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GIBBERELLIC ACID INDUCED MODIFICATION IN YIELD QUALITY, GRAIN BIOMASS AND BIOCHEMICAL ASPECTS IN DEVELOPED GRAINS OF SEA-WATER-TREATED WHEAT PLANTS

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The observed suppression in yield and yield attributes of wheat plants in response to sea-water treatments was relieved either partially or completely when grains were presoaked in gibberellic acid (GA_3, 50 mM). The alleviating effect of GA_3 for sea-water salinity was more pronounced at 25% sea-water.

Grain pretreatment with GA_3 increased carbohydrates and protein but decreased Na^+, Cl^- and phosphorus content in developed grains of sea-water-treated plants.

Sea-water treatments altered the balance of hormonal levels in developed grains of wheat plants. Thus, 10% or 25% sea-water induced a significant decrease in growth promoters levels with an increase in growth inhibitory substances equivalent to abscisic acid. On the other hand, grain priming with GA_3 increased the growth promotery substances and reduced abscisic acid level.

Key words: gibberellic acid, sea-water, wheat, yield

Introduction

The saltiness of sea-water has concerned man since early times. Accordingly the use of sea-water in irrigation of many crop plants is an increasing problem and is a significant factor in reducing crop productivity (Epstein 1985, Glenn et al. 1991, Aldesuquy 1998).

The response of crop plants to salinity stress is generally reflected in decreased growth and hence decreased productivity (Haroun 1985, Aldesuquy et al. 1998). Cereal crop plants have been found to be influenced by available soil water. Thus, Abdul-Halim et al. (1985, 1988), working on wheat found that as the soil water decreased from 1.7 to 11.0 dsm^{-1} the dry matter, plant height, tillers or heads per plant at different stages of plant growth as well as grain yield and root weight per plant were decreased significantly. Furthermore, Imamul-Huq and Larher (1983) reported that K^+ and Ca^{2+} concentrations in both shoots and roots of *Phaseolus*

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aureus decreased with increasing salinity where Na^+ and Mg^{2+} increased. Abo-Hamed et al. (1990) demonstrated that salinity furthered the accumulation of Na^+ and Ca^{2+} and decreased the accumulation of K^+.

The mechanism of salt adaptation by growth bioregulators was studied by many authors (Radi et al. 1989, Shaddad and El-Tayeb 1990, Aldesuquy 1995, 1998, Aldesuquy et al. 1998). Reports have shown that gibberellin application at the pollination stage increases the yield of *Zea mays* (Stuart and Cathey 1961). Furthermore, GA_3 and IAA increased the yield component including mobilization index and harvest index in rice plants (Ray and Choudhuri 1981). Irrigation of wheat plants with sea-water at 10% or 25% decreased reducing sugars with concomitant increase in sucrose and polysaccharide content in wheat leaves (Aldesuquy et al. 1998).

In the majority of cases, grain presoaking in GA_3 alleviated the inhibition of cumulative leaf area, improved water status of leaves by increasing their relative water content and, at the same time, reduced the accumulated free ABA in developing wheat leaves as well as the amino acids in yielded grains of sea-water-irrigated plants (Aldesuquy 1998). Thus, the present investigation was targeted to find out how GA_3 can improve the yield capacity and biochemical aspects of developed grains of sea-water-treated plants.

Materials and methods

Homogeneous *Triticum aestivum* L. (cultivar Sakha 69) grains were surface sterilized by soaking in 0.01 M $HgCl_2$ solution for 3 minutes, washed thoroughly with distilled water and divided into two sets which were soaked in distilled water or 50 mM GA_3, respectively, for about eight hours. After soaking, thoroughly washed grains were planted on 20 November, 1997 and 1998 (15 per pot) in earthenware pots (30 cm in diameter) filled with 3 kg soil (sand and clay 2/1 v/v). The pots were kept in the greenhouse under normal day/night regime and irrigated with tap water as required. After two weeks only five uniform seedlings were left in each pot. The plants of each set were divided into three groups, which were irrigated with 0, 10, 25% sea-water, respectively. The standard sea-water contained [kg m^{-3}] Cl^-: 21.6, Na^+: 11.1, SO_4^{-2} : 2.85, K^+: 0.49, P^{3+}: 16.6; salinity was 38.5 g/kg, pH = 8.1; its electrical conductivity 62 mS cm^{-1}. Following

thinning and before heading, the plants received 35 g (N) m^{-2} as ammonium nitrate and 35 g (P) m^{-2} as superphosphate.

At the end of the two seasons 1997–1998 (i.e. at harvest date), ten plants (main shoot) from each treatment were taken and the mean of two seasons per plant was calculated for the following parameters: shoot length (cm), spike length (cm), plant height (cm), shoot fresh and dry weights (g/shoot), spike fresh weight (g/spike), one hundred grains weight (g), crop yield/plant (g), harvest index (percentage of total aerial dry weight of the plant at maturity that represent grain yield) (Zelitch 1975), mobilization index (the ratio of crop and straw weight) (Ray and Choudhuri 1980). Also, ten grains from each treatment were collected for determining the grain fresh and dry weights. Furthermore, triplicate samples from dry powdered grains were taken for estimating ion concentration, carbohydrates and protein as well as fresh matter for determining phosphorus and growth bioregulators.

Estimation of elements

A known dry weight of plant sample was digested in concentrated HNO_3 and made up to volume with distilled water. K^+, Na^+ and Ca^{2+} concentrations were measured by flame emission spectrophotometry (Younis et al. 1994).

The procedure adopted for estimating Cl^- was essentially that described by Hansen and Munns (1988).

The procedure adopted for extraction of phosphorus was essentially that described by Barker and Mapson (1964). For quantitative determination of phosphorus in the extracts the method of Kuttner and Lichtenstein (1932) was adopted.

Estimation of carbohydrate

The carbohydrate content of developed grains was determined according to the procedure adopted by Younis et al. (1969).

Estimation of protein

Protein content was determined using the method of Lowry et al. (1951).

Estimation of growth bioregulators

The method of extraction of hormones was that originally described by Shindy and Smith (1975). For determination of abscisic acid (ABA) in extracts, the wheat coleoptile bioassay developed and adopted by Wright (1956) was used. The amounts of either acidic or neutral auxins were estimated according to straight growth test of barley coleoptile adopted by Foda and Radwan (1962). Gibberellic acid in extracts was determined by the lettuce hypocotyl bioassay developed and adopted by Frankland and Wareing (1960). Cytokinins content was estimated according to the method described by Esashi and Leopold (1969).

The results were first subjected to the analysis of variance (Anova). When Anova showed a significant ($p \leq 0.05$ at the least) effect, the least significant differences were used to compare treatments (Snedecor and Cochran 1967).

Results and discussion

Changes in yield and yield attributes

Grain yield in wheat is the end-product of complexity of assimilate-producing and consuming pathways. Yield as the final resultant of the different physiological processes in the plant is considered to reflect the plant's response to any change in salinity and or moisture content in soil (Rabie et al. 1985). Thus in the present investigation, irrigation of wheat plants with 10% or 25% sea-water, generally decreased yield and yield component of wheat plants (Table 1). The reduction in crop yield of sea-water-irrigated plants is a direct result of the reduction in grain yield and straw matter. In this connection Francois et al. (1984) found that, with high salinities, grain yield was significantly reduced. They attributed this to the reduction in seeds weight per spike and individual seed weight (expressed as the weight of 100 seeds). They also recorded that straw yield is more sensitive to salinity than grain yield.

The decrease in grain number per spike may be due to the reduction in spikelets number per spike (Table 1), and/or as a result of sterility induced by salinity so unfilled spikelets appeared where water stress during reproductive stage reduces grain set in grasses through the induction of male sterility but female fertility is unaffected unless the stress is more severe and prolonged (Skazkin and Lukomskaya 1962). The effect of salinity on grain yield of wheat plants was studied by Rabie et al. (1985). They found

Table 1

Effect of grain presoaking in gibberellic acid (GA_3) on yield components of wheat plants irrigated by two levels of seawater

Presoaking treatments	Seawater (%)	Plant height (cm)	Spike length (cm)	Spikelets number/spike	Crop yield (g/plant)	Grain yield (g/plant)	No of grains/main spike	100-Kernel weight (g)	Harvest index	Crop index	Mobilization index
No	0	87.12	15.44	17.0	7.86	5.2	40.1	6.0	102.00	0.52	1.31
	10	72.43	13.80	13.1	3.54	2.76	34.3	4.2	85.00	0.45	1.12
	25	54.23	11.21	9.3	0.50	0.18	17.0	1.3	31.00	0.25	0.59
GA_3	0	91.7	16.57	19.1	8.63	5.52	42.6	6.6	94.00	0.49	1.30
	10	80.62	15.30	16.2	5.43	3.45	39.2	5.2	99.00	0.51	1.35
	25	74.22	13.67	13.3	2.23	1.50	31.7	4.0	70.00	0.39	0.95
LSD	0.01	3.84	0.93	0.93	0.78	0.46	5.87	1.35	8.20	0.04	0.09
	0.05	5.12	1.23	1.27	0.11	0.61	7.79	1.90	10.90	0.05	0.13

that the number of grain per spike, the weight of 1,000 grains and the efficiency of grain yield production percent was generally decreased with each increase in soil salinity. Furthermore, the above-mentioned results were in accordance with those obtained by Abdul-Halim et al. (1985, 1988) working on wheat plants.

The reduction in plant height of sea-water-treated plants might be attributed to the reduction in the length of peduncle and/or spike. Water stress appeared to decrease the meristematic activity and elongation of cells, so plant height was decreased (Dorgham 1991). Salinity stress imposed by sea-water led to decrease harvest, mobilization and crop indices (Table 1). These results are in good conformity with those obtained by Feyerherm et al. (1984), and Aldesuquy et al. (1998).

Grain presoaking in GA_3 appeared to nullify either partially or completely the effects of stress induced by sea-water on yield components of wheat plants. The increase in wheat productivity may probably be due to the increased rate of translocation of photosynthates from leaves to developed grains by the effect of GA_3 application (Ray and Choudhuri 1981). The stimulative effect of GA_3 on crop productivity of sea-water-treated wheat plants could be explained on the basis that gibberellic acid increased the rate of transpiration under stress conditions (Livne and Vaadia 1965). Therefore, it may increase the rate of cytokinin supply from root to grains through transpiration stream, since the higher level of endogenous cytokinin improves yield in wheat (Herzog and Geisler 1976). This postulation may be quite decisive when looking to the findings recorded by Itai and Vaadia (1965) where they noticed a reduction in the level of cytokinin in the exudate of plants exposed to salinity stress. Furthermore, Sinel'nikova et al. (1972) indicated that the elimination of the inhibitory effect of NaCl salinization by GA_3 treatment is supposed to be due to the modified ratio between growth stimulators and inhibitors induced by GA_3 in the plant tissues. This suggestion become more decisive particularly after looking to the presented data in Table 3 which clearly shows that grain pretreatment with GA_3 increased the ratio of stimulators to inhibitors in developed wheat grains treated with seawater.

Changes in biochemical aspects of developed grains

Although much work has been done on the effect of salinity on various aspects of plant growth and development, very little attention has been paid to the effect of salinity on fruit synthesis and productivity. The mani-

fested decrease in grain biomass (fresh and dry matter) of the plants irrigated with sea-water may probably due to the stress imposed by sea-water which decreased the capacity of the sink (grain) to store the photosynthetic assimilates through reduced grain filling duration (Dorgham 1991). On the other hand, grain priming with GA_3 appeared to reduce the effect of sea-water on grain biomass (Table 2). This alleviation by gibberellic acid pretreatment may probably be due to the transport of photoassimilate from source (leaves) to sink (grain). This postulation becomes supported by the finding of Aldesuquy and Baka (1991) who found that grain pretreatment with IAA or GA_3 increased phloem thickness in conductive canals between flag leaf and the peduncle of main spike and in this way may increased the rate of translocation of assimilate from flag towards the developing grains increasing their dry matter.

Irrigation of wheat plants by sea-water at 10% and 25% induced a marked increase in phosphorus content of developing grains. Such increment was more pronounced in 25% sea-water-treated plants. It is well established that phosphorus is highly mobile element in plant moving readily between organs (Zabluda and Prosteva 1956). Thus, the high amount of phosphorus detected in leaves of salinized plants may be the reason of increment of phosphorus in developing grains (unpublished data). On the other hand, the interactive effect of sea-water and GA_3 led to marked reduction in phosphorus content of developing grains (Table 2). The reduction in phosphorus content may presumably due to the fact that GA_3 treatment increase the utilization of phosphorus during grain filling but the lack of data about the phosphorus changes during grains growth and development make this postulation not decisive.

Sea-water salinity at 10% increased K^+ and Cl^- content in the developing grains while Na^+ content appeared to be nonsignificantly affected. Irrigation with sea-water at 25% led to insignificant increase in Na^+ and K^+ content of developing grains. On the other hand, grain pretreatment with GA_3 reduced sea-water stress by eliminating Na^+, Cl^- and at the same time increase the K^+ level within the developing grains. The effect of GA_3 being more pronounced with the higher level of sea-water (Table 2). The increase of K^+ content in developing grains as a result of GA_3 pretreatment, might be explained on the assumption that this bioregulator increased K^+ absorption by the plants (Ibrahim and Khafaga 1986).

It is apparent from the data presented in Table 2 that irrigation of wheat plants with sea-water decreased the carbohydrate content of developing grains. The recorded decrease in polysaccharides and total carbohy-

Table 2

Effect of grain presoaking in gibberellic acid (GA_3) on grain biomass, minerals (mg g^{-1}d.wt), carbohydrates and protein content in developing grains of wheat plants irrigated by two levels of seawater

Presoaking treatments	Seawater (%)	Grain biomass (mg/grain)		Elements						Carbohydrates (mg g^{-1} d.wt)			Protein (mg g^{-1} d.wt)
		FW	DW	K^+	Na^+	Cl^-	Phosporus (mg g^{-1} f.wt)			Red .* Sugars	Sucrose	Polysaccharide	
							Inorg.	Org.	T.				
No	0	67.6	60.8	5.1	7.3	1.1	0.07	2.40	2.47	1.30	20.90	765.30	72.87
	10	60.8	54.5	6.0	7.9	1.3	0.09	2.45	2.54	1.50	27.30	666.70	89.34
	25	20.6	19.6	5.3	8.1	3.3	0.45	5.01	5.46	0.68	22.81	234.87	105.27
GA_3	0	66.8	60.2	5.0	6.5	0.9	0.08	1.40	1.48	1.42	20.46	743.10	98.19
	10	61.3	54.4	5.3	5.5	1.0	0.10	1.41	1.51	1.46	24.75	704.32	152.08
	25	57.9	51.6	5.6	6.1	1.4	0.23	2.60	2.8	0.52	30.48	532.65	201.64
LSD	0.05	3.1	2.9	0.3	0.2	0.4	0.19	0.03	0.45	0.19	5.15	91.56	11.15
	0.01	4.3	3.9	0.4	0.3	0.6	0.25	0.04	0.62	0.27	6.99	124.53	14.87

Red.* = reducing, f.wt = fresh weight, d.wt = dry weight

drates of wheat grains could be explained on the fact that, sea-water impaired utilization of carbohydrates during the vegetative growth and reduced the phloem thickness between leaves and spikes (unpublished data), therefore reduces the translocation of photoassimilates toward the developing grains. On the other hand, sea-water treatment at 10% increased the reducing sugars and sucrose values in developing grains. It is clear that increase in soluble sugars may increase at the expence of the insoluble ones. In accordance with these results, Jenner et al. (1991) concluded that stress curtails assimilate supply during grain filling.

Sea-water salinity increased the protein content of the developing grains supporting the view saying that salinity enhances protein synthesis in cereals and promotes the conversion of N into protein (Dorgham 1991). In various crop species, a decrease in protein level in salt-stressed plant parts is attributed to a decrease in protein synthesis, the decrease availability of amino acids, and denaturation of the enzymes involved in amino acids and protein synthesis (Dubey and Rani 1989). Interactive effect of sea-water and GA_3 increased the carbohydrate and protein content in the developing wheat grains. The stimulative effect of GA_3 on protein content of developing grains may be due to GA_3 enhancing the synthesis of protein in grains and thereby increasing the incorporation of amino acids into protein (Aldesuquy 1998). Another explanation may come from GA_3 enhancing the incorporation of free amino acids into conjugated protein or isoenzymes to increase the salt tolerance of developing grains, where a greater number of isoenzymes were observed in the embryoaxes of salt tolerant-sensitive varieties (Dubey and Sharma 1990).

The role of endogenous growth bioregulators during water and salinity stress has been a subject for much research nowadays. Irrigation of wheat plants with sea-water altered the balance of hormonal levels in developing wheat grains, where 10% or 25% induced a significant increase in ABA concurrently with a decrease in growth promotery substances (Table 3). This increase in ABA under stress condition could be contributed to an enzymatic conversion of a precursor to inhibitor-B during prolonged salinity exposure. One possible precursor could be the glucopyranoside of abscisic acid which was isolated from plant tissue (Koshimizu et al. 1968). Hiron and Wright (1973) suggested that the rapid increase in free levels of ABA in stressed tissues may arise from an inactive or bound form of ABA. The recorded decrease in auxins, gibberellins and cytokinins that accompanied with the massive increase in ABA in developed grains, in response to

Table 3

Effect of grain presoaking in gibberellic acid (GA_3) on growth bioregulators content ($\mu g\ g^{-1}$ f.wt) in developing grains of wheat plants irrigated by two levels of sea-water

Presoaking treatments	Sea-water (%)	Total auxins (equivalent to IAA)	Gibberellins (equivalent to GA_3)	Cytokinins (equivalent to kinetin)	Total inhibitors (equivalent to ABA)
No	0	10.38	3.92	2.85	2.66
	10	6.33	2.87	1.53	8.97
	25	1.63	0.09	1.02	14.67
GA_3	0	10.76	4.53	2.35	1.52
	10	7.84	3.54	2.05	4.46
	25	7.59	1.74	1.65	7.81
LSD	0.05	0.033	0.16	0.17	0.13
	0.01	0.063	0.31	0.31	0.24

sea-water salinity are in accordance with the results obtained by many workers (Abo-Hamed et al. 1990, Zevaart 1983).

