* L.) AND SOIL UNDER THE INFLUENCE OF VARIOUS MATERIALS

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The ecological conditions of Hungary are suitable for asparagus production. The humus content of the soils is about 1%, so a large amount of organic manure and fertilizer must be supplied before planting.

We studied the effect of alginit, rhyolite-tufa, secondary and tertiary biomass products and their combinations in non-irrigated sandy soil. The cultivars used were: UC-157 F_1, Brook-Imperial, Wiking KB3. In the period of the experiment, 1989–1993, the air temperature was higher, and the amount of precipitation less, than the many years' average. In the first year the stalk of asparagus contained the largest quantity of N (3.68%) in the plots treated with alginit and its combinations. The P content of the plants reached in the first year 0.23% on the average of the treatments. The rhyolite-tufa, peat and sewage-sludge increased the P uptake, compared to the control. The effect of alginit gradually decreased, compared to the other treatments. In the first years, the K supply of asparagus was sufficiently ensured by the combination of alginit and farmyard manure. In the fifth year of the experiment, the largest quantity of K was contained by plants in the plots treated with peat + farmyard manure, peat, sewage-sludge and rhyolite-tufa. On the average of five years, the Ca content of the plants was favourable in the plots treated with rhyolite-tufa, alginit and sewage-sludge. The Mg content was significantly more favourable, compared to the control, in each year of the experiment. The Fe, Mn, Cu and Zn contents of the stalk of asparagus were highest in the plots treated with sewage-sludge and its combinations. The heavy metal (Al, Cr, Ni, Pb, Hg, Cd) content of the shoots of asparagus varied with the treatments. The Pb content grew in the second year of the experiment. The As content was highest in the plots treated with sewage sludge. To satisfy the nutrient requirement of asparagus prior to planting, alginit, rhyolite-tufa, sewage-sludge can be used. The combination of these natural and organic origin materials has a favourable effect; therefore, they are suitable to replace farmyard manure in sandy soils. The heavy metal content of sewage-sludge, alginit and rhyolite-tufa accumulated in the asparagus below the permissible level.

Key words: *Asparagus officinalis,* alginit, zeolit, organic manure, peat, rhyolite-tufa, sewage-sludge, N, P, K, Ca, Mg, Cu, Mn, Zn, Cd, Hg, As, soil type, dry matter %, nutrient content of soil, meteorological factors

Introduction

The asparagus, due to the mineral substances accumulated in it, to its good dietary effect and earliness, and to the economic efficiency of its cultivation, belongs to the valuable vegetables. As it is well exportable, the ecological and economic conditions of Hungary make it reasonable to study the possibilities of its widest cultivation.

If the new up-to-date varieties are to produce large masses of shoots under the conditions of Hungary, the soils of poor productivity must be well supplied with organic matter and nutrient. Owing to the high price and quick decomposition of organic manure, we tried to find such materials of natural origin that, when worked into soils

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of low productivity, would improve the physical and chemical parameters of soil. Further, it is reasonable to use more extensively soil amelioratives of natural origin and secondary and tertiary biomass products rich in nutrient.

The asparagus is a nutrient intensive vegetable; it is therefore particularly important to know the extent of changes in the macro-, microelement and heavy metal contents of the stalk of asparagus in response to the materials used under non-irrigated conditions.

Materials and methods

The experiment was set up with the cultivars UC 157 F_1, Brook Imperial and Wiking KB3. Prior to planting the seedlings, the materials included in the experiment were worked into the soil by ploughing to a depth of 90 cm (September 1988). The soil was sterilized with Thimet 10G. In addition to the experimental materials 210 kg N, 435 kg P_2O_5 and 480 kg K_2O were supplied per ha. Planting was done between 24 and 28 March 1989 at a spacing of 150×30 cm. The area of a plot was 100 m^2, and the experiment was laid out in random block design with four replications. For the experiment alginit, rhyolite-tufa, peat, farmyard manure, communal sewage-sludge and their combinations were used.

The asparagus was planted on an area with no possibility of irrigation. We therefore studied the trend of temperature (°C) and precipitation (mm). In the first year of the experiment (1989), there was a +0.7 °C temperature surplus and 21 mm precipitation deficit in the period between January and October, compared to the many years' average. In the second year, the temperature excess was +0.9 °C and the precipitation deficit 102 mm. The third year was cooler and rainier than the many years' average. In the fourth year, the annual mean temperature was +1.5 °C higher, the precipitation 134 mm less; the last year was also characterized by warmer and drier weather.

Table 1

Chemical parameters of the soil of the experimental area before treatment and of the materials applied

Denomination	0–90 cm average	Farmyard manure	Peat	Alginit	Rhyolite-tufa	Sewage-sludge
pH (H_2O)	7.53	7.95^X	7.40^X	7.89^X	7.32^X	7.25
K_A (stickness)	33	63	84	90	72	–
Total salt %	0	–	–	–	–	–
$CaCO_3$ %	3.27	–	–	–	–	–
Humus	1.49	4.82	6.36	0.97	0.97	13.5
Soluble nutrients ppm						
$NO_3 + NO_2$	9.73	420	349	63	63	13669
P_2O_5	229.0	171	386	273	273	4057
K_2O	163.0	526	80	336	336	2330
Mg	146.0	10	35	14	14	9395
Na	25.0	82	84	32	32	100
Zn	1.0	–	31	53	53	622
Cu	1.4	–	–	–	–	110
Mn	39.5	0.8	6.0	3.9	3.9	356
SO_4	8.7	–	–	–	–	

x = KCl

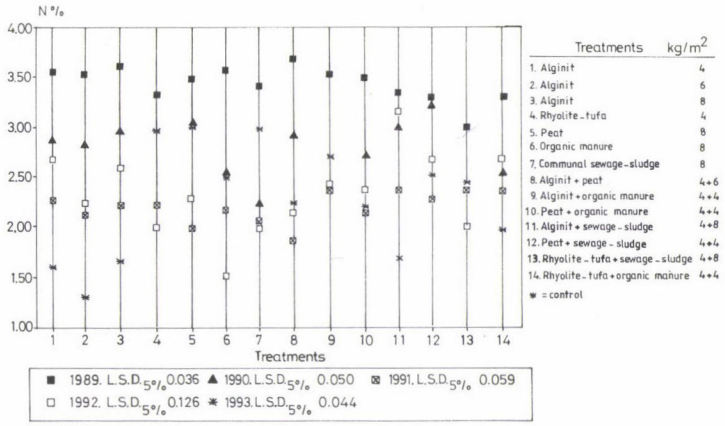

Fig. 1a. Components of the stalk of asparagus on % dry matter basis

The soil of the experiment area was a mildly alkali sandy loam of medium humus and low lime content, well supplied with P_2O_5, sufficiently with K_2O, abundantly with Mg; its Na content was of no harmful measure, the Zn content low, the Cu content satisfactory; Mn was contained in it in large SO_4 in medium quantities. The amount of Al-soluble nutrients in the materials applied was considerably larger than in the soil, except the P_2O_5 and Mn contents of the farmyard manure (Table 1). The P_2O_5, K_2O, Mg contents of the alginit were considerable, the Zn content of the rhyolite-tufa was high, which in the case of the soil of the experimental area was particularly advantageous. The sewage sludge contained a multiple of all nutrients that occurred in the other materials of natural origin. The nutrient supplying capacity of the soil was favourably influenced by the humus content of the materials tested; except the rhyolite-tufa.

Results

Nitrogen The volume and combination of materials worked in with the turning up of soil considerably influenced the nutrient content of the asparagus. In the first year, the stalk of asparagus contained the largest quantity of N (3.68%) in the case of the combination of alginit and peat used (Fig. 1a, treatment 8). The alginit treatments (1, 2, 3) also showed significant differences; further, alginit in every combination had a favourable effect on the N accumulation of asparagus. The joint application of rhyolite-tufa and sewage-sludge proved less favourable than the other treatments. In the second year of the experiment, the N content in the stalk of asparagus decreased except in one treatment, but a significant difference between the treatments was maintained. In the case of the treatments containing organic matter, the positive effect was unequivocal. Sewage-sludge and its combinations, as well as the rhyolite-tufa (treatment 4), had a favourable effect on the N content of the vegetation, compared to the control (treatment 10). In the third year, the N content in the stalk of asparagus further decreased on dry matter basis. Strikingly low values were obtained in plots treated with peat and with peat + alginit, respectively. The N content of plants on dry matter basis ranged between 1.95 and 2.35%. The measuring results of the next year, in the case of the alginit treatments (1, 2, 3), showed a more favourable trend than in the previous year. The best result was obtained with alginit and sewage-sludge (2.68%), at the same time the effect of rhyolite-tufa + sewage-sludge was lower by 0.16%, and that of farmyard manure

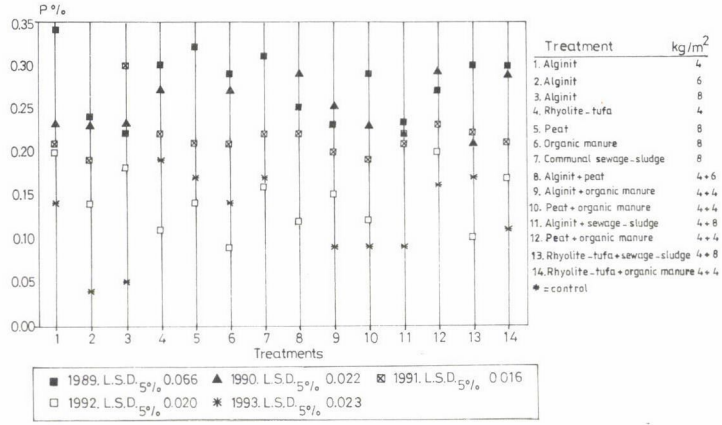

Fig. 1b. Components of the stalk of asparagus on % dry matter basis

by 0.64% compared to the control (treatment 10). According to the analysis results of the fifth year, the N content of the stalks of aspragus considerably decreased, compared to the first year. Particularly low values were obtained in the plots treated with alginit. The treatments combined with organic matter and sewage-sludge gave more N compared to the control. On the average of the five years, the N content of the stalk of asparagus on dry matter basis attained 2.44% (98% of the control) in the plot treated with organic manure (treatment 6), and 2.39% (96% of the control) in the alginit treated plot (treatment 2). The highest N percentage, which was 112.9% of the control, occurred in the plants of the plots treated with peat + sewage-sludge (treatment 12).

Phosphorus. The P content of the stalk of asparagus (Fig. 1b) attained 0.23% in the first year on the average of the treatments, which agreed with the result of the control. The plants in the plots treated with rhyolite-tufa, peat + sewage-sludge and farmyard manure contained more P than the control plants. In the second years, the P content was 0.28% on the average of the treatments; 0.01% less, compared to the control. There was no essential difference in effect between the 4 and the 8 kg/m² alginit, the effect of rhyolite-tufa, farmyard manure and peat was favourable. In the third year the P content of the plants decreased by 0.07%, compared to the second year, on the average of the treatments. The highest value, 0.23%, was obtained with peat + sewage-sludge (treatment 12). In the fourth year, the P content of the asparagus further decreased; the average of the treatments was 0.15%, 0.03% more than in the control. The alginit, the sewage-sludge and its combinations gave significantly better results, compared to the control. In the last year, the P content decreased by 0.15%, compared to the second year on the average of the treatments; the rhyolite-tufa showed a wide scatter; the most favourable results was attained by the joint application of rhyolite-tufa and sewage-sludge. The plants in the plot treated with 6 kg/m² alginit contained only 0.04% P, but in combined form alginit had a more favourable effect. On a five-year average, the best result was obtained with peat + sewage-sludge (treatment 12), 27.8% more P than in the control. The 6 kg/m² alginit (treatment 2) gave 5.6% less P, compared to the control.

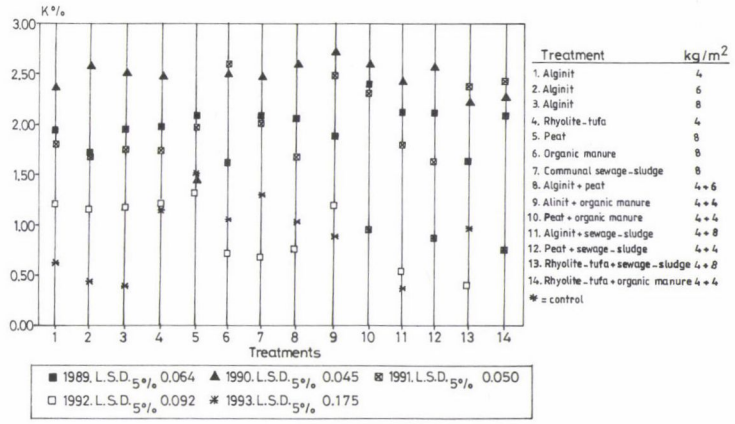

Fig. 1c. Components of the stalk of asparagus on % dry matter basis

Potassium. The potassium content on dry matter basis (Fig. 1c) attained 2.40% in the first year on the average of the treatments. The highest value was obtained with the combination of alginit and farmyard manure (treatment 9). In the other treatments this value was lower, compared to the control (treatment 10). In the second year the average of the treatments was 1.97%, compared to 2.38% in the control; in the case of all the other treatments, the K content of the plants was significantly reduced. In the subsequent years of the experiment, a similar tendency was observed. In the course of the years, the K supply of asparagus, however low rate, was best ensured by the combination of peat and farmyard manure. The most unfavourable values were obtained in the plots treated with alginit.

Calcium. In the first year, the calcium content (Fig. 1d) was 1.03% on the average of the treatments, 0.93% of the control, the largest quantity of calcium accumulated in the plants in the case of applying rhyolite-tufa nad sewage-sludge. The highest Ca

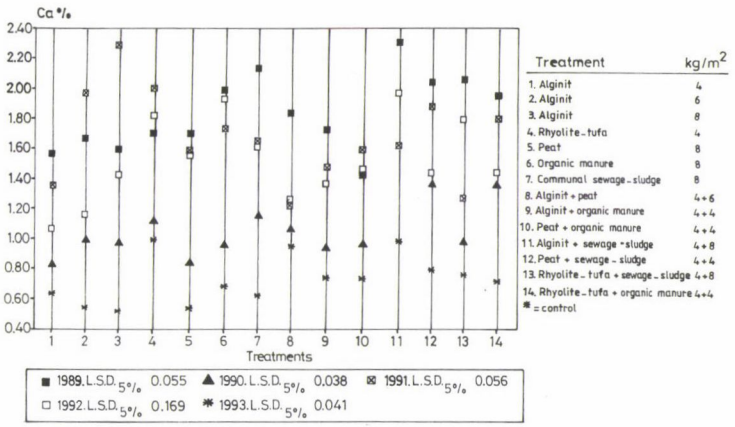

Fig. 1d. Components of the stalk of asparagus on % dry matter basis

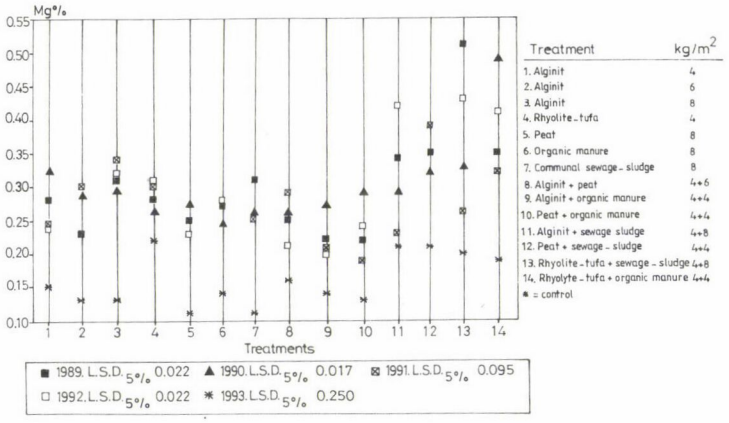

Fig. 1e. Components of the stalk of asparagus on % dry matter basis

content (1.83%) was measured in the second year, which fell to 0.72% by the fifth year on the average of the treatments. On a five-year average, the Ca content was in each case higher than in the control, with the exception of treatment 1.

Magnesium. As for magnesium supply (Fig. 1e), in the first year the rhyolite-tufa + farmyard manure treatment was the most favourable. The average of the treatments was 0.29%, which in the last year of the experiment fell to 0.16%. With the exception of the last year, the magnesium content of the plants was in each treatment significantly higher than in the control.

Iron. In the first year of the experiment, the iron content of the stalk of asparagus (Fig. 1f) was highest in the plants of plots treated with sewage-sludge. The effect of alginit and rhyolite-tufa was clearly demonstrable. According to the results of analyses, the iron content of the stalk of asparagus did not widely vary under the influence of the different treatments.

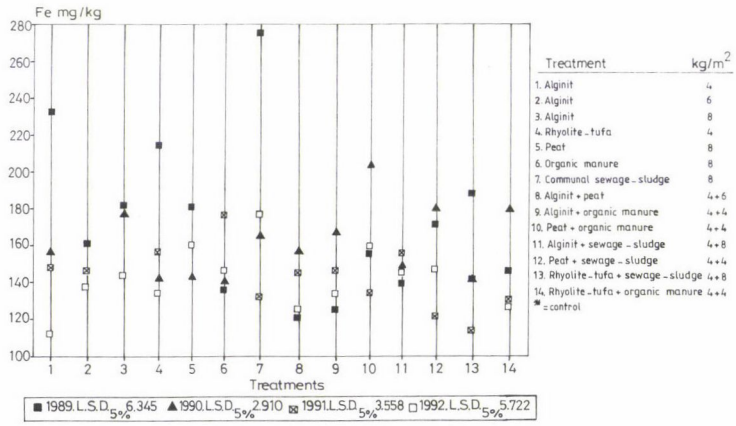

Fig. 1f. Components of the stalk of asparagus on % dry matter basis

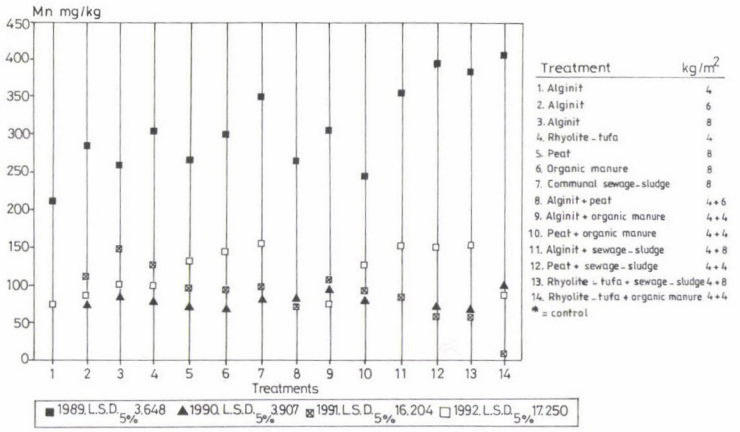

Fig. 1g. Components of the stalk of asparagus on % dry matter basis

Manganese. The manganese content was outstandingly high in the second year, compared to the other years, in particular in the plots treated with sewage-sludge and its combinations (Fig. 1g).

Copper. In a similar way, the copper content of the plants was highest in the second year. In the other years of the experiment, every treatment had a better effect than the control (Fig. 1h).

Zinc. The zinc content of the stalk of asparagus (Fig. 1i) greatly decreased in each treatment, compared to the first year. In the second year, essentially higher values were obtained, particularly in the case of rhyolite-tufa and farmyard manure application (treatment 14), as well as when sewage-sludge and its combinations were used. On a five-year average, each treatment gave a more favourable result than the control.

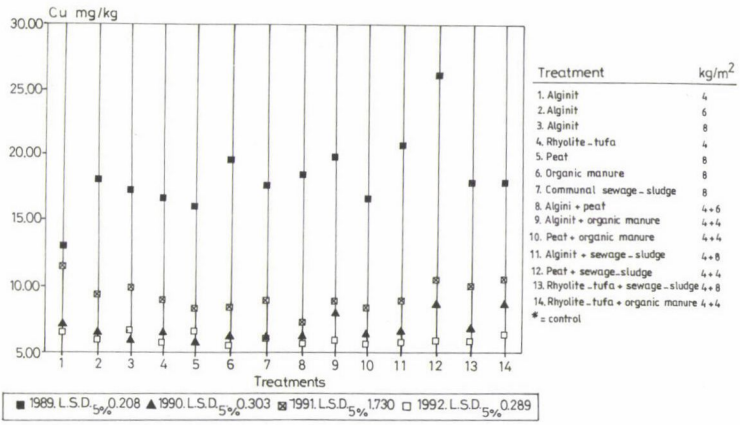

Fig. 1h. Components of the stalk of asparagus on % dry matter basis

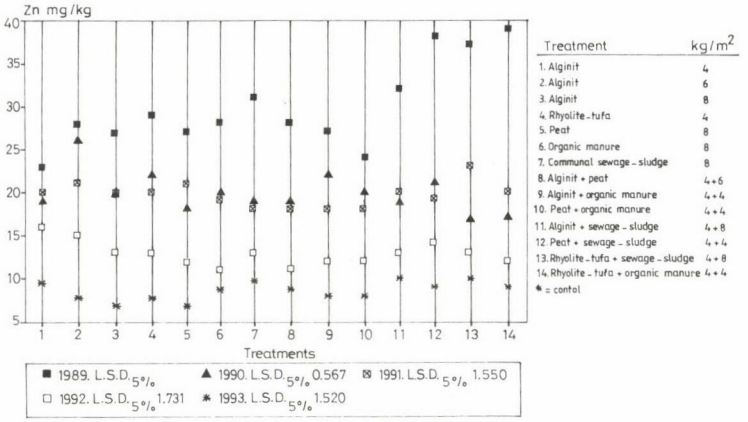

Fig. 1i. Components of the stalk of asparagus on % dry matter basis

Aluminium. The A_2 content, measured in the shoot of asparagus with an I. C. P. instrument, was lowest in the plot treated with sewage-sludge. In response to the joint application of peat and sewage-sludge, the shoots contained 51 mg/kg more A_2.

The value of Cr was below the limit of detection in each treatment.

Nickel. The Ni content was lowest in the control.

Chrome. The rhyolite-tufa increased the Cr content of the shoots.

Lead. The amount of Pb was also increased by the rhyolite-tufa treatment. In the plots treated with sewage-sludge, the shoots contained 1.49 mg/kg less Pb than in those treated with rhyolite-tufa + sewage-sludge (Table 2).

Cadmium. The Cd content of the stalks of asparagus increased in the second year of the experiment, but the effect of sewage-sludge was not more remarkable than in the case of other treatments. The amount of Pb was larger in the second year of the experiment, but was not more than with the other treatments.

Table 2

Heavy metal of asparagus shoot May 1991

Treatment	kg/m²	Al	Cr	Nv mg/kg	Pb	V
7. Sewage-sludge⁺	8	107	1.d.	3.04	3.43	1.11
10. Peat + Farmyard manure⁺⁺	4+4	122	1.d.	2.38	k.h.	0.41
11. Alginit + sewage-sludge⁺	4+8	132	1.d.	3.12	k.h.	0.59
12. Peat + sewage-sludge⁺	4+4	156	1.d.	2.41	k.h.	k.h.
13. Rhyolite-tufa + sewage-sludge	4+8	119	1.d.	3.10	4.92	0.76

1.d. = limit of detection
\+ = communal sewage-sludge
++ = control

Table 3

Heavy metal content of the stalk of asparagus ppm

Element	Communal sewage-sludge dose								In other treatments, ppm	
	8		8		4		8			
	–		+ Alginit 4		+ Peat 4		+ Rhyolite-tufa 4			
	kg/m^2									
	1992	1993	1992	1993	1992	1993	1992	1993	1992	1993
		7^X	11^X		12^X		13^X			
Cd	0.137	0.556	0.050	0.279	0.210	0.294	0.110	0.355	0.08–0.25	0.20–0.65
Pb	0.810	0.943	0.740	0.930	0.740	0.528	0.500	0.518	0.63–0.82	0.45–0.95
Hg	0.10	0.047	0.100	0.044	0.10	0.038	0.100	0.045	0.10	0.02–0.06
Ar	...	0.096	...	0.039	...	0.054	...	0.031	...	0.03–0.10

ppm = mg/kg raw material
= less than...
x = treatment

Mercury. The Hg content showed higher values, compared to the other treatments.

Arsenic. The plants of plots treated with sewage-sludge contained more As, compared to the combined treatment, but less of it could be detected than in the case of other materials applied (Table 3).

Changes in the nutrient content of the soil

Humus. In the 0–90 cm layer of the soil, the humus content decreased by 0.34% in the fifth year (Table 4), compared to the original status. The reducing effect of alginit and its combinations is clearly demonstrable, despite the fact that, at the time of working in, the highest percentage of humus was contained in the plot.

Phosphorus. The use of P_2O_5 was highly noteworthy, because the large amount of P_2O_5 introduced with the materials applied did not even counterbalance the amount taken up by the plant from the soil, as shown by the analysis results of the plots treated with peat, farmyard manure, alginit + sewage-sludge, peat + sewage-sludge, rhyolite-tufa + organic fertilizer. Under the influence of rhyolite-tufa + sewage-sludge, the P_2O_5 content of the soil gave the highest values compared to the initial quantity.

Potassium. The nutrient amount of K_2O introduced in the soil was used up in nearly all treatments; moreover, in some cases potassium deficiency could even be noted.

Table 4

Effect of various materials on the nutrient content of the soil of asparagus. Average of 1989–93 in the 0–90 cm soil layer

Treatment	Denomination	Nutrients						
		kg/m^2	Humus %	NO_3	P_2O_5	K_2O	Mg	Zn
1.	Alginit	4	1.33	17.3	236	165	99	0.93
2.	Alginit	6	1.45	14.4	231	162	133	1.12
3.	Alginit	8	1.44	14.3	187	149	137	0.97
4.	Rhyolite-tufa	4	1.44	13.6	246	138	114	0.81
5.	Peat	8	1.48	19.5	219	146	123	0.97
6.	Farmyard manure	8	1.33	13.7	188	163	122	0.97
7.	Communal sewage-sludge	8	1.27	20.7	196	181	124	2.95
8.	Alginit + peat	4+6	1.25	14.5	237	143	104	0.77
9.	Alginit + farmyard manure	4+4	1.19	10.5	215	193	104	0.79
10.^{x}	Peat + farmyard manure	4+4	1.23	22.3	200	213	115	1.01
11.	Alginit + sewage-sludge	4+8	1.15	22.8	178	132	93	1.58
12.	Peat + sewage-sludge	4+4	1.25	16.8	146	120	104	1.08
13.	Rhyolite-tufa + sewage-sludge	4+8	1.49	13.6	266	188	143	1.48
14.	Rhyolite-tufa + organic manure	4+4	1.49	18.4	166	199	139	0.76

x = control

Magnesium. The magnesium status of the experimental area worsened in the case of all treatments, particularly with treatments 1 and 11.

Zinc. With the exception of the sewage-sludge-, rhyolite-tufa + sewage-sludge treatment, the Zn content of the soil did not reach or only came close to the level preceding the experiment.

Discussion

The N content of the stalk of asparagus, on dry matter basis, was in the first year 3.68% in the case of alginit + peat, and the lowest was 3.00%, which reached the level considered optimum by Kaufmann (1967) and Bergmann (1983). In the second year, all treatments were still satisfactory, in agreement with the results published by Fehér (1974). In the subsequent years, the soil was less and less capable of supplying the asparagus with sufficient N.

The trend of the P content in the first years of the experiment confirmed the results obtained by Bergmann (1983) and Fehér (1967), though it did not reach the value published by Kaufmann (1967). So, it can be established that the P content of the plants was low or deficient in the case of each treatment.

The K content of the stalk of asparagus was highest (an average of 2.4%) in the first year in the case of each treatment, which according to Bergmann (1983) is satisfactory, though it does not reach the values given by Kaufmann (1967) and Fehér (1980, 1983). The K content of plants in the plots treated with 6 kg/m^2 alginit and laginit + organic manure, respectively, confirmed the results reported by Almási (1991). The results obtained in the plots treated with alginit, rhyolite-tufa, sewage-sludge and its combinations, respectively, agree with those published by Varju (1966), Mátyás (1974) and Pais (1988).

Each of the various treatment combinations verified, in the fifth year of the experiment, the Ca values considered optimum by Bergmann (1983). In the first years, on the other hand, we obtained essentially higher values which suggests, that the materials applied favourably influenced the Ca uptake of asparagus.

The Mg content of asparagus gradually decreased in the course of the years. The quantity of Mg absorbed verified the statements of Fehér (1983), Bergmann (1983), Almási (1991) and Liska (1991). In the case of peat, sewage-sludge, peat + organic manure applied, the Mg content of the asparagus was sharply reduced, which suggests that these materials do not fully satisfy the demand of the plant.