In this thesis, it is clear that grain presoaking in GA_3 induced great changes in endogenous bioregulators (Table 3). Thus, the manifested decrease in total auxins, gibberellins and cytokinins in developing grains was partially nullified in response to grain pretreatment with GA_3. Furthermore, a reduction in ABA was elicited and the magnitude of reduction was more pronounced with GA_3. Therefore, when GA_3 was applied to the plants under stress conditions, resulted in adding more enough gibberellins to counteract the high level of endogenous ABA already increased and presented in stressed plant tissues (Livne and Vaadia 1965, El-Antably 1975).

Now it is evident from this investigation that the drastic decrease in yield components and biochemical aspects of developed grains of sea-water-treated wheat plants alleviated and even promoted over control values by gibberellic acid pretreatment.

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REGULATING EFFECT OF SALICYLIC ACID ON GERMINATION OF *LUPINUS TERMIS* SEEDLINGS

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Salicylic acid at 2.5 or 5 mM increased the root length, fresh and dry matter of the whole seedlings while at all concentrations decreased the rate of germination and hypocotyl length. In the majority of cases, salicylic acid appeared to affect the composition of certain ions by increasing Na^+, decreasing K^+ (particularly at 5 or 10 mM), and consequently the K^+/Na^+ ratio in germinating lupin seedlings. Salicylic acid at 5 mM appeared to be the most effective dose in increasing the carbohydrate during germination of lupin seedlings. In general, salicylic acid at 2.5 or 5 mM caused significant increases in nitrogen and organic acid content of lupin seedlings, while the higher dose (10 mM) appeared to have an inhibitory effect. Salicylic acid treatments showed marked decrease in IAA level and enhanced IAA-oxidase activity during the germination of lupin seedlings. The growth inhibitory substances in terms of abscisic acid increased in response to salicylic acid treatments, while total gibberellins and cytokinins increased particularly on treatment with 2.5 or 5 mM salicylic acid.

Key words: germination, hormones, IAA-oxidase, lupin, organic acids, salicylic acid

Introduction

Various attempts have been carried out within the last few years to increase the yield and improve quality of several plants by various chemical treatments of seed before planting. Promising and positive results were obtained by several workers. Of particular interest, phenolic compounds have been shown to be of importance in the regulation of plant growth and metabolism, and they are longer considered to be passive by-product (Jain and Srivastava 1981). Salicylic acid a natural phenolic compound present in some plants. Salicylic acid is one of a range of chemicals that induce systemic resistance (Gorlach et al. 1996). In some cases, chemical treatment induces expression of the same genes and resistance against the same spectrum of pathogens as pathogen induced resistance (Lawton et al. 1996, Friedrich et al. 1996). The resistance induced by chemical treatment can be very effective (Gorlach et al. 1996) and may provide commercially useful broad-spectrum plant protection that is stable, long-lasting and environmentally benign.

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Salicylic acid (antitranspirant) was found to enhance growth of daughter fronds of *Lemna* (Dekock et al. 1975). Also, Jain and Srivastava (1981) reported that the relatively lower concentrations of salicylic acid stimulated the growth of maize seedlings whereas the relatively higher concentrations were of inhibitory action. Moreover, it has been demonstrated that salicylic acid accelerates leaching of soluble nitrogen from maize endosperm (Jain and Srivastava 1981).

In the light of the above, limited reviews of literature, it was thought of particular interest to study the effect of seed priming with salicylic acid on seedling growth observations, carbohydrates, nitrogen, organic acids, ionic composition, endogenous IAA level and IAA-oxidase activity, as well as the endogenous hormonal level in germinating lupin seedlings.

Material and methods

Plant material and experimental design

Seeds of *Lupinus termis* (var. Balady) were surface sterilized by soaking in 0.01 M $HgCl_2$ for 3 minutes, washed with sterile distilled water several times and then divided into four groups that were separately soaked in distilled water to serve as control, 2.5, 5 or 10 mM aqueous solution of salicylic acid for about three hours. After soaking period, the seeds were washed thoroughly with distilled water to remove any adhering chemicals. Samples were collected immediately for analyses of carbohydrates, nitrogen, organic acids, ionic composition and hormonal level, as well as IAA-oxidase as initial before being germinated in darkness. The rest of treated and untreated seeds were set to germinate in the dark for seven days on moist paper tissues in plastic trays at 25 °C. The germinating seeds were irrigated with water according to the usual practice.

Seedling growth observations

Fresh and dry matters were estimated for samples of ten seedlings. Fresh matters were determined immediately after sampling. These materials were dried at 80 °C until constant weights were attained. Ten seedlings were taken from each replicate to determine their length after 7 days germination. The length of both root and hypocotyl were recorded.

Analytical methods

The methods of extraction, clarification and determination were essentially those described by Younis et al. (1969).

Nitrogenous constituents were extracted from the dry powdered tissues by the method of Yemm and Willis (1956). Total soluble-N was determined in the extracts and total-N in the dry tissues by the conventional micro-Kjeldahl method. Subtraction of total soluble-N from total-N gave the value for protein-N. Aliquots of the extracts were used for estimation of amino-N according to the method described by Muting and Kaiser (1963).

The method adopted by Fridman and Haugen (1943) was used to determine total α-keto acids (in terms of α-keto-glutaric acid) in this study. For estimation of oxalic and citric acids, the methods used were essentially those described by Hasaneen et al. (1987).

For estimation of elements, a known amount of dry matter was digested in concentrated HNO_3 and made up to volume with distilled water. K^+, Na^+ and Ca^{2+} concentrations were measured by flame emission spectrophotometry (Younis et al. 1994).

Indole acetic acid (IAA) was extracted and assayed according to Gordon and Paleg (1957). IAA-oxidase was extracted and assayed according to Rabin and Klein (1957). Growth inhibitors equivalent to abscisic acid, total gibberellins equivalent to GA_3 and cytokinins were extracted and separated according to the method described by Shindy and Smith (1975). For determination of abscisic acid (ABA) in extracts, wheat coleoptile bioassay was used (Wright 1969). For measurement of gibberellin-like substances, the lettuce hypocotyl bioassay adopted by Frankland and Wareing (1960) was followed. The technique used to assay the activity of cytokinins was as in Esahi and Leopold (1969).

The results were subjected to an analysis of variance (ANOVA). When the effect was significant ($p \leq 0.05$), the least significant difference was used to compare the treatments (Snedecor and Cochran 1976).

Results and discussion

In control seeds, there was 100% germination after two days. On the other hand, seed presoaking in different concentrations of salicylic acid induced significant reduction ($p < 0.05$) in germination percentage (Table 1). In connection with these results, Aldesuquy (1990) found that coumarin at 1 and 10 ppm decreased the germination percentage of wheat seedlings,

while 100 ppm coumarin inhibited germination of seedlings. Moreover, Hasaneen et al. (1987) found that the germination of sorghum grains was inhibited by 500 ppm coumarin. Salicylic acid as a phenolic compound similar to coumarin which regarded as a "hormonal regulator of germination" (Mayer and Poljakoff-Mayer 1975).

Salicylic acid at all concentrations (except the 10 mM on the 7-d germination) induced significant increase in fresh matter of seedlings after one week germination (Table 1). It is clear that salicylic acid at 2.5 and 5 mM induced significant increase ($p < 0.05$) in dry matter of lupin seedlings at the beginning and after one week germination. The relatively higher dose of salicylic acid appeared to decrease ($p < 0.05$) the dry matter of lupin seedlings particularly at the beginning of germination. Moreover, salicylic acid at 2.5 or 5 mM caused significant increase ($p < 0.05$) in root length of germinating seedlings, while the higher concentration decreases ($p < 0.05$) this length. Salicylic acid at all concentrations appeared to reduce ($p < 0.05$) the hypocotyl length of germinating lupin seedlings (Table 1).

The increase in fresh matter of germinating seedlings in response to treatment with low and medium doses of salicylic acid may presumably be due to salicylic acid may increase the water uptake by lupin seedlings. Moreover, the observed increase in dry matter of lupin seedlings may result from the carbohydrate and nitrogen accumulation accompanied with salicylic acid treatments. Inhibition of root growth in lupin seedlings treated with 10 mM salicylic acid may be mediated via the effect of salicylic

Table 1

Effect of salicylic acid concentrations on growth observations of lupin seedlings

Growth observations		Germination (%)	Seedling f.wt (mg)		Seedling d.wt (mg)		Root length (cm)	Hypocotyl length (cm)
Germination period (days)		2	0	7	0	7	7	7
Presoaking treatments (mM)								
No		100.0	880.0	450.0	270.0	200.0	30.80	25.30
Salicylic acid	2.5	95.0	1210.0	470.0	279.0	230.0	32.70	20.8
	5	93.0	1325.0	480.0	291.0	235.0	33.40	22.60
	10	91.0	910.0	400.0	250.0	201.4	27.0	20.2
LSD ($p < 0.05$)		1.75	24.0	11.2	1.70	1.80	0.379	0.131

acid on endogenous IAA content. Salicylic acid decreases the level of IAA by increasing its destruction throughout the increase in the level of IAA-oxidase and may inhibit the IAA transport towards the root (see Fig. 5).

Salicylic acid at all concentrations used increased the Na^+ content in lupin seedlings during its germination, while only 5 or 10 mM decreased K^+ and consequently K^+/Na^+ ratio (Fig. 1). At the beginning of germination salicylic acid at all concentrations decreased ($p < 0.05$) Ca^{2+} content of lupin seedlings, whereupon after one week germination only 5 or 10 mM decreased ($p < 0.05$) significantly this content (Fig. 1). Salicylic acid appeared to affect certain ions compositions, where it caused marked increase in the amount of Na^+ and generally appeared to decrease the amount of K^+ and Ca^{2+} as well as K^+/Na^+ ratio. Salicylic acid may alter the permeability of cell membrane to specific ions (Glass 1974, Harper and Balke 1981) by depolarizing the membrane potential (Glass and Dunlop 1974). This may be the reason for the marked decrease in relative amounts of K^+ detected in germinating lupin seedlings particularly with 5 or 10 mM. The action of salicylic acid on membrane functions linked to the presence of the free phenolic hydroxyl on the benzene ring. Since the lack of this hydroxyl (benzoic acid) or estrification (ascorbic acid) abolishes its effects. In addition, salicylic acid as a phenolic compound possesses chelating properties (Clemeston and Anderson 1966). Thus it interferes with some important elements of cellular and organellar membranes, thereby increasing their permeability.

The data presented in Figure 2 showed that salicylic acid at all of doses applied caused marked increase ($p \leq 0.05$) in reducing sugars (throughout the germination period) and sucrose at the beginning of germination, and after one week germination, only the medium dose (5 mM) caused significant increase in sucrose. Furthermore, salicylic acid at 5 mM was found to be the more effective concentration in increasing the carbohydrate in germinating lupin seedlings (Fig. 2). The effect of salicylic acid on carbohydrate metabolism in germinating lupin seedlings may be mediated via phytohormones. Many phenolic compounds are known to enhance auxin oxidation (Schneider and Whitman 1974, Aldesuquy 1987). Therefore, the decrease in IAA content in lupin seedlings in response to salicylic acid treatment (see Fig. 5) may result in a decrease in invertase enzyme, as it has been demonstrated by Glasziou et al. (1966). This fact could explain the observed increases in sucrose content of germinating lupin seedlings particularly with the medium dose of salicylic acid. Alternatively, in such germinating seedlings, sucrose might be the main pool of sugars as it is the transportable form and part of sucrose could be transformed into polysaccha-

rides as it is obvious from the increased levels of such fraction in germinating seedlings treated with 5 mM salicylate.

In general, salicylic acid at 2.5 or 5 mM appeared to increase the total soluble nitrogen, protein-N and consequently the total nitrogen in germinating lupin seedlings. On the other hand, the higher level of salicylic acid appeared to have an inhibitory effect on nitrogen content of lupin seedlings. Furthermore, salicylic acid at all of doses seemed without significant effect on ammonia-nitrogen of lupin seedlings (Fig. 3). The observed in-

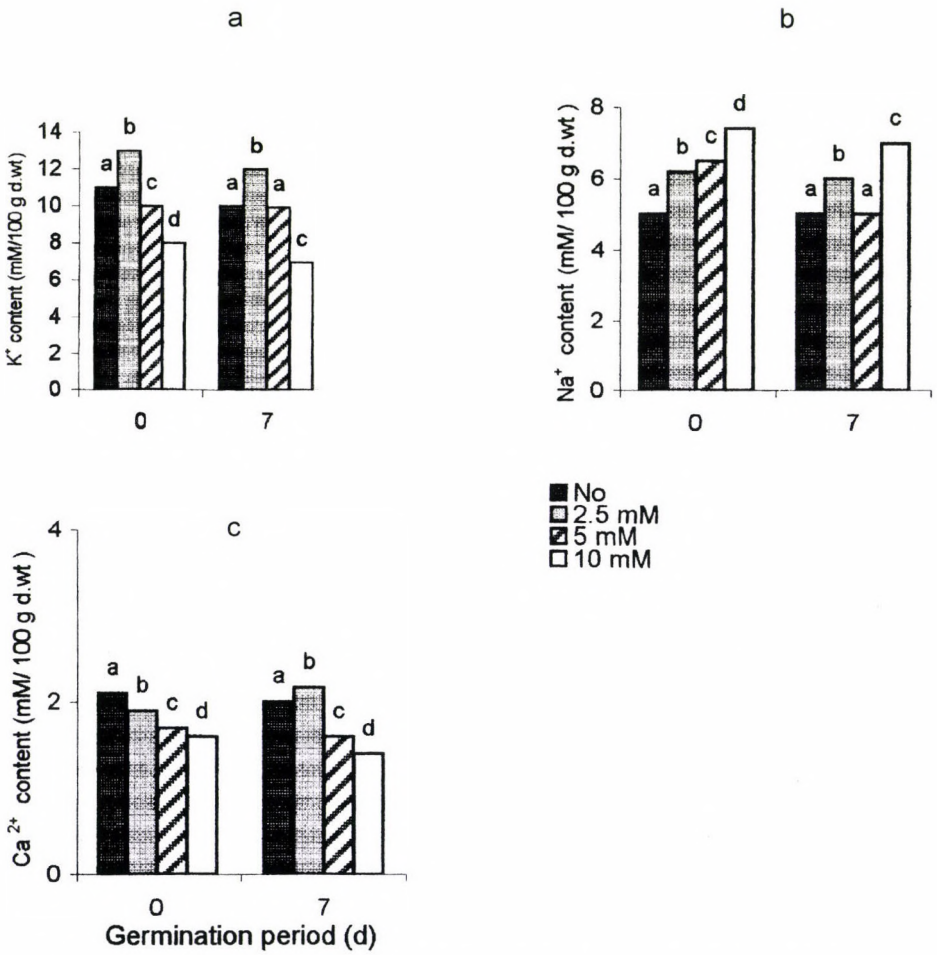

Fig. 1. Effect of salicylic acid concentrations on potassium (a), sodium (b) and calcium (c) content of lupin seedlings. Bars in a grouping labeled with the same letter are not significant as indicated by LSD ($p < 0.05$)

crease in nitrogen content of lupin seedlings resulted from the treatment with 2.5 or 5 mM salicylic acid may probably be due to salicylic acid may have an inhibitory effect on proteolytic enzymes but the lack of data about the protease level make this postulation not decisive.