The materials of natural origin had a positive effect on the iron content of the asparagus.The level considered optimum (Bergmann, 1983) could be noted in each treatment, and verified the data published by Liska (1991).

In the second year of the experiment, the Mn content of the asparagus was 2–3-times higher than either in the previous or in the subsequent years. In the case of a high rate alginit and rhyolite-tufa application, the amount of Mn measured was 30–40 mg/1000 g more than that reported by Fehér (1974).

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PRELIMINARY RESULTS ON THE EFFECT OF POLARIZED LIGHT ON THE CUTS ROOTING IN OLIVES

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The present study was performed to investigate the effect of polarized light on the rooting of different types of olive cuttings (Varianty Khodairi). Three types of cuttings (<4 mm, 4–6 mm, and < 6 mm in diameter), and five exposure times of polarized light ("0, "30, "90, "180, and "360) were used in this study. Rooting percentage, number of roots per cutting, and the mean length of the root were measured after 100 days of planting. The results showed an increase in rooting percentage, number, and length of the root. Exposure time of "180 was the best for top and middle types of cuttings, while "360 gave the best result with bottom type of cuttings followed by "180.

Key words: cuttings, exposure times, olive, polarized light, rooting, stimulation

Introduction

The olive tree is considered among the important crops in Syria, with a growing area estimated at 405 000 ha, comprising a total number of 48 million trees with and annual production of 343 000 tonnes of olive (1991). It is estimated that the annual increase in the number of olive trees is as much as one million.

Several studies have indicated that rooting rates of an olive cutting do not exceed 35% despite using the best rooting methods, such as appropriate collection period of the cutting, and the application of auxin (e.g. IBA), or other chemical (e.g. vitamins, sugar, etc.) (Dettori, 1981; Pannelli and Filippucci, 1982; Wareing and Phillipsi, 1982 Wali et al., 1985).

Exposing the cutting to low doses of X-rays (Bors and Zimmer, 1970), and low doses of gamma irradiation (Avrmov and Mihajlovic, 1965) stimulates callus formation and differentiation of roots.

Simon (1986); Szabó and Tejeda (1986); Szabó et al. (1989; 1992) showed that polarized light could stimulate the vegetative and rooting growth, and improve the germination of some crops.

The available data from a computer search indicate that there has been no published report regarding the use of gamma and X-rays or polarized light on rooting olive cutting.

Materials and methods

Olive cuttings of Khodairi variety were obtained from the Tartous area. The length of the cutting was 10–15 cm, which was divided into 3 types: top with diameter not exceeding 4 mm, middle 4–6 mm, buttom > 6mm.

Cuttings were treated with five exposure times of polarized light ("0, "30, "90, "180, and "360). Treatments were conducted using the Agrolux (Stimokomplux) technique, that generated the polarized light (Szabó et

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al., 1992). A hundred treated cuttings were divided into 4 replicates (25 each). Cuttings were irradiated on 29th and planted on 30th of June 1990 in a greenhouse. Planting was performed in trays containing sand, and greenhouse temperature ranged from 20 to 25 °C. Irrigation was performed by sprinkling. Rooting percentage, number of roots on cutting, and the mean length of the root were examined 100 days after planting.

Results and discussion

Rooting percentage

Table 1 illustrates the effect of polarized light on rooting percentage. There was an increase, albeit not significant, in rooting percentage of the top cuttings for all exposure times except "30 and "360 compared with the control.

Polarized light also significantly increased ($P < 0.05$) the rooting percentages of middle cuttings. Bottom cuttings responded similarly to the intreatment exposure times, except for "90. The rate of response was significant ($P < 0.05$).

Table 1

Rooting percentage in olive cuttings treated with polarized light

Exposure time	Rooting %		
	Top	Middle	Bottom
"0	3	7	8
"30	3	11	9
"90	4	18	2
"180	6	19	11
"360	2	8	18
$L.S.D._{5\%}$	4.6	9.2	10

Polarized light may have increased the concentration of materials, which may have some stimulating effect on root formation, particulary auxins. On the other hand, it may have reduced the percentage of inhibitory materials.

Table 2

Number of roots in olive cuttings treated with polarized light

Exposure time	Roots/cutting		
	Top	Middle	Bottom
"0	1.3	2.6	4.8
"30	3.5	6.2	5.2
"90	1.8	3.7	3.5
"180	5.1	6.6	6.6
"360	1.5	5.8	6.5
$L.S.D._{5\%}$	3.9	3.2	4.7

Number of roots

Table 2 shows that all exposure times used increased root formation on top and middle cuttings. For bottom cuttings, all exposure times except "90 increased significantly ($P < 0.05$) the number of roots on the cutting.

Polarized light may enhance callus formation, consequently increasing the number of root centers on the cutting, and result in a larger number of roots.

Mean length of the root

Table 3 shows that all exposure times, except "180, decreased, but not significantly, the mean length of the root for top cuttings. For the middle cuttings, exposure times at "30, "90, decreased the mean length of the roots. However, these differences were not significant. Treatments of the buttom cuttings resulted in non-significant increases in the mean length of the roots.

Table 3

Length of root in olive cuttings treated with polarized light

Exposure time	Length/mm		
	Top	Middle	Bottom
"0	15	19.5	10.1
"30	8.3	17.9	16.9
"90	11.2	19.8	20
"180	19.8	21.4	18
"360	10.2	14.6	16.2
$L.S.D._{5\%}$	18.3	11.6	19.7

Polarized light may have increased the number and size of cells in the root, consequently increasing the length of the formed roots.

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EFFECT OF LOW DOSES GAMMA RADIATION AND INDOLBUTYRIC ACID ON ROOT FORMATION OF APPLE CUTTINGS

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To improve the root formation of apple cuttings, Indolbutyric acid (IBA) (4000 ppm), low doses of gamma irradiation (1, 2, 3, 4, and 5 Gy), combined treatment of IBA followed by irradiation, and irradiation followed by IBA, were applied on the cultivar Golden delicious. The results indicated that irradiation treatments increased the callus formation, while IBA decreased it. The combined treatment of irradiation followed by IBA resulted in greater reduction in callus formation, when compared with IBA alone, while the treatment with IBA followed by irradiation reduced the negative effect of IBA when applied by itself.

Key words: apple cutting, callus, dose, IBA, (IBA + irradiation), (irradiation + IBA), treatment, stimulation

Introduction

The apple is considered one of the most important trees in Syria. The total number of apple trees is now 15 000 000, which occupy an area of 50 000 ha with an annual increase of one million trees (1991), and yield about 215 000 tons.

For the rooting of apple stock cutting, experiments indicated that optimum concentrations of the hormone indolbutyric acid (IBA) ranged from 1000–2500 ppm (Table 1).

After the discovery of X-rays by Roentgen, scientists have noticed that they have a stimulatory effect on plants, as first reported by Schober (1896).

Studies of 35 crops that responded to ionizing radiation (Simon and Bhattachariya, 1977). Avrmov and Mihailovic (1965) indicated that X-rays stimulated the rooting of grape cuttings. Similar results were also reported by Bors and Zimmer (1970), and Zimmer et al. (1971) when they treated flower cuttings.

Table 1

Effect of auxins on root formation of apple cuttings

Variety	Auxin	ppm	Treated	Control	Reference
M1	IBA	20	100	0	Wareing 1978
MM106	IBA	4000	No effect		=
MM106	IBA	4000	No effect		=
MM111	IBA	4000	No effect		=
M26	IBA	1250	High effect		=
Crabc	IBA	1250	High effect		=
Amara	IBA	1000	70		Al-Rawi 1989
MM106	IBA	1500	88		Velickovic 1985
MM106	IBA	2500	94		=
M26	IBA	2500	60		Eccher 1984

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In tissue culture, it has been shown that low doses of ionizing radiation increased the callus formation in tobacco (King, 1949), beans (Bajaj, 1970), shamotti orange (Spiegel-Roy and Padova, 1973) and carrot (Al-Safadi and Philipp, 1990).

The aim of this experiment was to assess the effect of gamma irradiation and IBA at a concentration of 4000 ppm, which is used by the Syrian Ministry of Agriculture and Land Reform on callus formation.

A computer search for the last ten years on gamma irradiation has shown no indication of any such use on apple cuttings.

Materials and methods

Apple cuttings of Golden Delicious were obtained from orchards farmed in the coastal Region. The length and diameter of the cuttings were 30–40 cm and 8–12 mm, respectively.

The IBA at a concentration of 4000 ppm, and gamma radiation (theratron 80 equipment 60 Co, dose rate of 0.11 Gy/min) at doses 1, 2, 3, 4 and 5 Gy were used. In addition to the IBA and irradiation treatment combined treatments of (IBA followed by irradiation then irradiation followed by IBA) have been used. The total number of treatments were 18, including control, as follows: control, IBA, (IBA + 1, 2, 3, 4, 5 Gy), (1, 2, 3, 4, 5 Gy + IBA). The experiment was arranged in a complete randomized block design with 8 replicates 25 each. After one day of irradiation (March 5th 1987), both control and treated cuttings were sown in greenhouse with temperatures ranging from 15 to 20°C. Planting was carried out in trays containing perlite, and irrigation was administered by sprinkling. Callus formation was evaluated after 45 days from planting.

Results and discussion

Table 2 shows that all doses of gamma irradiation, except the 1 Gy, increased the callus formation.

Doses of 2, 4 and 5 Gy show significant ($p < 0.05$), effects on callus formation while 1, and 3 Gy and no significant effect, in comparison with the control.

The increase of callus formation is an indicator of a stimulatory effect from these doses, which agrees with the findings of Woodham and Bedford (1970) and Revin and Berezina (1971) that showed an increase of callus formation in cuttings.

The IBA at a concentration 4000 ppm reduced callus formation to 28, as compared to the control (45%), which may be due to the high concentration of the hormone. As other studies indicated, 1000 to 2500 ppm were the optimum concentration (Table 1).

Irradiating samples with gamma-radiation, followed by the IBA treatment, decreased the rate of callus formation when compared with samples treated with IBA alone. However, such differences were not significant. The reduction in callus formation in this combined treatment may be attributed to the additional inhibitory effect of irradiation.

The treatment with IBA, followed by irradiation, increased the rate of callus formation in comparison to the effect of IBA alone. It is possible that irradiation destroyed some of the hormone (IBA), and reduced its inhibitory effect on callus formation.

Table 2

Effect of gamma irradiation and indolbutyric acid 4000 ppm on callus formation of apple cuttings (Golden)

Dose	Callus formation
Control	45
1 GY	39
2 GY	61
3 GY	52
4 GY	67
5 GY	72
IBA	28
IBA + 1 GY	26
IBA + 2 GY	36
IBA + 3 GY	40
IBA + 4 GY	32
IBA + 5 GY	41
1 GY + IBA	21
2 GY + IBA	29
3 GY + IBA	17
4 GY + IBA	20
5 GY + IBA	19
$L.S.D._{5\%}$	16
$L.S.D._{1\%}$	21

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Plant Cultivation

THE EFFECT OF LONG-TERM P APPLICATION ON THE YIELD OF MAIZE (*ZEA MAYS* L.) ON A CALCAREUS CHERNOZEM SOIL

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A long-term fertilizer trial has been set up on calcareous chernozem soil in maize-wheat diculture and in wheat-maize-maize-peas crop rotation to study the effect of fertilization, of P fertilization in particular, on soil, and maize yield as well as on the nutritive element content of the leaf being in the stage of flowering. Lime-ammonium nitrate, superphosphate and 60% potassium salt (KCl) were used as fertilizers.

During the years since 1968, on the average of altogether 35 maize trials, the treatments ($N_3K_1P_1$ and $N_4K_1P_1$) applying yearly 150 to 200 kg/ha N, 100 kg/ha K_2O and 50 kg/ha P_2O_5 proved to produce maximum yields. With the advance of years, dressings exceeding yearly 50 kg P_2O_5 were found to have an ever more unfavourable effect. To summarize the yields of ten maize years, in comparison to variant P_1, the variants P_2, P_3, P_4 resulted in 2-6-8 t/ha lower grain yield.

According to the results of investigations carried out in 1987 and 1988, on plots higher than 150 to 200 mg/kg AL-(ammonium lactate soluble)-P_2O_5 content (over 30 mg/kg Olsen-P), in the flowering-stage leaves, the Zn content dropped below 12 mg/kg and the P/Zn ratio rose above 250. As a consequence of P-induced Zn deficiency in both, a dry and a favourable year, maize grain yield fell by 1.5–2.0 t/ha in the P_4 level (200 kg/ha P_2O_5/year) in comparison to the P_1 variant (50 kg/ha P_2O_5/year), resp. The data obtained clearly indicate that maize yields are impeded by both a poor and an excessive P status. Soil and plant analyses may be useful means for monitoring the nutrient status of plants.

Key words: long-term field trial, P induced Zn deficiency, over-fertilization with P, soil and plant analyses, Hungary

Introduction

At the end of the last and beginning of this century, maize was considered to respond more to manures than to fertilizers (Cserháti, 1901); 'Sigmond and Flóderer, 1905). Maize hybrids, however, which were introduced into practice in the 1930's, provided a marked response to mineral fertilizers, too (Balla, 1960; Győrffy, 1979; Kádár, 1987; Sarkadi, 1975). On increasing the plant population density, the effect of fertilizers became even more pronounced (Győrffy, 1979; Jordan et al., 1950). Nowadays, maize with its high yields has become one of the field crops demanding the largest quantities of nutrients in Hungary (Győrffy, 1979; Kádár, 1987).

On the other hand, very few data exist in Hungary about the sensitivity of maize to overfertilization, especially of phosphorus over-dosage (Elek and Kádár, 1975).

In 1987, the maize practice in Hungary applied nearly the same P_2O_5-quantities (90–100 kg/ha on the average) on all soils, regardless of the level of the soils' P-supply. This caused an overfertilization with P on the soils, which were well or extremely well supplied with this nutrient. This was the case in about 3/4 of the total maize fields in Hungary according to national soil analyses (Buzás et al., 1988).

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At the same time, calcareous soils, which make up 40% of arable land are lower in zinc than the national average; this also is unfavourable (Elek et al., 1984; Várallyay et al., 1980). Maize, with its sensitivity to Zn-deficiency, is grown, however, on 22–24% of arable land in Hungary, and occupies nearly the same area as winter wheat (Csathó and Kádár, 1989).

Farmyard manure (FYM) containing the macro- and micro-nutrients in an adequate ratio has been applied annually on only 10–15% of arable land for a long time, and there are considerable areas which receive no FYM at all (Kádár, 1987).

In this paper, selected data from long-term field experiments are presented, which form a part of the National Uniform Fertilizer Experiment Network and have been set up at the experimental station of the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, at Nagyhörcsök, in mid-western Hungary.

Attention is mainly focused on the connection existing between the soil's P-supply and the grain yield of maize. The yield reducing effect of repeated P-overfertilization is also analysed, utilizing the soil and plant analysis data.

Materials and methods

The four experiments were started on the calcareous chernozem soil of the Experimental Station at Nagyhörcsök; two in 1968 and two in 1969. The ploughed layer had a $CaCO_3$ content of about 5% and a humus content of about 2.5–3%. Prior to starting the experimental work, the AL-(=ammonium lactate soluble) P_2O_5- and K_2O-contents were 60 and 160 mg/kg soil in two of the experiments, while in the other two experiments the corresponding values were 100 and 190 mg/kg soil, resp. According to the official analytical methods and limit values valid in Hungary, the Mn-supply of the soil was very good, the Mg- and Cu-supplies were satisfactory, and the Zn-supply was poor. The soil texture is a sandy loam (20% sand, 40% loess-like loam, 20% silt, 20% clay).

The experiments were set up in an incomplete block design with 20 fertilizer treatments with 4 replications. In two of the experiments, the gross area of the plots were 72 m^2 with a net area of 34 m^2, while in the other two experiments these areas were 88 m^2 and 56 m^2, resp. In two of the experiments, the crop rotation was winter wheat-maize-maize-peas, while in the other two experiments it was winter wheat-maize-maize-winter wheat. The maize hybrids were changed in the course of the years in the experiment: in the first three 4-year-cycles Mv 602, MvSC 580, Mv 59, SzeSC 444, and KSC 360 were sown, in the fourth cycle MvSC

Table 1

Yearly nutrient amounts applied in the experiments

Leve of nutrients	N	P_2O_5	K_2O	N	P_2O_5	K_2O
	Cycle 1			Cycle 2–5		
	kg/ha					
0	0	0	0	0	0	0
1	40	40	80	50	50	100
1	80	80	160	100	100	200
3	120	120	0	250	150	0
4	160	160	0	200	200	0
5	200	0	0	250	0	

580, SzeSC 444, Pioneer SC 3901 and Pioneer Sc 3732, while Pioneer SC 3901 and Pioneer SC 3732 were sown in the experiments of the fifth cycle. The plant population density per hectare was 47–48 000 in the first two cycles, 57 000 in the 3rd and 4th cycles, and 71 400 in the fifth cycle (the latter with a row space of 70 cm, and a plant space of 20 cm).

The amounts of NPK-nutrients applied yearly in the experiments are given in Table 1. The fertilizers used were calcium-ammonium-nitrate, potassium chloride and superphosphate (in granules). The P- and K-fertilizers were applied before ploughing, in autumn. The amount of N-fertilizer was split into two halves: The first part was given in autumn, the other part in spring.

During the 19 experimental years the crop results of 35 maize experiments were evaluated. Moreover, in 1987 and 1988, plant and soil samples were drawn from certain selected plots. To get composite plants samples a single leaf next to the maize ear was taken from 20 plants per plot for the purpose of a diagnostic plant analysis at the beginning of flowering. Following the harvest, composite soil samples were drawn from the ploughed layer of the net area of each plot. The composite samples consisted of 20 single samples of each plots net area.

The amount and distribution of precipitation in the winter of 1986/87 and in the vegetative period in 1987 were favourable (X. 1986 – III. 1987: 229 mm; IV. 1987: 58 mm; V: 86 mm; VI: 68 mm; VII: 26 mm; VIII: 74 mm; IX: 44 mm precipitation).

In the summer of 1988, however, a drought damaged maize plants in their most sensitive phase, at flowering (X. 1987 – III. 1988: 263 mm; IV. 1988: 25 mm; V: 11 mm; VI: 70 mm; VII: 30 mm; VIII: 97 mm; IX: 58 mm precipitation).

In 1987, maize was sown on April 28th, while in 1988 on April 26, and it was harvested on October 21, 1987 and on November 1, 1988, resp.

Results and conclusion

The grain yield of certain selected treatments of the five cycles are summed up in Table 2. The highest grain yields were obtained in the treatments $N_3K_1P_1$ and $N_4K_1P_1$ where 50 kg/ha P_2O_5 was given annually. On increasing the P-supply of the plants, the grain yield decreased, at first only as a tendency, but at the higher P-levels (P_3 and P_4) to a statistically significant degree. The yield reduction caused by the higher P-levels – when compared with that obtained at the P_1 level – became more and more expressed as the years passed. This is also illustrated if the cumulated yield reduction of the single P-treatments is compared with the mean yields of all the NKP_1 treatments. The total loss in grain yield of the 10 years of maize experiments made up about 2 t/ha at the P_2-level (where 80 kg/ha P_2O_5 was applied annually in the first, and 100 kg/ha in the following cycles), nearly 6 t/ha at the P_3-level and more than 8 t/ha at the P_4-level. This latter quantity is exactly as much as the yield loss in the NKP_0-treatment! (Table 2, Figure 1).

To reveal the causes of the above-mentioned reduction, an investigation was carried out in 1987 and 1988, the results of which are shown in Tables 3 and 4, resp. It should be noted that the 150 kg/ha N-doses applied in the experiment in that year were high enough to cover the N-demand of even the highest maize yields as no yield increases could be detected when higher N-doses were applied. Similarly, there were no real differences between the yields obtained at levels K_1 and K_2. To show the effect of P-fertilization on a NK base, yields of those treatments, where the amounts of NK fertilizers were sufficient, are given in Table 3.

As a result of the P-fertilization applied for nearly twenty years, the initial ammonium lactate (AL) soluble P-content of the soil grew about fivefold and the soil

Table 2

Effect of fertilization on grain yield of maize calcareous chernozem
(1970–1988. Nagyhörcsök, Hungary)

	Cycle						
	1	2	3	4	5	1–5	%
	Number of experiments						
	7	8	8	7	5	35	
Treatments	Grain, t/ha (86% dry matter/year)						
$N_0K_0P_0$	5.09	5.65	6.00	5.16	6.28	5.61	72
$N_3K_1P_0$	9.95	7.29	7.55	6.77	7.88	7.06	90
$N_3K_1P_1$	6.09	8.09	8.42	7.79	8.84	7.81	100
N_3K_1P2	5.84	7.75	8.37	8.08	8.30	7.65	98
$N_3K_1P_3$	5.97	7.62	8.02	7.41	8.08	7.41	95
$N_4K_1P_1$	6.27	8.17	8.45	7.69	8.84	7.85	101
N_4K_1P2	6.07	7.55	8.14	7.82	8.34	7.55	97
$N_4K_1P_3$	5.96	7.27	7.74	7.26	7.74	7.18	92
$N_4K_1P_4$	5.99	7.50	7.78	7.01	7.16	7.11	91
$N_5K_0P_3$	5.53	6.71	7.00	6.51	6.80	6.51	83
$N_5K_1P_3$	6.05	7.85	7.72	7.28	7.75	7.33	94
$N_5K_1P_4$	5.71	7.11	7.60	6.80	7.17	6.89	88
$N_5K_1P_4$	5.92	7.51	7.51	6.47	7.18	6.94	89
L.S.D. $_{5\%}$	0.39	0.47	0.62	0.52	0.74	0.25	3
Mean	5.88	7.39	7.72	7.08	7.72	7.15	–

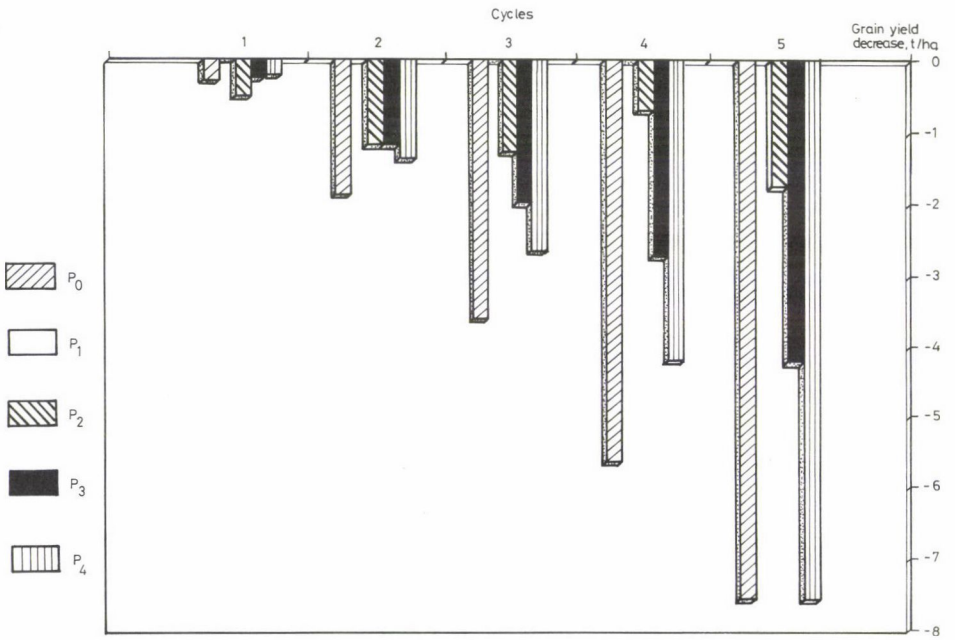

Fig. 1. Cumulative losses of maize grain yields due to under- or overfertilization with P, as compared with the best (P_1) variant (yearly 22 kg/ha P or 50 kg/ha P_2O_5). Calcareous chernozem. Nagyhörcsök, Hungary, 1968–1988

earlier poor in P became medium, well or very well supplied with phosphate. The changes were even more expressed with the Olsen-P amounts of the different P-treatments. The sulphate content of the ploughed layer increased together with the P-doses, i.e. the sulphate ions which got into the soil when superphosphate was applied, partly accumulated together with the phosphate ions in the upper layer of the soil. The EDTA soluble Zn-content of the soil showed a growing trend, though its increase could not be proved statistically.

Table 3

Effect of P-overfertilization on yield, nutritive element composition of flowering-stage leaf and on available nutritive element content of soil. Calcareous (lime coated) chernozem
(Nagyhörcsök, Hungary, 1987. Maize, Pioneer SC 3732)

	$N_0P_0K_0$	NKP_0	NKP_1	NKP_2	NKP_3	NKP_4	$L.S.D_{5\%}$
AL-P_2O_5 mg/kg	62	62	88	156	273	322	28
Olsen-P mg/kg	10.0	8.6	11.1	28.6	55.8	71.4	8.0
KCl-SO_4 mg/kg	5.5	5.7	8.5	13.9	18.3	18.4	4.8
EDTA-Zn mg/kg	1.5	1.7	1.5	1.8	1.9	2.0	0.6
Flowering-stage leaf, 20 pieces/plot							
Weight, g	60	72	73	61	64	58	7
N%	2.22	2.30	2.58	2.50	2.54	2.53	0.15
K%	1.02	1.49	1.66	1.65	1.60	1.71	0.20
Ca%	0.68	0.52	0.58	0.68	0.72	0.75	0.09
Mg%	0.53	0.32	0.40	0.48	0.50	0.49	0.05
P%	0.22	0.24	0.27	0.32	0.37	0.38	0.06
Fe mg/kg	201	207	230	306	357	402	41
Mn mg/kg	72	74	105	158	187	203	22
Zn mg/kg	14.9	20.2	17.4	12.0	10.4	9.7	2.7
Cu mg/kg	6.8	7.8	9.2	12.2	13.7	14.4	1.9
N/P	10.2	9.9	9.6	7.7	6.8	6.7	1.5
N/K	2.2	1.6	1.6	1.5	1.6	1.4	0.2
K/P	4.6	6.6	6.2	5.1	4.3	4.5	1.0
N/Cu	3110	3020	2940	2090	1880	1760	520
P/Zn	154	120	164	273	358	378	73
K/Mg	1.9	4.6	4.1	3.5	3.2	3.5	0.7
Fe/Zn	13.5	10.2	14.0	25.7	34.3	39.7	6.5
Plant number, 1000 pieces/ha	65.7	69.6	70.2	69.6	68.8	68.8	2.3
Percentage of infertile plants	1.6	0.7	0.8	0.8	0.8	1.1	0.6
Grain number/cob, piece	418	465	518	522	509	480	58
1000-seed mass, g	276	300	302	305	300	302	27
Grain number/m^2, piece	2940	3100	3620	3640	3260	3020	300
Grain mass/plant, g	134	144	168	163	153	142	10
Effectivity %	69	69	73	73	73	73	3
Stalk yield, t/ha	7.97	10.02	12.31	11.06	11.52	12.14	1.06
Grain yield, t/ha	8.69	9.95	11.70	11.27	10.47	9.70	0.45
D± NKP_1	–3.01	–1.75	0.00	–0.43	–1.23	–2.00	0.45

Table 4

Effect of P-overfertilization on yield, nutritive element composition of flowering-stage leaf and on available nutritive element content of soil. Calcareous (lime coated) chernozem, (Nagyhörcsök, Hungary, 1988. Maize, Pioneer SC 3732)

	$N_0P_0K_0$	NKP_0	NKP_1	NKP_2	NKP_3	NKP_4	L.S.D.$_{5\%}$
AL-P_2O_5 mg/kg	74	84	126	161	240	379	71
Olsen-P mg/kg	6.8	10.6	18.8	22.9	37.5	65.3	13.3
EDTA-Zn mg/kg	0.8	0.7	0.8	0.9	0.9	0.8	0.2
Flowering-stage leaf, 20 pieces/plot							
Weight, g	43.8	46.1	49.0	40.9	42.6	38.0	4.8
N%	2.52	2.95	2.87	2.95	2.91	2.80	0.17
K%	1.33	1.74	1.67	1.63	1.64	1.71	0.08
Ca%	0.54	0.53	0.63	0.65	0.62	0.58	0.05
Mg%	0.36	0.31	0.33	0.42	0.42	0.41	0.04
P%	0.22	0.23	0.28	0.32	0.34	0.34	0.03
Fe mg/kg	165	200	212	268	311	329	73
Mn mg/kg	82	78	107	155	176	194	27
Zn mg/kg	21.1	20.8	13.7	12.4	12.6	10.1	2.4
Cu mg/kg	11.3	12.2	13.7	16.0	17.0	16.8	1.2
N/P	11.7	12.9	10.4	9.2	8.7	8.3	0.7
N/K	1.9	1.7	1.7	1.8	1.8	1.6	0.1
K/P	6.2	7.6	6.0	5.1	4.9	5.0	0.6
N/Cu	2245	2429	2098	1849	1712	1673	170
P/Zn	104	111	212	259	271	337	52
K/Mg	3.7	5.7	4.8	3.9	3.9	4.2	0.5
Fe/Zn	8	10	16	22	25	33	6
Plant number, 1000 pieces/ha	63.6	67.2	68.1	66.3	61.4	64.1	6.0
Percentage of infertile plants	2.8	2.2	1.6	2.6	2.4	5.0	3.3
Grain number/cob, piece	398	362	353	360	374	290	65
1000-seed mass, g	263.2	298.9	279.5	264.3	276.2	293.1	25.7
Grain number/m^2,piece	2461	2380	2362	2317	2229	1766	318
Grain mass/plant, g	111	114	104	99	108	90	15
Effectivity %	81.1	79.0	85.3	85.3	84.4	81.6	3.8
Stalk yield, t/ha (86% DM)	5.77	4.51	4.86	4.93	4.82	4.49	1.23
Grain yield, t/ha (86% DM)	6.82	7.51	6.98	6.40	6.46	5.47	0.67
D± NKP_0	–0.69	–	–0.53	–1.11	–1.05	–2.04	0.67

The dry matter weight of the leaves next to the maize cobs was the highest in the approximately 90–120 mg/kg AL-P_2O_5 ranged soil. Improving the P-supply, the N-, K-, Ca-, Mg- and P-contents of the leaves generally reliably increased. Regarding the microelements in the leaves, their Fe-, Mn- and Cu-contents increased, while the Zn-content fell to one-half. The increase of P-supply can also be indicated (followed), by the changes of the nutrient ratios, e.g. the N/P, K/P and N/Cu ratios decreases, while the P/Zn and Fe/Zn ratios increased 2.5 and 3.0 fold., resp., when the P-level rose from P_0 to P_4 (Tables 3 and 4).

Relying upon data found in literature as well as on those obtained in earlier experiments in Hungary, the conclusion can be drawn that the P/Zn ratio in maize leaves is optimum between 80 and 150 (Elek and Kádár, 1980). If P gets into a considerable predominance over Zn, i.e. the P/Zn ratio markedly exceeds the value of 200,

Zn deficiency cannot be ignored. In such cases P-fertilization may be ineffective, or it can even cause a reduction in yield. On the calcareous soil of the experiments, the availability of Zn to the plants is limited, which is why higher P-doses may cause P-Zn antagonisms. The higher P-doses also exerted an unfavourable effect on the single factors of crop yield, e.g. the number and the weight of the total maize grains per cob showed a decreasing trend.

In these experiments, each maize plant produced only one cob. In the favourable year of 1987, the average number of grains per cob ranged between 520 and 420, while in the dry year of 1988 between 400 and 290, resp. Overfertilization with P, and the Zn deficiency induced by it, hindered the growth of maize cobs to reach an appropriate length and suppressed the numbers of maize grains per cob.

The connection between the grain yield of maize and the soil's Olsen-P values call attention to the fact that both the low (poor) and the too high P-supply of the soil (under and over the optimum range of 10–30 mg Olsen-P/kg soil, i.e. under and over 150–200 mg/kg AL-P_2O_5) are equally dangerous for soil fertility. Leaf-analysis may provide useful information about P-excess, P/Zn ratios (high if over 150–200), and about, P-induced Zn-deficiency. Soil and plant analyses may be a good means for controlling the nutrient status of plants and for determining the possible occurrence of under- or overfertilization (Figure 2).

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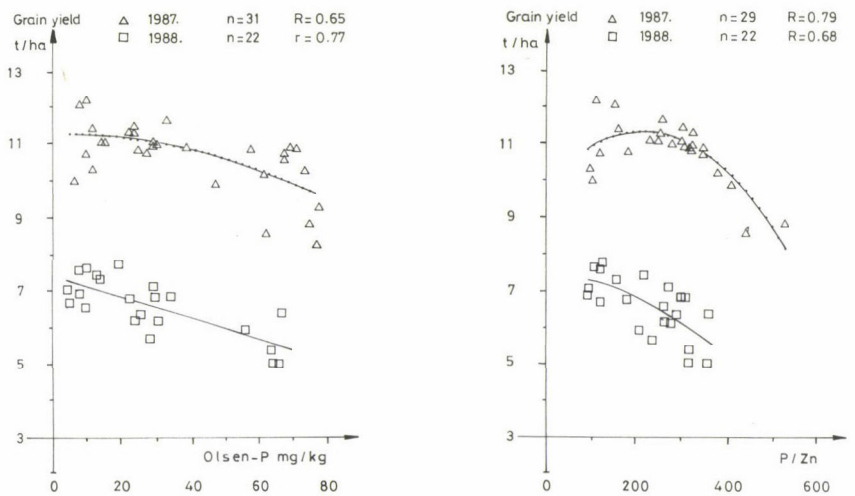

Fig. 2. Connection between maize grain yield and Olsen-P values, and P/Zn content of leaves at flowering, resp.

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Plant Protection

FAUNAL INVESTIGATION OF GROUND-BEETLES (*CARABIDAE*), IN THE ARABLE SOILS OF HUNGARY. II

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Before our survey started in 1975, we carried out mechanical soil sampling on large-scale farms cultivated (altogether on 25589 ha) mainly on plain territories of Hungary in 1976–78. The 26 090 samples (2609 sample units) represented about 820 m². During this work 1506 carabid adults and 1175 larvae belonging to 65 species were collected. According to our data it has been found that:

– Besides the ground-beetles found earlier we could establish the presence of 20 additional species on the arable soils (they are represented mainly by 1 beetle per species), but none of these can be regarded to be frequent. Nevertheless 45 species are identical with the species found in 1975.

– From the 13 species (*Zabrus tenebrioides* (Goeze), *Platynus dorsalis (Pontopp.), Pseudophonus rufipes* (De Geer), *Anisodactylus signatus* (Panz.), *Trechus quadristriatus* (Schrank), *Harpalus distinguendus* (Duft.), *Poecilus cupreus* (Duft.), *Clivina fossor* (L.), *Bembidion properans* (Steph.), *Pterostichus longicollis* (Duft.), *Pseudophonus griseus* (Panz.), *Amara familiaris* (Duft.), *Poecilus sericeus* (Frisch.) exceeding the dominance of 1% of the most frequent 9 species are identical with the dominant species of the survey of 1975, purely their order of dominance is different. The total dominance of this 9 species is almost at the same high level (79.31% and 79.79%, respectively) in the two survey periods. This refers to the fact that invariance of the more frequent species is considerable on arable soils.

– There is a high level of invariance regarding the density of ground-beetles and their larvae as well (1.74 and 1.52 per m² respectively in 1975 and 1.73 and 1.35 per m² respectively in 1976–78). Imagos were found at a higher rate in every case.

– Within the area sampled the border areas of "Alföld" (Hungarian Plain) (east and south-east-Sárrét, north-east-Tiszahát, Szatmár-Bereg) and the southern part of Transdanubia are extremely rich in species, since these areas have more diversified, proportioned and humid circumstances.

– Regarding forecrop the insect frequency of the most frequent species (*Clivina fossor* (L.), *Bembidion properans* (Steph.), *Anisodactylus signatus* (Panz.), *Pseudophonus rufipes* (De Geer), *Harpalus distinguendus* (Duft.), *Poecilus cupreus* (L.) are the lowest in the stubble-field of cereals (winter wheat), higher in the stubble-field of hoed plants (mainly maize) and the highest after perennial papilionaceae (lucerne). The same frequency is observable at the total insect frequency of ground-beetles. This order is probably in connection with plant cover and with the more humid microclimate of the area. Preferring of hoed plants is observable in the case of *Trechus quadristriatus* (Shrank) while *Platynus dorsalis* (Pontopp.) in contradiction with the majority of the species, hardly occurs after lucerne and its presence was the most significant after winter wheats. The attraction of *Zabrus tenebrioides* (Goeze) towards graminaceous plants is well known. *Amara familiaris* (Duft.) and *Amara similata* (Gyll.) are also worthy of note because of their more significant rate of occurrence in culture of perennial papilionaceae. The number of species occurring in cultures of perennial papilionaceae is presumably larger, but the small number of samples of these cultures does not make its proving possible. The number of species per cultures is in correlation with the number of samples.

– In cases of the more frequent species, a high rate of elasticity is observable regarding the overwintering (this is proved mainly at autumn breeders). At the same species in some proportion – depending on the weather – both overwintering types (in form of larva and imago) can occur.

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– The highest rate of frequency of insects and species can be observed on the smallest fields.

– Summarizing the connections with protection of nature it can be ascertained as a fact that rather high number of endangered species of ground-beetles occur also on the agricultural areas: *Acupalpus interstitialis* (Reitt.), *Asaphidion pallipes.* (Duft.), *Bembidion obtusum,* Audinet-Serville, *Calosoma auropunctatum* (Herbst), *Harpalus politus(* Dej.), *Harpalus pygmaeus* (Dej.), *Harpalus tenebrosus(* Dej.), *Harpalus zabroides* (Dej.), *Masoreus wetterhalli* (Gyll.), *Ophonus diffinis* (Dej.), *Parophonus maculicornis* (Duft.), *Parophonus mendax* (Rossi), *Poecilus pucticollis* (Dej.), *Poecilus punctulatus* (Shall.).

Key words: *Carabidae,* arable soils of Hungary, soil sampling, frequent species, dominance, forecrop, overwintering, protection of nature

Introduction

Some years ago we (Horvatovich and Szarukán, 1986) published the results of our researches referring to ground-beetles of Hungarian arable soils on the basis of material collected by soil sampling. In our present work we report on further experiences of our investigation.

Materials and methods

The insect material evaluated has been collected from arable fields of 36 farms in Hungary (Tables 1, 2, Fig. 1).

The samplings were carried out by soil sampling machines, type TVG-2, of Hungarian made mounted on a tractor and operated by the hydraulic of the tractor in farms belonging to the agricultural system of KITE (Co-operation for Cultivation of Maize and Industrial Plants) of Nádudvar. The insect material of the cylinder of soil of about 50–60 cm of depth and of 20 cm of diameter (314 cm^2) lifted by the machine out was assorted by hand and then the insect material was taken into a sampling glass filled with alcohol up. The material of ten samples were collected into the same sampling glass. The farms took one sample from each hectare or they raised the number of samples to take ten samples to one sampling glass. One sample unit that is the insect material of 10 soil cylinder represents about one-third m^2 of soil surface. Samples were taken in uniform distribution in the fields in autumn (September–October) and in spring (April) of 1976–78 (Table 1). The most important data of the fields and the reports containing sketches of sampling were enclosed to the sample glasses. After determination of the insect material of the samples we prepared suggestions for protection for each field of the farms (in the most cases we suggested to stop the use of pesticides).

For the determination of ground-beetles, besides the material for comparison of the Museum of Natural Sciences, the works of Csiki (1946), Horvatovich (1974) and Freude (1976) were also used.

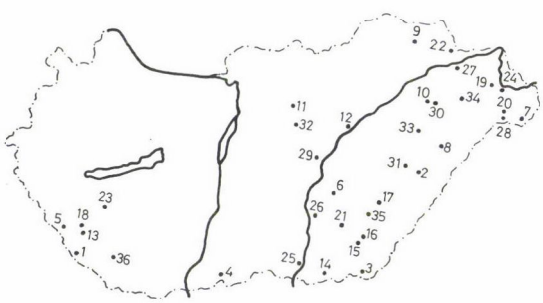

Fig. 1. Distribution of sampling sites in Hungary (1976–1978)

Results and discussion

In comparison with the sampling of 1975 (Horvatovich and Szarukán, 1986) this time we had only 2609 sample units (that was the material of samples coming from 25 589 ha, representing about 820 m^2 of soil surface) at our disposal (Tables 1 and 2). In this connection the number of species collected is also less, thus contrary to the 74 species of 1975 we could determinate only 65 species of ground-beetles. From these species 45 occurred in both survey periods (they are marked with* in Table 1) and that means that further 20 species of ground-beetles were observable in the arable soils of Hungary. Thus we could determinate altogether 94 species and these species represent almost the one-fifth (19.1%) of the species of carabids (492 species) known to occur in Hungary at this moment. On the other hand we should note that none of the species determinated recently can be regarded to be frequent, the majority of them (12 species) were represented only by 1 beetle per species and it also proves their rarity on cultivated soils. First of all these rare species have importance in nature protection since their occurrence on cultivated soils gives information about the species that are allowed to exist by the agriculture under certain circumstances (soil cultivation, fertilization, crop plant, non-cultivated areas of the surrounding etc.).

The dominant 13 species (exceeding the dominance of 1.0%) (Table 3) represent almost 86% of the ground-beetles collected. The most dominant 9 species correspond to the species sampled in 1975 purely their order of dominance is different. The total dominance of these 9 species was almost at the same level (79.31% and now 79.69%) in the two survey periods. This refers to the fact that invariance of the more frequent species is considerable on large-scale fields of arable soils. In "Bácska" of Yugoslavia similar conditions of dominance were observed on fields of winter wheat (Sekulic et al., 1987). There only two species could not get into the group of the most frequent 9 species (*Bembidion properans* and *Clivina fossor*, appearing at the 12th and 17th places, respectively). According to Lővei (1989) the total dominance of the most frequent 9 species of ground-beetles is 96.7% in maize monoculture. These 9 species are as follows in decreasing order of their dominance: *Harpalus rufipes, Pterostichus melanarius, Anisodactylus signatus, Harpalus distinguendus, Dolichus halensis, Laemostenus terricola, Broscus cephalotes, Pterostichus sericeus, Calathus ambiguus.* From these species only 3 occur amongst the 9 most frequent species determinated by us (*Pseudophonus rufipes, Anisodactylus signatus* and *Harpalus distinguendus*). In maize cultures cultivated by rotation of crops from the most frequent species determinated by Lővei (*Harpalus rufipes, Pterostichus sericeus, Pterostichus melanarius, Calathus ambiguus, Broscus cephalotes, Dolichus halensis, Trechus quadristriatus, Agonum dorsale* and *Laemostenus terricola*) only 3 (*Platynus dorsalis, Pseudophonus rufipes* and *Trechus quadristriatus*) correspond with the species from the 9 species regarded to be the most frequent by us. It is to be noted that dominance of the 9 most frequent species is less in case of rotation of crops (81.9%). Comparing with both of the maize cultivation methods only one species (*Pseudophonus rufipes*) shows high level of invariance. The difference between the two surveys can be explained by the Transdanubian climate richer in precipitation and by the difference between the conditions of soil.

Table 1a

Number of individuals of ground-beetle imagos in 1976–78, in the individual farms (Species marked with were found in 1975 as well)

Period of sampling	Serial number	Place-name	Area sampled (ha)	A	*Carabus granulatus* (L.)	*Clivina collaris* (Herbst)	*Clivina fossor* (L.)*	*Oyschirius globosus* (Herbst)*	*Broscus cephalotes* (L.)*	*Trechus quadristriatus* (Schrank)*	*Bembidion biguttatum* (F.)	*Bembidion inoptatum* (Schaum)	*Bembidion properans* (Steph.)*	*Bembidion quadrimaculatum* (L.)*	*Bembidion striatum* (F.)	*Asphidion flavipes* (L.)*	*Aniscodactylus signatus* (Panz.)*	*Diacromus germanus* (L.)*	*Ophonus signaticornis* (Duft.)*
Spring of 1976	1	Babocsa	605	60	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–
	2	Bakonyszeg	481	48	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	Battonya	1084	117	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	Bácsborsod	868	90	–	–	–	–	–	–	–	–	1	1	–	–	–	–	–
	5	Berzence	427	44	–	–	2	–	–	–	–	–	25	–	–	1	10	–	2
	6	Békésszentandrás	486	51	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–
	7	Csengerújfalu	364	38	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	8	Debrecen	621	65	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–
	9	Encs	211	22	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	10	Hajdúnánás	575	60	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	11	Hatvan	518	53	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	12	Kisköre	124	13	–	–	1	–	–	–	–	–	–	–	–	–	1	–	–
	13	Lábod	407	43	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	14	Makó	813	83	–	–	4	–	–	–	–	–	3	–	–	–	6	–	–
	15	Magyarbánhegyes	606	60	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	16	Medgyesegyháza	562	59	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–
	17	Mezőberény	922	90	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–
	18	Nagyatád	519	54	–	–	8	–	–	–	–	–	2	–	–	–	7	–	1
	19	Nagydobos	288	22	–	–	–	–	–	–	–	–	3	–	–	–	8	–	–
	20	Nagyecsed	852	98	–	–	–	–	–	–	–	–	1	–	–	–	8	–	–
	21	Orosháza	373	38	–	–	2	–	–	–	–	–	3	–	–	–	–	–	–
	22	Sárospatak	271	26	–	–	–	–	–	–	–	–	–	–	–	–	6	–	–
	23	Somogysárd	436	46	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	24	Szamosszeg	286	30	–	–	9	–	–	–	–	–	–	1	–	–	14	–	–

Table 1a (cont'd)

Period of sampling	Serial number	Place-name	Area sampled (ha)	A	*Carabus granulatus* (L.)	*Clivina collaris* (Herbst)	*Clivina fossor* (L.)*	*Oyschirius globosus* (Herbst)*	*Broscus cephalotes* (L.)*	*Trechus quadristriatus* (Schrank)*	*Bembidion biguttatum* (F.)	*Bembidion inoptatum* (Schaum)	*Bembidion properans* (Steph.)*	*Bembidion quadrimaculatum* (L.)*	*Bembidion striatum* (F.)	*Asaphidion flavipes* (L.)*	*Aniscodactylus signatus* (Panz.)*	*Diacromus germanus* (L.)*	*Ophonus signaticornis* (Duft.)*
	25	Szeged	182	16	1	–	2	–	–	–	–	–	–	2	–	–	1	–	–
Spring of	26	Szentes	1542	162	1	–	4	–	–	1	–	–	2	–	–	–	6	–	–
1976	27	Tiszabercel	180	12	–	–	4	–	–	–	–	–	–	–	–	–	3	–	–
	28	Vállaj	313	34	–	–	2	–	–	–	–	–	1	–	–	–	36	–	–
Autumn of	29	Abony	491	40	–	–	–	–	1	–	–	–	–	–	–	–	4	–	–
1976	5	Berzence	404	18	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–
	8	Debrecen	387	67	–	–	–	–	–	2	–	–	3	–	–	–	–	–	–
	30	Hajdúdorog	512	46	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–
	31	Sárrétudvari	827	82	–	–	–	–	–	87	–	–	11	–	–	–	6	–	–
	23	Somogysárd	813	92	–	–	–	–	–	2	–	–	2	1	–	–	–	–	1
	26	Szentes	420	44	–	–	–	–	–	1	–	–	1	–	–	–	1	–	–
	32	Szentmártonkáta	150	18	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–
	27	Tiszabercel	277	16	–	–	2	–	–	1	–	–	–	–	–	–	2	–	–
Spring of	33	Balmazújváros	760	72	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1977	3	Battonya	227	20	–	–	1	–	–	6	–	–	–	–	–	–	1	–	–
	10	Hajdúnánás	288	30	–	–	1	–	–	–	–	–	–	–	–	–	7	–	–
	34	Nagykálló	411	46	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	35	Telekgerendás	1039	103	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–
Autumn of	22	Sárospatak	1100	111	–	–	5	–	–	1	–	–	1	–	1	–	11	2	–
1978	24	Szamosszeg	104	19	–	3	15	1	–	10	1	1	–	–	–	–	3	–	–
Spring of	2	Bakonszeg	386	37	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1978	36	Nagypeterd	1160	118	–	1	7	–	–	2	–	–	6	3	–	–	5	–	–
	22	Sárospatak	837	88	–	–	6	–	–	–	–	–	1	3	–	–	10	1	–
		Total	25509	2609	2	4	76	1	1	116	1	1	70	11	1	1	162	3	4
		Dominance %			0.13	0.27	0.07	0.07	0.07	0.07	0.07	0.07	4.65	0.72	0.07	0.07	10.76	0.20	0.27

A: Number of sample units

Table 1b

Number of individuals of ground-beetle imagos in 1976–78, in the individual farms (Species marked with were found in 1975 as well)

Period of sampling	Serial number	Place-name	Area sampled (ha)	A	*Pseudophonus calceatus* (Duft.)*	*Pseudophonus griseus* (Panz.)*	*Pseudophonus rufipes* (De Geer.)	*Harpalus affinis* (Schrank)*	*Harpalus autumnalis* (Duft)*	*Harpalus dimidiatus* (Rossi)*	*Harpalus distinguendus* (Duft.)	*Harpalus flaviocornis* (Dej.)	*Harpalus hospes Sturm**	*Harpalus pygmaeus Dej.*	*Harpalus servus* (Duft.)	*Harpalus tardus* (Panz.)*	*Stenolophus teutonus* (Schrank)*	*Acupalpus interstitialis* Reitt.*	*Acupalpus meridianus* (L.)*
Spring of 1976	1	Babocsa	605	60	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	Bakonszeg	481	48	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	Battonya	1084	117	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–
	4	Bácsborsod	868	90	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–
	5	Berzence	427	44	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–
	6	Békésszentandrás	486	51	–	3	14	–	–	–	5	–	–	–	–	–	–	–	–
	7	Csengerújfalu	364	38	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	8	Debrecen	621	65	–	–	4	–	–	–	3	–	1	–	–	–	–	–	–
	9	Encs	211	22	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–
	10	Hajdúnánás	575	60	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–
	11	Hatvan	518	53	–	1	4	–	–	–	1	–	–	–	–	–	–	–	–
	12	Kisköre	124	13	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–
	13	Lábod	407	43	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	14	Makó	813	83	–	1	1	–	–	–	4	–	–	–	–	–	–	–	–
	15	Magyarbánhegyes	606	60	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–
	16	Medgyesegyháza	562	59	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	17	Mezőberény	922	90	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–
	18	Nagyatád	519	54	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–
	19	Nagydobos	208	22	1	–	2	–	–	–	1	–	–	–	–	–	–	–	–
	20	Nagyecsed	852	98	–	–	2	1	–	–	–	–	–	–	–	–	–	–	–
	21	Orosháza	373	38	–	1	3	–	–	–	4	–	–	–	–	–	–	–	–
	22	Sárospatak	271	26	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–
	23	Somogysárd	436	46	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	24	Szamosszeg	286	30	–	–	3	–	–	–	1	–	–	–	–	–	–	–	1

Table 1b (cont'd)

Period of sampling	Serial number	Place-name	Area sampled (ha)	A	*Pseudophonus calceatus* (Duft.)*	*Pseudophonus griseus* (Panz.)*	*Pseudophonus rufipes* (De Geer)	*Harpalus affinis* (Schrank)*	*Harpalus autumnalis* (Duft)*	*Harpalus dimidiatus* (Rossi)*	*Harpalus distinguendus* (Duft.)	*Harpalus flaviocornis* (Dej.)	*Harpalus hospes Sturm**	*Harpalus pygmaeus Dej.*	*Harpalus servus* (Duft.)	*Harpalus tardus* (Panz.)*	*Stenolophus teutonus* (Schrank)*	*Acupalpus interstitialis* Reitt.*	*Acupalpus meridianus* (L.)*
Spring of	25	Szeged	182	16	–	–	3	–	–	–	1	–	–	–	–	–	–	–	–
1976	26	Szentes	1542	162	1	1	5	–	–	–	3	–	–	–	–	–	–	–	–
	27	Tiszabercel	180	12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	28	Vállaj	313	34	–	–	1	–	–	–	3	–	–	–	–	–	–	–	–
Autumn of	29	Abony	491	48	7	8	23	1	–	–	3	–	–	–	–	–	–	–	–
1976	5	Berzence	404	18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	8	Debrecen	387	67	–	–	9	–	–	–	5	–	–	–	–	–	–	–	–
	30	Hajdúdorog	512	46	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	31	Sárrétudvari	827	82	1	1	36	8	–	–	32	2	–	1	3	2	1	–	1
	23	Somogysárd	813	92	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	26	Szentes	420	44	–	1	2	–	–	–	–	–	–	–	–	–	–	–	–
	32	Szentmártonkáta	150	18	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–
	27	Tiszabercel	277	16	–	1	2	–	–	–	7	–	–	–	–	–	–	–	–
Spring of	33	Balmazújváros	760	72	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1977	3	Battonya	227	20	–	–	12	–	–	–	–	–	–	–	–	–	–	–	–
	10	Hajdúnánás	288	30	–	1	2	–	–	–	1	–	–	–	–	–	–	–	–
	34	Nagykálló	411	46	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	35	Telekgerendás	1039	103	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–
Autumn of	22	Sárospatak	1100	111	–	–	13	–	–	–	2	–	–	–	–	–	–	–	–
1977	24	Szamosszeg	184	19	–	–	5	–	2	–	9	–	1	–	–	–	–	10	7
Spring of	2	Bakonszeg	386	37	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1978	36	Nagypeterd	1160	118	–	2	9	–	–	1	1	–	–	–	–	–	1	–	–
	22	Sárospatak	837	88	–	–	5	–	–	–	3	–	–	–	–	–	–	–	2
		Total	25589	2609	10	21	173	15	2	3	91	2	2	1	3	2	2	10	12
		Dominance %			0.66	1.39	11.40	1.00	0.13	0.20	6.04	0.13	0.13	0.07	0.20	0.13	0.13	0.66	0.80

A: Number of sample units

Table 1c

Number of individuals of ground-beetle imagos in 1976–78, in the individual farms (Species marked with were found in 1975 as well)

Period of sampling	Serial number	Place-name	Area sampled (ha)	A	*Stomis pumicatus* (Panz.)*	*Poecilus cupreus* (L.)	*Poecilus lepidus* (Leske)*	*Poecilus punctticollis* (Dej.)*	*Poecilus sericeus* Fisch.*	*Poecilus versicolor* (Sturm)*	*Pterostichus inquinatus* (Sturm)	*Pterostichus longicollis* (Duft.)	*Pterostichus macer* (Marsch.)*	*Pterostichus melanarius* (Illig.)*	*Pterostichus ovoideus* (Sturm)	*Pterostichus vernalis* (Panz.)*	*Calathus melanocephalus* (L.)*	*Dolichus halensis* (Schall.)*	*Agonum sexpunctatum* (L.)*
Sampling of 1976	1	Babocsa	605	60	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	Bakonszeg	481	48	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	Battonya	1084	117	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	Bácsborsod	868	90	–	3	–	–	2	–	–	–	–	–	–	–	–	–	–
	5	Berzence	427	44	–	3	3	–	–	8	–	–	–	–	–	1	–	–	–
	6	Békésszentandrás	486	51	2	1	–	–	–	–	1	–	–	–	–	–	–	–	–
	7	Csengerújfaalu	364	38	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	8	Debrecen	621	65	–	4	–	–	2	–	–	–	–	–	–	–	–	–	–
	9	Encs	211	22	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	10	Hajdúnánás	575	60	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–
	11	Hatvan	518	53	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	12	Kisköre	124	13	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	13	Lábod	407	43	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	14	Makó	813	83	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–
	15	Magyarbánhegyes	606	60	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	16	Medgyesegyháza	562	59	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–
	17	Mezőberény	922	90	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	18	Nagyatád	519	54	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	19	Nagydobos	288	22	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	20	Nagyecsed	852	98	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–
	21	Orosháza	373	38	3	1	–	–	–	–	–	–	–	–	–	–	–	–	–
	22	Sárospatak	271	26	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–
	23	Somogysárd	436	46	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	24	Szamosszeg	286	30	–	3	–	–	–	–	–	–	–	–	–	–	–	–	1

Table 1c (cont'd)

Period of sampling	Serial number	Place-name	Area sampled (ha)	A	*Stomis pumicatus* (Panz.)*	*Poecilus cupreus* (L.)	*Poecilus lepidus* (Leske)*	*Poecilus punctricollis* (Dej.)*	*Poecilus sericeus* Fisch.*	*Poecilus versicolor* (Sturm)*	*Pterostichus inquinatus* (Sturm)	*Pterostichus longicollis* (Duft.)	*Pterostichus macer* (Marsch.)*	*Pterostichus melanarius* (Illig.)*	*Pterostichus ovoideus* (Sturm)	*Pterostichus vernalis* (Panz.)*	*Calathus melanocephalus* (L.)*	*Dolichus halensis* (Schall.)*	*Agonum sexpunctatum* (L.)*
Spring of	25	Szeged	182	16	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–
1976	26	Szentes	1542	162	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	27	Tiszabercel	180	12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	28	Vállaj	313	34	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–
Autumn of	29	Abony	491	40	–	16	–	–	8	–	–	–	–	4	–	–	–	1	–
1976	5	Berzence	404	18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	8	Debrecen	387	67	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–
	30	Hajdúdorog	512	46	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	31	Sárrétudvari	827	82	–	9	–	1	–	–	–	3	–	2	–	–	1	–	–
	23	Somogysárd	813	92	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	26	Szentes	420	44	1	–	–	–	1	–	–	–	–	–	–	–	–	–	–
	32	Szentmártonkáta	150	18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	27	Tiszabercel	277	16	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–
Spring of	33	Balmazújváros	760	72	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1977	3	Battonya	227	20	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	10	Hajdúnánás	288	30	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	34	Nagykálló	411	46	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	35	Telekgerendás	1039	103	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–
Autumn of	22	Sárospatak	1100	111	–	9	–	–	–	–	–	–	–	–	–	–	–	–	–
1977	24	Szamosszeg	184	19	–	9	–	–	–	–	–	30	1	1	2	–	–	–	–
Spring of	2	Bakonszeg	386	37	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1978	36	Nagypeterd	1160	118	–	3	–	–	–	–	–	–	–	2	–	–	–	–	–
	22	Sárospatak	837	88	–	10	–	–	–	–	–	–	–	–	–	–	–	–	–
		Total	25589	2609	11	86	3	1	17	8	1	33	1	9	2	1	1	1	1
		Dominance %			0.73	5.71	0.20	0.07	1.13	0.53	0.07	2.19	0.07	0.60	0.13	0.07	0.07	0.07	0.07