Greenway and Munns (1980) suggest that higher concentration of organic solutes in cytoplasm play a double role (i) they can contribute to the osmotic balance when electrolytes are lower in cytoplasm than in vacuole and they have a protective effect on enzymes in the presence of higher elec-

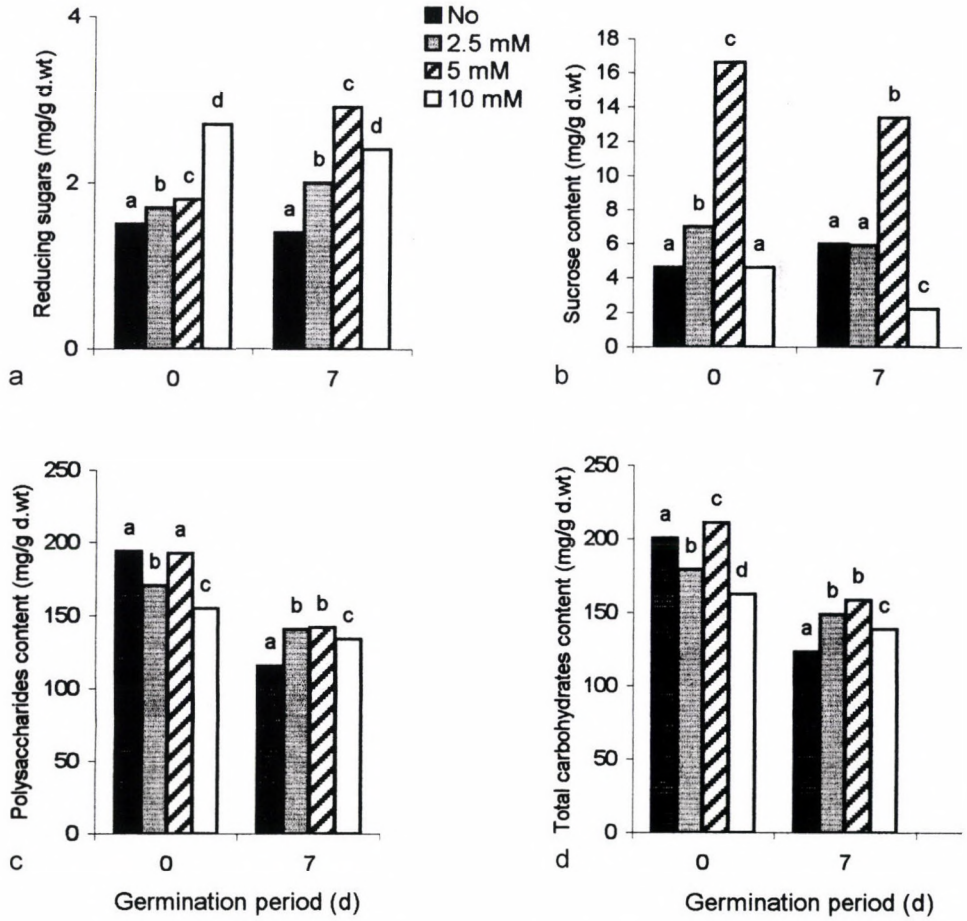

Fig. 2. Effect of salicylic acid concentrations on carbohydrate content of lupin seedlings. Bars in a grouping labeled with the same letter are not significant as indicated by LSD ($p < 0.05$)

trolytes in cytoplasm. Thus in the present investigation, the resulted increase in organic acid content of lupin seedlings treated with salicylic acid at 2.5 or 5 mM is in agreement with the increase in protein (i.e. amino acids) (Fig. 4). This is to be expected since the two groups of substances are known to be readily inter-convertible by the widely distributed transaminases. On the other hand, the higher concentration of salicylic acid (10 mM) decreased ($p < 0.05$) the organic acid content particularly oxalic and α-ketoglutaric acids. This may be presumably due to the higher concentration of salicylic acid which acts in a reverse manner by inactivation of transaminases.

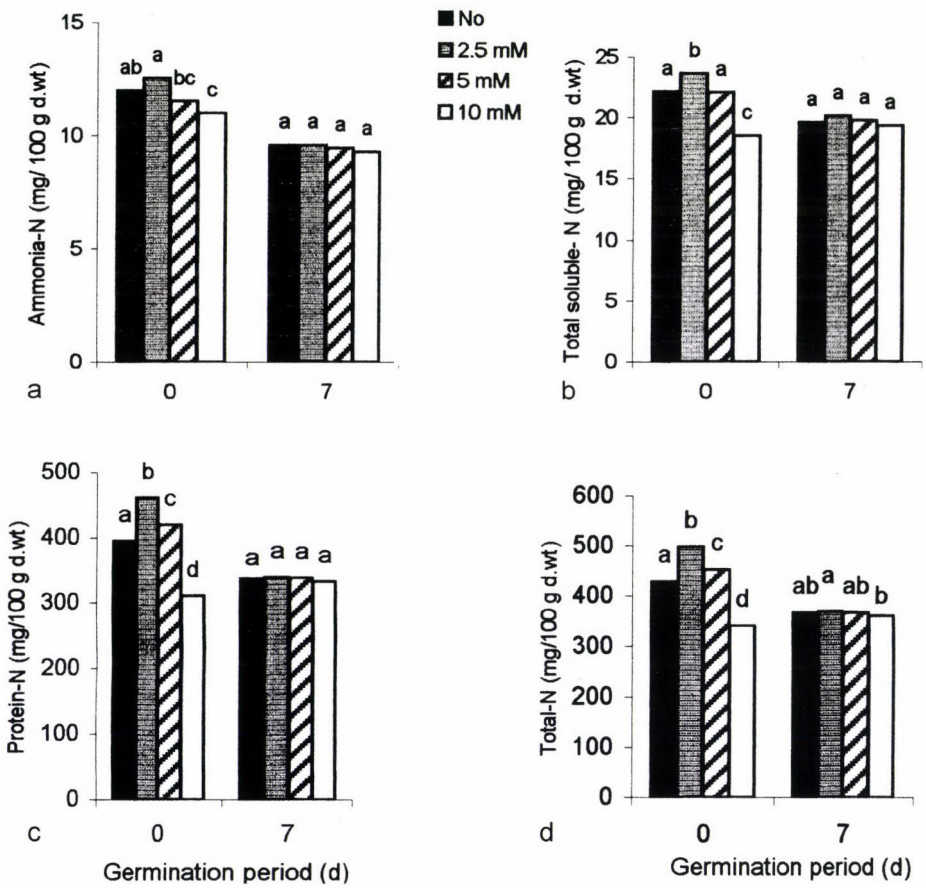

Fig. 3. Effect of salicylic acid concentrations on nitrogen content of lupin seedlings. Bars in a grouping labeled with the same letter are not significant as indicated by LSD ($p < 0.05$)

Although hormones have been shown to promote germination, it is by no means clear-cut. In this connection, salicylic acid caused remarkable decline in IAA content of lupin seedlings and at the same time enhanced IAA-oxidase activity which mainly responsible for the oxidation of IAA leading to the low level of IAA in lupin seedlings (Fig. 5). These results were in accordance with those obtained by several authors (Lee and Skoog 1965, Sharma and Kaushik 1983, Aldesuquy 1987).

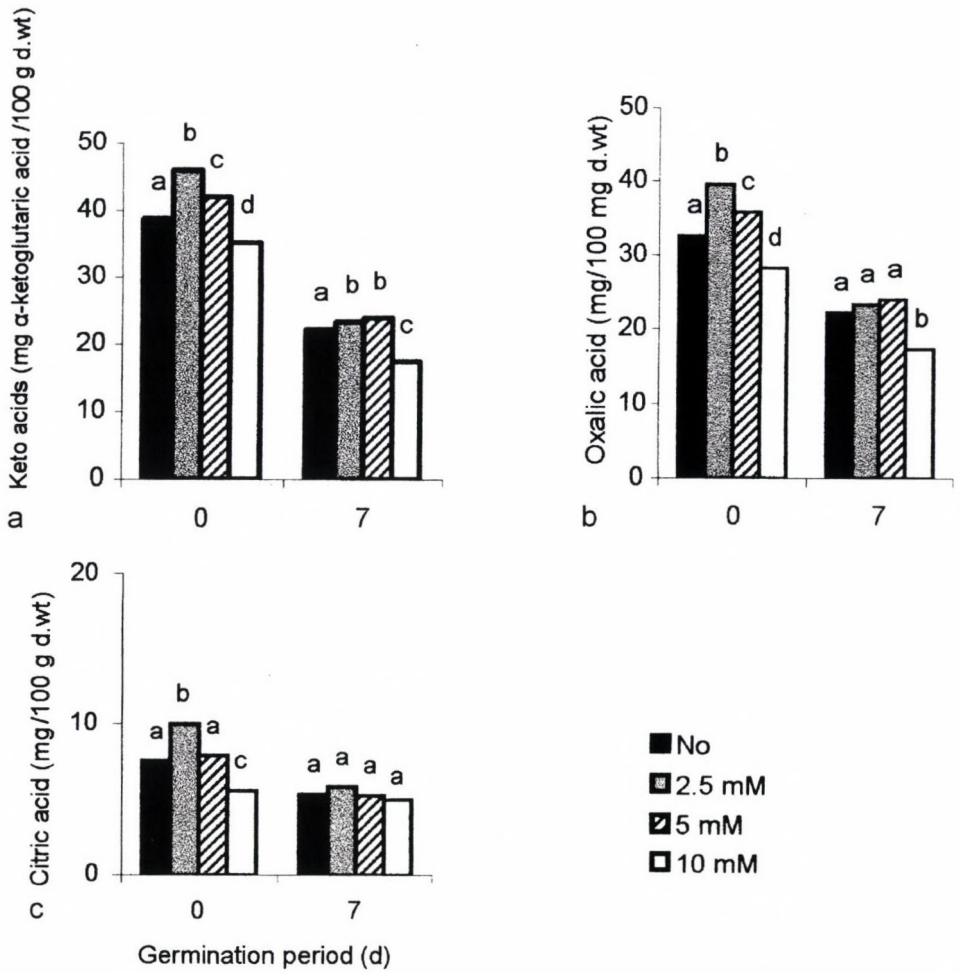

Fig. 4. Effect of salicylic acid concentrations on organic acids content (keto acids (a), oxalic acid (b) and citric acid (c)) of lupin seedlings. Bars in a grouping labeled with the same letter are not significant as indicated by LSD ($p < 0.05$)

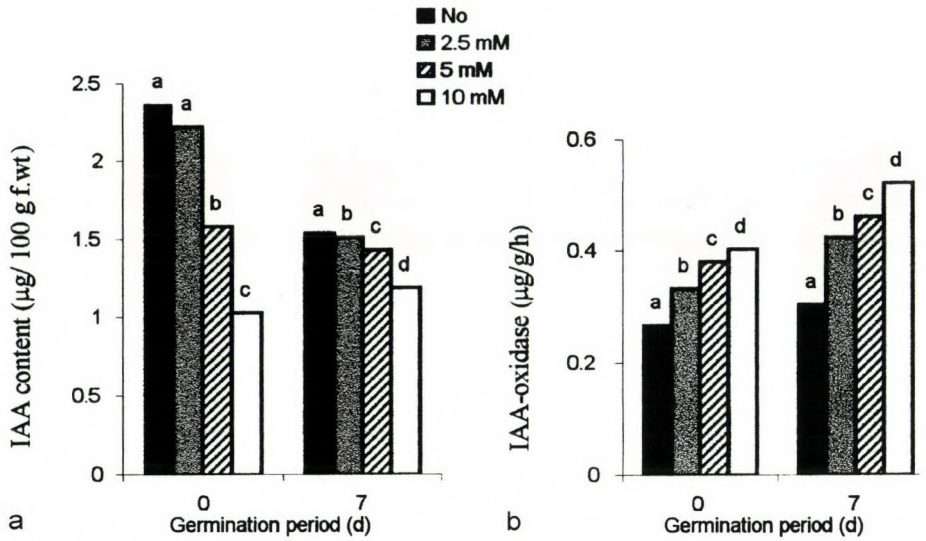

Fig. 5. Effect of salicylic acid concentrations on endogenous indole acetic acid level (a) and indole acetic acid oxidase activity (b) of lupin seedlings. Bars in a grouping labeled with the same letter are not significant as indicated by LSD ($p < 0.05$)

Salicylic acid treatments (5 or 10 mM) enhance the accumulation of growth inhibitory substances in terms of ABA in lupin seedlings, the greater the salicylic acid concentration the higher the rate of ABA level. Throughout the germination period there was a decline in ABA in response to salicylic acid treatments (Fig. 6). The observed increase in ABA in lupin seedlings treated with salicylic acid suggest that salicylic acid may act as inducer for the production and/or accumulation of ABA in germinating seedlings. As the germination progress the accumulated ABA may be inactivated by glucosylation or changed to bound form by the effect of salicylic acid treatments.

Concerning the changes in cytokinins and total gibberellins equivalent to gibberellic acid in germinating lupin seedlings, the lower levels of salicylic acid was obviously able to enhance the cytokinin and gibberellin production during germination, while the higher dose led to marked reduction in cytokinins and gibberellins. This may probably be due to the demand for cytokinin and gibberellins during germination, since gibberellins act as inducer for some hydrolyases which play an important role during germination (Jacobsen et al. 1995, Jones et al. 1998, Lenton et al. 1994).

Based on the afore-mentioned pattern of results, it emerged out that salicylic acid at relatively higher concentration (10 mM) decreased germi-

nation of lupin seedlings. This could presumably be due to salicylic acid caused an increase in the ABA level and IAA-oxidase activity with a simultaneous decrease in IAA, gibberellins and cytokinins which may interfere with seedlings growth by limiting water uptake of embryo rather than by inhibiting energy metabolism (Schopfer and Plachy 1985). Salicylic acid caused certain metabolic changes, i.e. carbohydrate metabolism where it increased soluble sugars in developing seedlings and at the same time reduced the respiration rate (Gaber et al. 1991). Furthermore, salicylic acid at higher dose reduced protein-N, total soluble-N and organic acids, consequently due to these event of results, the germination of lupin seedlings was significantly decreased.

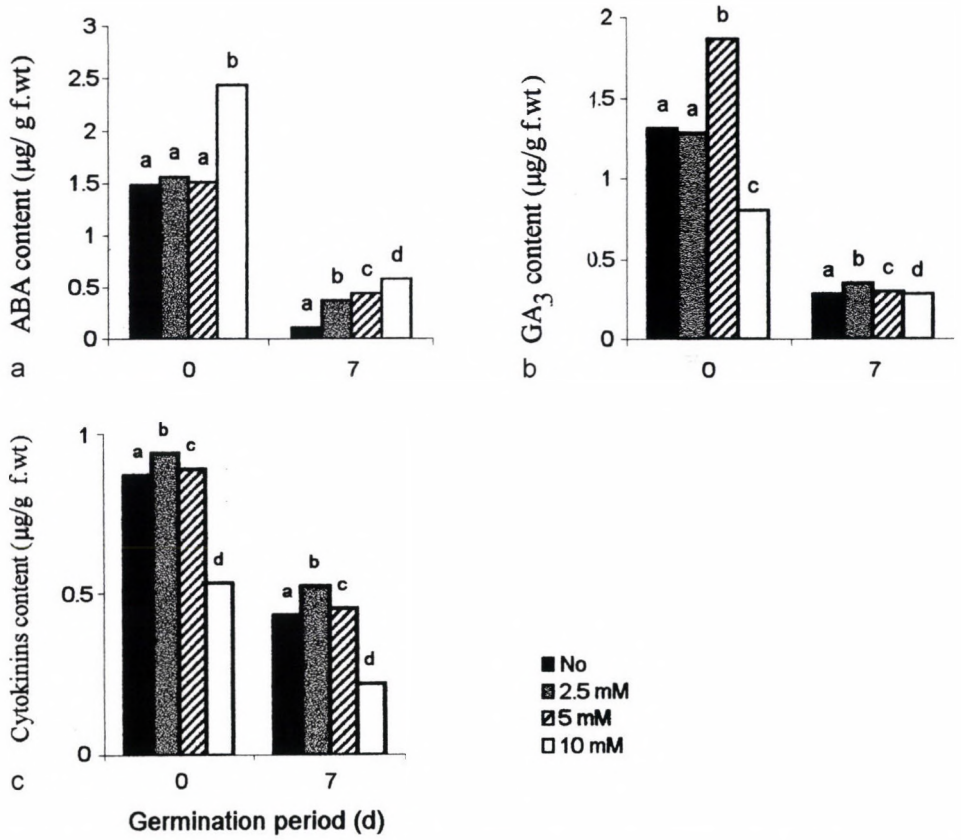

Fig. 6. Effect of salicylic acid concentrations on total growth inhibitors equivalent to ABA (a) and total gibberellins equivalent to GA_3 (b) and cytokinins (c) of lupin seedlings. Bars in a grouping labeled with the same letter are not significant as indicated by LSD ($p < 0.05$)

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THE EFFECT OF POLYETHYLENE GLYCOL ON ISOPEROXIDASES IN WHEAT (*TRITICUM AESTIVUM* L.)

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The effect of polyethylene glycol (PEG) on the activity and expression of peroxidases (EC 1.11.1.7) in both the root and shoot systems of *Triticum aestivum* var. *ERA* was investigated. The roots of the PEG-treated plants showed 46% reduction in peroxidase activity after 6 hrs of treatment, while the shoots showed 44% and 105% increase in peroxidase activity after 6 and 24 hrs, respectively. The pattern of isoperoxidases resolution by isoelectric focusing (IEF) polyacrylamide gel electrophoresis indicated that the decrease in peroxidase activity in the PEG-treated roots was due to a reduction in the expression of isoperoxidases under such a condition and excluded the possibility of de novo synthesis of new isoperoxidases in the PEG-treated shoots. The presented differences in the expression of isoperoxidases in both the control and PEG-treated plants represent one of the biochemical changes plants undergo in coping with stressing conditions.

Key words: polyethylene glycol, isoperoxidases, wheat (*Triticum aestivum*)

Introduction

Higher plants are constantly exposed to stressing factors that affect their physiology and metabolism via the induction and/or repression of certain proteins (Sachs and Ho 1986). As part of their protective machinery against such factors, plants usually exhibit significant and characteristic changes in the activity of their antioxidant enzymes. Water shortage represents one of the most important stressing factors which has been shown to increase guaiacol peroxidase, decrease catalase and superoxide dismutase activities in different wheat genotypes (Zhang and Kirkham 1994) and increase ascorbate peroxidase activity in *T. aestivum* primary leaf segments (Baisak et al. 1994). The clear association between the activity of peroxidases and changes in environmental conditions and the simplicity of assay procedures recommend peroxidase enzymes as good and useful markers for plant stress (Baga et al. 1995, Lagrimini et al. 1990).

In higher plants, the expression of different peroxidase isozymes is tissue specific, developmentally regulated and influenced by environmental

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factors (Lagrimini and Rothstein 1987). There are at least 12 anionic, moderately anionic and cationic isoperoxidases in tobacco (Lagrimini et al. 1987), 7 anionic and cationic isoperoxidase types in rice (Reimers et al. 1992), and 13 soluble cationic forms in wheat germ (Converso and Fernandez 1995). However, in spite of the great interest in isoperoxidases and their great multitude, little is known about their role in the adaptation of plants to changing environmental conditions (Lagrimini et al. 1990, Liu et al. 1990).

In the present study, polyethylene glycol (PEG) which acts as an inert osmoticum in penetrating cell membranes and induces water stress in various plant tissues (Dell'Aquila 1992, Dell'Aquila and Bewley 1989, Stewart and Voetberg 1987) has been used as a stressing factor to investigate the effects of its treatment on the activity and expression of guaiacol peroxidases in the shoot and root systems of *T. aestivum* L. var. *ERA*.

Materials and methods

Plant material and treatment

Seeds of wheat (*Triticum aestivum* L. var. *ERA*) were rinsed, sterilised with 1% $CuSO_4$ and 10% commercial chlorox and then planted in pots containing perlite. Plants were grown under the greenhouse conditions and watered for the first five days of growth with tap water, as necessary, then with half strength Hoagland's solution. Test seedlings were grown for ten days before they were uprooted. The roots of the test seedlings were rinsed with tap water then incubated for one hour in a solution of 50 mM sucrose and 1 mM glutamic acid (Stewart and Voetberg 1987) before the PEG treatment which was accomplished by immersion of the roots of three seedlings in 20 ml solution of 100 g l^{-1} PEG-1500, sucrose and glutamic acid. Control seedlings were prepared in the same way as test plants except that they were not treated with PEG. Control and PEG-treated seedlings were harvested after 2, 6, and 24 hrs of incubation in the proper solutions in the laboratory. Shoot and root tissues of each treatment were collected, weighed, and either used immediately for determination of the relative water content or stored at –70 °C until they were used for enzyme assays.

Determination of the relative water content (RWC)

The relative water content of the root and shoot systems of the control and PEG-treated plants was calculated according to the following relationship (Ristic and Cass 1992):

$$RWC = (\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight}) \times 100$$

where dry weight was the tissue weight after incubation at 80 °C for 48 hrs and the turgid weight was the tissue weight after incubation in distilled water for 3–4 hrs at room temperature followed by overnight incubation at 4 °C.

Peroxidase extraction

Root and shoot tissues were ground in liquid nitrogen. The thawed powdered tissues were then suspended in cold sodium phosphate buffer (10 mM, pH 6.0) at a weight to volume ratio of 1 : 10 for enzymatic assay and 1 : 1 for electrophoretic studies. The suspension was centrifuged at 20,000 g for 15 min. The supernatant containing soluble peroxidases was dialysed against 10 mM sodium phosphate buffer for two hours before use in enzymatic assay. All the steps of peroxidase extraction were performed under cold conditions.

Peroxidase assay

Peroxidase activity was assayed using a method based on that of Garraway et al. (1989). Each 2 ml reaction volume contained 6.5 mM sodium phosphate buffer (pH 6.0), 12.5 mM guaiacol (Janssen Chimica, Belgium) and 50 µl tissue extract. The reaction was started at room temperature by the addition of 100 µl of 5 mM H_2O_2 (Fluka, Switzerland) to the above reaction mixture. Peroxidase activity, represented by the rate of tetraguaiacol formation, was determined as the change in absorbance at 470 nm per min per mg protein. The absorbance was recorded using a Pye Unicam SP6–550 UV/VIS spectrophotometer (Philips). Three series of experiments, each with three replicates, were conducted. The peroxidase activity of each replicate was assayed twice and the mean values obtained for the individual experiments are presented.

Protein assay

Protein concentrations of the enzyme extracts were determined by the Bio-Rad protein assay using bovine albumin as a protein standard (Bradford 1976).

Electrophoresis

Soluble peroxidases were separated by horizontal isoelectric focusing (IEF) polyacrylamide gel electrophoresis. The gel (11 × 11 cm) containing 5% carrier ampholyte of pH 3–10 (Pharmacia) was prefocused for 1.5 hrs at 300 V and the current was decreased to 1–3 mA using an LKB 2301 Macro Drive-1 power supply. After application of the control and PEG-treated samples to points 1 cm away from the cathodic end of the gel, the electrophoresis was run at 1–3 mA and constant voltage of 490 V for 5.5 hrs. A 1.0 cm wide strip from one end of the gel in the electrofocusing direction was cut into 0.5 cm segments starting from the cathodic end of the gel. Each segment was incubated in a separate test tube containing 2 ml double distilled water for 2–3 hrs. The pH of each segment extract was measured and plotted versus its distance from the cathodic end of the gel. The gel containing the separated isoperoxidases was then stained for activity as described by Reimers et al. (1992). The distance of each isoperoxidase band from the cathodic end was measured and its pI was estimated based on those of the extracts of the gel segments.

Results

Colorimetric determinations of peroxidase activity

Soluble peroxidase activities, RWC, and total protein concentrations in the roots and shoots of *T. aestivum* are presented (Table 1). Peroxidase activities of the root and shoot systems yielded opposite responses to PEG treatment. After 2 and 6 hrs of PEG treatment, the roots exhibited a noticable reduction in peroxidase activity reached to 42% and 46% of that observed in the corresponding controls, respectively. Compared to the controls, the shoots of the PEG-treated seedlings showed a higher activity of soluble peroxidases; this enhanced percent activity was noticed after 2 hrs (17%) and increased to reach about 105% of controls after 24 hrs of PEG treatment.

Table 1

Soluble peroxidase activities, relative water contents, and protein concentrations in the root and shoot systems of both the control and PEG-treated seedlings of *T. aestivum* ±SE

Time (h)	Peroxidase activity (Δ Abs. min^{-1} mg^{-1} protein)		Relative water content		[Protein] (mg ml^{-1})	
	Control	PEG	Control	PEG	Control	PEG
I. The root system						
2.0	1400.2±70.34	808.97±87.24	80.63±1.63	75.25±18.25	0.0253±0.0013	0.0444±0.0015
6.0	1512.08±52.69	812.14±35.01	74.42±9.45	67.80±7.32	0.0283±0.0032	0.0434±0.0020
II. The shoot system						
2.0	148.08±16.98	173.36±17.37	78.35±5.36	57.54±8.0	0.259±0.011	0.298±0.017
6.0	114.57±25.86	164.80±17.08	73.17±2.41	51.89±1.08	0.336±0.039	0.314±0.004
24.0	107.91±30.68	223.84±42.34	81.20±4.50	40.85±0.24	0.431±0.039	0.260±0.051

The RWC of the root and shoot systems did not significantly change over time in the control samples but was decreased in the shoots of the PEG-treated plants; the roots of such plants were not as affected.

The root and shoot systems of the PEG-treated seedlings also exhibited opposite effects in terms of their protein content. Compared to the controls, protein concentration (mg protein ml^{-1} extract) in the PEG-treated roots was increased after 2 and 6 hrs of treatment; while that of the shoots was increased after 2 hrs then slightly and markedly decreased after 6 and 24 hrs, respectively.

Peroxidase isozymes

The pattern of separation of peroxidase isozymes by IEF polyacrylamide gel electrophoresis is shown in Figure 1. There are clear differences between the roots of the PEG-treated and those of the control seedlings as the former exhibited lower intensities of all of the expressed isoperoxidases (Fig. 1a). The shoots of the PEG-treated seedlings (Fig. 1b) showed no qualitative differences from controls as both exhibited the main isoperoxidase bands in the basic and neutral regions of the pH gradient. However, the PEG-treated shoots exhibited an increase in the intensity of the isoperoxidase bands with pIs 8.7 and 7.0, and a decrease in the intensity of that with pI 8.2. In the acidic region, differences between the isoperoxidase

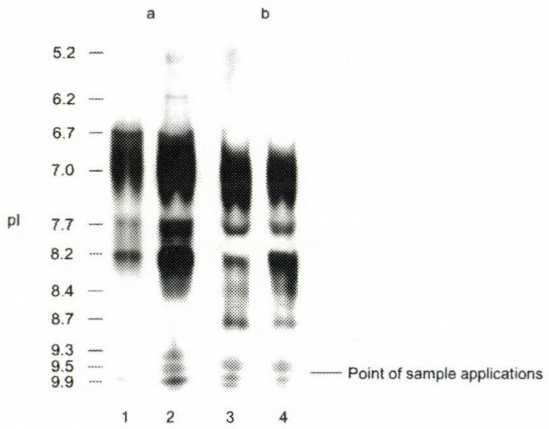

Fig. 1. An IEF gel electrophoresis of the shoot and root systems of wheat after 24 hrs of PEG treatment. a: Lanes 1 and 2 represent PEG-treated and control roots, respectively (1.0). b: Lanes 3 and 4 represent PEG-treated and control shoots, respectively (8.0). The numbers in brackets represent the amount of proteins (μg) added to the gel

bands were very minor and hard to detect even with higher concentrations of proteins.

In spite of the similarities in expression of their isoperoxidases, the control root and shoot systems showed few differences (Fig. 1) as the roots expressed additional isoperoxidases with pIs 9.3, 6.7, and 6.2, but did not express that with pI 8.7 which was found only in the shoots.

Discussion

Alterations of activity of certain enzymes such as peroxidases are among the metabolic changes that occur in plants to cope with stressing factors. The root and shoot systems of *T. aestivum* expressed different peroxidase activities upon treatment with PEG (Table 1). Compared to the controls, the PEG-treated roots showed 46% reduction after 6 hrs of treatment, while the shoots exhibited 44% and 105% increase in peroxidase activity after 6 and 24 hrs, respectively. A similar trend of peroxidase activity in the shoots was obtained by Zhang and Kirkham (1994) after exposing wheat seedlings of different ploidy levels to water stress caused by water withholding instead of PEG treatment. This increase in peroxidase activity in the shoot systems of *T. aestivum* was not reflected qualitatively by the expression of peroxidase isozymes (Fig. 1b). The possibility of de novo syn-

thesis of new isoperoxidases in the PEG-treated shoots does not seem likely to occur as indicated by their resolution in the IEF gel electrophoresis and the reduction in protein concentration compared to the controls. The increase in peroxidase activity in the PEG-treated shoot systems could thus be attributed mainly to the stimulation of the expression of some of the already existing isoperoxidases and the accumulation of peroxidase substrates such as phenolic compounds (Zhang and Kirkham 1994) and H_2O_2 (Moran et al. 1994). Amako et al. (1994) reported a higher expression of peroxidase activity in the root than the leaf tissues of certain dicots under normal conditions. In this study using the monocot *T. aestivum*, a similar expression of peroxidase activity was found as the control roots exhibited about ten times more activity than the shoot systems. Despite this, the PEG-treated roots showed a reduction of peroxidase activity compared to the controls which is clearly indicative of the reduction of the expression of isoperoxidases under such conditions.

Quantitative changes in total proteins were also observed upon PEG treatment in both the shoot and root systems of *T. aestivum*. Imbibition of pea seeds in PEG reduced protein synthesis in the axes of the seeds but no qualitative changes in protein synthesis were observed (Dell'Aquila and Bewley 1989). A reducing effect of the osmotica mannitol and carbowax-4000 on protein synthesis was also detected after 3–5 hrs of the osmoticum treatment of *Avena sativa* coleoptile tissues (Dhindsa and Cleland 1975). A similar effect of PEG on protein synthesis in *T. aestivum* shoots was detected as protein synthesis was reduced (Table 1) but the quality of proteins represented by peroxidases was not affected (Fig. 1b). The reduction in protein concentration in the PEG-treated shoots could thus be attributed to a reduction in the overall rate of protein synthesis after such a treatment. However, the increase of protein concentrations in the PEG-treated roots was neither reflected by an increase in peroxidase activity (Table 1) nor by the quantity and quality of the isoperoxidases (Fig. 1a), since the number and intensity of these isoperoxidases were less in the PEG-treated than in the control roots. The osmoticum treatment might have caused a differential inhibition of the synthesis of root proteins by inhibiting the synthesis of peroxidases at the expense of other proteins that participated in maintaining higher RWC in the roots. Protein synthesis also might have been affected by other metabolic changes occurred under the effect of PEG treatment.

The control roots and shoots showed shared isoperoxidases as expected and the few differences between them (Fig. 1) represent a confirmed evidence that isoperoxidases in *T. aestivum* var. *ERA* are also tissue specific.

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SOME MORPHOLOGICAL CHANGES IN TWO ORNAMENTAL PLANT SPECIES DUE TO AUTOMOBILE POLLUTION

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A comparative study of leaf surface traits of two ornamental plant species namely, *Asparagus racemosus* L. and *Bougainvillea spectabilis* Comm. ex Juss. growing in high traffic density area (HTDA) and low traffic density area (LTDA) have been undertaken along with leaf and soil metal content. In leaves sampled from HTDA, damaged patches of tissues were observed along with the changes in configurations of wax pattern, surface pattern, size and frequency of stomata and trichomes, striations were also disintegrated in polluted environment. The damage patterns are almost similar in both plants. A high metal accumulation was noticed in both plants in HTDA areas. Our observations showed that these plants, although showing severe damage on the surface, were growing well at the heavily polluted (HTDA) sites. These plant species may, therefore, be used as indicators and mitigators of automobile pollution.

Key words: automobile pollution, trichomes, wax pattern

Introduction

Air and soil pollution resulting from automobile exhaust fumes in the environment is recognised as a major problem in most of the Indian cities and towns. With the rapid increase in the number of motor vehicles on Indian roads, proportionately, a considerably higher quantity of lead and SO_2 is now being emitted annually. Lead concentrations are sharply elevated in the roadside soils with heavy traffic density (Yassoglou et al. 1987, Ho and Tai 1988). Consequently, plants growing along the roadsides accumulate excess lead in their tissues (Ward et al. 1977, Ho and Tai 1988, Cook et al. 1994). Plants, because of their large leaf areas relative to the ground on which they stand, and the physical properties of their surfaces, can act as biological filters, removing large numbers of airborne particles. Therefore, they can be used as passive biomonitors in urban environments (Wittig 1993, Beckett et al. 1998). Response of plants can be used to obtain information on changes in the environment, and foliar analysis has often been used

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to detect elements which may be present temporarily in the air in a tiny concentration (Caselles 1998). Earlier works were mostly concentrated on the effect of urban air pollution on roadside vegetation but studies on the relationship between the leaf surface morphology and pollutants originating from automobile exhaust are very few.

The present study was undertaken to detect the effects of automobile pollution on two roadsides ornamental plants, i.e. *Asparagus racemosus* and *Bougainvillea spectabilis* grown on the roads of Lucknow city, where the vehicular traffic is the main anthropogenic source of atmospheric pollution.

Materials and Methods

Study sites

Lucknow, a major north Indian city with a population of over 2.5 million, situated between 26° 52″ N latitude and 80° 52″ E longitude, 120 m above sea level. The climate is tropical with a marked monsoonal effect. In recent years, the number of vehicles has increased manifold and now stand at 0.4 million. Vehicular pollution is the main source of atmospheric pollution in the city.

For the study, two sites have been selected, one is control (LTDA), i.e. NBRI garden campus and the other is busy highway with very high traffic density (HTDA), i.e. Air Port Road (Fig. 1).

Air quality monitoring

The study was conducted in all the three seasons (summer, rainy, winter). Monitoring of air quality, sampling of plant material and counting of traffic density was carried out on normal working days between 9.00 hrs to 11.00 hrs, a period of maximum traffic load. Measurements of suspended particulate matter (SPM) and sulphur dioxide (SO_2) were carried out, using Anderson High Volume Sampler, Pulsed Fluorescent SO_2 Gas Analyser (Singh et al. 1997).

Pb content in the air, plant and soil samples were estimated using Perkin Elmer 2380 Atomic Absorption Spectrophotometer (AAS) at 283.3 nm (Singh et al. 1997).

For the study, mature and healthy leaves of *Asparagus racemosus* L. and *Bougainvillea spectabilis* Comm. ex Juss. were collected from the mentioned sites. Leaves were washed and dried for constant weight for chemical anal-

ysis. Dry leaves (0.5 g) were digested with mixed acid (cca HNO_3 : cca H_2SO_4 : 60% $HClO_4$ = 3 : 0.5 : 1) on an aluminium digestion block. Pb content in the diluted digest was estimated as above by AAS. For estimation of Pb in the soil one gram of air-dried soil was taken. The digestion and estimation method was the same as of plant samples.

Microscopic study (light and SEM)

Size and frequency of epidermal cells, stomata and trichomes were observed under light microscope and their surface structural details were seen with scanning electron microscope (SEM), too. For SEM studies, leaf samples were thoroughly and repeatedly washed with tap water followed by deionized water to eliminate all loose dust from the leaf surface and fixed in 2.5% glutaraldehyde solution in phosphate buffer at pH 7.0, dehydrated in ethanol series, and further drying was carried out in critical point drier with liquid CO_2 at 1072 psi pressure and 31.4 °C temperature. Materials were then coated with thin conductive film of gold about 200 Å in thickness in an ion sputter coater (JFC 1100), and examined and photographed under scanning electron microscope (Philips XL20).