A: Number of sample units

Table 1d

Number of individuals of ground-beetle imagos in 1976–78, in the individual farms

Period of sampling	Serial number	Place-name	Area sampled (ha)	A	*Agonum viridicupreum* (Goeze)	*Platynus dorsalis* (Pontopp.)*	*Europhilus micans* (Nic.)	*Zabrus tenebrioides* (Goeze)*	*Amara aenea* (De Geer)	*Amara antiqua* Villa	*Amara apricaaria* (Payk.)*	*Amara consularis* (Duft.)*	*Amara eurynota* (Panz.)*	*Amara familiaris* (Duft.)*	*Amara incognita Fassati*	*Amara similata* (Gyll.)*	*Amara tricuspidata* Def.	*Masoreus wetterhallii* (Gyll.)	*Microlester maurus* (Sturm.)
Spring of 1976	1	Babocsa	605	60	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	Bakonszeg	481	48	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	Battonya	1084	117	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–
	4	Bácsborsod	868	90	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–
	5	Berzence	427	44	1	2	–	1	6	–	–	–	–	–	–	1	8	–	–
	6	Békésszentandrás	486	51	–	5	–	6	–	–	–	1	–	–	–	–	–	–	–
	7	Csengerújfalu	364	38	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	8	Debrecen	621	65	–	5	–	1	–	–	–	–	–	–	–	–	–	–	–
	9	Encs	211	22	–	5	–	–	1	–	–	–	–	–	–	–	–	–	–
	10	Hajdúnánás	575	60	–	–	–	4	–	–	–	–	–	–	–	–	–	–	–
	11	Hatvan	518	53	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	12	Kisköre	124	13	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–
	13	Lábod	407	43	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	14	Makó	813	83	–	–	–	1	–	–	–	–	–	–	–	–	–	1	–
	15	Magyarbánhegyes	606	60	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–
	16	Medgyesháza	562	59	–	2	–	5	–	–	–	–	–	–	–	–	–	–	–
	17	Mezőberény	922	90	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	18	Nagyatád	519	54	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–
	19	Nagydobos	288	22	–	–	–	–	1	–	–	–	–	2	–	–	–	–	–
	20	Nagyecsed	852	98	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–
	21	Orosházaa	373	38	–	8	–	–	–	–	–	–	–	–	–	–	–	–	–
	22	Sárospatak	271	26	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–
	23	Somogysárd	436	46	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	24	Szamosszeg	286	30	–	–	–	–	–	–	–	–	–	2	–	1	–	–	–

Table 1d (cont'd)

Period of sampling	Serial number	Place-name	Area sampled (ha)	A	*Agonum viridicupreum* (Goeze)	*Platynus dorsalis* (Pontopp.)*	*Europhilus micans* (Nic.)	*Zabrus tenebrioides* (Goeze)*	*Amara aenea* (De Geer)	*Amara antiqhobia Villa*	*Amara apricaaria* (Payk.)*	*Amara consularis* (Duft.)*	*Amara eurynota* (Panz.)*	*Amara familiaris* (Duft.)*	*Amara incognita Fassati*	*Amara similata* (Gyll.)*	*Amara tricuspidata* Def.	*Masorreus wetterhallii* (Gyll.)	*Microlester maurus* (Sturm.)
Spring of	25	Szeged	182	16	–	2	1	–	–	–	–	–	–	–	–	–	–	–	–
1976	26	Szentes	1542	162	–	10	–	1	–	–	–	–	–	–	–	1	–	–	–
	27	Tiszabercel	180	12	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–
	28	Vállaj	313	34	–	1	–	–	–	–	–	–	–	1	–	–	–	–	–
Autumn of	29	Abony	491	48	–	1	–	103	–	–	1	1	–	–	–	–	–	–	–
1976	5	Berzence	404	18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	8	Debrecen	307	67	–	1	–	2	1	–	–	–	–	–	–	–	–	–	–
	30	Hajdúdorog	512	46	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	31	Sárrétudvari	827	82	–	142	–	84	–	–	2	5	1	–	–	–	–	–	1
	23	Somogysárd	813	92	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	26	Szentes	420	44	–	–	–	10	–	–	–	–	–	–	–	–	–	–	–
	32	Szentmártonkáta	150	18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	27	Tiszabercel	277	16	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–
Spring of	33	Balmazújváros	760	72	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1977	3	Battonya	227	20	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–
	10	Hajdúnánás	288	30	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	34	Nagykálló	411	46	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	35	Telekgerendás	1039	103	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Autumn of	22	Sárospatak	1100	111	–	–	–	9	–	–	–	–	–	5	2	2	–	–	–
1977	24	Szamosszeg	184	19	–	–	–	1	1	1	–	–	–	8	1	10	–	–	–
Spring of	2	Bakonszeg	386	37	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1978	36	Nagypetend	1160	118	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–
	22	Sárospatak	837	88	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–
		Total	25589	2609	1	192	1	237	14	1	4	7	1	19	3	15	8	1	1
		Dominance %			0.07	12.75	0.07	15.74	0.93	0.07	0.27	0.46	0.07	1.26	0.20	1.00	0.53	0.07	0.07

A: Number of sample units

Table 1e

Number of individuals of ground-beetle imagos in 1976–78, in the individual farms

Period of sampling	Serial number	Place-name	Area sampled (ha)	A	*Microlestes minutulus* (Goeze)*	*Orypta dentata* (Rossi)	*Brachinus crepitans* (L.)*	*Brachinus explodens* Duft *	*Brachinus ganglbaueri* Apfb.**	B		
										C	D	E
Spring of 1976	1	Babocsa	605	60	–	–	–	–	–	1	0.06	1
	2	Bakonszeg	481	48	–	–	–	–	–	–	–	–
	3	Battonya	1084	117	–	–	–	–	–	3	0.09	2
	4	Bácsborsod	868	90	–	–	–	–	–	9	0.30	5
	5	Berzence	427	44	–	–	–	–	–	77	5.25	18
	6	Békésszentandrás	486	51	–	–	–	–	–	41	2.40	12
	7	Csengerújfalu	364	38	–	–	–	–	–	–	–	–
	8	Debrecen	621	65	–	–	–	–	–	21	0.96	8
	9	Encs	211	22	–	–	–	–	–	8	1.08	4
	10	Hajdúnánás	575	60	–	–	–	–	–	6	0.30	3
	11	Hatvan	518	53	–	–	–	–	–	7	0.39	4
	12	Kisköre	124	13	–	–	–	–	–	5	1.17	4
	13	Lábod	407	43	–	–	–	–	–	–	–	–
	14	Makó	813	83	–	–	–	–	–	22	0.81	9
	15	Magyarbánhegyes	606	60	–	–	–	–	–	4	0.21	2
	16	Medgyesháza	562	59	–	–	–	–	–	11	0.57	4
	17	Mezőberény	922	90	–	–	–	–	–	2	0.06	2
	18	Nagyatád	519	54	–	–	–	–	–	24	1.32	6
	19	Nagydobos	208	22	–	–	–	–	–	18	2.46	7
	20	Nagyecsed	852	98	–	–	–	–	–	20	0.63	7
	21	Orosháza	373	38	–	–	–	–	–	25	1.93	8
	22	Sárospatak	271	26	–	–	–	–	–	9	1.05	4
	23	Somogysárd	436	46	–	–	–	–	–	–	–	–
	24	Szamosszeg	286	30	–	–	–	–	–	36	3.60	10

Table 1e (cont'd)

Period of sampling	Serial number	Place-name	Area sampled (ha)	A	*Microlestes minutulus* (Goeze)*	*Orypta dentata* (Rossi)	*Brachinus crepitans* (L.)*	*Brachinus explodens* Duft.*	*Brachinus ganglbaueri* Apfb.**	B C	B D	B E
Sampling of	25	Szeged	182	16	–	–	–	–	–	14	2.64	9
1976	26	Szentes	1542	162	–	–	–	–	–	40	0.85	13
	27	Tiszabercel	180	12	–	–	–	–	–	8	2.19	3
	28	Vállaj	313	34	–	–	–	–	–	48	4.23	8
Autumn of	29	Abony	491	48	–	–	–	–	–	182	11.37	15
1976	5	Berzence	404	18	–	–	–	–	–	1	0.18	1
	8	Debrecen	387	67	–	–	–	–	–	26	1.17	8
	30	Hajdúdorog	512	46	–	–	–	–	–	1	0.06	1
	31	Sárrétudvari	827	82	–	–	–	–	–	452	16.53	29
	23	Somogysárd	813	92	–	–	–	–	–	7	0.24	5
	26	Szentes	420	41	–	–	–	–	–	18	1.23	8
	32	Szentmártonkáta	150	18	–	–	–	–	–	4	0.66	2
	27	Tiszabercel	277	16	–	–	–	–	–	17	3.18	8
Spring of	33	Balmazújváros	760	72	–	–	–	–	–	–	–	–
1977	3	Battonya	227	20	–	–	–	–	–	22	3.30	5
	10	Hajdúnánás	288	30	–	–	–	–	–	12	1.20	5
	34	Nagykálló	411	46	–	–	–	–	–	–	–	–
	35	Telekgerendás	1039	103	–	–	–	–	–	5	0.15	3
Autumn of	22	Sárospatak	1100	111	–	–	–	–	–	67	1.80	15
1977	24	Szamosszeg	184	19	–	–	–	–	–	141	22.26	28
Spring of	2	Bakonszeg	386	37	–	–	–	–	–	–	–	–
1978	36	Nagypetend	1160	118	–	–	–	–	–	48	1.65	16
	22	Sárospatak	837	88	–	–	–	–	–	44	1.14	10
		Total	25589	2609	5	2	8	4	3	1506		
		Dominance %			0.33	0.13	0.53	0.27	0.20		1.73	

A: Number of sample units
B: Number of individuals
C: Total
D: pcs/m^2
E: Number of species

Table 2

Number of individuals of ground-beetle larvas in 1976-78, in the individual farms

Period of sampling		Place-name	*Cicindela* spp.	*Clivina* spp.	*Broscus cephalotes*	*Trechus* spp.	*Anisodactylus signatus*	*Harpalus et Pseudophonus*	*Pterostichus et Poecilus* spp.	*Zabrus tenebrioides*	*Amara* spp.	A	B	C
Spring of 1976	1	Babocsa	–	2	–	3	–	–	7	3	–	–	15	0,25
	2	Bakonszeg	–	–	–	–	1	5	2	–	–	–	8	0,17
	3	Battonya	1	3	–	1	2	75	8	–	–	–	90	0,77
	4	Bácsborsod	–	–	–	–	–	–	1	–	–	–	1	0,01
	5	Berzence	–	–	–	–	–	5	1	3	–	–	9	0.21
	6	Békésszentandrás	–	–	–	–	1	84	3	5	9	–	102	2,00
	7	Csengerújfalu	–	6	–	4	–	21	–	–	–	–	31	0,82
	8	Debrecen	–	–	–	–	–	14	1	–	–	–	15	0,23
	9	Encs	–	–	–	–	–	1	–	–	–	–	1	0.05
	10	Hajdúnánás	1	–	–	–	–	4	2	–	–	–	7	0,12
	11	Hatvan	–	–	1	–	–	19	7	–	–	–	27	0,51
	12	Kisköre	–	–	–	–	–	–	–	–	–	–	–	–
	13	Lábod	–	–	–	–	–	5	–	–	–	–	5	0,12
	14	Makó	2	1	–	38	3	8	2	7	–	–	61	0,73
	15	Magyarbánhegyes	5	–	–	6	–	32	4	–	–	–	47	0,78
	16	Medgyesegyháza	–	–	–	1	–	3	1	3	–	–	8	0,14
	17	Mezőberény	1	–	–	–	–	23	10	1	–	–	35	0,39
	18	Nagyatád	–	–	–	1	–	43	3	9	–	–	56	1,04
	19	Nagydobos	2	–	–	–	–	6	1	–	–	–	9	0,41
	20	Nagyecsed	–	1	–	1	–	4	3	1	–	1	11	0,13
	21	Orosháza	–	–	–	–	–	8	2	–	–	–	10	0,26
	22	Sárospatak	–	–	–	–	–	–	–	–	–	–		
	23	Somogysárd	–	–	–	–	–	2	–	1	1	–	4	0,09
	24	Szamosszeg	–	11	–	5	–	4	3	–	–	–	23	0,77

Table 2 (cont'd)

Period of sampling		Place-name	*Cicindela* spp.	*Clivina* spp.	*Broscus cephalotes*	*Trechus* spp.	*Anisodactylus signatus*	*Harpalus et Pseudophonus*	*Pterostichus et Poecilus* spp.	*Zabrus tenebrioides*	*Amara* spp.	A	B	C
Spring of	25	Szeged	–	2	–	8	–	36	2	–	–	–	48	3,00
1976	26	Szentes	3	4	1	3	–	43	6	11	–	–	71	0,65
	27	Tiszabercel	–	–	–	–	1	7	1	–	1	–	10	0,91
	28	Vállaj	–	–	1	–	–	14	3	–	–	–	18	0,53
Autumn of	29	Abony	–	–	–	–	6	–	–	7	–	–	13	0,27
1976	5	Berzence	–	–	–	–	–	–	–	–	–	–	–	–
	8	Debrecen	–	–	–	–	1	–	1	1	–	–	3	0,05
	30	Hajdúdorog	–	–	–	–	–	–	–	2	–	–	2	0,04
	31	Sárrétudvari	–	–	–	–	11	9	1	91	–	–	112	1,37
	23	Somogysárd	1	–	–	–	–	–	2	–	–	–	3	0,03
	26	Szentes	–	–	–	–	–	4	4	55	–	–	63	1,43
	32	Szentmártonkáta	–	–	–	–	–	–	–	4	–	–	4	0,22
	27	Tiszabercel	–	–	–	–	4	1	–	–	–	–	5	0,31
Spring of	33	Balmazújváros	–	–	–	–	2	–	4	–	–	–	6	0,08
1977	3	Battonya	–	2	–	2	2	2	14	4	–	–	24	1,20
	10	Hajdúnánás	–	–	–	–	3	–	2	–	–	–	5	0,17
	34	Nagykálló	1	1	–	2	2	10	2	–	–	–	26	0,57
	35	Telekgerendás	1	1	–	7	26	7	17	–	–	–	59	0,57
Autumn of	22	Sárospatak	1	1	–	1	2	3	4	–	–	–	12	0,11
1976	24	Szamosszeg	–	4	–	–	17	9	8	1	–	–	39	2,05
Spring of	2	Bakonszeg	–	–	–	–	–	15	1	1	2	1	20	0,54
1978	36	Nagypeterd	2	–	–	–	–	1	9	3	–	6	21	0.24
	22	Sárospatak	–	–	–	2	11	18	5	–	–	–	36	0,31
		Total	21	39	3	90	90	571	131	209	13	8	1175	
		Dominance %	1.79	3.33	0.26	7.68	7.68	48.72	10.92	17.83	1.11	0.68	100 %	1.35

A: Other *Carabidae*
B: Total
C: Density of individuals (individual/m^2)

Table 3

Order of ground-beetle species exceeding the dominance value of 1 %

Serial number	Name	D%
1.	*Zabrus tenebrioides* (Goeze)	15.74
2.	*Platynus dorsalis* (Pontopp.)	12.75
3.	*Pseudophonus rufipes* (De Geer)	11.49
4.	*Anisodactylus signatus* (Panz.)	10.76
5.	*Trechus quadristriatus* (Schrank)	7.70
6.	*Harpalus distinguendus* (Duft.)	6.04
7.	*Poecilus cupreus* (L.)	5.71
8.	*Clivina fossor* (L.)	5.05
9.	*Bembidion properans* (Steph.)	4.65
10.	*Pterostichus longicollis* (Duft.)	2.19
11.	*Pseudophonus griseus* (Panz.)	1.39
12.	*Amara familiaris* (Duft.)	1.26
13.	*Poecilus sericeus* Fisch.	1.13
	Total	85.86

Rather stable and constant conditions of dominance of the arable soils are explained properly by the surveys of choice of habitat (Wallin, 1987). According to these surveys the dominant species establish high population levels on new plough-lands as well.

Considerable constancy can be observed regarding the density of ground-beetles and their larvae as well: in 1975 we registrated the frequency of 1.74 and 1.52 per m^2, respectively while in the new period (1976–78) their frequency was 1.73 and 1.35 per m^2, respectively.

According to our data of several years we make an attempt at outlining the species composition of *Carabidae* on Hungarian cultivated areas. We got the most part of our data from the territory of "Alföld" (the Hungarian Plain) but we have data at our disposal from samples taken by some farms (4 in 1975 and 6 in 1976–78) of the southern part of the Transdanubia. "Alföld" does not seem to be homogeneous since from its northeastern part ("Tiszahát", "Szatmár" and "Bereg") besides the dominant species far more colouring elements were found (Szamosszeg – 28 species, Sárospatak – 20 species, Mátészalka (1975) – 24 species etc.) "Sárrét" (south-south-eastern part of Hungary) shows different conditions from the "Alföld" as well, since in "Sárrét" the fauna of plough-lands is richer in species as well (Sárrétudvari – 29 species, Kötegyán (1975) – 35 species, Komádi (1975) – 24 species). The "colouring" elements of these two areas are not the same. Thus the species characteristic of east-south-eastern part of "Alföld" are for example: *Trichotichnus maculicornis, Pterostichus melas, Leistus ferrugineus, Harpalus nitidulus, Brachinus ganglbaueri* while the colouring elements of north-eastern part of "Alföld" are *Chlaenius spoliatus, Harpalus modestus, Agonum sexpunctatum, Harpalus dimidatus, Brachinus crepitans, Diachromus germanus, Bembidion biguttatum, Bembidion inoptatum* etc.

The southern part of Transdanubia is also characterised by the higher rate of occurrence of colouring elements (Kutas (1975) – 13 species, Nagyatád (1975) – 15

species, Somogysárd (1975) – 13 species, Berzence – 18 species) but the species of this area are different from the species of "Alföld" (*Acupalpus teutonus, Clivina contracta, Agonum viridicupreum, Poecilus lepidus, Pterostichus vernalis, Ophonus signaticornis*).

The areas outside "Alföld" and the border areas of "Alföld" have different and richer fauna than "Alföld" has. It reflects more diversified, proportioned and humid circumstances of these areas.

The effects of preceding crops cannot be evaluated reliably considerint that the sampling was aimed at planned areas of cultures sensitive to soil-inhabiting pests and not at the forecrop and it follows that the samplings were carried out some months or for example after cereals in spring of next year, a half year after harvesting the culture given. During this period considering their high degree of mobility the ground-beetles could move to biotopes being more favourable for them and thus the composition of ground-beetles characteristic of the single cultures could change basically.

Sampling is carried out after cereals and perennial papilionaceae in general after a longer period than after hoed plants (cereal was mainly winter wheat, hoed plant was mainly maize and perennial papilionaceous plant was lucerne in general).

Results of the surveys are distorted also by the fact that the number of samples was not identical in different plants (hoed plants – 10 410 samples, 44 species; cereals – 8660 samples, 38 species and perennial papilionaceae – 1830 samples, 25 species) and as it is visible if the number of samples was less the number of species collected was also less. It contradicts somewhat to data of the former survey (Horvatovich and Szarukán, 1986) since in 1975 in spite of the hoed culture (maize) the density of insects and the number of species were less than after winter cereals. The low level of species and insect density can be explained unanimously by the far less samples in both cases.

In spite of the methodological problems mentioned and considering the results of the former survey we try to sketch the affinity relations of the most frequent species towards the single cultures (Table 4). Attraction of *Zabrus tenebrioides* towards cereals is well known but in this case it is not proven by our data (nor the adults nor the larvae; Table 5). Similarly to the earlier years *Platynus dorsalis* occurred in cultures having higher number of samples (hoed plants, cereals) with more considerable number. Dominance of *Pseudophonus rufipes* is high in every of the three cultures in this case as well. It is extremely high after perennial papilionaceae and almost at the same level in cultures of corn in the ear and of hoed plants (10.66 and 9.97 D%, respectively) similarly to data of 1975 proving the claim of this species to humidity and wide ecological valency and good capability of this species to change of place. *Anisodactylus signatus* shows similar capabilities to the former species in 1976–78. *Harpalus distinguendus* occurred with about the same similarity in every of the three cultures (5.87–7.69%) but regarding its density it has a great importance in perennial papilionaceae (similarly to *Pseudophonus rufipes* and to *Anisodactylus signatus*). Data of density and dominance of *Poecilus cupreus* are similar to the former species but with lower values and with some preference to perennial papilionaceae. Clivina fossor shows similar tendency to the former species but with somewhat lower value of density and dominancy. We should stress the attachment of *Trechus quadristriatus* to hoed

Table 4

Number of individuals of ground-beetle imagos grouped according to the preceding crops

Name of species	Cereals			Hoed plants (maize)			Perennial papilion		
	I	I/m^2	D%	I	I/m^2	D%	I	I/m^2	D%
Carabus granulatus L.	–	–	–	2	0.0058	0.29	–	–	–
Clivina collaris (Herbst)	–	–	–	–	–	–	1	0.164	0.77
Clivina fossor (L.)	18	0.0623	4.08	30	0.0864	4.40	9	0.1475	6.92
Broscus cephalotes (L.)	–	–	–	2	0.0058	0.29	–	–	–
Trechus quadristriatus (Schrank)	19	0.0658	4.31	74	0.2133	10.85	5	0.0820	3.85
Bembidion properans (Steph.)	19	0.0658	4.31	39	0.1123	5.72	7	0.1147	5.38
Bembidion quadrimaculatum (L.)	4	0.0138	0.91	4	0.0115	0.59	4	0.0656	3.08
Bembidion striatum (F.)	–	–	–	1	0.0029	0.15	–	–	–
Asaphidion flavipes (L.)	–	–	–	1	0.0029	0.15	–	–	–
Anisodactylus signatus (Panz.)	41	0.1420	9.30	70	0.2016	10.26	20	0.3279	15.38
Diachromus germanus (L.)	1	0.0035	0.23	1	0.0029	0.15	1	0.0164	0.77
Ophonus signaticornis (Duft.)	–	–	–	2	0.0058	0.29	1	0.0164	0.77
Pseudophonus calceatus (Duft.)	1	0.0035	0.23	9	0.0259	1.31	–	–	–
Pseudophonus griseus (Panz.)	8	0.0277	1.81	12	0.0346	1.76	1	0.0164	0.77
Pseudophonus rufipes (De Geer)	47	0.1628	10.65	68	0.1959	9.97	23	0.3770	17.69
Harpalus affinis (Schrank)	5	0.0173	1.13	8	0.0230	1.17	2	0.0328	1.54
Harpalus dimidiatus (Rossi)	1	0.0035	0.23	–	–	–	1	0.0164	0.77
Harpalus distinguendus (Duft.)	27	0.0935	6.12	40	0.1152	5.87	10	0.1639	7.69
Harpalus flaviocornis Dej.	2	0.0069	0.45	–	–	–	–	–	–
Harpalus hospes Sturm	–	–	–	1	0.0029	0.15	–	–	–
Harpalus pygmaeus Dej.	–	–	–	1	0.0029	0.15	–	–	–
Harpalus servus (Duft.)	3	0.0104	0.68	–	–	–	–	–	–
Harpalus tardus (Panz.)	2	0.0069	0.45	–	–	–	–	–	–
Stenolophus teutonus (Schrank)	–	–	–	1	0.0029	0.15	1	0.0164	0.77
Acupalpus meridianus (L.)	5	0.0173	1.13	–	–	–	–	–	–
Stomis pumicatus (Panz.)	2	0.0069	0.45	6	0.0173	0.88	–	–	–
Poecilus cupreus (L.)	28	0.0970	6.35	34	0.0980	4.99	11	0.1803	8.46
Poecilus lepidus (Leske)	1	0.0035	0.23	2	0.0058	0.29	–	–	–

Table 4 (cont'd)

Name of species	Cereals			Hoed plants (maize)			Perennial papilion		
	I	I/m^2	D%	I	I/m^2	D%	I	I/m^2	D%
Poecilus puncticollis (Dej.)	–	–	–	1	0.0029	0.15	–	–	–
Poecilus sericeus Fisch.	6	0.0208	1.36	8	0.0230	1.17	3	0.0491	2.31
Poecilus versicolor (Sturm)	–	–	–	8	0.0230	1.17	–	–	–
Pterostichus inquinatus (Sturm)	1	0.0035	0.23	–	–	–	–	–	–
Pterostichus longicollis (Duft.)	1	0.0035	0.23	2	0.0058	0.29	–	–	–
Pterostichus macer (Marsch.)	2	0.0069	0.45	–	–	–	–	–	–
Pterostichus melanarius (Illig.)	–	–	–	7	0.0202	1.03	1	0.0164	0.77
Pterostichus vernalis (Panz.)	1	0.0035	0.23	–	–	–	–	–	–
Calatnus melanocephalus (L.)	–	–	–	1	0.0029	0.15	–	–	–
Dolichus halensis (Schall.)	–	–	–	–	–	–	1	0.0164	0.77
Agonum sexpunctatum (L.)	–	–	–	–	–	–	1	0.0164	0.77
Agonum viridicupreum (Goeze)	–	–	–	1	0.0029	0.15	–	–	–
Platynus dorsalis (Pontopp.)	83	0.2875	18.82	95	0.2737	13.93	2	0.0328	1.54
Europhilus micans (Nic.)	–	–	–	1	0.0029	0.15	–	–	–
Zabrus tenebrioides (Goeze)	83	0.2875	18.82	126	0.3631	18.48	19	0.3115	14.61
Amara aenea (De Geer)	4	0.0138	0.91	5	0.0144	0.73	1	0.0164	–0.77
Amara apricaria (Payk.)	3	0.0104	0.68	2	0.0058	0.29	–	–	–
Amara consularis (Duft.)	5	0.0173	1.13	2	0.0058	0.29	–	–	–
Amara eurynota (Panz.)	–	–	–	1	0.0029	0.15	–	–	–
Amara familiaris (Duft.)	5	0.0173	1.13	2	0.0058	0.29	2	0.0328	–1.54
Amara incognita Fassati	1	0.0035	0.23	1	0.0029	0.15	–	–	–
Amara similata (Gyll.)	2	0.0069	0.45	–	–	–	2	0.0328	1.54
Amara tricuspidata Def.	4	0.0138	0.91	3	0.0086	0.43	–	–	–
Masoreus wetterhallii (Gyll.)	1	0.0035	0.23	–	–	–	–	–	–
Microlestes meurus (Sturm.)	1	0.0035	0.23	–	–	–	–	–	–
Microlestes minutulus (Goeze)	–	–	–	3	0.0086	0.43	–	–	–
Drypta dentata (Rossi)	1	0.0035	0.23	1	0.0029	0.15	–	–	–
Brachinus crepitans (L.)	2	0.0069	0.45	1	0.0029	0.15	–	–	–
Brachinus explodens Duft.	1	0.0035	0.23	1	0.0029	0.15	1	0.0164	0.77
Brachinus ganglbaueri Apfb.	–	–	–	2	0.0058	0.29	–	–	–
Total	441	1.5277	100	682	1.9654	100	130	2.1312	100
Number of species	38			44			25		
Number of samples	8660			10410			1830		

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Table 5

Number of individuals of ground-beetle larvae grouped according to the preceding crops

Name of species	Cereals			Hoed plants (maize)			Perennial papilion		
	I	I/m^2	D%	I	I/m^2	D%	I	I/m^2	D%
Cincidela spp.	8	0.0276	1.58	7	0.0201	1.73	2	0.0327	2.50
Clivina spp.	13	0.0450	2.57	11	0.0317	2.72	8	0.1311	10.00
Broscus cephalotes	–	–	–	4	0.0115	0.99	1	0.0164	1.25
Trechus spp.	38	0.1317	7.51	10	0.0288	2.48	6	0.0984	7.50
Anisodactylus signatus	32	0.1110	6.32	32	0.0921	7.92	5	0.0819	6.25
Harpalus et Pseudophonus spp.	282	0.9768	55.73	234	0.6743	57.93	8	0.1311	10.00
Pterostichus et Poecilus spp.	42	0.1455	8.30	47	0.1354	11.63	8	0.1311	10.00
Zabrus tenebrioidcs	79	0.2736	15.61	52	0.1498	12.87	41	0.6721	51.25
Amara spp.	10	0.0345	1.98	3	0.0086	0.74	–	–	–
Others	2	0.0069	0.40	4	0.0115	0.99	1	0.0164	1.25
Total	506	1.7529	100	404	1.1643	100	80	1.3116	100

Table 6

Density of indivuals and species of ground-beetles according to size of fields

A	B	C	D	E	F	G
24 ha >	62	116	1.58	1.29	2.20	3.78
25–48 ha	135	502	1.08	0.78	1.30	2.38
49–72 ha	106	633	1.33	0.67	1.46	2.79
73 ha <	132	1358	1.43	0.62	1.98	3.41
Total	1506	2609	1.35	0.84	1.73	3.09

A: Size of field
B: Number of fields
C: Number of sample units
D: Density of individuals of ground-beetle larvae (number of individuals/m^2)
E: Density of species of ground-beetles imagos (number of species /m^2)
F: Density of individuals of ground-beetle imagos (number of individuals/m^2)
G: Density of individuals of ground-beetles (larvae + imagos/m^2)

plants. From the species having lower level of dominance and density *Bembidion properans* is worthy for mention but in this case its attachment to hoed plants is not perceptible. In present case *Amara familiaris* (in 1975 *Amara aenea*) was the most frequent species of the genus (with lower values) that is characterized by its affinity to perennial papilionaceae, similarly to *Amara similata.*

Our knowledge concerning overwintering of more frequent species (survive the winter in form of imago that means that they breed in spring: *Platynus dorsalis, Amara aenea, Amara familiaris, Clivina fossor, Anisodactylus signatus, Harpalus distinguendus, Poecilus cupreus* survive the winter in form of larva that means that they breed in autumn: *Pseudophonus rufipes, Ps. griseus, Trechus quadristriatus, Poecilus sericeus, Zabrus tenebrioides, Bembidion properans*) cannot be managed rigidly since these insects are very adaptable in this respect. Since of all of the larvae were not determinated exactly to species this can be proven first of all in autumn breeders because their imagos can be found also in spring. From the spring breeders it is proven too in cases of *Anisodactylus signatus* and *Clivina fossor* regarding their larvae collected in autumn and in spring. (Actual overwintering in form of larvae we could experience only at some species of lower frequency for example at *Cicindela germanica* L., *Broscus cephalotes* (L.), and *Dolichus halensis* (Shall.). Presumably it can be stated in general that specimens of dominant species belonging to both of types of overwintering can survive the winter in more or less rate by the form characteristic of the other type as it is proved in cases of some species by others (Kadocsa, 1941, Lindropth, 1945, Kasandrova and Sharova, 1971 etc.).

We should touch upon the connection between the size of field and the number of specimens in this case as well (Table 6). If we investigate the fields sampled by 24 ha in growing groups it is striking that fields smaller than 24 ha show the highest value by far considering their insect density and species number as well in spite of that relatively less fields belong to this category (62) and because of the method of the fields (because the number of samples taken is also higher) but neither the insect density nor the species number reach the value experienced on the smallest fields.

Table 7

Species found only during the sampling of 1975

Serial number	Name of species	Habitat	Area	Name of farms	Number of specimens
1.	*Amara communis*	wet meadows	Europe, N. Asia	Baja	4
2.	*Amara convexior*	wet meadows	Europe, N. Asia	Hajduszoboszló, Kötegyán	3
3.	*Amara crenata*	sandy soils	S. Europe	Kötegyán	1
4.	*Amara lucida*	wet soils	Europe, N. Asia	Kötegyán, Gyöngyöspata	8
5.	*Amara plebeja*	wet meadows	N. and C. Europe	Sárrétudvari	1
6.	*Asaphidion pallipes*	water sides	Europe, Asia Minor, W. Siberia	Somogysárd	1
7.	*Bembidion lampros*	shaded soils	Holarctic species	Sárrétudvari	1
8.	*Bembidion obtusum*	dry meadows, lucerne	W. and C. Europe	Kötegyán	1
9.	*Calosoma auropunctatum*	dry meadows	Europe, Asia Minor	Hajdúböszörmény	1
10.	*Carabus cancellatus*	wet meadows	Europe, Siberia, Mongolia	Debrecen, Komádi	9
11.	*Chlaenius spoliatus*	subhalobiontic, water-sides	S. and C. Europe	Mátészalka	1
12.	*Harpalus anxius*	sandy soils	Palearctic species	Komádi	2
13.	*Ophonus azureus*	chalky, sandy soils	Europe, W. Asia	Komádi, Kötegyán, Szentes	6
14.	*Ophonus rufibarbis*	groves, fallow lands	Palearctic species	Kötegyán	2
15.	*Ophonus diffinis*	dry soils	S. Europe, Asia Minor	Kötegyán	3
16.	*Harpalus froelichi*	sandy soils	Europe, Siberia	Derecske, Nyírtelek	17
17.	*Harpalus melletii*	dry soils	W. and C. Europe	Kötegyán	2
18.	*Harpalus modestus*	sandy soils	Europe, Siberia, Japan	Mérk	1
19.	*Harpalus politus*	wet meadows	Europe, Siberia	Kötegyán	1
20.	*Harpalus punctatulus*	dry soils	Europe, Siberia	Kötegyán	1
21.	*Harpalus rupicola*	dry soils	Europe, Asia Minor	Gyula, Komádi	2
22.	*Harpalus tenebrosus*	dry soils	N. Africa, Europe, Trans-Kaspia	Gyöngyöspata	1
23.	*Harpalus zabroides*	chalky soils	S. Europe, W. Siberia	Sárrétudvari	1
24.	*Leistus ferruguineus*	dry meadows	Europe	Sárrétudvari	1
25.	*Parophonus mendax*	dry soils	S. Europe	Kötegyán	1
26.	*Pterostichus melas*	warm and wet soils	S. and C. Europe	Komádi	1
27.	*Poecilus punctulatus*	sandy soils	C. and E. Europe	Sárrétudvari	1
28.	*Tachys bistriatus*	water sides	N. Africa, Europe	Kötegyán	1
29.	*Trichotichnus maculicornis*	chalky, sandy soils	S. Europe, W. Asia	Kötegyán	1

Table 8

Species found only in 1976–78

Serial number	Name of species	Habitat	Area	Name of farms	Number of specimens
1.	*Carabus granulatus*	wet meadows	Europe, Siberia	Szeged, Szentes	2
2.	*Bembidion biguttatum*	water sides	Europe, W. Siberia	Szamosszeg	1
3.	*Bembidion inoptatum*	water sides	S.-W. Europe	Szamosszeg	1
4.	*Bembidion striatum*	river sides	Europe, Siberia	Sárospatak	1
5.	*Harpalus flavicornis*	heath	S.-W. Europe	Sárrétudvari	2
6.	*Harpalus pygmaus*	heath	S. Europe	Sárrétudvari	1
7.	*Harpalus servus*	sandy soils	Europe, Siberia	Sárrétudvari	3
8.	*Acupalpus interstitialis*	chalky soils	Europe, Asia Minor	Szamosszeg	10
9.	*Poecilus puncticollis*	heath	S. Europe	Sárrétudvari	1
10.	*Poecilus versicolor*	dry meadows	Europe, Siberia	Berzence	8
11.	*Pterostichus inquinatus*	heath	S.-W. Europe	Békésszentandrás	1
12.	*Pterostichus ovoideus*	wet meadow	Europe, Siberia	Szamosszeg	2
13.	*Agonum sexpunctatum*	water sides	Europe, Siberia	Szamosszeg	1
14.	*Agonum cupreum*	water sides	S. Europe, Morocco	Berzence	1
15.	*Europhilus micans*	water sides	Europe, Siberia	Szeged	1
16.	*Amara anthobia*	heath	Europe	Szamosszeg	1
17.	*Amara incognita*	heath	S.-W. Europe	Sárospatak, Szamosszeg	3
18.	*Amara tricuspidata*	wet meadows	Europe	Berzence	8
19.	*Masoreus wetterhallii*	sandy soils	N. Africa, Europe	Makó	1
20.	*Microlestes maurus*	clayey soils	Europe	Sárrétudvari	1

The insect density and species number seem to be growing by further increase of the size of the fields (because the number of samples taken is also higher) but neither the insect density nor the species number reach the value experienced on the smallest fields. The smallest fields are striking mainly considering their species density, their characteristic value (1.29 species/m^2) is almost twice higher than of the larger fields. Relatively larger edge and more diversified surroundings of the smaller fields explain this phenomenon experienced by us earlier as well. It is inconsistent with the observations of Lővei (1989) who could not demonstrate edge-effect in maize fields.

This phenomenon can be analysed also from nature protectional point of view. More specimens and more species live on the smaller fields and thus diversity of smaller fields is larger that is important because of preservation the original state of fauna.

We presented 29 species accounted colouring elements in Table 7 (according to our survey of 1975) with their typical habitat preferences and with their ranges. From the analysis of habitat and ranges it emerges that we can find species living on humid soils (8 species) and species living in arid habitats as well but the rate of drought-resistant species was a bit more than twice of the number of species preferring humid soils.

The species shown in Table 8 are also colouring elements since they were found in plough-lands only on the occasion of the last succession of surveys (1976–78). Amongst the 20 species listed there the rate of species living on humid and on arid soils (9 and 11, respectively) is almost the same because of the different conditions of soils of the individual farms.

The following species of the 94 species are considered as rare ones in Hungary: *Acupalpus interstitialis* (Reitt.), *Asaphidion pallipes* (Duft.), *Bembidion obtusum* Audinet-Serville, *Calosoma auropunctatum* (Herbst), *Harpalus politus* (Dej.), *Harpalus pygmaeus* (Dej.), *Harpalus tenebrosus* (Dej.), *Harpalus zabroides* (Dej.), *Masoreus wetterhalli* (Gyll.), *Ophonus diffinis* (Dej.), *Parophonus maculicornis* (Duft.), *Parophonus mendax* (Rossi), *Poecilus puncticollis* (Dej.), *Poecilus punctulatus* (Shall.).

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SEASONAL ABUNDANCE OF CABBAGE LOOPER *TRICHOPLUSIA NI* AS RELATED TO TEMPERATURE, RELATIVE HUMIDITY AND THE NATURAL INCIDENCE OF A NUCLEAR POLYHEDROSIS VIRUS

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Infection rates of a nuclear polyhedrosis virus (NPV) in the cabbage looper, *Trichoplusia ni* (Hb.) larvae were assessed during the years 1985 and 1986 in El-Behiera, El-Fayoum and El-Gharbia governorate, Egypt. The incidence of the pathogen in El-Gharbia larval population significantly surpassed its occurrence in El-Fayoum and El-Behiera populations. Infection rates of NPV were highest as larval population peaked indicating the close relationship of natural virus incidence with density of larvae. Certain micro-climatic factors appeared to dictate the magnitude and duration of the epizootic after first incidence of disease was observed. Effects of temperature and relative humidity on pathogen virulence and spread were found to be locality and seasonality dependent. Natural termination of NPV epizootic occurred when environmental temperature rose above a temperature threshold of 28 °C.

Key words: *Trichoplusia ni*, NPV, cabbage, infection rate

Introduction

The cabbage looper, *Trichoplusia ni* (Hb.) is a major pest of cruciferous plants throughout the world and causes usually considerable yield loss (Harrison and Brubaker, 1943; Geizin, 1962; Prasad, 1963; Whalfenbarger, 1967; Martin et al., 1976; Ali et al., 1984). Hazards resulting from the application of chemical control on crucifers, particularly fresh marketing vegetables such as cabbage and cauliflower, created the search for a new powerful non-toxic protection for consumers that efficiently suppresses the pest population. The biological control has become a great priority for the control of such serious insect pests. Among the biological agents, insect viruses which showed a bio-control potential against insect pests particularly lepidopterous larvae (Abul-Nasr, 1965; Hall, 1957; Tanada and Omi, 1974; Hassan and Moawad, 1974).

The nuclear polyhedrosis virus has been long recognized as an important natural mortality factor affecting populations of the cabbage looper *Trichoplusia ni* in United States of America (Semel, 1956; McEwen and Harvey, 1958; Hofmaster, 1961; Jaques, 1974). Elmore (1961) reported that a nuclear polyhedrosis virus disease of the cabbage looper appears to be quite virulent under a wide variety of weather conditions. The disease was effective during April as September when temperatures were much higher. Since chemical control measure is the only available for controlling this pest in cruciferous crops in Egypt, an intensive work have been initiated to assess the natural incidence of NPV disease in different populations of the cabbage worm larvae collected from different localities of unsimilar environmental conditions.

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Materials and methods

Larvae of the cabbage looper were sampled from cabbage fields located at three different governorates during the period extending from July to November of 1985 and 1986. In El-Fayoum and El-Beheira governorates, two cabbage fields, one feddan each (feddan = 0.75 hectare = 4200 m^2), were chosen for sampling. In El-gharbia governorate, larger area of cabbage was available therefore two fields of cabbage, 3–4 feddans each, were chosen in 1985 while two fields, five feddan each, were assigned for sampling in 1986. Five randomized plots, 42 m each, were selected from each cabbage field where 10 cabbage plants in each plot were inspected for cabbage looper presence. Field-collected larvae were preserved with cabbage leaves in glass jars covered with muslin and transferred to the laboratory where they were fed individually on cabbage leaves in 1/4 lb. glass jars and kept under laboratory conditions of 26 + 1 C and 70 + 5% relative humidity. Larvae were daily inspected for the occurrence of virus disease. Isolation of virus-infected larvae, identification and calculating percentages of larval mortality were carried out as described in detail by Harrap et al. (1977) and Ali et al. (1991). Records of environmental temperature and relative humidity were supplied by the meteorological station located at each locality.

Results and discussion

(1) Seasonality and locality trends

El-Fayoum governorate

Larvae of the cabbage looper were first observed in the field in appreciable numbers on the first of July (Tables 1 and 2).

After that date, the population density of larva increased gradually showing two peaks by early September and mid-October during the first season, while these peaks occurred on mid-August and October 1st in 1986. The highest populations of larvae were found on early September 1985 and mid-August 1986. It was noticed that the population density of larvae gradually declined towards the end of cabbage season.

July was somewhat hot and dry, therefore virus was unprevalent throughout the entire period of July in both cabbage seasons. The nuclear polyhedrosis virus was first observed in cabbage fields during the first two weeks of August and the percentage of virus infection averaged 17.2 and 20 in 1985–1986, respectively. By the increase of relative humidity from mid-August and through October, virus disease became widespread and virulent bearing a peak of looper infection reaching 37.5 in 1985 and 26.8% in 1986. A close relation between relative humidity and virus infection was found in nature. The rate of virus infection ranged between 21.89 and 24.6% with an average of 23.3% for both seasons.

El-Gharbia governorate

In El-Gharbia governorate, the population of *T. ni* larva started with a few numbers by early July in 1985 and 1986 (Tables 1 and 2). Larval population peaked three times during the first cabbage season on early August, September and October, respectively during 1985, while in 1986 two peaks were only found on mid-August and October 1st. The heaviest cabbage infestation was shown by early September. Looper population gradually decreased towards the end of cabbage season.

Table 1

Biweekly numbers of collected and rates of virus infection of Trichoplusia ni infesting cabbage in relation to some weather factors in different governorates, Egypt (Season, 1985)

Date of inspection	Governorate	Total No. of collected larvae	No. of infected larvae	% Infection	Weather factors	
					Temp. °C	R. H. %
July 1		4	0	0.00	28.3	52
15		9	0	0.00	28.3	53
Aug. 1		15	0	0.00	27.3	53
15		29	5	17.24	28.9	56
Sept. 1	Fayoum	62	19	30.64	29.6	59
15		39	11	28.20	29.3	59
Oct. 1		24	9	37.50	26.5	62
15		37	13	35.13	26.7	64
Nov. 1		33	5	15.15	23.1	65
	Total	252	62	–		
	Mean	28	6.89	24.60		
July 1		4	0	0.00	26.5	66
15		9	0	0.00	27.9	74
Aug. 1		16	9	56.25	26.2	77
15		12	7	58.33	25.4	77
Sept. 1	Gharbia	30	15	50.00	26.1	75
15		10	6	60.00	26.1	71
Oct. 1		24	9	37.50	25.0	70
15		11	4	36.36	23.1	68
Nov. 1		5	0	0.00	21.7	77
	Total	121	50	–		
	Mean	33.49	6.56	41.32		
July 1		8	0	0.00	28.0	62
15		21	0	0.00	27.0	67
Aug. 1		37	5	13.50	27.7	62
15		29	6	20.68	28.4	69
Sept. 1	Behiera	21	4	19.40	27.2	67
15		49	9	18.36	26.0	70
Oct. 1		30	4	13.33	23.7	66
15		34	5	14.70	21.4	70
Nov. 1		22	3	13.63	20.8	79
	Total	251	36	–		
	Mean	27.88	4.00	14.34		

Table 2

Biweekly numbers of collected and rates of virus infection of Trichoplusia ni infesting cabbage in relation to some weather factors in different governorates, Egypt (Season, 1986)

Date of inspection	Governorate	Total No. of collected larvae	No. of infected larvae	% Infection	Weather factors	
					Temp. °C	R. H. %
July 1		3	0	0.00	28.3	49
15		9	0	0.00	28.3	54
Aug. 1		25	5	20.00	30.2	56
15		82	22	26.82	29.5	59
Sept. 1	Fayoum	38	9	23.68	28.2	58
15		30	8	26.66	28.4	57
Oct. 1		33	5	15.15	23.5	58
15		20	3	15.00	23.6	62
Nov. 1		9	1	11.11	18.2	64
	Total	249	53	–		
	Mean	27.67	5.89	21.89		
July 1		11	0	0.00	26.5	62
15		13	0	0.00	28.7	70
Aug. 1		17	0	0.00	26.6	70
15		33	12	36.36	28.1	70
Sept. 1	Gharbia	19	7	36.84	27.1	63
15		27	9	33.33	27.8	67
Oct. 1		66	21	31.81	23.3	72
15		32	8	25.00	23.4	72
Nov. 1		28	2	7.14	22.8	69
	Total	246	59	–		
	Mean	27.33	6.56	23.98		
July 1		4	0	0.00	27.6	60
15		15	0	0.00	28.3	66
Aug. 1		32	6	18.75	28.2	62
15		27	8	29.62	28.1	65
Sept. 1	Behiera	33	9	27.27	25.1	70
15		35	9	25.71	22.1	65
Oct. 1		25	3	12.00	22.5	64
15		27	4	14.81	22.9	65
Nov. 1		20	1	5.00	22.2	69
	Total	218	40	–		
	Mean	24.22	4.44	18.38		

Nuclear polyhedrosis virus was first noticed in the field by August 1st, 1985 and two weeks later in 1986. Virus infection percent averaged 36.4%. The pathogen widely spread and became more virulent by increasing of relative humidity rather than temperature particularly during the first season when rates of virus infection eventually enumerated that of the second season. Population density on the other side showed pronounced association with degree of virus infectivity. This phenomenon was quite apparent as reflected by the presence of the highest rates of virus infection in nature when large population of loopers occur. The maximum rates of virus incidence occurred on mid-September 1985 (60%) and one month earlier in 1986.

El-Behiera governorate

Cabbage loopers were first observed in the field on first of July when an average of 4–8 larvae per 10 cabbage plants were recorded (Tables 1 and 2). The period extending from August 1st to mid-October was characterized by prevailing of mild temperature (21–27 °C) and high relative humidity (62–70%) which created the development of cabbage looper. Two peaks of looper population were noticeable either in 1985 or in 1986, and occurred resembly on early August and mid-September.

The aforementioned results declare that August and particularly September had the highest populations of larvae in the different inspected localities. These results are of great correspondence with results reported by Hofmaster (1961) and El-Khouly (1979). They also denoted the presence of the highest population density of *T. ni* in cabbage fields during August and September.

Cabbage fields that were inspected during July 1985 and 1986 showed low infestation by the cabbage looper and the field-collected larvae were quite free of virus infection (Tables 1 and 2). On early August of the same seasons, cabbage loopers were found in the field in appreciable numbers when the first virus-infected larvae were detected. Percentages of virus infection averaged 13.5 and 18.8 on August 1st 1985 and 1986, respectively. During August and September, evidences on spread and virulence of the pathogen were noticed indicating to the association between humid weather and natural incidence of virus, since relative humidity exhibited considerable increase during these two months. High temperature and excess of humidity were also found to be the most important factors affecting the spread and virulence of *T. ni* – NPV in the United States of America (Elmore, 1961; Getzin, 1962).

(2) Environmental conditions

Concerning the influence of some environmental factors on virus infection in the cabbage looper worm population, a positive and highly significant correlation of larval density and natural incidence of NPV was found in 1985 and 1986 seasons as shown in Table 3.

Relative humidity, on the other hand, showed positive and significant effect on virus infection in El-Fayoum, while this effect was insignificantly positive in El-Gharbia governorate, in El-Behiera, a negative correlation was detectable. Heavy populations of larvae and high relative humidity increased the rates of virus infection under El-Fayoum environment, while larval density was the key factor regulating virus incidence

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Table 3

Correlation and partial regression values on the relationship between infestation rates of NPV and each of larval density of T. ni and certain climatic factors for sampling periods in 1985 through 1986

Locality	Season	Larval density		Daily mean temperature			Daily mean relative humidity (%)				Explained variance (%)		
		r	p	b	r	p	b	r	p	b	F value	p	E.V.
El-Fayoum	1985	0.782	5	–0.090	–0.059	NS	5.934	0.818	5	4.643	30.86	1	94.9
	1986	0.685	5	0.145	0.026	NS	1.609	0.603	5	2.541	4.72	5	73.5
El-Gharbia	1985	0.590	5	1.546	0.068	NS	–0.331	0.167	NS	0.428	0.82	NS	35.5
	1986	0.502	5	0.981	–0.143	NS	2.043	0.125	NS	–1.454	1.56	NS	48.4
El-Behiera	1985	0.640	5	0.558	0.643	5	0.313	–0.043	NS	0.733	1.65	NS	49.7
	1986	0.914	1	1.226	0.537	NS	0.185	–0.112	NS	–0.247	8.79	1	84.1

in El-Gharbia governorate. Temperature and population density of larvae were the most pronounced factors in El-Behiera (Table 3). The previously mentioned factors seem to be the most responsible factors for virus incidence in El-Fayoum where values of explained variance (effective rate) ranged 73–94%. Lower values (35.5–48.8%) were obtained under El-Gharbia conditions indicating that there are other environmental factors than these undertaken in the present study still responsible for virus incidence in this locality. In this respect, studies of McEwen and Harvey (1963), Getzin (1962), Heimpel et al. (1973) and Jaques (1974) showed that temperature, relative humidity and pest density are the most factors influencing the incidence and virulence of NPV in the cabbage looper populations. These studies are in harmony with our achieved data in the present investigation as shown in the different localities. However, the superiority of each factor showed big difference from one locality to the other one.

It was also noticed that the highest rate of virus incidence occurred in El-Gharbia governorate. This finding suggests the possibility of using virus disease as biological agent against cabbage looper in this locality. Because El-Fayoum governorate lies in the southern-west part of Egypt, temperature is usually higher which oppose the natural incidence and spread of the pathogen, while this effect was denoted by relative humidity in El-Behiera. However, the role played by the virus in suppressing the pest population still possible by increasing virus rate in the field by spraying cabbage fields with virus preparations.

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Plant Genetics and Breeding

LEAF-NRA IN CULTIVARS OF TRITICALE, WHEAT AND RYE AND ITS RELATIONSHIP WITH YIELDS OF GRAIN AND GRAIN CARBOHYDRATE AND PROTEIN

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A factorial randomized field experiment was conducted with four triticale cvs. (TL 419, Tigre "S", Muskox "S" and Delfin) and one cv. each of bread wheat (HD 1982) and rye (Russian Rye) at Aligarh, India, to determine the effect of nine combinations, comprising three doses each of nitrogen (150, 200 and 250 kg N ha^{-1}) and phosphorus (30, 40 and 50 kg P ha^{-1}) on nitrate reductase activity (NRA) in flag and penultimate leaves at three stages of crop development. Of the fertilizer doses, 200 kg N + 40 kg P ha^{-1} proved optimum for leaf-NRA at each stage. Moreover, leaf-NRA was highest in Delfin and lowest in HD 1982 wheat, and declined with the successive growth stages (tillering > heading > milky grain) as well as with the leaf age (unexpanded flag leaves > expanded flag leaves > penultimate leaves) at the corresponding stage.

The cvs. categorically exhibited consistent strong correlations between different yields and NRA levels at (i) the tillering stage (penultimate leaf-NRA of Muskox "S" and Tigre "S") and (ii) the milky grain stage (penultimate leaf-NRA of Russian Rye as well as expanded flag leaf-NRA of TL 419, Delfin and HD 1982 wheat). Thus, NRA levels in penultimate/flag leaves could be utilized as a reliable physiological parameter during the screening of improved cvs. of triticale.

Key words: triticale, wheat and rye, N, P and K fertilizers, nitrate reductase activity, expanded and unexpanded flag leaves, penultimate leaf, grain protein and carbohydrate yields, correlation studies

Introduction

Triticale, an intergeneric hybrid of wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.), combines high productivity of the former with high protein and lysine content of grain and disease resistance of the latter (Villegas et al., 1970) and has been claimed to be the future staple food for mankind (Zilinsky and Borlaug, 1971). However, rigorous in-depth research will be required to enrich triticale with genes that may enhance its grain yield and quality before cvs. capable of replacing traditional wheats are evolved. The easiest way for identifying such genes is to study the gene products (particularly enzymes that regulate plant metabolism) and their influence on grain yield and quality of crop. For cereals, inorganic nitrogen metabolism is second in importance only to photosynthesis. The key enzyme regulating inorganic nitrogen metabolism is nitrate reductase (NR) which has been shown to be heritable and subject to manipulation by classical breeding techniques (Hageman, 1990). NR (E. C. 1.6.6.1)

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is a substrate inducible enzyme and reflects potential input of reduced nitrogen into its products (Hewitt and Afridi, 1959; Afridi and Hewitt, 1962). Thus, NR meets the requirements for a physiological trait useful for selection of superior genotypes. The present investigation was, therefore, undertaken to study the association between nitrate reductase activity (NRA) in specified leaves of four triticale cvs. and their yield and quality, so as to ascertain whether NRA could be used as a tool for breeders during screening of improved triticale cvs.

Materials and methods

The experiment was conducted during the winter of 1984/1985 at the University Farm of Aligarh Muslim University, Aligarh, India (27°52'N, 78°51'E and 187.5 m altitude). The soil of the experimental field was sandy loam (73% sand, 8% silt, 19% clay), having pH (1 part soil: 2 parts distilled water): 7.5, E. C.: 0.35 m mhos cm^{-1}; N: 201 kg ha^{-1}, P: 35.5 kg ha^{-1} and K: 765 ha^{-1}. The nine NP combinations, comprising three doses each of N viz., 150, 200 and 250 kg ha^{-1} and P viz., 30, 40 and 50 kg ha^{-1} were randomly factorized with four triticale cvs. (TL 419, Tigre "S", Muskox "S" and Delfin) and one cv. each of wheat (HD 1982) and rye (Russian Rye) as checks. A uniform dose of 30 kg K ha^{-1} was also supplied. All fertilizers were applied in furrows at the time of sowing in 10 m^2 plots. The sources were urea for N, monocalcium superphosphate for P and muriata of potash for K. Each treatment had three replicate plots. Seeds were sown on 10th November, 1984 at the rate of 125 kg ha^{-1} (Moinuddin et al., 1985). Row to row distance was 20 cm. The crop was irrigated five times and standard agricultural practices were employed as required before harvesting on 20th April, 1985.

The *in vitro* leaf-NRA, assayed according to Jaworski (1971), was studied in the penultimate leaves at tillering, heading and milky grain stages as well as in unexpanded flag leaves at heading stage and in expanded flag leaves at heading and milky grain stages. The selection of these leaves was made bacause of their well-documented role in ear development and grain filling (Thorne, 1966; Yoshida 1972). Total nitrogen content in grain was determined by adopting the method of Lindner (1944), while grain protein content was computed by multiplying percent grain nitrogen with a factor of 5.7 (A.O.A.C., 1970). Grain carbohydrate was extracted using the method of Yih and Clark (1965) and estimated according to Dubois et al. (1956). The per hectare protein and carbohydrate yield, expressed in quintals (q ha^{-1}, q = 100 kg), was computed using these data.