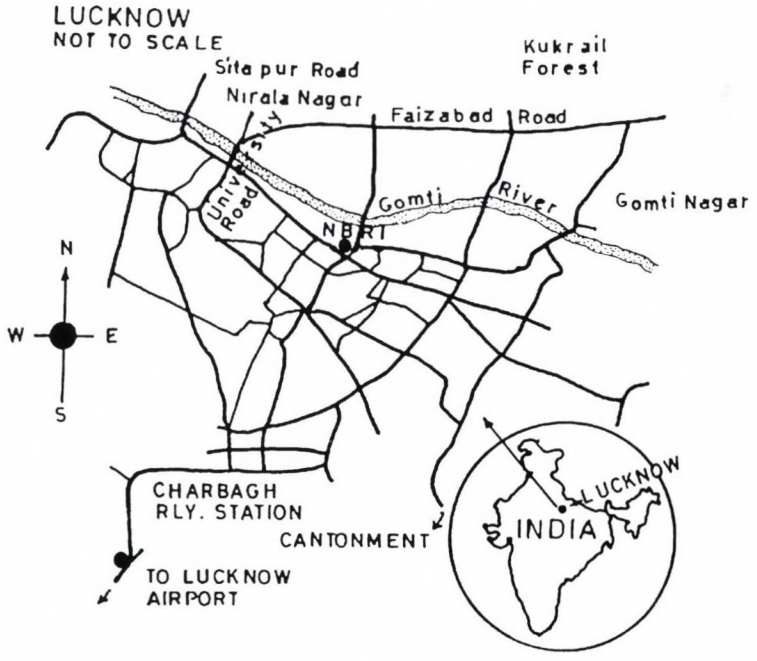

Fig. 1. Location of the study sites. (NBRI = National Botanical Research Institute)

Observations

Asparagus racemosus

In leaves collected from LTDA, wax was absent or inconspicuous, smooth cuticle, clear cell boundaries, stomata slightly elevated from the surface, peristomatal rim entire and smooth (Fig. 2a). On the other hand, in leaves from HTDA sites normal structure was disrupted, cuticle was wrinkled, full of damaged patches and laden with trapped dust. Because of raised cell boundaries stomata appeared sunken (Fig. 2b–d).

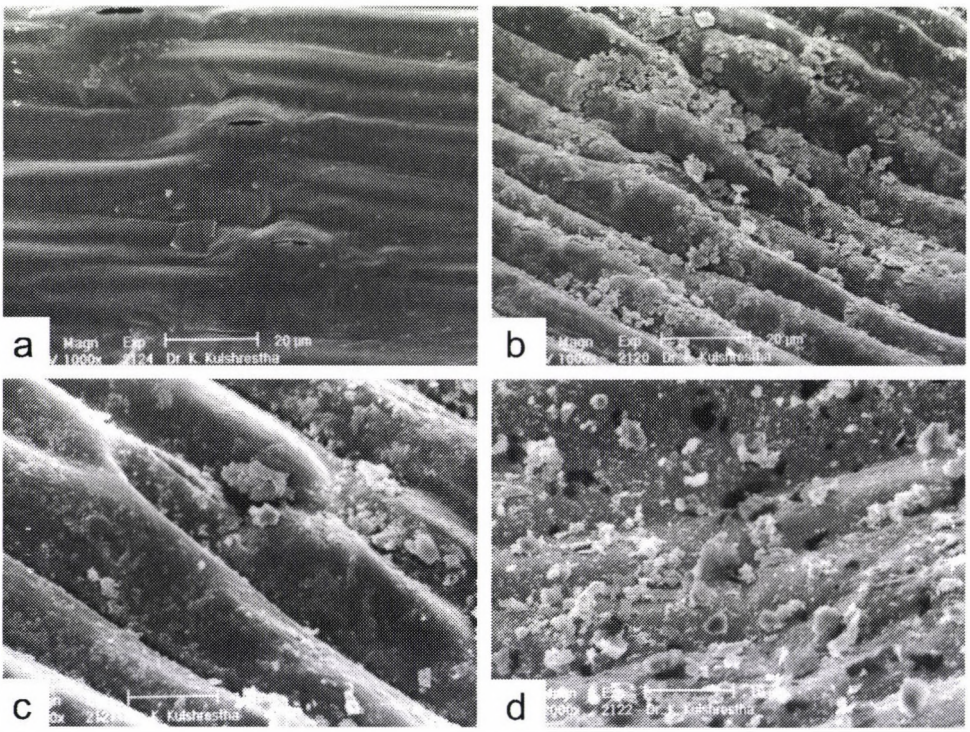

Fig. 2. Scanning micrographs of leaf surface of control (a) and polluted population (b, c, d) of *Asparagus racemosus*. – a = smooth surface with clear cell boundaries, b = surface laden with trapped dust, c = partially damaged surface, d = completely damaged surface with burnt patches and holes

Bougainvillea spectabilis

In case of LTDA population (Fig. 3a, c, e) cuticle was smooth with inconspicuous wax, at certain places it was granular and aggregate type, too. Sometimes cuticle was striated, striae radiated from guard cells. Stomata globose, present at the level of rest of the cells. Cell boundaries of subsidiary cells clear and slightly raised. Outer stomatal ledge and peristomatal rim were clear. Non-glandular trichomes uniseriate, multicellular, bulbous base and globular tip. Trichome surface smooth, cell demarcation clear. But in HTDA population (Fig. 3b, d, f), wax was aggregated (both granular and crusts), fused with dust particles over the surface, cuticle wrinkled abnormally. No trace of striae visible. Cell boundaries were not clear, irregularly fused. Stomata raised from the surface. A twofold increase in the stomatal frequency observed. Trichome shorter in length, frequency highly increased, cuticle over the trichome surface dirty and broken.

Results and discussion

Air quality of Air Port Road (Table 1) showed that the atmospheric Pb, SPM and SO_2 levels are higher than the permissible limits sets by the Central Pollution Control Board, New Delhi (Goel and Sharma 1996). This could be due to the fact that a large number of vehicles are passing on this road every day. Both plants, *A. racemosus* and *B. spectabilis* are growing on the road divider in the middle of the street and therefore directly exposed to exhaust fumes of automobiles from both sides and accumulate excess amount of Pb.

Table 1

Traffic density and air quality of Air Port Road and NBRI garden of Lucknow city (9.00–11.00 hrs*)

Sites	Petrol vehicles	Diesel vehicles	SO_2 μg m^{-3}	SPM μg m^{-3}	Pb μg m^{-3}
Air Port Road (HTDA)	1575	2402	667.60	635.00	4.47
NBRI garden (LTDA)	29	03	187.00 (80.00)	123.60 (200.0)	0.67 (1.00)**

* Year 1998, values are average of three seasons
** Values in parentheses are recommended by CPCB, New Delhi for residential and other areas

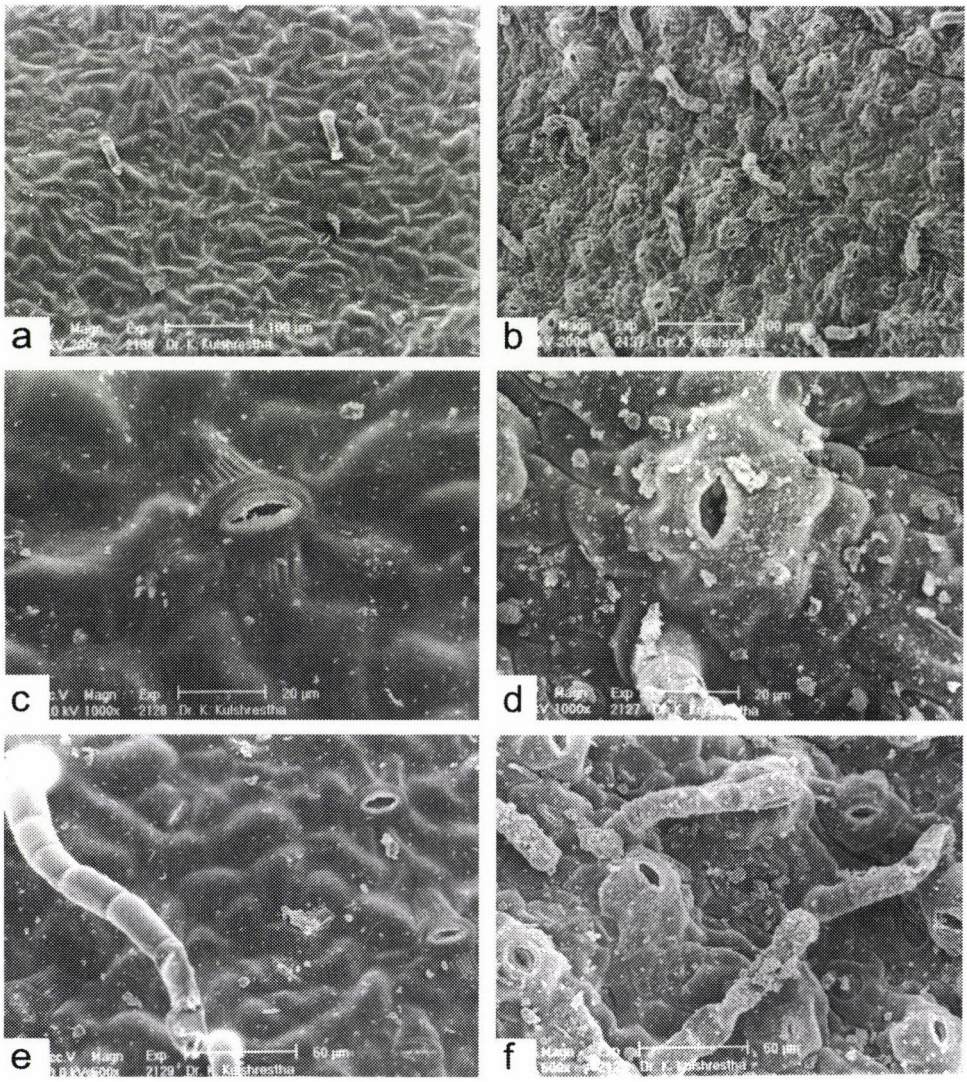

Fig. 3. Scanning micrographs of leaf surface of control (a, c, e) and polluted population (b, d, f) of *Bougainvillea spectabilis*. – a = leaf surface showing well-defined cell boundaries, b = higher stomatal and trichome frequency, stomatal elevation, c = a normal stoma with striae radiating from guard cells, d = damaged stoma with wide stomatal opening, e = multicellular, uniseriate non-glandular trichome, f = disruption in epidermal cell outline and trichome structure

Table 2

Lead accumulation in *A. racemosus* and *B. spectabilis* leaves in HTDA and LTDA sites (μg g^{-1} dw)

Sites	*A. racemosus*	*B. spectabilis*
Air Port Road (HTDA)	36.86	43.1
NBRI garden (LTDA)	13.10	18.3

* Year 1998, values are average of three seasons

Several heavy metals, such as Pb, Cr, Ni, Zn etc. are toxic to man and animals. Vehicle exhaust emit these toxic elements, so that soils, plants and residents along roadsides with heavy traffic loads are subjected to increasing levels of contamination with heavy metals. Soils, due to their cation exchange capacity, presence of organic substances, oxides and carbonates, have high retention capacity for the heavy metals. Therefore, metal concentration levels increase continuously as long as the nearby sources remain active. Table 3 showed the comparative soil characteristics and metals content of HTDA and LTDA sites.

The present study showed significant amount of Pb content (Table 1) in ambient air of Lucknow city as well as the roadside plant (Table 2) species. Deleaded petrol though introduced in several metropolitan cities in India, but Lucknow still not able to get such type of facilities. Therefore, atmospheric Pb is the major problem in around city. Automobiles which are responsible for line sources of pollution emissions in rural and suburban areas have a more far-reaching impact on roadside vegetation, already under considerable stress, in urban areas (Seaward and Richardson 1990).

Table 3

Comparative soil characteristic of HTDA and LTDA sites in upper layer of soil (0–6 cm)

Parameters	HTDA	LTDA
pH	8.43	8.05
EC (mMhs)	0.28	0.32
OC (%)	0.75	1.20
Pb (μg g^{-1} dw)	51.2	17.6
Cr (μg g^{-1} dw)	1.5	1.2
Ni (μg g^{-1} dw)	127.8	109.5
Zn (μg g^{-1} dw)	88.3	120.9

* Year 1998, values are average of three seasons

Most of the earlier studies on automobile pollution were on the deposition of heavy metals on roadside soil and vegetation (Smith 1976, Peterson 1978, Rovinsky et al. 1993, Singh et al. 1995, Suo and Huang 1996, Monaci and Bargagli 1997). Deposition of Pb on leaves and the uptake and subsequent transport of metals depend on their availability at the plant surface, whether it be root, stem or leaf. Aubazhagan et al. (1990) reported the accumulation of particulate Pb on the surface of leaves from the ambient source, that is, auto exhaust. Entry of Pb into the plant system is reported to be mostly through stomatal pores (Lort et al. 1979), although it could enter also through the roots (Little and Martin 1974). The foliar surface is the most important receptor of atmospheric pollutants in which it undergoes several structural and functional changes. Godzik and Sassen (1978), who examined the leaves of *Aesculus hippocastanum* L. from controlled and polluted areas under scanning electron microscope noted that the small folds present in the outer epidermal wall of normal leaves had disappeared in polluted leaves and stomata had also an abnormal appearance. Kulshreshtha et al. (1994) made a comparative study of the cuticular and epidermal features of *Syzygium cuminii* L. and *Lantana camara* L. growing close to a diesel generating set and a control site. They observed very significant differences in trichome frequency, stomatal opening and callus formation in the two sets of populations. Our study also reveals significant differences in leaf surface structures including cuticular and epidermal features in both species as a result of automobile pollution. These changes indicate the environmental stress and can, therefore, be used as bioindicators of pollution.

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COMPARATIVE MORPHOLOGICAL AND ANATOMICAL STUDY ON LEAVES OF TWO CUBAN *RONDELETIA* TAXA

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Some morphological and anatomical leaf characteristics of two evergreen Cuban Rubiaceae shrubs were studied. Differences in the epidermis and venation justified the separation of the recently described subspecies *Rondeletia pachyphylla* Krug et Urb. subsp. *myrtilloides* Fernandez et Borhidi from the *Rondeletia pachyphylla* Krug et Urb. subsp. *pachyphylla*.

Key words: *Rondeletia*, leaf anatomy, epidermis, venation

Introduction

A series of taxonomic studies has been carried out by A. Borhidi (Institute of Ecology and Botany, Hungarian Academy of Sciences, Vácrátót) and Maira Fernandez Zequeira (Institute of Ecology and Taxonomy Academy of Science of Cuba, La Habana) on different genera and species belonging to the Rondeletieae tribe of *Rondeletia*, for the purposes of the New Flora of Cuba Project (Borhidi 1993, Fernandez 1995, Fernandez and Borhidi 1985).

Within the frame of the series "Studies in *Rondeletieae* (Rubiaceae)" in the article number VIII. – among others – the variability range of the species *Rondeletia pachyphylla* Krug et Urb. was studied. The species belongs to the section Pedicellares (Fernandez 1995). The authors found that the individuals living in the humid lowland pine woodland of the Sierra de Moa differ in some morphological features of the leaves from those of the montane pine woodland of the much drier Nipe Mts (Fernandez and Borhidi 1985). Since the type was described from the Nipe Mts, the populations of Moa Mts have been separated under the name subsp. *myrtilloides*. Being the main differences of the two taxa in the size and shape and the venation of leaves, Dr Babos was asked by them to make some anatomical studies in addition to confirming or rejecting the validity of the new taxon.

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Material and methods

Leaf samples of both taxa were taken for measuring the length and width of leaf-blade, length of petiole, further on, the shape of the leaf-blade and the formation of the main rib on the abaxial surface were studied.

The fine structure of the leaf veins and their anatomical characteristics were examined on refined preparations. For these examinations three parallel refined leaf preparations of both subspecies were made. The leaves were refined and the preparations made with the commonly used microtechnical methods (Sárkány and Szalai 1964).

The microscopic examination of the refined leaves was performed after the description of Hickey (Metcalfe and Chalk 1979; chapter 4).

Of the preparations of refined leaves and of the places marked out suitably magnified micrographs were taken.

Results

Size and morphology of the leaves

The shape of the leaf-blade in *Rondeletia pachyphylla* subsp. *pachyphylla* is mostly ovate with rounded or truncate base, while that of subsp. *myrtilloides* is usually obovate with shortly attenuate blade to the base. In *R.*

Table 1

Size of leaf-blades and petioles

Species and subspecies		Length of leaf-blade (mm)	Width of leaf-blade (mm)	Length of petiole (mm)
Rondeletia pachyphylla subsp. *pachyphylla*	minimum	22.0	8.0	2.0
	average	25.0	9.2	2.7
	maximum	30.0	10.0	3.0
Rondeletia pachyphylla subsp. *myrtilloides*	minimum	16.0	6.0	4.0
	average	18.5	7.0	5.0
	maximum	20.0	8.0	4.0

Note: The measurements were taken of fully developed foliage leaves. Average values were calculated based on 10 parallel measurements of each characters. The leaves were selected from herbarium specimens. Since leaves are extremely variable organs of woody plants, the values given in the table only are of informative nature

pachyphylla subsp. *pachyphylla* the main rib is emergent on the abaxial surface and clearly visible. While in *R. pachyphylla* subsp. *myrtilloides* the main rib is slightly impressed on the abaxial surface and inconspicuous. The average values of the measurements obtained from the two materials examined are found in Table 1. They show that the average leaf-blade size of *R. pachyphylla* subsp. *myrtilloides* is smaller in both length (18.5 mm) and width (7.0 mm), while its petiole is longer (5.0 mm) than in the *R. pachyphylla* subsp. *pachyphylla* (length of leaf-blade: 25.0 mm, width of leaf-blade: 9.2 mm, length of petiole: 2.7 mm).