Statistical analyses were done according to Panse and Sukhatme (1985), using analysis of variance (ANOVA) method. Significance of data was assessed at $P = 0.05$, applying "F" test. If the data were found significant at $P < 0.05$, the critical difference (CD), which is the equivalent of Fisher's L.S.D, was computed to compare means of NP combinations, cvs. and NP combination × cv. interaction. Correlation coefficients and regression equations for straight lines were determined to ascertain the association of leaf-NRA with yields of grain, grain carbohydrate and grain protein.

Results and discussion

The application of combined NP doses significantly affected NRA levels in both penultimate and flag leaves at different growth stages, with 200 kg N + 40 kg P ha^{-1} proving optimum (Tables 1 and 2). It may be admitted that the source of applied nitrogen was urea (and not nitrate) that yields ammonium ions on hydrolysis. However, it is well established that ammonium ions are rapidly oxidized to nitrate by soil microorganisms (Beevers and Hageman, 1980). The nitrate, thus formed, is taken up by plants, resulting in induction and stabilization of NR (Hewitt and Afridi, 1959; Afridi and Hewitt, 1962). Therefore, such response of leaf-NRA to soil applied urea-N in the present study would not be unexpected.

Table 1

Effect of basal nitrogen and phosphorus combinations on nitrate reductase activity (μ mol NO_2^- g^{-1} h^{-1}) in the penultimate leaves of triticale, wheat and rye cvs. at three growth stages

Cultivar	Treatments (kg ha^{-1})									Mean
	$N_{150}P_{30}$	$N_{200}P_{30}$	$N_{250}P_{30}$	$N_{150}P_{40}$	$N_{200}P40$	$N_{250}P_{40}$	$N_{150}P_{50}$	$N_{200}P_{50}$	$N_{250}P_{50}$	
					Tillering stage					
TL 419	0.279	0.287	0.286	0.279	0.368	0.363	0.316	0.373	0.372	0.325
Tigre "S"	0.214	0.398	0.358	0.263	0.384	0.370	0.272	0.368	0.280	0.323
Muskox "S"	0.175	0.264	0.273	0.148	0.380	0.313	0.128	0.306	0.319	0.256
Delfin	0.180	0.383	0.287	0.229	0.353	0.331	0.185	0.372	0.379	0.300
HD 1982 (wheat)	0.107	0.303	0.367	0.248	0.315	0.321	0.202	0.202	0.248	0.257
Russian Rye	0.217	0.363	0.327	0.210	0.344	0.287	0.288	0.307	0.340	0.298
Mean	0.195	0.333	0.316	0.230	0.358	0.331	0.232	0.321	0.323	
CD at P=0.05	Treatment 0.033			Cultivar 0.025			Treatment × Cultivar 0.075			
					Heading stage					
TL 419	0.142	0.264	0.297	0.115	0.232	0.250	0.235	0.212	0.274	0.229
Tigre "S"	0.206	0.280	0.313	0.283	0.361	0.318	0.292	0.310	0.223	0.287
Muskox "S"	0.107	0.311	0.267	0.170	0.312	0.203	0.189	0.215	0.170	0.216
Delfin	0.169	0.308	0.200	0.211	0.372	0.204	0.201	0.258	0.300	0.247
HD 1982 (wheat)	0.107	0.293	0.221	0.248	0.268	0.232	0.232	0.200	0.202	0.223
Russian Rye	0.217	0.227	0.244	0.210	0.340	0.307	0.287	0.288	0.240	0.262
Mean	0.158	0.281	0.257	0.213	0.314	0.252	0.239	0.247	0.235	
CD at P=0.05	Treatment 0.043			Cultivar 0.030			Treatment × Cultivar 0.090			
					Milky grain stage					
TL 419	0.106	0.111	0.113	0.089	0.165	0.095	0.058	0.139	0.110	0.110
Tigre "S"	0.130	0.170	0.156	0.129	0.213	0.202	0.204	0.179	0.152	0.171
Muskox "S"	0.128	0.241	0.260	0.155	0.250	0.150	0.197	0.270	0.162	0.201
Delfin	0.214	0.260	0.214	0.166	0.319	0.254	0.200	0.249	0.219	0.233
HD 1982 (wheat)	0.043	0.055	0.070	0.052	0.062	0.052	0.056	0.072	0.047	0.057
Russian Rye	0.122	0.155	0.174	0.173	0.183	0.153	0.171	0.181	0.166	0.160
Mean	0.124	0.159	0.164	0.127	0.179	0.151	0.148	0.182	0.143	
CD at *P*=0.05	Treatment 0.016			Cultivar 0.013			Treatment × Cultivar 0.039			

Cvs. of triticale, wheat and rye exhibited marked differences in NRA at various stages of sampling (Tables 1 and 2). Delfin maintained high levels of NRA in both penultimate and flag leaves through the milky grain stage, whereas Tigre "S" and Russian Rye exhibited high NRA only in the penultimate leaves at heading stage. Also, NRA in penultimate leaves of TL 419 and Tigre "S" at tillering stage and expanded flag leaves of Muskox "S" at milky grain stage were on par with those of Delfin at the corresponding stages. The lowest enzyme activities were recorded in wheat leaves at all stages, being on par with those of the penultimate leaves of Muskox "S" at tillering and heading stages. NRA levels of the penultimate leaves in all cvs. declined stage-wise progressively (tillering>heading>milky grain); but NRA levels in the flag leaves generally remained unaltered. However, in Delfin, wheat and rye, the NRA levels in flag leaves were higher at the milky grain stage than at the heading

Table 2

Effect of basal nitrogen and phosphorus combinations on nitrate reductase activity (μ mol NO_2^- g^{-1} h^{-1}) in the unexpanded and expanded flag leaves of triticale, wheat and rye cvs. at heading and milky grain stages

Cultivar	Treatments (kg ha^{-1})									Mean
	$N_{150}P_{30}$	$N_{200}P_{30}$	$N_{250}P_{30}$	$N_{150}P_{40}$	$N_{200}P40$	$N_{250}P_{40}$	$N_{150}P_{50}$	$N_{200}P_{50}$	$N_{250}P_{50}$	
A. Unexpanded flag leaf					Heading stage					
TL 419	0.207	0.343	0.230	0.261	0.342	0.246	0.291	0.315	0.230	0.274
Tigre "S"	0.278	0.340	0.330	0.340	0.433	0.340	0.342	0.412	0.357	0.352
Muskox "S"	0.251	0.318	0.276	0.258	0.322	0.359	0.243	0.343	0.270	0.293
Delfin	0.355	0.448	0.400	0.348	0.489	0.462	0.301	0.401	0.436	0.404
HD 1982 (wheat)	0.152	0.208	0.341	0.207	0.289	0.218	0.225	0.361	0.240	0.249
Russian Rye	0.355	0.338	0.438	0.457	0.496	0.402	0.438	0.411	0.416	0.417
Mean	0.260	0.332	0.336	0.312	0.395	0.338	0.307	0.374	0.325	
CD at P=0.05	Treatment 0.018			Cultivar 0.013			Treatment × Cultivar 0.037			
B. Expanded flag leaf										
TL 419	0.140	0.257	0.205	0.216	0.300	0.237	0.101	0.250	0.288	0.222
Tigre "S"	0.227	0.316	0.300	0.254	0.362	0.251	0.246	0.309	0.342	0.290
Muskox "S"	0.127	0.123	0.201	0.129	0.298	0.304	0.237	0.220	0.263	0.222
Delfin	0.221	0.316	0.360	0.316	0.362	0.300	0.296	0.350	0.310	0.315
HD 1982 (wheat)	0.099	0.176	0.149	0.174	0.231	0.183	0.148	0.249	0.221	0.181
Russian Rye	0.250	0.362	0.239	0.276	0.371	0.302	0.215	0.350	0.311	0.297
Mean	0.177	0.278	0.242	0.228	0.321	0.262	0.207	0.285	0.289	
CD at P=0.05	Treatment 0.013			Cultivar 0.011			Treatment × Cultivar 0.032			
Expanded flag leaf					Milky grain stage					
TL 419	0.187	0.237	0.234	0.142	0.252	0.262	0.143	0.286	0.195	0.215
Tigre "S"	0.261	0.273	0.321	0.274	0.372	0.309	0.270	0.252	0.310	0.293
Muskox "S"	0.200	0.316	0.271	0.180	0.316	0.231	0.240	0.267	0.315	0.260
Delfin	0.243	0.261	0.300	0.258	0.317	0.299	0.250	0.306	0.314	0.283
HD 1982 (wheat)	0.080	0.126	0.164	0.137	0.185	0.121	0.090	0.200	0.173	0.142
Russian Rye	0.241	0.257	0.260	0.219	0.319	0.294	0.256	0.300	0.260	0.267
Mean	0.202	0.245	0.258	0.202	0.294	0.253	0.208	0.268	0.261	
CD at *P*=0.05	Treatment 0.013			Cultivar 0.011			Treatment × Cultivar 0.033			

stage. Considering leaf age and position, unexpanded flag leaves showed the highest NRA level followed by expanded flag leaves and penultimate leaves in that order at the comparable stages of samplings.

Such variations in NRA levels in different genotypes of triticale are expected and corroborate the observations made on maize by Shrader et al. (1966) and Warner et al. (1969) indicating that the level of NRA varies within a species and is, thus, under genetic control. The observed decrease in NRA levels with aging of plants is compatible with the observations of Croy and Hagman (1970) on wheat cvs. and of Deckard et al. (1973) on maize cvs. Enhanced NRA levels in unexpanded and expanded flag leaves over that in penultimate leaves at comparable stages are presumably due to the former being younger compared with the latter. The situation is analogous to the decreased NRA noted in the penultimate leaves with advancing stages of sampling.

Further, a significant positive correlation of leaf-NRA with grain yield and quality in all cvs. tested was noted. Variations in the degree of correlation between penultimate leaf-NRA and the three yields of various cvs. were, however, noted at different growth stages. Thus, at tillering stage, penultimate leaf-NRA in Muskox "S" and Tigre "S" could be depended upon to monitor yield of grain, grain carbohydrate and grain protein as it exhibited the strongest correlations at this stage ($P<0.001$). Among the other triticales, Delfin, followed by TL 419, showed consistent stronger correlations ($P<0.01$ and 0.001) than the other cvs., particularly at the last stage. A broad classification of the cvs. selected for this trial may, therefore, be made on the basis of the behaviour of each noted above. Thus, Muskox "S" and Tigre "S", showing the lowest and highest NRA levels, respectively, in the penultimate leaves among the triticales (Table 1), exhibited the strongest correlation ($P<0.001$) with the three yields at tillering stage. This would mean that they proved to be the most efficient storer and supplier of reduced soluble nitrogen to the developing grains long after its absorption and reduction by the plant.

The wheat check held a unique position in this classification. Its NRA in penultimate leaves was as low as that of Muskox "S" (Table 1); but it showed weak correlation ($P<0.05$) with one or the other yield at the tillering and milky grain stages. Thus, HD 1982 wheat as well as Delfin (inspite of its highest NRA levels) maintained a similar degree of correlations between NRA and various yields. On the other hand, TL 419 showed the highest NRA levels in its penultimate leaves at tillering stage (Table 1) but had no significant correlations with the three yields. Similarly, Russian Rye, among the checks, possessed the highest NRA levels in the penultimate leaves at tillering and heading stages (Table 1) but exhibited inconsistent weak correlations.

This implies that efficient partitioning of reduced nitrogen between vegetative and reproductive organs is as important as the reduction of available nitrogen itself by NR. The situation is analogous to that cited by Beevers and Hageman (1980), where during a preliminary investigation the same level of NRA was noted in 50 cvs. each of high and low yielding maize.

As for correlation of NRA levels at heading stage with three yields the trend in unexpanded flag leaves was: Muskox "S" = HD 1982 wheat > Delfin = Tigre "S" > Russian Rye and in expanded flag leaves, Delfin HD 1982 wheat = Tigre "S" > Muskox "S" = TL 419. Similarly, at milky grain stage, the order of this correlation was: TL 419 > HD 1982 wheat > Delfin > Muskox "S" > Russian Rye.

Therefore, the selected cvs. may be categorized into two groups, showing consistent strong correlations ($P < 0.01$ and $P < 0.001$) between all the three yields and leaf-NRA levels at (i) early (tillering) stage (Muskox "S" and Tigre "S") and (ii) late (milky grain) stage (TL 419, Delfin, HD 1982 wheat and Russian Rye). Moreover, NRA levels of penultimate leaves of Muskox "S" and Tigre "S" (at the tillering stage) and Russian Rye (at the milky grain stage) as well as of expanded flag leaves of TL 419, Delfin and HD 1982 wheat (at the milky grain stage) could be utilized as a dependable physiological parameter during screening of improved cvs. by triticale breeders. Besides, these findings also establish that, in addition to the contribution of flag leaves (Yoshida, 1972), the penultimate leaves also play a consistent major role in the development of ears and grains, particularly at the growth stage when the flag leaves are not properly expanded.

However, the positive correlation of leaf-NRA with grain yield and quality indicates a parallel increase of both grain yield and grain quality along with that of leaf-NRA. However, it is generally known that among cultivars threre exists a negative correlation between grain yield and grain quality (Terman et al., 1969; Kramer, 1979) because under certain environmental conditions, when grain yield is increased, grain N concentration (grain protein content) is decreased due to dilution effect. Nakhtore and Kewat (1989) and Sah et al. (1990) have observed such an effect in case of wheat by increasing the number of irrigations in the soils requiring optimum moisture level for maximum yield potential. However, progressive increase in nitrogen fertilization of the soil could have different impact on grain yield and grain protein content relationship. Use of N in excess of the amount needed for maximum yield would generally result in increased grain N concentration (Kramer, 1979; Fowler and de la Roche, 1984; Holford et al., 1992). This view is supported by the findings of Hibbered and Hal (1990) who found a positive linear relationship between grain yield and grain N content in maize hybrids as a result of increasing applied N rates. The possible reason for this is that photosynthetic rate does not increase as the N content in the plant (Lawlor et al., 1989). A parallel increase in grain yield and grain N content (grain protein content) of wheat with the increasing N or combined NPK doses has been reported by several workers (Hucklesby et al., 1971; Velayudhan and Seth, 1987; Nakhtore and Kewat, 1989; Cooper and Blakeney, 1990; Vaughan et al., 1990; Pol et al., 1991), which justify the positive correlation of leaf-NRA with grain yield as well as grain quality of triticale in the present investigation, where grain yield and grain quality are increased in accordance with increasing NP doses.

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Animal Husbandry and Physiology

THE ROLE OF GROUP SIZE IN DEVELOPING HOUSING MANAGEMENT OF DAIRY COWS III. USE OF MILKING PARLOUR

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In two 2×2 factorial experiment – using different group size – the effect of varying milking time, milking interval, stability of entry order and side preference at milking in herringbone parlour were investigated. Holstein-Friesian cows kept in sheds with deep litter and Hungaro-Friesians housed in sheds with free stalls were used in Expt. 1 and Expt. 2, respectively. Results indicate that stability of milking order, although occasionally defined, is weak when cows are milked in herringbone parlours under loose housing conditions. From the point of view of milk yield per milking varying milking order, time and interval are not primary factors. Depending on milking procedure certain side preference in cows can be recorded when entering the milking parlour and those cows having side preference tend to react with decrease of milk yield when milked in the opposite side.

Key words: dairy cow, loose housing, group size, milking, milk yield, milking parlour, entry order, side preference

Introduction

It is easy to see that the technological requirement relatively easily fulfilled in the stanchion barn, namely that the cows have to be milked every day and on each occasion at the same strictly fixed points of time, can hardly be respected in loose housing systems. It is particularly true in the case when a group consists of many animals and they enter the milking parlour at random, that is, the milking time of the individuals and the intervals between milkings vary irregularly.

The mentioned rather strict technological prescription was earlier explained with the supposition that departure from it may cause decreased production. Recent experiments carried out with cows kept untied and milked in a herringbone parlour milking did not verify this supposition.

Professionals engaged in milk production are of the opinion, confirmed by our earlier experiences too, that in loose housing system some cows enter the milking parlour at the head, others at the tail-end of the group. Many experts of the subject are convinced that the phenomenon is in connection with the level of milk production, too. All these opinions are mostly based on the rich practical observations made during their professional activity. It is also known that, in the case of free choice, some individuals may prefer one or the other – left or right – side of the herringbone milking parlour. It is easy to imagine that choosing the side of the milking parlour is due either to accustoming or to the natural inclination of the animal. In the latter case it is possible that, if the cow cannot stand on the side it is used to, it reacts with reduced milk production.

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From a technological point of view the question is further complicated by the size of the group. Namely, with the fluctuation of the milking interval, the place mistaken on entering the milking parlour, further, in the case of using a herringbone milking parlour with the side unsteadily chosen, a reduction of milk production can be expected.

Kovalčik and Kovalčikova (1975) think that the group size is primarily determined by the technical equipment of the milking house. For smaller dairy plants (with the capacity of 520 head) they recommend 30 animals, for larger farms (700 head) 40 individuals to be contained in a group. In the case of a rotolactor used continuously, it is more advantageous to form groups with a larger number of cows. In a herringbone milking house, the group size is a function of the number of milking units. In grazing periods larger groups can be composed. In keeping Slovakian spotted and Pinzgau cows, untied groups of 30–40 are regarded as optimum, while with dairy-type cows 50 individuals is the maximum. Grouping can be determined either by the reproduction cycle or by the feeding system. The composition of the groups should not ideally change, this is, however, hardly feasible. According to Kaiser (1971) – as opposed to the earlier conception that reckoned with units of 30 – larger groups of dairy-cows are easier to fit in the technological processes. He found that groups even composed of 65–70 cows are not disadvantageous from the standpoint of production and state of health. A group of cows must not include more than 50–60 individuals – thinks Facsar (1981) – and they had better be milked separately. So, in the case of cows milked at almost the same time, 0.5–1.5 litre of milk can be saved a day, and the amount of milk left in the udder is not more than 100–150 ml. In dairy farms with accommodation 1000–1200 head, the group size is not a matter of indifference at all. Namely, it matters a good deal whether to handle 24 groups of 50 or 12 groups of 100 individuals (Admin and Zyukina, 1977). More groups make the work of milking more difficult, the work organization becomes more complicated and the labour force requirement also increases. Forming larger groups does not exclude the possibility of composing sub-groups. Points of view to be taken into consideration on setting up groups: daily milk production, stage of lactation or lactation milk production. Accordingly, latter authors recommend groups of 100 cows for farms with the capacity of 1000 head. Clark, et al. (1977) placed Guernsey cows in milk in three groups according to their production level expressed in FCM, then regrouped them every 30 days. The proportion of the population left over was placed in the same group throughout the examination. In regard to milk production, periodical regrouping was not found to be disadvantageous. They emphasize that the number of groups adapting themselves best to the technological operations must be determined in further experiments. To establish the optimum system, such factors as farm size, feeding and milking system, labour supply and feeding cost must also be taken into cosideration. Andreiev (1980) kept Simmenthal cows in cow-shed with 4:1 ratio of resting and feeding space in groups of 24, 48, 64 and 80. Since, in the technological system in question, the feeding space and feeding time were limited, the group size was suggested to be reduced to 64 individuals. In Hungary the use of herringbone milking parlours is a general practice. Usually 80–100 cows are driven up to the milking house. In the dairy farm examined by Czakó (1977) only 4% of the cows were milked steadily in the same group, while half of the animals were placed always in a different group. As a consequence of mistaking the place, the

milking interval became irregular, causing a loss of milk. Namely, if a cow of medium milk yield is milked one hour later than the usual time of milking, it gives 0.25–0.30 kg more milk, while in the case of a one-hour earlier milking the reduction of milk is 0.10–0.50 kg.

Because the probability of shorter intervals is 50%, a loss of milk will be the consequence of irregular milking. In a large group the uniform milking interval is almost impossible to realize (Czakó, 1981). In a smaller group (30–32 individuals) compared to the large group (80–100 individuals) the proportion of cows milked at the same time increases from 5–8% to 48–59%, and the milking interval will be nearly uniform. According to Czakó, 15–25 cows form the group in which the ability of the animals' orientation is good, the social order of rank is linear and so the steady time of milking can be realized.

As regards the effect of factors of technological nature, Horn (1973) says that, besides the actual milking technique, the waiting time before the milking room, the cows' order on entering the milking room and their choosing the side of milking parlour are also important.

With these ideas presented and our earlier investigations kept in view, the present analysis is aimed at elucidating the following question, taking into consideration their possible correlations with the group size:

– Is the order of milking stable in the unbound system of keeping milk-cows – in connection with the group size?

– Can the order and time of entering the milking parlour, the milking interval and the milk yield per milking be brought into interrelation in groups with a varying number of cows?

– Do the high and the low yielding cows enter the milking box at the head or at the tail-end of the group?

– Are there individuals among milk-cows that prefer one or the other side of the herringbone milking parlour?

– In groups with a varying number of individuals, is the per milking milk yield of the so-called "one-side" cows influenced by the fact that they are not milked on the side they are used to?

Materials and methods

The effect of changing time and interval of milking was examined in model experiments carried out with a cow population of different quality breeding material, supplied with less favourable feed and technology.

The plan and arrangement of the experiment and the execution of the examinations were totally identical in the two experiments. The animals were kept tied, the milker worked with two milking machines, and the milk was collected in pails. Throughout the experimental period, i.e. over 14 days, the time of beginning and ending both the morning and the evening milking process was recorded and the amount of the milk produced measured for each cow. The number of measuring was 112 per group and milking, a total of 672 in an experiment. Altogether 1344 in the two experiments.

The experiments were set up each with three groups of cows in the 100–130th day of lactation. The cows were of Hungarian Red spotted × Holstein-Frisian breed with various proportions of Holstein genes. The animals were placed in groups on the basis of milk records preceding the experiment, and according to the stage of lactation, as shown in Table 1.

Table 1

Size of group and number of measurings in the experiment

Experiment		Part of day	A	B	C
			group		
Number of cow/group	I		8	8	8
	II		8	8	8
Number of measurings	I.	Morning	112	112	112
		Evening	112	112	112
	II	Morning	112	112	112
		Evening	112	112	112

A group = milking begins every day at the same time
B group = cows milked second on odd days and third on even days
C group = cows milked third on odd days and second on even days

The statistical evaluation of the data was carried out by F-test and analysis of variance, respectively, with the help of the following model:

$$SQ_t = SQ_{k_1} + SQ_{k_2} + SQ_{k_3} + \ \ldots \ + SQ_{k_i} \times SQ_{k_j} + SQ_e$$

where SQ_t = total variance, k_1 = effect of groups, k_2 = effect of examination days, k_3 = effect of daily milking times, k_i x k_j = double interactions, e = rest and error.

Analysis of the correlation system of milking characteristics, stability of entering the milking parlour and laterality of milk-cows.

The experiments were carried out with two dairy-cow populations in the same arrangement as described in paper I.

The correlation system of the milk production and milking data was evaluated by means of a computer with factor analysis after Weber (1974) and Sváb (1979). In cases when the result of the factor analysis suggested positive effects the statistical analysis was continued with analysis of variance, e.g. when the milk production of cows entering first or last the milking parlour. For the examination of the laterality of cows on entering the milking parlour and its effect on milk production analysis of variance was used again.

Results

Examination of the effect of changing milking time and interval in model experiments.

Milking of the 8 cows placed in each group took about half an hour. On the *average times of beginning the milkings,* information is given in Table 2. In the case of either the morning or the evening milking, the table shows the extreme values of the individual averages, that is the first figure is the average beginning time of milking for the first cow, the second figure for the last – eigth – cow of the group. The values of scattering suggest that in the first experiment – as a result of a higher technological discipline – milking of the individuals of group A generally began at a time strictly fixed in the order of the cow-house, while in the second experiment the higher values of scattering found in group A indicate beginning times of milking suggesting a somewhat looser technological discipline, smaller or larger daily differences. In groups A, B and C – in accordance with the original purpose – of the individual mean

Table 2

Average values and standard deviations of individual milking times

Experiment	Treatments	Beginning of milking, hour			
		Morning		Evening	
		$\bar{x}$	s	$\bar{x}$	s
I.	A	5.01–5.43	0.01–0.07	15.53–15.92	0.03–0.05
	B	5.81–6.16	0.3	16.26–16.58	0.3
	C	5.77–6.20	0.2	16.22–16.61	0.2
II	A	5.39–5.82	0.1	17.22–17.64	0.2
	B	6.17–6.62	0.3	18.00–18.43	0.4–0.5
	C	6.25–6.73	0.4	18.04–18.51	0.3

values of the beginning times of milking a wider scattering was generally obtained, about 0.2–0.3 hour in the first and 0.3–0.5 hour in the second experiment.

The outlined tendencies are clearly reflected by the average values of milking intervals and the standard deviations shown in Table 3. The mean values are almost constant, in the first experiment the longer night milking interval takes 56–57%, the shorter daytime interval 43–44% of the daily 24 hours; in the second experiment the night- and daytime milking intervals are almost the same (51 and 49%). The group A

Table 3

Trend of milking intervals and significance of variance differences

Experiment	Groups	Milking intervals, hour				
		Night		Daytime		significance
		$\bar{x}$	s	$\bar{x}$	s	
I	A	13.5	0.04	10.5	0.05	NS
	B	13.5	0.52	10.5	0.10	xxx
	C	13.6	0.42	10.4	0.09	xxx
	significance					
	A/B		xxx		xxx	
	A/C		xxx		xxx	
	B/C		NS		NS	
II	A	12.2	0.16	11.8	0.21	xx
	B	12.2	0.61	11.8	0.31	xxx
	C	12.2	0.57	11.8	0.30	xxx
	significance					
	A/B		xxx		xxx	
	A/C		xxx		xxx	
	B/C		NS		NS	

Significance of variance differences

NS = $P > 0.05$
xx = $P < 0.01$
xxx = $P < 0.001$

of the first experiment is found to show relatively stable milking intervals, while the larger variances observed in group A of the second experiment indicate the looser technological discipline of the poorer management. In the first experiment the variances between the parts of day in group A do not differ significantly, while in the second experiment the difference is statistically proved. The variances of the night milking intervals in groups B and C of both experiments are significantly larger. As to the differences between the groups, the variances of the milking intervals in groups B and C are, in accordance with the treatments, significantly larger in each case than in the case of the group A.

Concerning the average amount of milk produced per milking. Table 4 offers information on the effects of the treatments. The average amount of milk obtained per milking was 11.68 kg in the first and 8.29 kg in the second experiment, which means 23.36 kg and 16.59 kg daily milk production, respectively. In the case of both experiments the differences between groups A, B and C are not significant ($P > 0.05$). There was no difference between the days of observation concerning the amount of milk per milking either. The significant differences of milking according to the parts of day in the first experiment are due to the longer night- and the shorter daytime milking interval. The proportion of milk produced in the morning is 56%, while that of the

Table 4

Amount of milk produced per milking (kg)

		I	II
	Treatments	experiment	
Main average		11.68	8.29
Group average	A	11.57	8.41
	B	11.87	8.21
	C	11.59	8.25
	significance	NS	NS
Averages of examination days	1	12.18	8.58
	2	11.99	8.40
	3	11.79	8.15
	4	11.89	8.40
	5	12.01	7.79
	6	11.78	8.57
	7	11.75	8.46
	8	11.59	8.28
	9	11.47	8.42
	10	11.30	8.31
	11	11.31	8.46
	12	11.30	8.40
	13	11.56	8.02
	14	11.56	7.87
	significance	NS	NS
Average values of milking times	morning	13.08	8.15
	evening	10.28	8.44
	significance	xxx	xx

NS = $P > 0.05$
xx = $P < 0.01$
xxx = $P < 0.001$

amount of milk obtained in the evening is 44%, in proportion with the milking intervals. In the second experiment less milk was obtained in the morning and more in the evening, still, in spite of the significant differences the difference can be considered slight. The ratio is 49:51%.

Analysis of the correlation system of the milking characteristics by factor analysis

Considering the possible role of the size of group the situation is particularly critical with milking, the most sensitive phase of the whole technological process. Although in some opinions, differences between the milking intervals or their irregular changing hardly if at all influence the milk production. Yet, many think that the group driven up to the milking house forms by chance, whereby the order of milking changes, and all this may unfavourably affect the milk production of the whole population.

In the factor analysis natural and derived variables were equally taken into account. The variables were:

x_1 = amount of milk produced in the evening
x_2 = amount of milk produced in the morning
x_3 = deviation of milking order from the average in the evening
x_4 = actual milking order in the evening
x_5 = deviation of milking order from the average in the morning
x_6 = actual milking order in the morning
x_7 = time of milking in the evening
x_8 = time of milking in the morning
x_9 = milking interval

The mean values and standard deviations of measured and calculated parameters originating from the two replications of the *first experiment* and evaluated in combination are shown in Table 5. As regards the tendencies the varying size of group, and in the case of the same size of group the place occupied within the cow-house did not seem to influence either the evening (x_1) or the morning milk production (x_2). The ratio of evening to morning milk production was 40:60 irrespective of the size of group and the place occupied within the house; the relative scattering around the averages was 20–23% and 18–20%, respectively. Whether we consider the evening or the morning data, the mean values of deviation from the average milking order (x_3 and x_5 variables) hardly deviate from zero, in accordance with the expectations. Increase in the standard deviations as a function of the size of group suggests, however, the possibility that the probability of mistaking the place increases in a larger group. On the other hand, the standard deviations indicates that certain individuals are milked usually in the first part, others in the second part of the group in the milking parlour. The mean values of the actual milking order (x_4 and x_6 variables) determined on the evening and morning milking, respectively, follow from the group size. Standard deviations increases as a result of the growing size of group, the relative scattering, on the other hand, hardly changes, it ranges from 54 to 57%. Standard deviations around the average point of time of evening milking (x_7) and morning milking (x_8) varies from 22 to 28 and from 28 to 34 minutes, respectively, it increases only in the group

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Table 5

Mean values and standard deviations of the variables examined in cow groups of different size (First experiment, A and B replications together)

Designation	Variables	Size of group									
		30		40e		40m		50		80	
		average	s	average	s	average	s	average	s	average	s
Amount of milk, kg evening	x_1	7.44	1.60	7.00	1.57	7.16	1.67	7.19	1.45	7.24	1.64
morning	x_2	10.78	2.02	10.69	2.09	10.74	2.11	10.94	1.96	10.67	1.92
Order of milking, evening	x_1										
deviation from average	x_3	0.09	8.00	–0.26	9.49	–0.04	9.17	–0.17	11.82	–0.29	16.87
actual	x_4	15.87	9.13	19.88	10.98	19.16	10.74	24.25	13.70	37.22	20.96
Order of milking, morning											
deviation from average	x_5	–0.02	8.01	0.23	9.78	0.04	9.29	–0.56	10.09	–0.04	16.66
actual	x_6	16.21	9.04	19.59	11.11	18.33	10.49	24.62	13.26	37.57	21.11
Milking time, hour											
Milking time, hour evening	x_7	19.79	0.41	20.75	0.36	21.21	0.36	20.12	0.46	20.74	0.73
morning	x_8	10.14	0.57	11.31	0.49	11.83	0.50	10.62	0.47	11.34	0.58
Milking interval, hour	x_9	14.35	0.58	14.56	0.58	14.62	0.59	14.50	0.49	14.60	0.61

e = groups placed at the edges of the cow-house

m = groups placed in the middle of the cow-house

Table 6

Mean values and standard deviations of variables examined in cow groups of different size (Second experiment, A and B replications together)

Variables		Size of group, n: 15		25		30		40		45		55		70	
		average	s	average	s	average	s	average	s	average	s	average	s	average	s
Amount of milk, kg, evening	x_1	6.89	1.55	5.95	1.45	6.39	1.53	6.49	1.57	6.24	1.44	7.16	1.75	6.59	1.56
morning	x_2	6.84	1.29	6.75	1.48	6.63	1.60	6.80	1.57	6.60	1.49	7.22	1.56	6.80	1.56
Milking order, evening															
deviation from average	x_3	–0.11	3.87	1.26	5.62	0.40	6.70	–0.79	8.91	–1.62	9.83	–0.58	12.00	0.08	14.25
actual	x_4	7.93	4.19	12.40	6.42	14.90	8.00	18.56	10.61	21.65	13.08	27.51	15.52	33.05	19.26
Milking order, morning	x_5														
deviation from average		–0.21	4.08	0.83	5.79	0.25	6.57	–0.50	8.21	–2.11	9.87	–0.24	11.95	–0.34	14.37
	x_6	7.84	4.32	12.85	7.06	15.70	8.00	17.95	10.81	20.69	12.31	27.61	15.55	33.81	19.44
Milking time, hour evening	x_7	20.58	0.54	19.83	0.28	20.39	0.49	20.60	0.46	20.23	0.19	20.12	0.60	20.67	0.43
morning	x_8	8.23	0.34	7.82	0.23	8.29	0.76	8.64	0.49	8.31	0.34	8.07	0.83	8.73	0.57
Milking interval, hour	x_9	11.65	0.38	12.00	0.30	11.90	0.62	12.04	0.36	12.08	0.32	11.95	0.44	12.06	0.43

of 80 to 44 minutes in the case of the evening milking. The longer, evening milking interval (x_9) ranged between 14 hours 21 minutes and 14 hours 37 minutes, accordingly, the average time of the shorter, daytime interval fluctuated between 9 hours 23 minutes and 9 hours 39 minutes. There are no signs indicating any relationship between changes in the average values and the size of group. The order of magnitude of the standard deviations was also independent from the group size, being 29–37 minutes, and the coefficient of variation accordingly 3–4%.

The mean values of the measured and calculated parameters and their scattering in the *second experiment* are contained in Table 6. A combined evaluation of the two replications depending on what size of group the cows were placed it does not show the significant differences; no considerable difference was found in the amount of milk produced in the evening (x_1) and in the morning (x_2) either, the ratio generally was 50:50 or 49:51 with an only exception when it shifted to 47:53. The order of coefficient of variation was 22–24% and 19–24%, respectively. Deviation from the average milking order is hardly more than zero in the case of either the evening or the morning milking (variables x_3 and x_5). The order of magnitude of standard derivation – like in the first experiment – gradually increases with the growing number of cows in the group, suggesting the possibility of mistaking the place when entering the milking parlour. The mean values of the actual milking order follow from the number of cows per group (variables x_4 and x_6). Standard derivation increases with the size of group, the coefficient variation on the other hand, persists around 50–60%. Standard deviations the evening and morning milking time (x_7 and x_8) is 11–36 and 14–50 minutes, respectively. It is not, however, supposed to be connected with the group size. The night milking interval (x_9) is more or less steady, its fluctuation is between 11 hours 39 minutes and 12 hours 5 minutes. Standard derivation ranges from 18 to 37 minutes, the coefficient variation is 3–5%, similar to that in the first experiment.

In this paper we disregard the correlation matrix of the variables examined, although on the interrelations of the variables even the paired correlation coefficients supply some primary information. The analysis only covered those correlation coefficients that more or less deviated from zero. The relatively less close correlation between the amounts of milk produced in the evening and in the morning (x_1 and x_2 variables) is surprising; it may partly be explained by the unequal milking intervals and the relatively wide standard derivation. Only the correlations between the deviation from the average milking order and the actual milking order (x_3 and x_4, x_5 and x_6) were found to be close, when examining the correlation coefficients determined either for the evening or for the morning milking. The reason for the phenomenon is the moderate standard derivation with a stable milking order supposed. The reciprocalness between the parameters examined is otherwise rather loose, and their orders of magnitude generally are increasing, in some cases decreasing or varying, depending on the group size. The correlations between the evening and morning milking orders (x_4 and x_6) – no matter how loose they are – deserve special mentioning, as they are independent from the group size of and suggest the moderate stability of the milking order. The same is suggested by the $r = 0.19–0.51$ coefficients of correlation between the milking order and the milking time (x_4 and x_7, and x_6 and x_8, respectively). The correlations between milking time and milking interval (x_7 and x_8, and x_9) suggest

that the cows, even when in larger groups, try to get into the milking parlour at about the accustomed time.

The correlation system of the variables examined was evaluated by factor analysis; out of the results we only give in Table 7 the numerical data of groups placed at the edges and in the middle of the house in replications A and B of the first experiment. From the mutual part of the total variances generally 91–96% could be explained with the examined variables, the cumulative eigenvalue changes with the size of group. The number of the retained background variables is four, not much less than the number of the original parameters.

Table 7

Analysis of the correlation system of the variables examined with factor analysis in groups of differenctsize (First experiment, A and B replications)

Designation		Factors			
		I	II	III	IV
		factor loadings			
Group size, $n=40_e$					
Eigenvalue		3.18	2.08	1.33	1.10
Cumulative eigenvalues %		35.28	58.43	73.26	85.52
Variables					
	x_1	–0.1867	0.016	0.1788	0.7164
	x_2	0.1152	0.0374	0.0667	0.7436
	x_3	0.3032	–0.6290	0.4564	–0.0259
	x_4	–0.2916	0.7799	–0.4969	0.0280
	x_5	–0,3192	–0.5702	–0.5593	0.0637
	x_6	0.3328	0.7186	0.5973	–0.1172
	x_7	–0.5242	0.3952	–0.0075	0.0657
	x_8	0.7543	0,2847	–0.2694	0.0992
	x_9	0.9730	–0.0075	–0.2239	0.0427
Reciprocalness		0.7618	0.7703	0.8635	1.0028
Individualities		0.6479	0.6377	0.5043	0.0742
Group size, $n=40_m$					
Eigenvalue		3.26	2.01	1.48	1.00
Cumulative eigenvalue %		36.17	58.54	75.03	86.19
Variables					
	x_1	–0.2528	0.0561	0.0691	0.6410
	x_2	0.0782	0.0079	–0.1169	0.6441
	x_3	0.3494	–0.5855	0.4500	0.1575
	x_4	–0.3688	0.7468	–0.4777	–0.1616
	x_5	–0.3246	–0.5435	–0.6149	0.0022
	x_6	0.3069	0.6999	0.6432	–0.0396
	x_7	–0.5696	0.4342	–0.0524	0.2819
	x_8	0.7327	0.3650	–0.3367	0.2131
	x_9	0.9659	0.0426	–0.2521	0.0074
Reciprocalness		0.7160	0.7528	0.8639	1.0029
Individualities		0.6981	0.6583	0.5037	0.0765

e = groups placed at the edges of the cow-house
m = groups placed in the middle of the cow-house

The content of artificial variables, factors easy to separate in the course of the analysis modifies according to the size of the group concerned:

	Nuber of cows per grup				
	30	40_e	40_m	50	80
Factor of milking interval	I	I	I	I	I
Factor of milk production	IV	VI	IV	III	III

The content of the highest 2.94–3.26 eigenvalue background variable I determines first of all the milking interval (x_9), which up to a size of group consisting of 40 individuals forms a common factor with the time of morning milking (x8). The picture is clearest in the case of cow groups of 30, where – as was expected – the content of factors II and III (eigenvalue: 2.02 and 1.52, respectively) fills in the content of the variables x_3 (deviation from the evening milking order) and x_4 (actual evening milking order), and x_5 (deviation from the morning milking order) and x_6 (actual morning milking order), respectively and forms a background variable common with them. In the case of larger groups these factors determine the variables with different weights, with an increase in the size of group the picture becomes more diversified. Yet, the cumulative eigenvalues, which besides the factor of milk production make 71–75% of the mutual part of total variance in smaller groups, and 60% in larger groups indicate the importance of background variables filling in these variables, although in the case of smaller groups they precede in order the generally 1.00–1.6 eigenvalue factor that determines the milk production. However, with groups of 80 the eigenvalue of the background factor that determined the milk production rises to 1.53, and so milk production advances in the order of factors. However, it is characteristic that the production variables (x_1 and x_2) are usually filled in with the content of the same, common background variable. This means at the same time that the amount of milk produced in the evening and in the morning is determined by a separate factor.

For the fact that the actual evening and morning milking order (x_4 and x_6 variables) in groups of different size is always the same, except in the group of 30, the reason can be found in factor II, the eigenvalue of which is 2–2.1 in the case of 30 and 40 cows, and 2.4–2.6 in groups of 50 and 80; it suggests certain moderate stability of the milking order, though the variables occasionally are filled in simultaneously with the content of various factors of different weight.

When analysing the extent of the effect of the amount of milk produced in the evening and in the morning, as factors acting on the mutual part of the x^1 and x^2 variables, and the effect of the $x_3 \ldots x_9$ explanatory variables with special orthonormalized rotation (Tables 8 and 9) we can establish that in the case of any group size the factors of milk production are independent from the other background variables. Furthermore, in the trend of the factors of milk production, the role of factors outside the ones examined by us may be of increased importance.

Examination of the stability and role of entering the milking parlour. Since in both main experiments the cows were milked in milking parlours with 2'8 stalls, as a first step we recorded for each individual of the groups of 8 how many times a given

Table 8

Extent of the effect of the amount of milk produced in the evening and in the morning, as explanatory variables acting on the reciprocal part of variables x_1 and x_2 after special orthonormalized rotation (First experiment, A and B replications together)

Designation		Size of group, n									
		30		40_e		40_m		50		80	
		x_1	x_2	x_1	x_2	x_1	x_2	x_1	x_2	x_1	x_2
Factor		V	V	IV	IV	IV	IV	III	III	III	III
Variables	x_1	0.7360	0.7024	0.7618	0.6768	0.7160	0.5956	0.8616	0.7961	0.9668	0.4558
	x_1	0.6985	0.7320	0.6844	0.7703	0.6262	0.7628	0.6138	0.6643	0.4558	0.9668
	x_1	0.0693	0.0672	0.0109	–0.0126	–0.0410	–0.0171	–0.0919	0.0728	0.0298	0.0636
	x_1	0.0064	0.0067	–0.0215	0.0263	0.0561	0.0214	–0.0067	–0.1753	–0.0122	–0.0433
	x_1	–0.0417	–0.0182	0.0070	–0.0760	–0.0071	0.0147	–0.0563	–0.1323	0.0153	0.0058
	x_1	0.0386	0.0169	–0.0516	0.0391	–0.0109	–0.0628	–0.0543	0.0037	–0.0105	0.0006
	x_1	0.0375	–0.0855	0.2021	–0.1387	0.3288	–0.1116	–0.0157	–0.0708	0.3687	–0.0772
	x_1	–0.0115	0.1944	–0.1455	0.1010	–0.1719	0.1150	–0.1140	0.1760	0.2096	0.0460
	x_1	–0.0380	0.2494	–0.2515	0.1736	–0.3457	0.1656	–0.0952	0.2374	–0.2393	0.1362

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Table 9

Extent of the effect of the amount of molk produced in the evening and in the morning, as explanatory variables acting on the reciprocal part of variables x_1 and x_2 after special orthonormalized rotation (Second experiment, A and B replications together)

Designation	Size of group, n													
	15		25		30		40		45		55		70	
	x_1	x_2	x_1	x_2	x_1	x_2	x_1	x_2	x_1	x_2	x_1	x_2	x_1	x_2
Factor	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	III	III	IV	III
Variables	0.9720	0.4505	0.8156	0.6591	0.7604	0.7280	0.9744	0.4750	0.9004	0.4626	0.9679	0.5178	0.9746	0.4720
x_2	0.4509	0.9730	0.5988	0.7410	0.7204	0.7524	0.4713	0.9668	0.4778	0.9300	0.5183	0.9689	0.4721	0.9749
x_3	0.0586	–0.0306	0.0053	–0.3341	–0.0572	–0.0086	–0.0580	–0.0747	0.0363	0.0553	–0.0478	0.0445	–0.0586	0.0460
x_4	0.0588	0.1315	–0.0508	0.2565	–0.0298	–0.0888	0.0348	0.0134	–0.2088	–0.2065	–0.0212	–0.1128	0.0359	–0.0459
x_5	0.0490	–0.1142	–0.0732	0.2550	–0.0518	0.0703	–0.0641	–0.0389	–0.0715	–0.0856	–0.0467	–0.0686	0.0294	–0.0556
x_6	0.0560	0.2213	–0.1323	–0.4553	–0.0059	–0.1315	0.0473	–0.0105	–0.0800	–0.0675	–0.0651	–0.0519	–0.0098	0.0633
x_7	–0.0088	–0.1836	0.1274	0.1643	–0.0346	0.0618	0.0563	0.0683	–0.3040	–0.0445	0.0532	0.0542	0.0362	0.0298
x_8	–0.0179	–0.0771	–0.0641	–0.1458	–0.1640	0.0546	0.0271	0.1246	–0.2122	0.0970	0.0473	0.0683	–0.0233	0.1459
x_9	–0.0043	0.1914	–0.1693	–0.2678	–0.1753	0.0179	–0.0367	0.0829	–0.0218	0.1153	0.0169	0.0556	–0.0659	0.1655

cow enters the parlour in the first, second etc. group, and calculated the percentage frequency, too. On the basis of the frequency data the cows were placed in classes. Five classes were formed according to the order of entering the milking parlour:

1. categorically first in the group
2. first in tendency in the group
3. entering at random
4. last in tendency
5. categorically last.

The milk production data of groups of different size were compared by these categories in the case of both replications of both experiments. The experiments were evaluated separately after combining the data of the two replications. Processing was carried out with variance analysis with the milk production data of evening and morning milking handled separately.

The results of the first and second experiments were evaluated together. On the percentage of individuals of the different size cow groups entering first, last or at random the milking box information is given in Table 10. According to the data in the first experiment half to two-third of the cows entered the milking box at random, in the second experiment two-third to three-quarter of them entered in chance order. In tendency, the proportion of cows entering the milking parlour head of the group was one-tenth to one-fifth of the total number of cows in the two experiments. Somewhat less was in tendency the proportion of cows entering at the tail-end the group. The proportion of categorically first and last cows in the group was relatively small, 4–7 and 1–7% in the first experiment, while in the second experiment their proportion was surprisingly insignificant. The mean values of milk produced per milking are contained in Table 11. Between the two factors examined, the milk production of cows

Table 10

Cows' entering the milking parlour

Designation	Size of group n	Percentage distribution of cows according to their entering the milking parlour				
		categorically first	tendenciously first	at random	tendenciously last	categorically last
First experiment	80[1]	7	28	50	12	3
	50[1]	4	9	72	8	7
	40[1]	7	9	73	10	1
	30[1]	4	20	56	18	2
Second experiment	70[1]	6	21	62	9	2
	55[2]	4	19	60	15	2
	45[3]	–	22	76	2	–
	40[1]	1	25	69	5	–
	30[1]	2	16	76	6	–
	25[3]	–	11	78	11	–
	15[2]	–	47	40	13	–

[1] = A and B replications together
[2] = A replication
[3] = B replication

Table 11

Effect of the order of entering the milking parlour on milk production per milking (kg)

Designation	Time of milking	
	Evening	Morning
First experiment		
head of group	7.26	10.79
tail-end of group	6.98	10.56
significance	NS	NS
Second experiment		
head of group	6.56	8.86
tail-end of group	6.47	6.71
significance	NS	xx

NS = P > 0.05
xx = P < 0.01

entering first and last the milking parlourinteraction could not in a single case be pointed out. Although the milk production of cows milked first compared to the milk production of those milked last is about 1–4% higher, the phenomenon with a single exception could not be statistically verified. The exception was the morning milking of the second experiment. Thus, although the tendency is perceptible, it does not definitely prove that the higher yielding individuals of the group enter first the milking parlour, the low yielding ones only later, at the tail-end of the group.

Laterality of cows and its effect on milk production. When processing the experiment data the following two questions had to be examined: (1) whether laterality can be pointed out for milk-cows in case they have free choice of the two sides of the milking parlour, and if so, of what extent it is, (2) whether it influences the milk production of cows milked at the side opposite to the one they are used to. When processing the experiment data we followed the same procedure as used when analysing the role of the milking order. We recorded for each animal how many times it went to the left and how many times to the right side of the milking parlour, then expressed the values in percentage. Relying on the individual results we classified the cows with the following categories taken into consideration:

1. entering categorically to the left side,
2. in tendency to the left side,
3. at random.
4. in tendency to the right side,
5. categorically to the right side.

The results of the first and second experiment were evaluated together. According to the data of Table 12 in the first experiment, 61–67% of the cows used both sides of the milking parlour, while in the second experiment only 11–35% of them did so. Yet, there were cows in the first experiment that preferred either the left or the right side. In the second experiment we found that there were categorically left- and right-side cows (11–33%), and even ones tendenciously preferring one or the other side

Table 12

Laterality of cows on entering the milking parlour

Designation	Size of group n	Percentage distribution of cows on entering the milking parlour				
		categorically on the left side	tendenciously on the left side	alternately	tendenciously on the right side	categorically on the right side
First experiment	80[1]	1	16	65	18	–
	50[1]	1	16	67	16	–
	40[1]	1	19	61	19	–
	30[1]	–	19	62	19	–
Second experiment	70[1]	23	23	17	9	28
	55[2]	15	23	22	19	21
	45[3]	5	36	22	22	15
	40[1]	24	23	12	18	23
	30[1]	11	38	11	22	18
	25[3]	13	17	35	22	13
	15[2]	27	13	14	13	33

[1] = A and B replications
[2] = A replications
[3] = B replications

(13–38%). The role of the group size in this question seems to be negligible, but more conspicuous, on the other hand, is the unambiguous difference between the data of the two experiments. The cause of the phenomenon is – highly probably – of technological origin. If in the waiting place the animal has possibility and enough time to decide which side to choose, it will give preference to the side it has been used to. If with the view of an increased performance the technology hastens the cows to enter the milking parlour, they have no possibility to choose the accustomed side. It is a question whether the mentioned phenomenon has any role in milk production. To be able to answer this question, in the course of processing the data we compared the average milk production per milking of the so-called "one-side" cows when they entered the milking parlour on the usual side to the average milk production they showed when milked on the accustomed side. The results of the first experiment are given in Table 13. Out of the additive effects the effect of laterality in this experiment could only be pointed out in the case of the evening milking, the effect of the group size – in accordance with the earlier evaluation – is not consistent. The significant interactions of laterality × group size are supposedly caused by the fact that the amount of milk produced on the usual or the opposite side of the milking parlour is statistically proved only for the individuals of the group of 30, and in the two categories the result of comparison between the number of animals per group is not unequivocal.

The results of the second experiment are also summarized in Table 13. In the second experiment a considerable proportion of the cows proved to be "one-sided". The categorically "one-sided", unilateral individuals gave 4.4–6.5% less milk on the side of the milking parlour they had not been used to. Similar was the situation with the tendenciously "one-sided" cows, though the difference was smaller, only 3–3.5%. According to the results the habit to use one or the other side of the milking parlour may

Table 13

Effect of the cows' laterality on entering the milking parlour on milk production per milking (kg)

Designation	Time of milking	
	Evening	Morning
First experiment		
usual side	7.38	11.02
opposite side	7.33	11.01
significance	xx	NS
Second experiment		
Categorically one-sided cows		
usual side	6.93	6.84
opposite side	6.51	6.55
significance	xxx	xxx
Tendenciously one-sided cows		
usual side	6.86	6.79
opposite side	6.66	6.56
significance	xxx	xxx

NS = $P > 0.05$
xx = $P < 0.01$
xxx = $P < 0.001$

have some importance for the animals in regard to milk production. It is therefore reasonable to develop the technology so as to satisfy this demand of the cows, occasionally making a compromise with the requirement of increasing the capacity of the milking house.

Discussion

Evaluating the results of the experiments, we can state that in the amount of milk obtained per milking, the size of the group can hardly be a decisive factor. On the other hand, the effect of the time of milking, i.e. that of the uneven milking interval is clearly demonstrable. The shift of proportions in connection with the uneven milking intervals has been observed several times (Czakó and Guba, 1956; Balika, 1969; Ichikawa and Fujishima, 1976; 1982). The reduction in milk- and butterfat production – in comparison to the uniform milking intervals – is only minor (Balika, 1969; Linnerud et al., 1962; Spahr and Ormiston, 1966; Ichikawa and Fujishima, 1976; 1982), some authors found no difference (McMeekan and Brumby, 1956; Linnerud et al., 1964; Garcia and Brito, 1972; Czakó et al., 1968), while again others even reported a slight increase in milk production in the case of uneven milking intervals (Turner, 1955). Hannson et al. (1958) found the milk production to be maximum when the night interval was somewhat longer than the daytime interval of milking. Data by Kocsis (1970) are not unequivocal, while according to Schmidt and Trimberger (1963) the 16/8 ratio of milking interval is disadvantageous only with high yielding cows, though they emphasize that the evidence of this statement is not conclusive. In the experiment of Czakó and Illés (1962) the irregular fluctuation of the milking interval was in connection with the fluctuation of milk production ($r = 0.47$). According to Czakó et al. (1968) in industrial

dairy plants there is no biological objection to the 16/8 hour milking interval, although in earlier investigations they found that the daily fluctuation of the butterfat content depended on the milking interval; they emphasize that in the case of uniform milking intervals the butterfat content of the morning milk may also reach the required level. Ormiston et al. (1967) are of the opinion that a change in the butterfat content in the case of uneven milking intervals is due to the so-called complementary milk left in the udder after milking. The butterfat content in the latter is usually higher than in the normal milk. Its amount is proportionate with the amount of milk to be found in the udder at the time of milking. Thus, after a longer interval more is left of it in the udder, and this increases the average butterfat content of milk given after a shorter interval. At the same time they do not exclude the possibility that in the course of a longer milking interval the synthesis of fat declines. According to the results of our own investigations, with dairy-type cows kept either in smaller or in larger groups, in an intensive housing system – unlike the earlier supposition – there is little fear of minor irregular fluctuations of milking time and interval.

Keeping the group size in view, we did not find the role of the milking order to be important in the trend of milk production either. At the same time Czakó and Enyedi (1965) found that the groups driven up to the milking house were formed by chance; accordingly, the order of milking varies and all this may have an unfavourable effect on the milk production of the whole population. The relevant literature also shows the correlation between milking order and milk production to be rather low (Lamb, 1976; Czakó, 1977, 1978; Willems and Lampo, 1964), or at least not too close (Tschirch and Sommer, 1970).

To the voluntary choice of the side of the milking parlour and its effect on milk production, no reference was found in the literature.

The definite though low stability of the milking order detected in larger groups too was experienced in our investigations as well. The literary data are not unequivocal in this question either. Czakó and Enyedi (1966) observed a chance order of milking in loose housing system; Suzuki et al. (1982) with milking in rotolactor, also found that in the waiting room there was no place preferred by the cows on the one hand, and the order of entering the milking parlour was influenced by the size of the group (n = 39) on the other, and it proved to be a chance order. Mácha et al. (1980) found the order of entering the milking parlour to be highly variable, and pointed out differences in genotype, too. In their opinion the milking order reflects the social status of the cow in the group, its motory activity, and its adaptability to the given technology. Many authors give account of the stability of the milking order, e.g. Willems and Lampo (1964) in their classical work; many other publications (Porzig et al., 1969; Lamb, 1976; Odai et al., 1981) report a definite milking order, though minor differences from milking to milking may occur – they emphasize –, and in the order of entering the milking parlour the leading-following group effect may be felt. In an experiment Czakó (1978) found that about 50% of a cow-group of 60–100 entered the milking parlour together with other individuals, and 96% of them were not milked at the same time. Only 15–20% of the cows occupy the same position (Gandbury, 1975), but towards the end of the lactation period the order becomes more and more stable. According to Odai et al. (1981) the milking order of cows transferred to another place

was upset, but later it was restored. Adaptation took two weeks. Bünger and Bünger (1978) pointed out the order of milking even in larger groups (n = 58 and n = 63). Our results primarily support those observations in which the stability of the milking order, whether in a smaller or in a larger group of cows, while rather moderate, can be most clearly seen. Observations of this nature seem thus to be proved for smaller groups of cows, and according to the results they are valid for larger groups as well.

What is the biological explanation of the phenomenon that in the trend of the amount of milk produced per milking the role of the milking order, time and interval is not primary compared to the other possible circumstances in groups of any size?

According to Turner (1955) the rate of milk secretion though somewhat slows down during a longer milking interval; still, in the rate of milk- and butterfat synthesis there is hardly any change during the day. Moreover, McMeekan and Brumby (1956) found the rate of secretion to be constant for 20–24 hours following milking. Ormiston et al. (1967) pointed out that the rate of milk- and butterfat synthesis is linear for 14–16 hours. Wheelock et al. (1966) studied the effect of 6-, 12-, 18-, 24-, 30- and 36-hour milking intervals and found that, simultaneously with an increase in their length, there was a curvilinear decrease in the secretion of milk and milk components, the extent of which varied with the component. Kárpáti and Várkonyi (1981) state that milk secretion in high yielding cows stops only 18–24 hours following milking. Milk secretion is for 15 hours ascendent at an angle of 30–40°, it is descendent (Csiszár, 1957). According to Turner (1953) the supposition and the earlier standpoint concerning the inhibition of milk secretion by the long milking interval require revision. In the case of uneven intervals between successive milkings, the hypothesis as to the milk obtained on milking, formed in the period immediately preceding the milking, is not reasonable. The amount of residual milk left in the udder after milking following a longer interval is significant and in positive correlation with the total amount of milk present is in the udder on milking. After milking the rate of milk secretion remains thus constant for a considerable time, and the increased pressure caused by the accumulation of milk does not check it, as was thought earlier. Witzel and McDonald (1965) point out that the pressure measured in the udder does not necessarily reflect the pressure developed in the alveoles or in the small collecting tubules in the course of milk secretion. The secreted milk remains there until the pressure in the udder becomes normal. Changes in the milking order and time of milking as well as their minor fluctuations do not – probably for biological reasons – make their effects felt in the trend of milk production in connection with the cow groups of different size. Moreover, the tolerance of cows to these technological characteristics, and their adaptability are partly due to this. In the case of the so-called "one-sided" cows the decrease in the amount of milk given can be explained by the fact that milking on the side the cow is not used to act as a disturbing stress factor in the neurohormonal control of milk secretion, and the animal, irrespective of the group size, is not able to tolerate this fully.