Leaf structure

The comparison of the morphological and anatomical characteristics of the leaf-blades in the two taxa studied are compiled in Table 2. It can be established that there are differences in some major characteristics between them as follows:

Characteristics	*R. pachyphylla*	
	subsp. *pachyphylla*	subsp. *myrtilloides*
Leaf base	asymmetrical, rounded or truncate	symmetrical, shortly attenuate
Leaf apex	pointed	blunt
Trend and size of main rib	remarkably inclining, moderate	straight, weak
Trend of secondary veins	reclinate, veins inside the margin rarely form curves (Fig. 1)	slightly reclinate, veins along the margin join in curves over several sections (Fig. 2)
Branching of veinlets	double (Fig. 3)	triple (Fig. 4)
Shape of areoles	quadrangular or pentagonal (Fig. 3)	triangular or quadrangular (Fig. 4)

Discussion

According to the results of the comparative examination of some morphological and anatomical leaf characteristics of both *Rondeletia pachyphylla* subsp. *pachyphylla* and *R. pachyphylla* subsp. *myrtilloides*, differences

Table 2

Characteristics of leaf anatomy

Characteristics	*R. pachypylla* subsp. *pachyphylla*	*R. pachyphylla* subsp. *myrtilloides*
Leaf	simple	simple
Leaf-blade	symmetrical	symmetrical
Shape	oblong-ovate	narrow elliptic to oblong-obovate
Length-width ratio of leaf-blade	2.71 : 1	2.64 : 1
Base of leaf blade	asymmetrical, rounded or truncate	symmetrical, attenuate decurrent
Leaf apex	acute, pointed	rounded, blunt
Leaf margin	entire, convex	entire, convex
Leaf texture	thick and stiff, coriaceous	thick and stiff, coriaceous
Petiole	normal	normal
	(without perceptible thickening or projection)	
Venation	pinnate	pinnate
Main rib	remarkably protruding	plate
Joining of secondary	moderately strong,	moderately strong,
Veins to the main rib	45–56°	45–56°
Relative thickness of secondary veins	thick	moderate
Trend of secondary veins	reclinate (veins inside the leaf margin rarely forming loops	slightly reclinate (veins inside the leaf margin join forming loops
Looping branches of secondary veins	joint at acute angles	joint at blunt or right angles
Arrangement of tertiary veins	irregular reticulate, forked	irregular reticulate, forked
Veinlets	start at right angles from the primary, secondary and tertiary veins	
Branching of veinlets	double	triple
	the veinlets form well-developed areoles of relatively uniform size and shape	
Shape of areoles	quadrangular or pentagonal	triangular or quadrangular
Size of areoles	small, less than 0.3 mm	small, less than 0.3 mm

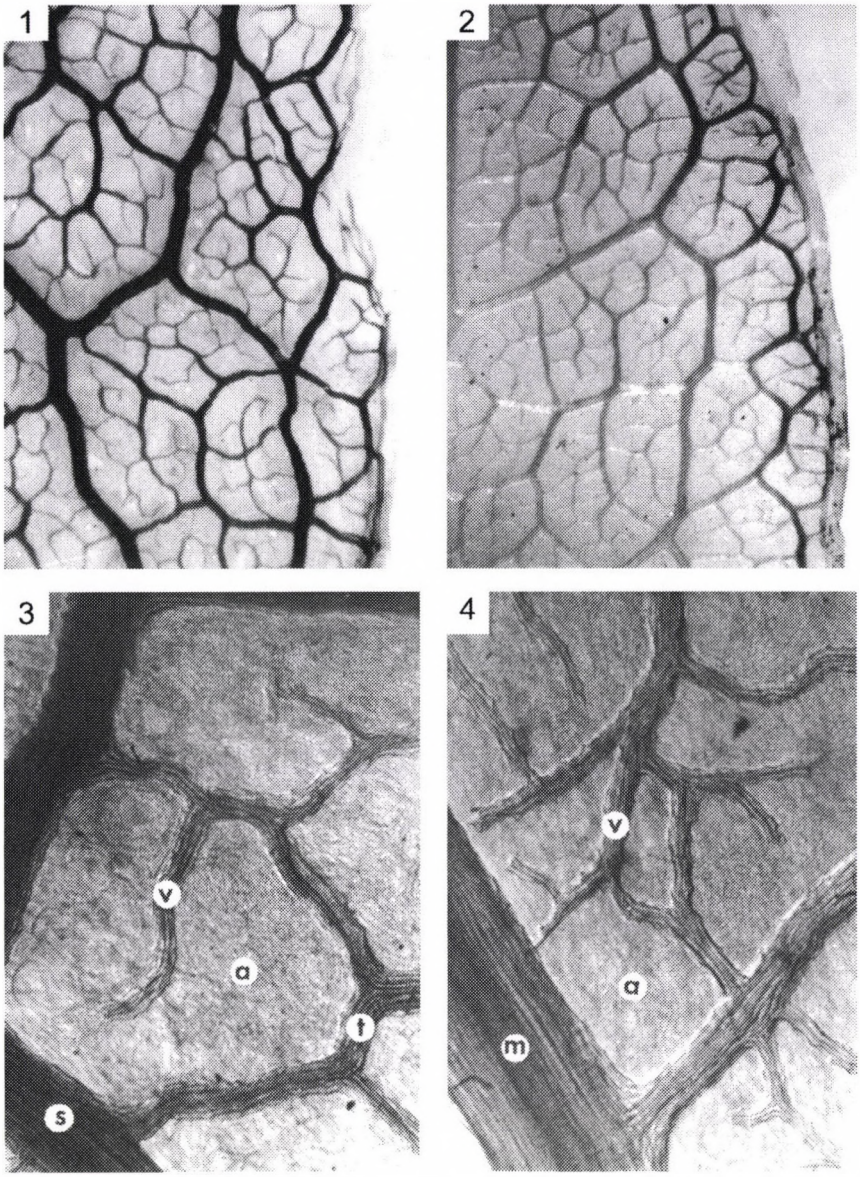

Fig. 1. Rondeletia pachyphylla Krug et Urb. subsp. *pachyphylla*, part of leaf-blade. LM micrograph: ×6.25. Secondary, tertiary veins and veinlets. *Fig. 2. Rondeletia pachyphylla* Krug et Urb. subsp. *myrtilloides* Fernandez et Borhidi, part of leaf-blade. LM micrograph: ×6.25. Secondary, tertiary veins and veinlets. *Fig. 3. Rondeletia pachyphylla* Krug et Urb. subsp. *pachyphylla*, part of leaf-blade. LM micrograph: ×120. Ramifying of veinlets and the areoles. s = secondary vein; t = tertiary vein; v = veinlet; a = areole. *Fig. 4. Rondeletia pachyphylla* Krug et Urb. subsp. *myrtilloides* Fernandez et Borhidi, part of leaf-blade. LM micrograph ×120. Multiple branches of veinlets and areoles. m = main rib; v = veinlet; a = areole

in several features were found which confirm the separation of the two subspecies within the frame of the species *R. pachyphylla*.

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USING CONCENTRATION ANALYSIS FOR OPERATING WITH INDICATOR VALUES: EFFECT OF GROUPING SPECIES

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Précsényi (1995) suggested the use of concentration analysis for studying the pattern of indicator values. During the analysis the researcher has to make decisions in some questions and these judgements may influence the final results. The effect of two decisions will be discussed in this paper: 1. How many species-groups to be used? 2. Which indicator values should belong to the same group? The different partitions of species, which seem to be biologically meaningful in theory, may yield different results. We concluded that the use of fewer groups may be more favourable, because the preliminary conditions of χ^2 test are fulfilled more frequently in this case. Concerning grouping, the distribution of F values in any relevé-groups should be unimodal, but in non-equilibrium cases the bimodality may be biologically meaningful.

Key words: indicator values, concentration analysis, grouping, random models

Introduction

The use of indicator values in vegetation research became widespread following Ellenberg's works (Ellenberg 1950, Ellenberg et al. 1991). Précsényi called attention several times (Zólyomi and Précsényi 1964, Précsényi 1996) to the fact that the indicator numbers are just symbols and are not real values. The equal, smaller, bigger relations on the ordinal scale could be applied, but we may not do any other mathematical operation (for example: averaging them) with these "numbers". Zólyomi and Précsényi (1964), and Précsényi (1995, 1996) gave information about the right statistical analysis with the indicator values. Some examples for the correct application of the indicator values were given in Zólyomi et al. (1988), Précsényi (1995), Borhidi and Dénes (1997), Morschhauser and Salamon-Albert (1997).

Generally, the aim of the researchers is describing the changes of indicator value patterns with as few variates as possible. The use of average indicator values may be strongly criticized from a mathematical point of

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view (see above). To solve this problem Précsényi (1995) suggested the use of concentration analysis. This method was developed by Feoli and Orlóci (1979) for analysing the relationship between the groups of species and the groups of relevés. Précsényi (1995) pointed out that if species are divided into groups based on their indicator values, the pattern of indicator values can be analysed by concentration analysis.

During the analysis the researcher has to make decisions which may influence the final results. The effect of the following two decisions will be discussed in this paper:

– How many species-groups to be used?

– Which indicator values should belong to the same group?

The data set that consists of relevés which were made in fen associations at the Malom Valley (near Cluj-Napoca, Romania) in different years (Ruprecht and Botta-Dukát 2000) are used to answer these questions. The groups of relevés applied in these analyses are listed in Table 1. The indicator values for moisture (W) and acidity (R) were developed by Borhidi (1995). In case of species which did not occur in Hungary the work of Sanda et al. (1983) was used.

Table 1

The data set used in this paper. All relevés were made at the Malom Valley (near Cluj-Napoca, Romania)

Groups of relevés	Association	Year	No of relevés	Source
I	CE	1940–44	11	Soó 1949
II	CE	1956	2	Pop et al. 1962
III	CE	1961	5	Pop et al. 1962
IV	CE	1998	14	Ruprecht and Botta-Dukát 2000
V	JS	1956	5	Pop et al. 1962
VI	JS	1961	2	Pop et al. 1962
VII	JS	1998	8	Ruprecht and Botta-Dukát 2000
VIII	C	1961	2	Pop et al. 1962
IX	C	1998	4	Ruprecht and Botta-Dukát 2000
X	CEcp	1956	3	Pop et al. 1962

Description of the area and associations see Ruprecht and Botta-Dukát (2000).
Abbrevations: CE = *Carici flavae-Eriophoretum latifolii*, JS = *Junco obtusiflori-Schoenetum nigricantis*, C = *Cladietum marisci*, Cecp = *Carici flavae-Eriophoretum latifolii caricosum paniceae*

Concentration analysis

The main point of the method is the following: let f_{jk} be the total number of occurrences of species belonging to species-group j in relevés belonging to relevé-group k. The value of f_{jk} depends on the sizes of species-group j and relevé-group k. Eliminating this effect the corrected values (F_{jk}) have to be used in the analysis (Orlóci and Kenkel 1985):

$$F_{jk} = \frac{f_{jk}}{p_j q_k} \cdot \frac{\sum_{g=1}^{n} \sum_{h=1}^{m} f_{gh}}{\sum_{g=1}^{n} \sum_{h=1}^{m} \frac{f_{gh}}{p_g q_h}}$$

where: n = number of species-groups, m = number of relevé-groups, p_j = number of species belonging to group j, q_k = number of relevés belonging to group k.

In the analysis we regard **F** as a contingency table although this matrix may contain fractions. First the independence of species-grouping and the grouping of relevés is statistically tested. If they are independent from each other **F** will not differ from $\mathbf{F}^0$ significantly:

$$F^0_{jk} = \frac{\sum_{j=1}^{n} F_{jk} \sum_{k=1}^{m} F_{jk}}{\sum_{j=1}^{n} \sum_{k=1}^{m} F_{jk}}$$

This hypothesis can be tested by χ^2 (Feoli and Orlóci 1979) or G^2 test (Précsényi 1995). If **F** significantly differs from $\mathbf{F}^0$ the matrix **F** will be analysed by correspondence analysis. The scores of species- and relevé-groups in the same min{m, n}–1 dimensional ordination space and the canonical correlation coefficients between scores are obtained this way. The sum of canonical correlation coefficients are connected with the χ^2 value computed earlier:

$$\chi^2 = F_{..} R_1^2 + F_{..} R_2^2 + \ldots + F_{..} R_S^2$$

where: S = min{m, n}–1, R_1 = canonical correlation between first scores of species-groups and first scores of relevé-groups, $F_{..}$ = the grand total of F.

The χ^2 value can be taken to components. The values of canonical correlation coefficients show the importance of the axes. If the value of $F..R_j^2$ is smaller than the appropriate critical value of χ^2 distribution with $(m-1)(n-1)$ degree of freedom, the jth axis may be left out of consideration.

The number of species-groups

The indicator values for moisture (W) are used here to demonstrate the effect of number of species-groups (on the analysis). In the case of indicator values for soil reaction (R) we got similar results.

Two different numbers of groups were compared. In the first case only the extremely small groups (WB1 and WB2) are amalgamated. So the number of species-groups was nine. In the second case the species are divided into three groups in the following way:

- xerofrequent group (WB1, WB2, WB3, WB4)
- mesofrequent group (WB5, WB6, WB7)
- hygrofrequent group (WB8, WB9, WB10)

We were showing above that the concentration analysis strongly corresponded with the χ^2 test. The preliminary condition of the test is that the empirical distribution of any F_{jk} value must not differ from the normal distribution substantially. F_{jk} has a binomial distribution with two parameters distribution p and $F..$. If $F..$ is large enough and p is not too small the binomial distribution will not differ from the normal distribution substantially. This fact is the theoretical basis for using χ^2 test (Yule and Kendall 1957). This preliminary condition of the test is likely not to be fulfilled entirely.

$F..$ was about 1000 in the data set used here. Our preliminary studies showed that if $F.. = 1000$ and $p < 0.005$ the asymmetry of binomial distribution would not be negligible. The exact value of p was not known, but $p^* = F_{jk}/F..$ is an undistorted estimation of p. When the species were divided into nine groups there were 17 cells where p^* was less than 0.005. However, when only three species-groups formed the preliminary conditions of the test were fulfilled entirely in all cases.

The effect of the lack of preliminary conditions can be examined by null-models. In our case two different null-models could be used. In both cases the group memberships of species were randomized. In the first case the size of groups did not change (fixed group size method), in the other case only the number of groups was fixed (random group size method). In both cases 10,000 permutations of species-groups were made.

First the appropriate null-model had to be selected from the two possibilities discussed above. For this reason the significance levels based on hypothetical χ^2 distribution and based on randomization were compared in the case of three species-groups. Since the preliminary conditions were fulfilled entirely here, the results should not differ. The significance levels based on χ^2 distribution and randomization with random group sizes were similar. The randomization with fixed group sizes proved to be more rigorous because here an insufficient condition (size of groups) was used which diminished the number of possible different groupings. Therefore, only the random group method was used further.

When the number of groups is nine, the difference between significance levels of G^2 statistics based on randomization and hypothetical distribution were negligible despite the fact that the preliminary conditions of the test are not fulfilled entirely as seen above. But there are several differences between significance levels of canonical correlation coefficients which could be mentioned. Only the first canonical correlations were higher than the critical value based on the hypothetical distribution. Whereas the first four canonical correlations proved to be significant based on the randomization test. This means that four axes should be used, which cannot be regarded as an effective information compressing. On the other hand if only three species-groups were applied there was only one significant canonical correlation coefficient, therefore it was sufficient to interpret only the first axis. Moreover, the number of species-groups had little effect on the values of first canonical correlation coefficients. Its value was 0.4008 in the case of nine species-groups and 0.3705 in the case of three species-groups.

Shortly summarizing the main results of this section we can say that the appropriate randomization test is the random group sizes method. The fact that preliminary conditions did not fulfil entirely had only little effect on the significance level of G^2 statistics, but the robustness of the statistical test of canonical correlation coefficients was significantly smaller. The effectiveness of the method may be increased by the decrease of number of species-groups.

Which indicator values should belong to the same group?

Sometimes the answer to this question is not so trivial. For example we wanted to study R indicator values of the data set used in the previous sec-

Table 2

The F matrix of the first case in the analysis of R indicator values

	I	II	III	IV	V	VI	VII	VIII	IX	X
1	26.7	58.2	47.8	29.8	31.1	40.4	25.8	35.5	17.7	63.6
2	17.6	42.1	38.8	33.2	24.5	29.1	34.7	45.3	24.2	36.6
3	24.6	43.9	42.4	44.4	33.6	47.6	38.4	65.9	34.7	51.2

The species-groups are the following: 1. acidophilous and acidofrequent species (R3, R4, R5), 2. plants living mostly neutral soils (R6), 3. basiphilous and basifrequent species (R7, R8, R9). It can be seen that in six groups of species (I, II, III, V, VI and X) the importance values of both acidofrequent (R1) and basifrequent (R3) higher than the groups of neutral reaction indicators

tion. Based on the results of the previous section we tried to establish three species-groups. There were two solutions which were acceptable from a biological point of view. The first: 1. acidophilous and acidofrequent species (R3, R4, R5), 2. plants living mostly on neutral soils (R6), 3. basiphilous and basifrequent species (R7, R8, R9); the second: 1. extremely acidophilous species (R3, R4), 2. plants living mostly on neutral soils, including slightly acidophilous and slightly basiphilous species (R5, R6, R7), 3. extremely basiphilous species (R8, R9).

The two possibilities were compared. First it can be stated that in the first case the preliminary conditions of χ^2 test are entirely fulfilled, but in the other case there are cells whose values are smaller than the critical value. That is why the significance levels were computed by randomization test. In first case $G^2 = 27.229$, which does not prove to be significant. When the second partition of species was regarded, the G^2 value was sig-

Table 3

The F matrix of the second case in the analysis of R indicator values

	I	II	III	IV	V	VI	VII	VIII	IX	X
1	2.930	80.57	77.35	4.604	25.78	48.34	0.00	64.46	16.11	85.94
2	23.24	46.88	39.20	30.29	26.85	33.02	28.50	34.09	18.64	48.30
3	22.82	40.71	39.35	41.19	31.21	44.10	35.62	61.06	32.23	47.49

The species-groups are the following: 1. extremely acidophilous species (R3, R4), 2. plants living mostly on neutral soils including slightly acidophilous and slightly basiphilous species (R5, R6, R7), 3. extremely basiphilous species (R8, R9). In this case there are only two relevé-groups (VI, VIII), where the importance values of both extremely acidophilous (1) and basiphilous species (3) are higher than the groups of neutral reaction indicators (2)

Table 4

An artificial data matrix with 3 species-groups (a, b, c) and 3 relevé-groups (I, II, III)

	I			II			III		
	1	1	1	1	1	1	0	0	0
a	1	1	1	1	1	1	0	0	0
	1	1	1	1	1	1	0	0	0
	1	1	1	0	0	0	1	1	1
b	1	1	1	0	0	0	1	1	1
	1	1	1	0	0	0	1	1	1
	0	0	0	1	1	1	1	1	1
c	0	0	0	1	1	1	1	1	1
	0	0	0	1	1	1	1	1	1

The relevés are in the columns, the species are in the rows. The groups are separated by lines. The data matrix is well structured ($G^2 = 43.79$, $p < 0.1\%$). The bimodality in the second group yields that the two canonical correlation coefficients are equal ($R_1 = R_2 = 0.5$)

nificantly higher ($G^2 = 169{,}541$, $p < 0.1\%$). The cause of differences could be understood if the two F matrixes were examined in detail. In the case of the first partitioning of species there were six relevé-groups where both the first (acidophilous) and the third (basiphilous) groups have high values (Table 2). It can be explained first of all by the fact that sligthly acidofrequent and slightly basifrequent species can co-occur on soils with nearly neutral pH (see Table 3). Therefore, it is proper that in the second partition they are regarded as indicators of neutral pH. This example shows, that the partitioning of species, which seems biologically meaningful in theory, may prove to be inappropriate in practice.

In general, if there are many relevé-groups, where distribution of F values is bimodal (e.g. in the case of three groups both first and third F values are higher than the second) the partitioning of species is probably inappropriate. In spite of bimodality the contingency table may be well structured (e.g. Table 4). In such cases the second canonical correlation is not significantly smaller than the first, so it is not enough to use the first axis. It is connected with using correspondence analysis, which method is applicable to analyse unimodal distributions (ter Braak and Prentice 1988). In non-equilibrium cases, of course, the bimodality of distribution may be biologically meaningful, when the species of two successional phases tempo-

rarily co-occur. The two types of bimodality (biologically meaningful or consequence of wrong partitioning of the species) cannot be distinguished by any statistical test, only by the personal judgement of the researcher.

Conclusion

The partitioning of species strongly influences the final results. We recommend that if the F value is small, the number of species-groups must be decreased. Due to the decreasing number of groups the probability of fulfilling the preliminary conditions of χ^2 test increase.

If the conditions of statistical test do not fulfil the significance levels can be established by random models. We compared two possible random models and concluded that the "random group size" method is more appropriate.

If there are more than one possible partition of species with the same number of groups, the one with rare data bimodality should be chosen. In non-equilibrium communities the bimodality of distribution may be biologically meaningful. Of course, this type of bimodality should not be eliminated from the analysis.

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A GLOSSARY OF PLANT CUTICLE TERMINOLOGY IN RUBIACEAE

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The use of scanning electron microscopy has led to the realisation that the traditional terminology for ornamentation types of leaf cuticle in Rubiaceae is inexact and insufficiently descriptive for purposes of critical systematic comparisons.

Until the present work, however, there has been no readily available dictionary of terms for identification of the primary and secondary ornamentation of the cuticle present in the Rubiaceae family.

The following brief survey on cuticular ornamentation of Rubiaceae is based on SEM studies of 120 species belonging to subfamily Cinchonoideae. Some examples of patterns of cuticle are illustrated and a terminology is suggested.

Key words: SEM, primary/secondary cuticular ornamentation, Rubiaceae

Introduction

Since Heywood (1971) drew attention to the importance and impact of SEM on the study of systematic problems, most valuable information has been provided by using this technique. Micromorphological and ultrastructural data (e.g. Cole and Behnke 1975) have contributed invaluable information to our understanding of the evolution and classification of seed plants and play an important role in the modern synthetic systems of angiosperms (Dahlgren 1979–80).

A general observation is that plant surfaces are normally sculptured, smooth or non-sculptured epidermis is rare. The cuticular striations or cuticular fold patterns show high micromorphological diversity. Cuticular striations and micropapillations seem to be characteristic features of angiosperms (Barthlott 1981). Although the systematic significance of cuticular sculptures for delimitation of categories above the species level is rather limited, they may serve as excellent diagnostic characters in the lower taxa (Barthlott and Voit 1979).

It is well-known that the sculpturing of a chemically hydrophobic surface (cutin, wax, etc.) increases the water repellency (Martin and Juniper

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1970, Rentschler 1971). Connected to the reduced wettability appears to be the decreased ability of surfaces to contaminate (Rentschler 1971, 1977). The ecological advantage of decreased wettability is obvious: wettable surfaces are always highly polluted and colonized by microorganisms, while in water repellent taxa it is usually quite hard to find any trace of contamination (Barthlott and Wollenweber 1981).

Although the results of cuticular studies by SEM can be of use in taxonomic, palaeobotanical and ecological points of view, there is no standardised terminology and often no structural interpretation of the characters illustrated.

So far the most important surveys on this subject were made by Wilkinson (1979) and Barthlott (1990). The descriptive vocabularies for the cuticular ornamentation use words mainly not derived from Latin and are inconsistent in application of these words. Our present study is intended to amplify these terminologies from additional material and to establish new types of detailed surface ornamentation with descriptive-diagnostic value. The nomenclature for descriptions of pollen surface sculpturing by Tschudy (1969), for example, is excellent in this regard and in fact is model that might be followed throughout.

In the course of the maturation the cuticle may undergo changes that involve the addition of secondary ornamentation. Such ranges of variability cannot be ignored in dealing with the identification and classification of the cuticle pattern types, a distinction should be made between the degree of ornamentation.

Beyond purely descriptive use, comparative data may be important for studies of relationships not only in the family Rubiaceae. They can be useful in investigation of the roles of cuticle ornamentations in various aspects of physiological and ecological adaptation as well.

Synopsis of common cuticle ornamentation types in Rubiaceae:

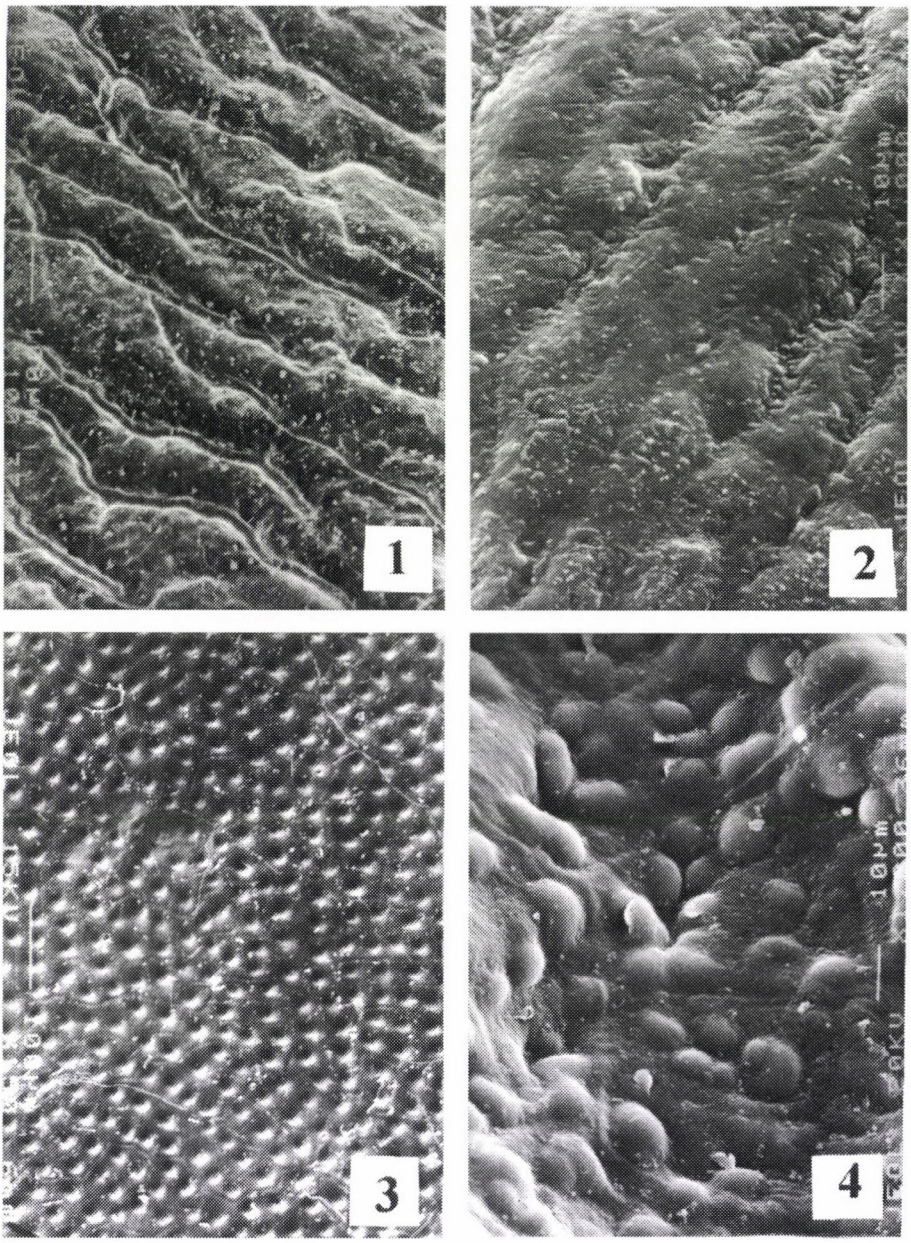
Table 1
Principal types of primary ornamentation

Type of primary pattern	Examples of species where noted
canaliculate – small channels parallel to others	*Rondeletia nipensis* Urb. (Fig. 1)
corrugate – widely spaced alternating ridges and grooves	*Exostema brachycarpum* (Sw.) Schult. (Fig. 2)
foveolate – rather plain surface with small depressions	*Rondeletia alaternoides* A. Rich. (Fig. 3)

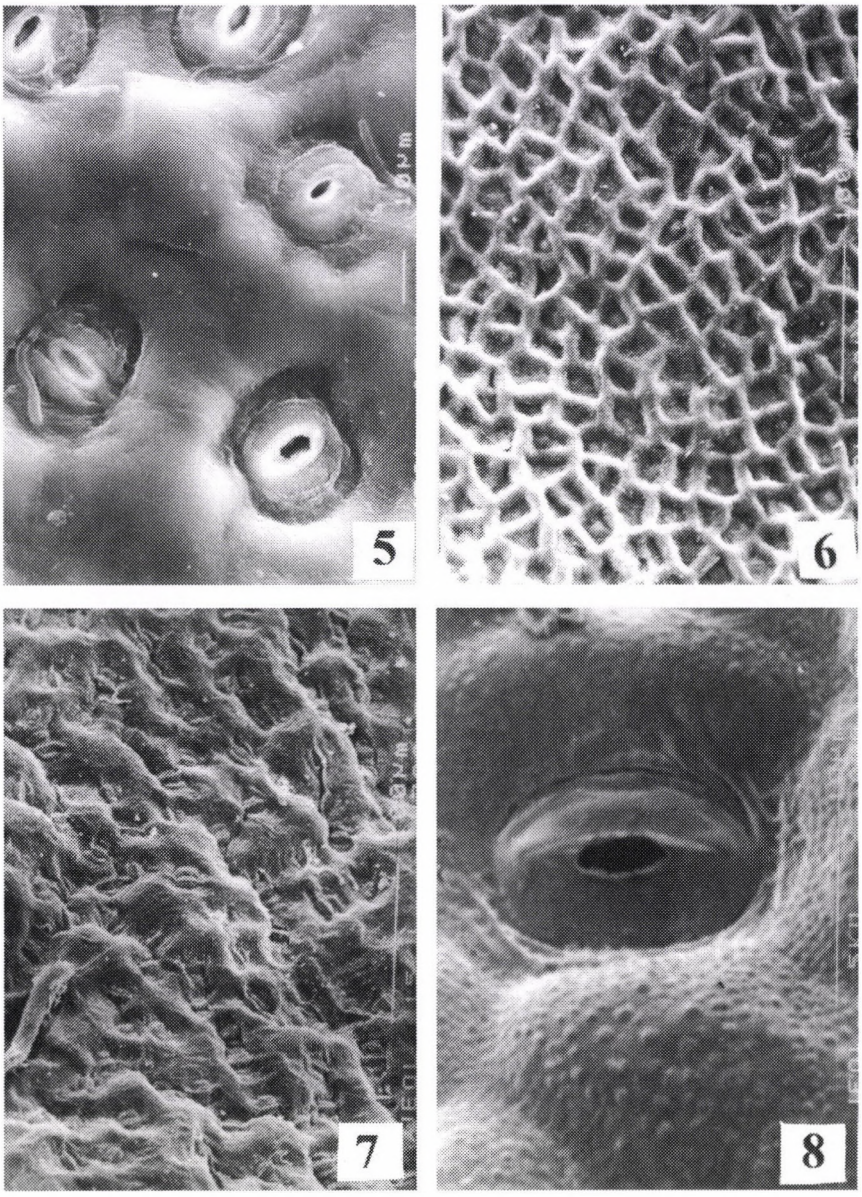
gemmate – rather plain surface having spherical protrusions	*Rondeletia longibracteata* Alain et Acuna (Fig. 4)
plain – flat surface without ornamentations	*Exostema purpureum* Griseb. (Fig. 5)
reticulate – net-like structure having ridges of uniform width crossing each other	*Exostema floribundum* Roem. et Schult. (Fig. 6)
rugulate – full of wrinkles of irregular crossing, wider than ordinary striae	*Rondeletia portoricensis* Kr. et Urb. (Fig. 7)
scabrate – rough surface with projecting small dots	*Exostema velutinum* Standl. (Fig. 8)
striate – fine streak, furrow, or thread-like line, usually parallel to others	*Exostema peruvianum* (Poepp.) Endl. *Exostema caribaeum* (Jacq.) Schult. *Rondeletia glauca* Griseb. (Figs 9–11)
verrucate – rough surface having wart-like elevations	*Rondeletia subcanescens* Fernandez et Borhidi (Fig. 12)

Table 2
Principal types of secondary ornamentation

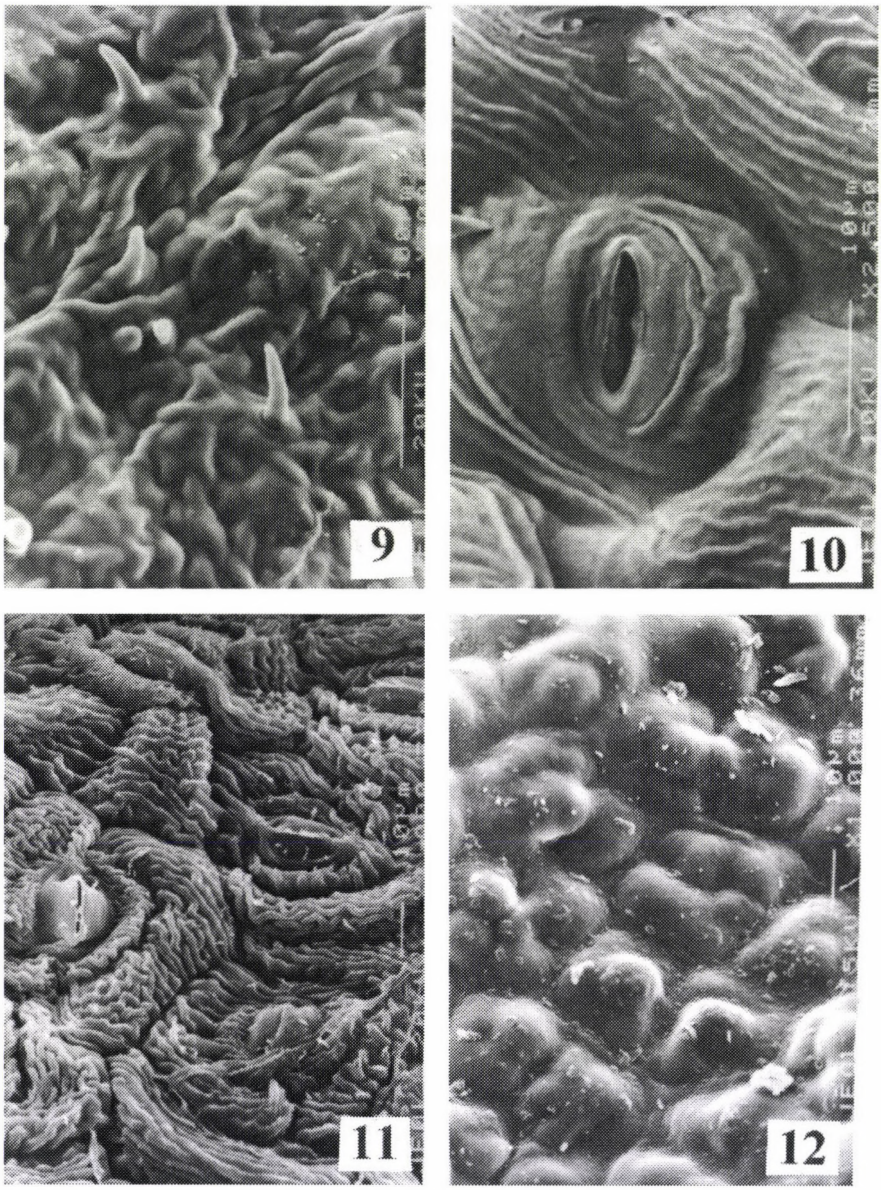
Type of secondary patterns	Examples of species where noted
foveolate/reticulate – ridges of uniform width between the sinkings show the primary foveolate surface	*Exostema maestrense* Borhidi et Fernandez (Fig. 13)
foveolate/striate – the primary foveolate structure wrinkles into striae	*Rondeletia clarendonensis* Britton ex S. Moore (Fig. 14)
gemmate/striate – the gemmate primary surface wrinkles into striae	*Rondeletia longibracteata* Alain et Acuna (Fig. 15)
reticulate/rugulate – the primary ornamentation shows net-like structure which wrinkles into folds with irregular orientation	*Rondeletia portoricensis* Kr. et Urb. (Fig. 16)
reticulate/striate – the primary ornamentation is net-like structure which wrinkles into slender folds usually parallel to others	*Exostema floribundum* Roem. et Schult. (Fig. 17)
scabrate/reticulate – ridges of uniform width between the sinkings show the primary rough scabrate surface	*Arachnothryx izabalensis* (Standl. et Steyerm.) Borhidi (Fig. 18)
scabrate/striate – the primary rough scabrate surface wrinkles into slender folds usually parallel to others	*Rondeletia savannarum* Britton (Fig. 19)