We think that the cited papers closely connected with our subject, and the basic investigations referred to and supporting the results of our experiments make it possible to draw the following major conclusions:

– In the loose system of dairy type cows unbound, in the case of milking in milking-parlour the stability of the milking order is – irrespective of the group size – low, though occasionally definite. Earlier observations in this question made in smaller cow groups seem to be valid in the case of larger groups too. Depending on the technological system applied, half to two-thirds or two-thirds to three-quarters of the cows enter the milking parlour in a chance order drifted away from their mates. There are, however, individuals categorically or tendenciously first or last (1–7 and 4–7%, respectively). The milking routine employed may be an influencing factor in this respect. The moderate stability indicated in the order of milking can supposedly be explained with the above phenomenon.

– In regard to milk production per milking, the interrelation of slight changes in the order, time and interval of milking is not a primary factor. The tolerance of milk-cows to the mentioned technological factors studied by us indicates their adaptability.

– And although the milk production of cows first to enter the milking parlour is 1–4% higher compared to those milked last, with the exception of a single case, this statement could not be statistically proved. In connection with the group size, the order of entering the milking parlour is not thought to be of decisive importance from the standpoint of milk production.

– With dairy type cows, whether kept in larger or smaller groups, in an artificial keeping system sharply differing from the natural one, minor fluctuations of the order, time and interval of milking can hardly be reckoned with – as opposed to earlier suppositions.

– Depending on the technology of milking – driven up to the milking parlour or entering it voluntarily – three to four-fifths of the cows or one to three-tenths of them use the two sides of the milking parlour alternately. In a given technological system, certain degrees of definite laterality on entering the milking parlour appear in a large proportion of the cows. The role of the group size in this respect seems to be negligible.

– In case the "one-sided" cows are milked on the side of the milking parlour they are not used to, cows categorically choosing the same side give 4.4–6.5% less milk, while the tendenciously one-sided cows gave 3–3.5% less milk.

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Reviews

PROTOPLAST ISOLATION, CULTURE AND PLANT REGENERATION IN WHEAT AND OTHER CEREAL CROPS: REVIEW AND UPDATE

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Protoplasts of wheat and other cereal crops can be isolated directly from meristematic tissues or from cultured cells using various combinations of hydrolytic enzymes. The best source of dividing cereal protoplasts is the finely dispersed, fast growing, embryogenic and regenerable cell suspension. Plant regeneration from suspension cells can also provide good information about the regeneration system of the derived protoplasts. The incubation time required for protoplast release depends upon a number of factors, e.g. the enzyme type, concentration and composition of the digestion mixture, the osmoticum and osmotic pressure, the mechanical conditions during incubation, the pH and the incubation temperature, and the ratio of tissue: enzyme volume.

Plant genotype, physiological status of the protoplast source, isolation and purification conditions all can exert marked effects on yield, survival and division capacity of the cereal protoplasts in culture. Besides viability, there are different factors influencing capacity of the isolated protoplasts to dedifferentiate and proliferate, e.g. protoplast density, composition of culture medium and culture conditions. Although cereal protoplasts are regarded as being recalcitrant, plants have already been regenerated from cell suspension-derived, cultured protoplasts of wheat and other cereal species, but in the case of wheat only a few could be raised to maturity in soil. In addition, nothing was mentioned about the fertility of plants regenerated from wheat protoplasts, except one case, when a single regenerant produced seven fertile ears. Cytological instability of source cell suspension cultures should be minimized for successful use of the derived protoplasts in crop improvement programmes.

Key words: cell suspension, cereals, plant regeneration, protoplast culture, protoplast isolation, *Triticum aestivum* L., wheat

Introduction

Somatic hybridization of different species or varieties by protoplast fusion can overcome the natural barriers of sexual incompatibility that prevent successful crossing between unrelated varieties and species (Gamborg et al., 1981; Morrish et al., 1987; Hayashi and Shimamoto, 1988). The ability of plant protoplasts to uptake foreign DNA molecules, integrate them into the cell genome and to regenerate complete transformed plants, opened a new way for transferring desirable traits into a superior cultivar, without a concomitant transfer of undesirable traits, as in the classical breeding methods (Power et al., 1970; Gamborg et al., 1981; Morrish et al., 1987; Simmonds 1991). Be-

Abbreviations: ECS = embryogenic cell suspension cultures, RECS = regenerable embryogenic cell suspension cultures.

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sides genetic transformation and somatic hybridization, plant protoplasts can also be utilized for biochemical, physiological, physical and pathological studies also (Jones, 1985).

Reproducible and effective regeneration of plants from protoplasts is indispensable for successful application of such techniques (Potrykus, 1991; Simmonds, 1991). Although cereal protoplasts are regarded as being recalcitrant, plants have already been regenerated from cultured protoplasts of various cereal species (see review of Lazzeri and Lörz, 1988; Lörz et al., 1988; Vasil, 1987; 1988; Roest and Gilissen, 1989). In a wheat protoplast culture, between 1970 and 1993, 26 publications reported successful protoplast isolation from various plant parts and cultured cells, but the first cell division, and colony formation, was reported by Maddock in 1987. Harris et al. (1988) regenerated the first plantlet from wheat protoplast cultures. From 1990 to 1993, 11 publications from 7 different laboratories gave an account of green plant regeneration from wheat protoplasts (Ren et al., 1990; Sun et al., 1990; Vasil et al., 1990; Wang et al., 1990; Chang et al., 1991; Guo et al., 1991; He et al., 1992; Li et al., 1992a, b; Qiao et al., 1992; Ahmed and Sági, 1993). Five of them reported regeneration of mature plants, but only one announced the production of a protoplast-derived, fertile wheat plant (Ahmed and Sági 1993). From rice, barley and maize protoplasts, fertile plants have already been regenerated (see later).

In this review, recent progress in regenerable protoplast culture of wheat and other cereals will be surveyed.

(1) Source of protoplasts

Cereal protoplasts can be isolated directly from the plant or from cultured cells. For isolation of protoplasts from the plant, meristematic tissues containing morphogenetic or competent cells, including immature embryos and inflorescences, young leaves, leaf sheaths, stems, nodes, roots and apices, microspores and ovules have been used (Potrykus et al., 1976; Jones, 1985; Lazzeri and Lörz, 1988). However, isolation of protoplasts from these tissues is difficult and even if good yields can be obtained from mesophyll tissue, for instance, these protoplasts rarely divide, and are incapable of regeneration (Evans et al., 1972; Potrykus et al., 1976; Lazzeri and Lörz, 1988). More recently, promising results has been reported by Gupta and Pattanayak (1993) about regenerated plants from mesophyll protoplasts isolated from the leaf base and sheath of rice seedling. The protoplasts were cultured in a modified N6 medium in the presence of a feeder layer prepared from young rice cells. The feeder layer plays probably a significant role in this protoplast system, since without in the protoplasts did not divide.

Wheat protoplasts have been isolated from various tissues and plant parts (Table 1), including root tips (Power et al., 1970), mature leaf mesophyll tissue (Evans et al., 1972; Potrykus et al., 1976; Okuno and Furusawa, 1977), aleurone layer of immature grains (Norman et al., 1983; Lee et al., 1988, 1989) and endosperm of developing kernels (Lee et al., 1988; Keeling et al., 1989). Schulze et al. (1989) isolated protoplasts from the scutellum of immature wheat embryos, which were similar to protoplasts obtained from embryogenic cell suspension cultures (ECS). Unfortunately, none of the wheat protoplasts isolated in these studies were capable of division.

Table 1

List of published reports about cell division and regeneration from protoplast culture of wheat (Triticum aestivum L.), up to the beginning of 1993

Starting material	Cell division	Level of regeneration	Reference
Rm	–	–	Power et al., 1970
M	–	–	Evans et al., 1972
M	–	–	Potrykus et al., 1976
M	–	–	Okuno and Furusawa, 1977
Al	–	–	Norman et al., 1983
CS (Sm and Rm)	–	–	Potrykus and Petruska, 1983
ECS (IE →C)	+	calli	Maddock, 1987
ECS (A and C)	+	plantlets	Harris et al., 1988
C and IE	+	albino plantlets	Hayashi and Shimamoto, 1988
CS (II and C)	+	Sm and leaves	Lee et al., 1988
Al and En	–	–	Lee et al., 1988, 1989
Sc	–	–	Schulze et al., 1989
ECS (ME and C)	+	plants	Wang et al., 1988, 1990
En	–	–	Keeling et al., 1989
C	+	plants	Ren et al., 1990
ECS (IE and C)	+	plants	Sun et al., 1990
RECS (IE and C)	+	mature plants	Vasil et al., 1990
RECS (IE and C)	+	mature plants	Chang et al., 1991
C	+	plants	Guo et al., 1991
ECS (IE and A C)	+	roots	Djardemaliev et al., 1992
RECS (IE and C)	+	mature plants	He et al., 1992
ECS (II and C)	+	plants	Li et al., 1992a
ECS (IE and C)	+	plants	Li et al., 1992b
RECS (IE and C)	+	mature plants	Qiao et al., 1992
RECS (IE and C)	+	fertile plant	Ahmed and Sági, 1993

Abbreviations: Rm = root meristems, M = Mesocotyl, Al = Aleurone, CS = Cell suspension, Sm = Shoot meristems, ECS = Embryogenic cell suspension culture, IE = Immature embryo, C = Callus, A = Anther, II = Immature inflorescence, En = Endosperm, Sc = Scutellum, ME = Mature embryo, RECS = Regenerable embryogenic cell suspension cultures. Abbreviations in parentheses refer to the source of cell suspension cultures.

There are a few reports on isolation of dividing protoplasts from fast-growing and friable callus of cereals (Kyozuka et al., 1987; Hayashi and Shimamoto, 1988; Wang et al., 1988; Ren et al., 1990; Guo et al., 1991; Wu and Zapata, 1992), but the best source of dividing cereal protoplasts seems to be the finely dispersed, fast growing cell suspension cultures (Jones, 1985; Morrish et al., 1987; Lazzeri and Lörz, 1988; Lörz et al., 1988; Vasil, 1987, 1988; Simmonds, 1991; Datta et al., 1992). There are numerous reports on isolation and sustained division of protoplasts from non-morphogenic suspensions, but these protocalli remained non-morphogenic in all

cases (see review of Lazzeri and Lörz, 1988; Vasil, 1987). However, from embryogenic cereal suspensions, somatic embryos and plantlets were successfully obtained (see review of Jones, 1985; Morrish et al., 1987; Lazzeri and Lörz, 1988; Lörz, 1988; Vasil, 1987, 1988; Roest and Gilissen 1989).

Hayashi and Shimamoto (1988) isolated wheat protoplasts directly from immature embryos or from embryogenic callus formed from the embryos. Later, Ren et al. (1990) and Guo et al. (1991) also obtained viable protoplasts from immature embryo-derived embryogenic calli of bread wheat.

Wheat ECS have also provided sources of rapidly-dividing, competent cells for numerous protoplast isolations (Table 1). Potrykus and Petruska (1983) isolated wheat protoplasts from suspension cultures of shoot- or root-meristem origin. Harris et al. (1988) and Djardemaliev et al. (1992) used anther-derived embryogenic calli to initiate fine cell suspension cultures for obtaining totipotent protoplasts. Maddock (1987), Vasil et al. (1990), Chang et al. (1991), He et al. (1992), Li et al. (1992b), Qiao et al. (1992) and Ahmed and Sági (1993) also obtained high yields of viable protoplasts from rapidly growing ECS initiated from immature wheat embryos. From young inflorescence-derived nodular embryogenic calli of winter wheat, Lee et al. (1988) and Li et al. (1992a) established cell suspension cultures suitable for protoplast isolation. Recently, Yang et al. (1993) also established embryogenic cell suspensions from scutellar callus of durum wheat and isolated regenerable protoplasts from them.

Haploid suspension cells derived from anther cultures or microspores were also successfully used for initiating regenerable protoplast cultures of cereals. In barley, regenerable suspension-cultured cells initiated from embryogenic anther-callus was used by Jähne et al. (1991) for obtaining protoplasts capable of plant regeneration. Haploid suspension cells of rice also served for establishment of regenerable protoplast cultures (Toriyama et al., 1986; Su et al., 1992). Embryogenic haploid calli and microspores were the sources of maize protoplast cultures as reoported by Cai et al. (1991) and Mitchell and Petolino (1991), respectively.

(2) Isolation and purification of wheat protoplasts

Wheat protoplasts are most commonly isolated from plant organs or cultured cells by enzymatic digestion (see the publications cited in Table 1). There are no standard methods for protoplast isolation and purification: these differ from one laboratory to the another at least in some details.

For protoplast isolation of several cereal species, numerous enzyme mixtures have been applied. Most of them contain (w/v) cellulase (1–2%), pectinase (0.5–2%) and pectolyase (0.1–0.5%), dissolved in an osmoticum (giving 400–800 mOsm/kg H_2O), at pH 5.2–5.6. Mannitol, sorbitol, glucose and sucrose are the major osmotica used in the digesting solutions at a concentration of 0.5–0.6 M (Chung, 1988). For preparing wheat protoplasts from suspension cells, glycerol was found to be superior to mannitol as an osmoticum (Djardemaliev et al., 1992; Ahmed and Sági, 1993). Optimum osmotic condition is very important for obtaining viable protoplasts and for reducing spontaneous fusions during isolation (Potrykus and Shillito, 1988; Schulze et al., 1989; Funatsuki et al., 1992). Addition of calcium, MES buffer and bovine serum albumin

to the enzyme solution increases yield and viability of the protoplasts and prevents them from deterioration during isolation (Gamborg et al., 1981; Chung, 1988; Potrykus and Shillito, 1988).

The incubation time required for cereal protoplast release depends upon a variety of factors (Gamborg et al., 1981), e.g. physiological state of the source (Guo et al., 1991; Datta et al., 1992; He et al., 1992; Qiao et al., 1992), the enzyme type, concentration and composition of the digestion mixture (Gamborg et al., 1981; Schulze et al., 1989; Guo et al., 1991; Lyznik et al., 1991; Diaz and Carbonero, 1992; Ahmed and Sági, 1993), the osmoticum and osmotic pressure (Schulze et al., 1989; Djardemaliev et al. 1992; Ahmed and Sági, 1993), the mechanical conditions during incubation (Jones, 1985; Wang et al., 1990), the pH and the incubation temperature (Keeling et al., 1989), and the ratio of tissue: enzyme volume (Keeling et al., 1989; Lee et al., 1989). In general, incubation time for cereal protoplast isolation varies between two and twenty-four hours.

After appropriate incubation, the protoplasts must be removed from the enzyme solution and separated from the incompletely digested tissues. By sieving the enzyme solution/cells mixture through a stainless steel sieve or nylon filters (40–100 μm), the cereal protoplasts can easily be separated from the debris. Washing and collection of the protoplasts can be done with a salt solution, e.g. W5 (Ahmed and Sági, 1993) of relatively low buoyant density, and by subsequent low-speed centrifugation (700–1000 rpm, for 3–10 min). For obtaining completely purified protoplasts, flotation or density gradient centrifugation methods may be required, using sugars, sugar alcohols or colloids (e.g. sucrose, mannitol, sorbitol, Ficoll, Percoll at 0.3–0.6 M) with buoyant densities higher than that of the washing solution (Larkin, 1976). Protoplasts should be washed at least three times by repeated sedimentation or flotation. During protoplast harvest and purification, care must be taken to maintain a similar osmotic pressure, low-speed centrifugation, gentle handling and keeping the protoplasts always wet. Lazzeri et al. (1991) stored barley protoplasts at 7 °C for 5 h in LW washing solution. Viability of the protoplasts can be checked using a number of methods, among which the fluorescein diacetate (FDA) staining is the most popular (Widholm, 1972; Larkin, 1976).

(3) Protoplast culture in cereals

Plant genotype, physiological status of the protoplast source, isolation and purification conditions all can exert marked effects on yield, survival and division capacity in culture of the cereal protoplasts (Potrykus et al., 1976; Gamborg et al., 1981; Jones, 1985; Morrish et al., 1987; Lazzeri and Lörz, 1988; Lörz et al., 1988; Vasil, 1988; Chung, 1988; Guo et al., 1991). An ECS as source of totipotent protoplasts is very sensitive to inconsistencies in the culture conditions, e.g. the time interval of subcultures, the dilution ratio with fresh culture medium, etc. (Wang et al., 1988; Redway et al., 1990; Schmitz and Lörz, 1990; Jähne et al., 1991; Datta et al., 1992; Qiao et al., 1992). Isolation of viable protoplasts is often possible only in the exponential growth phase of the culture (2–6 days after subculture, Gamborg et al., 1981; Jones, 1985; Vasil, 1988).

Besides viability there are numerous factors influencing capacity of the isolated protoplast to dedifferentiate and proliferate, e.g. protoplast density, composition of the culture medium, undefined factors in protoplast culture medium, and culture conditions (Gamborg et al., 1981; Jones, 1985; Potrykus and Shillito, 1988).

Protoplast density

The ratio of protoplasts to volume of culture medium is important (Funatsuki et al., 1992). Wang et al. (1990) reported that first division time and division frequency of wheat protoplasts in KM_{8p} medium were closely related to the protoplast density. The usual range of cereal protoplast density is between 1×10^5– 1×10^6 protoplasts/ml of culture medium.

Composition of culture medium

The nutritional requirements of cultured cells and protoplasts are very similar. Cereal protoplast culture media are low in ammonium (Li an Murai, 1990), iron and zinc, but higher in calcium, vitamin, sugar and sugar alcohol than the cell culture media (Chung, 1988). For cereal and grass protoplasts, a range of modified nutrient media based on MS (Murashige and Skoog, 1962), B5 (Gamborg et al., 1968), KM (Kao and Michayluk, 1975), C17 (Dudits et al., 1977), N6 (Chu et al., 1975) and AA (Abdullah et al., 1986) were used. Recently, different research groups used protoplast culture media composed of various culture media components, e.g. N6M (Mórocz et al., 1990), NMB (Guo et al., 1991) and L_1 (Jähne et al., 1991).

Osmotic pressure of the culture medium is critical and incorrect osmotic pressure can be detrimental because of protoplast fragility. Type, quality and quantity of the osmoticum are very important (Potrykus and Shillito, 1988). Mannitol and sorbitol are the compounds most frequently used. Usual osmotic pressure of cereal and grass protoplast culture media is between 400 and 700 mOsm/kg H_2O (Junker et al., 1987; Chang et al., 1991; Jähne et al., 1991; Funatsuki et al., 1992; Jørgensen et al., 1992; Ahmed and Sági, 1993). However, for successful culture of protoplasts, the osmotic potential of the initial culture medium may need to be reduced gradually after inoculation as suggested by Chung (1988), Sun et al. (1990) and Wang et al. (1990).

Sugar type and concentration can considerably affect protoplast division and colony formation as reported by Wang et al. (1990) in *Triticum aestivum* L. Glucose at 0.4–0.6 M seems to be the best carbon source for cereal protoplasts (Fujimura et al., 1985; Prioli and Söndahl, 1989; Vasil et al., 1990; Lazzeri et al., 1991, Ahmed and Sági, 1993). KM, L_1 and N6M media contain a mixture of different carbon sources, e.g. glucose, sucrose, fructose, ribose and raffinose.

Growth regulators added to protoplast culture media are also essential for inducing divisions and plant regeneration. 2,4-D (2,4-dichlorophenoxyacetic acid) and NAA (1-naphthaleneacetic acid) proved to be effective auxins, and kinetin, BAP (6-benzylaminopurine) and zeatin were effective cytokinins (Prioli and Söndahl, 1989; Mórocz et al., 1990; Vasil et al., 1990; Wang et al., 1990; Baset et al., 1991; Datta et al., 1992; Ahmed and Sági, 1993). Reduction of the hormone content after a few days of plant protoplast culture can be beneficial (Potrykus and Shillito, 1988).

Culture conditions

Numerous protoplast culture methods have been developed to increase plating efficiency and subsequent plant regeneration. The "liquid thin layer" cultures in Petri-dishes and culture on semisolid media, solidified with agar, agarose or alginate are the most frequently used (Vasil et al., 1990; Wang et al., 1990; Chang et al., 1991; Ahmed and Sági, 1993). Agarose gives better plating efficiencies with sensitive protoplasts (Chung, 1988; Potrykus and Shillito, 1988; etc.). The type and purity of agarose also greatly influence plant regeneration from cultured protoplasts (Torrizo and Zapata, 1993). Hayashi and Shimamoto (1988) and Wu and Zapata (1992) pointed out that presence of the nurse cells was indispensable for induction of wheat and rice protoplast division, respectively. Rhodes et al. (1988), Shillito et al. (1989) and Mitchell and Petolino (1991) confirmed that nurse culture improved plating efficiency in maize. Funatsuki et al. (1992) and Qiao et al. (1992) made similar observations for barley and wheat protoplast culture, respectively. However, Abdullah et al. (1986), Datta et al. (1990), Mórocz et al. (1990), Vasil et al. (1990), Jähne et al. (1991), Datta et al. (1992), He et al. (1992) and Ahmed and Sági (1993) obtained good protoplast division and recovery of green plants in rice, maize, wheat and barley without nurse cells. Using conditioned media from the donor ECS culture improved colony formation of rice (Datta et al., 1992) and barley protoplasts (Jørgensen et al., 1992). Using the feeder cells was the most important single factor for success of Gupta and Pattanayak (1993) to induce sustained divisions, colony formation and plant regeneration from mesophyll protoplasts of rice (*Oryza sativa* L.) seedlings. Su et al. (1992) also applied the feeder cell method for protoplast culture of rice.

The environmental factors, e.g. temperature and light also have to be considered in plant protoplast culture (Potrykus et al., 1976; Potrykus and Shillito, 1988). For cereal and grass protoplast culture, a constant temperature between 24 and 28 °C is usual. Darkness at the initial phase is normally required for induction of division, as high light intensities inhibit the early development of cereal protoplasts (Gamborg et al., 1981). However, light has been found to promote their further development (Potrykus and Shillito, 1988; Wu and Zapata, 1992).

Plating efficiency

Within 2–3 days of plating, cell wall regeneration is completed in most cases and during 3–7 days of the culture the newly regenerated cells usually undergo their first divisions. Within 3 weeks, protocolonies and somatic embryos can be seen in the culture (Gamborg et al., 1981; Jones, 1985; Morrish et al., 1987; Lazzeri and Lörz, 1988). For wheat protoplasts, plating efficiency (expressed as the percentage of individual protoplasts in the vessel, giving rise to colonies) ranged from 0 to 46% (Maddock, 1987; Vasil et al., 1990; Wang et al., 1990; Chang et al., 1991; Guo et al., 1991; He et al., 1992; Li et al., 1992a, b, Qiao et al., 1992; Ahmed and Sági, 1993).

Li and Murai (1990) compared 5 basal media: MS, RY-2, modified R_2, AA, and general medium for rice cell culture, and found that suspension cell growth rate, protoplast yield and plating efficiency were all about 30% higher in general medium than in the second-best R_2 medium. When wheat protoplasts were cultured in MS,

C17, D2a, and KM_{8p} media, Wang et al. (1990) found that KM_{8p} medium supported best the cell division and colony formation. Vasil et al. (1990) and Chang et al. (1991) also found that KM_{8p} medium was superior for recovery of wheat protocalli. In agreement with the above findings, Ahmed and Sági (1993) also obtained higher wheat cell division frequency and plating efficiency with KM_{8p} and general media than with MS and L_1 media.

Potrykus et al. (1976) isolated wheat protoplasts from different plant organs at every developmental stage, Potrykus and Petruska (1983) from shoot- or root-meristem suspension culture and cultured the purified protoplasts under a wide range of nutritional, hormonal and environmental conditions, but could not induce divisions. Norman et al. (1983), Lee et al. (1988, 1989) and Keeling et al. (1989) also failed to obtain divisions in protoplasts released from aleurone or endosperm layers of immature wheat seeds. Wheat protoplasts isolated from plant organs have limited ability to divide, and sustained divisions have never been demonstrated, although the protoplasts remained viable for several weeks (Table 1). Potrykus (1980) suggested that protoplasts derived from cereal plant organs cannot be induced to divide due to a block in the nuclear divisions. However, rice leaf protoplasts could recently be re-enter the mitotic cycle under suitable *in vitro* growth conditions of the donor plant, method of isolation and culture of protoplasts (Gupta and Pattanayak, 1993).

Plant regeneration from wheat protoplast culture

Somatic embryo and plant regeneration generally take place upon transfer of the protocolonies and the embryos to solid regeneration media. Sustained division of wheat protoplasts was first reported by Maddock (1987), who isolated protoplasts from two rapidly growing suspension cultures, but the protocalli were not capable of regeneration. Lee et al. (1988) also obtained protocalli, showing limited morphogenesis from protoplasts of an inflorescence-derived suspension cell line. Hayashi and Shimamoto (1988) produced a few protocolonies from protoplasts isolated from immature embryos or from embryogenic calli which could regenerate rudimentary albino shoots and roots only. According to Harris et al. (1988), division frequency of wheat protoplasts derived from a cell suspension of another origin was low and only a few green plantlets could be regenerated.

Recently, Ren et al. (1990) and Guo et al. (1991) reported plant regeneration from wheat protoplasts isolated from embryogenic callus. Protoplast-plant regeneration has also been accomplished from callus-derived ECS initiated from mature embryos (Wang et al., 1988, 1990), immature embryos (Vasil et al., 1990; Chang et al., 1991; He et al., 1992; Li et al., 1992b; Qiao et al., 1992; Ahmed and Sági, 1993) and young inflorescences of wheat (Li et al., 1992a, Table 1).

The more recent protocols depend upon regenerable embryogenic cell suspension (RECS) as a prerequisite for recovery of mature plants from cereal protoplasts. In the first reports on regeneration of fertile maize (Prioli and Söndahl, 1989; Shillito et al., 1989) and barley plants (Jähne et al., 1991; Funatsuki et al., 1992) from protoplasts, RECS were used as protoplast sources. In rice (Fujimura et al., 1985; Jenes and Pauk, 1989; Datta et al., 1992; etc.), napier grass (Vasil et al., 1983) and ryegrass (Dalton,

1988), green plants were also regenerated from RECS-derived protoplasts. Successful plant regeneration from wheat protoplasts using RECS has already been reported (see Table 1).

Before starting any protoplast isolation from ECS in a genetic improvement program, it is desirable to test the regenerative capacity of ECS. Plant regeneration from suspension cells can provide good information about the regeneration system (media, culture conditions etc.), which can be utilized when preparing a protoplast-plant protocol (Vasil et al., 1990; Jähne et al., 1991; Ahmed and Sági, 1993).

Suspension cells and plants derived from cereal cell suspension and protoplast cultures can show considerable chromosomal anomalies, e.g. chromosome elimination, genomic rearrangements, existence of polytene chromosomes, giant nuclei, minute and double minute chromosomes and granular chromatin particles (Karp et al., 1987; Chang et al., 1991; Shang and Wang, 1991; Wang et al., 1992; Ahmed and Sági, 1993). In contrast, Yang et al. (1991) and He et al. (1992) reported that cultured cells of bread wheat, and the plants regenerated from protoplasts derived from these suspensions had the normal chromosome complement ($2n = 6x = 42$), as experienced by workers with other cereals. Probably, the explants used to initiate the cell culture play an important role in this respect (Lee et al., 1988).

Vasil et al. (1990), Chang et al. (1991), He et al. (1992) and Qiao et al. (1992) transferred the protoplast-regenerated plants to soil, but they did not describe fertility or sterility level in the mature plants. As these regenerated plants were probably not free from some chromosomal abnormalities (see above), their fertility seems to be questionable. We could obtain a large number of wheat plants from protoplasts isolated form RECS of GK Ságvári winter bread wheat, most of them showing chromosomal abnormalities and rooting problems and only one plant grew to maturity and set seed (Ahmed and Sági, 1993). The R_1 generation plants of this fertile plant showed protoclonal variation in plant height, days to heading, spike number and fertility of the spikes.

It is clear from the above results, that in cereal protoplast culture, protocols allowing more efficient and reproducible, fertile plant regeneration have to be developed. Selection of responsive genotypes and optimal culture conditions certainly will assist to solve this particular problem, as happened formerly in the callus and anther culture of small grain cereals.

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