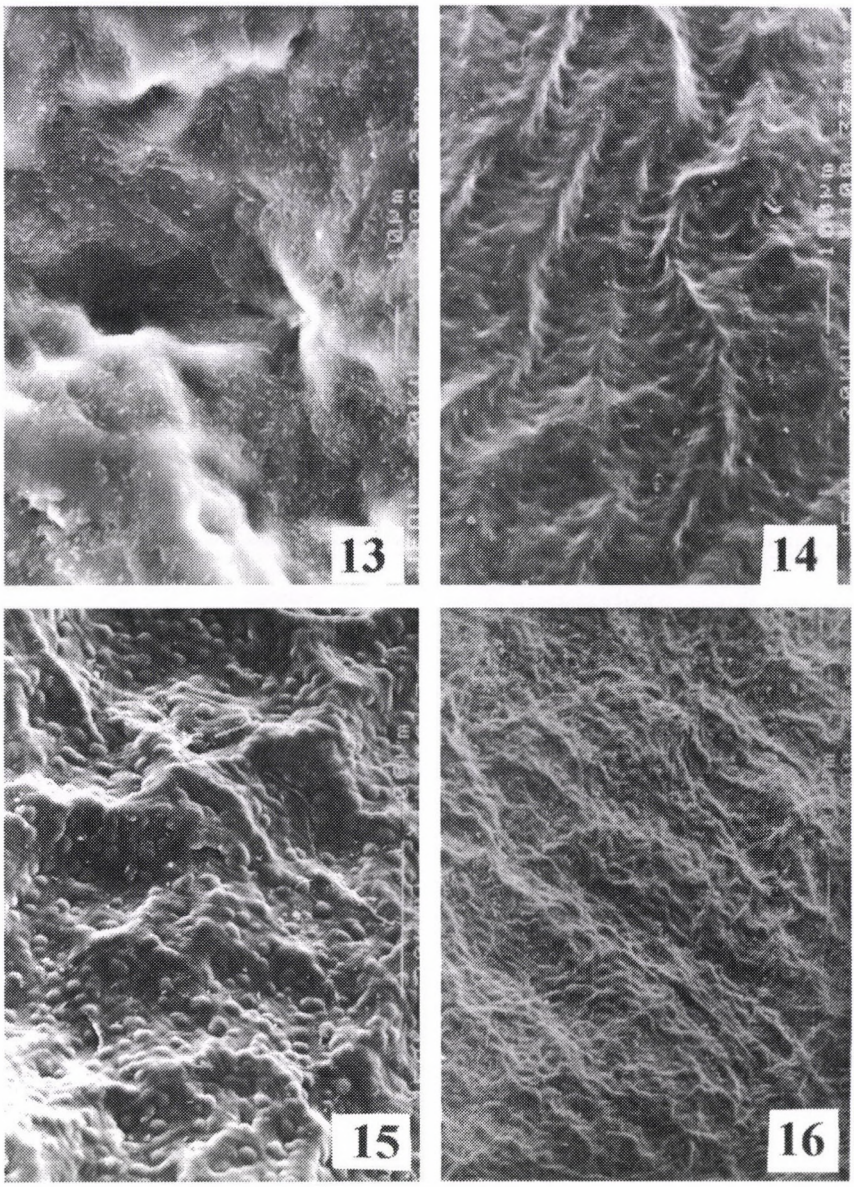
Figs 1–4. Primary cuticular ornamentation on the adaxial (ADS) and abaxial (ABS) leaf surface. – *Fig. 1.* canaliculate, *Rondeletia nipensis* Urb., ADS; *Fig. 2.* corrugate, *Exostema brachycarpum* (Sw.) Schult., ADS; *Fig. 3.* foveolate, *Rondeletia alaternoides* A. Rich., ADS; *Fig. 4.* gemmate, *Rondeletia longibracteata* Alain et Acuna, ADS



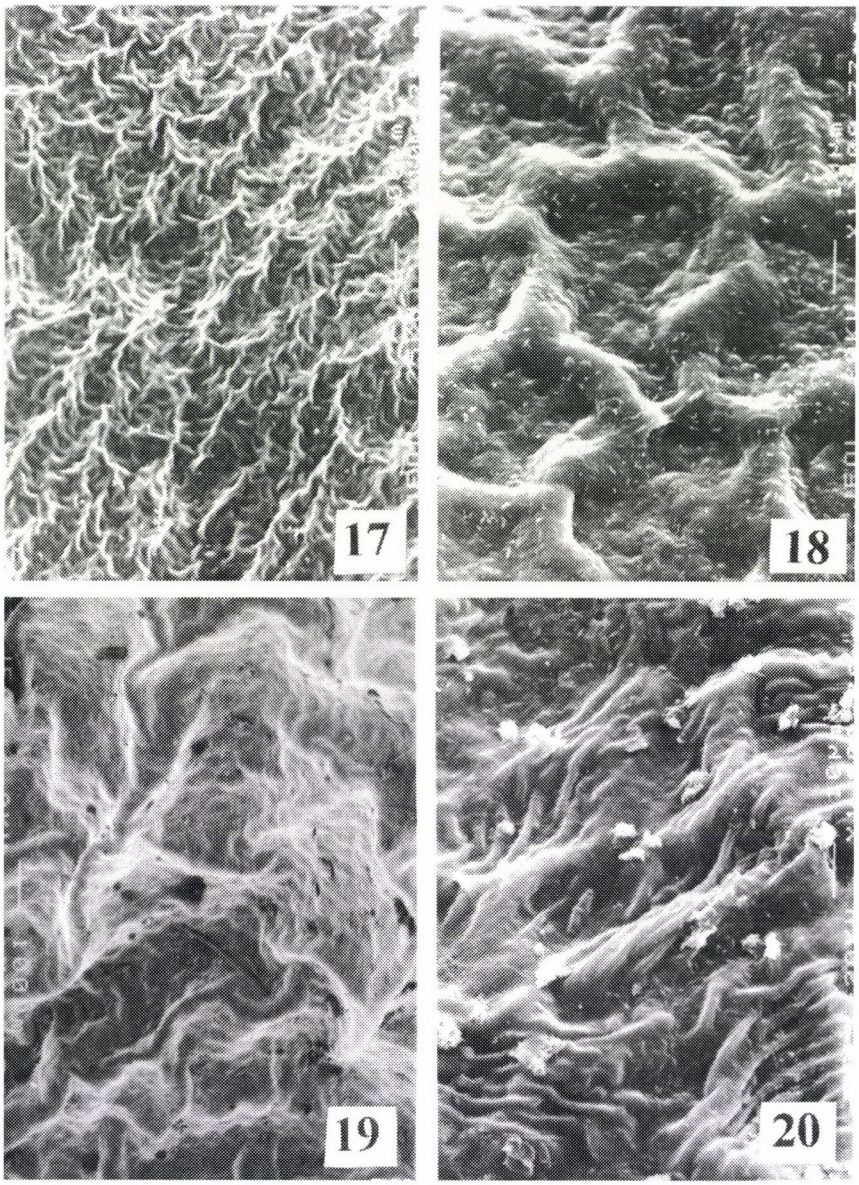
Figs 5–8. Primary cuticular ornamentation on the adaxial (ADS) and abaxial (ABS) leaf surface. – *Fig. 5.* plain, *Exostema purpureum* Griseb., ABS; *Fig. 6.* reticulate, *Exostema floribundum* Roem. et Schult., ADS; *Fig. 7.* rugulate, *Rondeletia portoricensis* Kr. et Urb., ABS; *Fig. 8.* scabrate, *Exostema velutinum* Standl., ABS



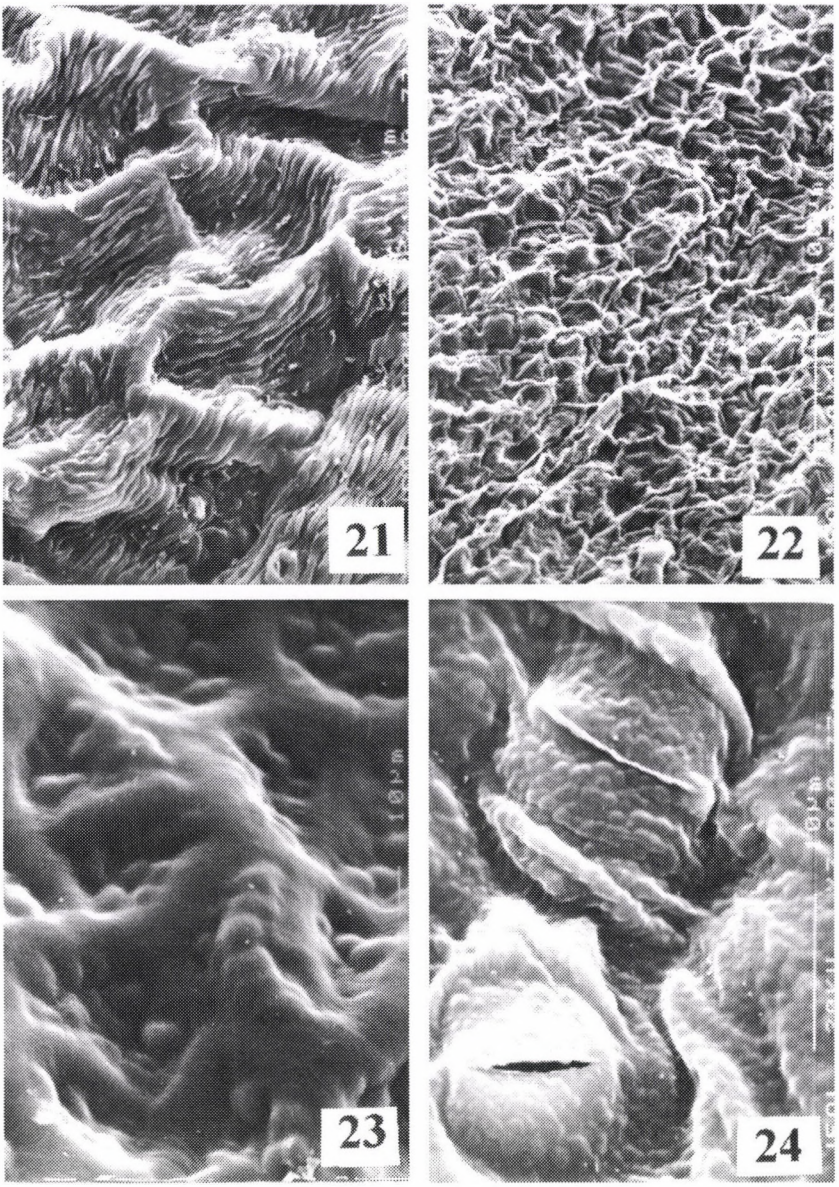
Figs 9–12. Primary cuticular ornamentation on the adaxial (ADS) and abaxial (ABS) leaf surface. – *Fig. 9.* striate, *Exostema peruvianum* (Poepp.) Endl., ADS; *Fig. 10.* striate, *Exostema caribaeum* (Jacq.) Schult., ABS; *Fig. 11.* striate, *Rondeletia glauca* Griseb., ABS; *Fig. 12.* verrucate, *Rondeletia subcanescens* Fernandez et Borhidi, ADS



Figs 13–16. Secondary cuticular ornamentation on the adaxial (ADS) and abaxial (ABS) leaf surface. – *Fig. 13.* foveolate/reticulate, *Exostema maestrense* Borhidi et Fernandez, ADS; *Fig. 14.* foveolate/striate, *Rondeletia clarendonensis* Britton ex S. Moore, ADS; *Fig. 15.* gemmate/striate, *Rondeletia longibracteata* Alain et Acuna, ADS; *Fig. 16.* reticulate/rugulate, *Rondeletia portoricensis* Kr. et Urb., ADS



Figs 17–20. Secondary cuticular ornamentation on the adaxial (ADS) and abaxial (ABS) leaf surface. – *Fig. 17.* reticulate/striate, *Exostema floribundum* Roem. et Schult., ADS; *Fig. 18.* scabrate/reticulate, *Arachnothryx izabalensis* (Standl. et Steyerm.) Borhidi, ADS; *Fig. 19.* scabrate/striate, *Rondeletia savannarum* Britton, ADS; *Fig. 20.* striate/corrugate, *Rondeletia sylvestris* S. Moore, ADS



Figs 21–24. Secondary cuticular ornamentation on the adaxial (ADS) and abaxial (ABS) leaf surface. – *Fig. 21.* striate/reticulate, *Rondeletia saxicola* Britton, ADS; *Fig. 22.* striate/rugulate, *Rondeletia saxicola* Britton, ABS; *Fig. 23.* verrucose/reticulate, *Rondeletia combsioides* Fernandez et Borhidi, ADS; *Fig. 24.* verrucose/striate, *Rondeletia hirta* Sw., ADS

striate/corrugate – the primary striate structure wrinkles into thick folds usually parallel to others	*Rondeletia sylvestris* S. Moore (Fig. 20)
striate/reticulate – ridges of uniform width between the sinkings show the primary striate ornamentation	*Rondeletia saxicola* Britton (Fig. 21)
striate/rugulate – the primary striate structure wrinkles into thick folds with irregular orientation	*Rondeletia saxicola* Britton (Fig. 22)
verrucose/reticulate – ridges of uniform width between the sinkings show the primary rough verrucose surface	*Rondeletia combsioides* Fernandez et Borhidi (Fig. 23)
verrucose/striate – the primary verrucose structure wrinkles into folds usually parallel to others	*Rondeletia hirta* Sw. (Fig. 24)

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APPLICATION OF LEAF EPIDERMAL MORPHOLOGY TO TAXONOMIC DELIMITATIONS IN THE GENUS *JAVORKAEA* BORHIDI ET J. KOMLÓDI (RUBIACEAE)

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Three species of the recently described genus *Javorkaea* were examined with the aid of light microscopy and SEM, for characterising their epidermal cell structure and to find leaf anatomical features useful for their taxonomic separation.

Key words: cell shape, cuticular ornamentation, indumentum, *Javorkaea*, stomata

Introduction

An investigation of leaf microcharacters of the genus *Javorkaea* was conducted, using LM and SEM, in order to evaluate their reliability as taxonomic markers. Examinations of the epidermis of mature leaves of three species have provided information consistent with an earlier revision of the genus by Borhidi et al. (1999).

Shape of the epidermal cells, direction of anticlinal walls can be used as distinguishing characters. The stomata appear to be of two types: paracytic or hemiparacytic stomata. Species can also be distinguished by the presence of hairs. Similarities and dissimilarities of cuticular ornamentations can be used as distinguishing characters, usually significant at the species level.

Material and methods

Species used in this study are from the National Herbarium of Mexico.
Javorkaea hondurensis (Donn.-Smith) Borhidi – Hjalmarsson S. s. n. 1852
Javorkaea acuminata (Oerst. ex Standl.) Borhidi – Rafael Torres 10937
Javorkaea uxpanapensis (Lorence et Castillo-Campos) Borhidi – Tom Wendt 5251

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Dried mature leaves were revived in boyling water after that in Schultze solution until loosing the colour. After thorough washing in water epidermal peelings were stained methylen blue and mounted in Canada balsam.

To examine the cuticular ornamentation the herbarium material was cleared with ultrasound (20–30 KHz frequency), coated with gold, observed with JEOL-6300 scanning electron microscope at 300–1000× magnification.

Results

The results of the observations are shown in Table 1.

The unspecialised cells of the epidermis are irregular to polygonal as far as the shape is concerned. Irregular are on the adaxial surface of the *J. hondurensis* and *J. acuminata*, polygonal on the adaxial surface of the *J. uxpanapensis* and on the abaxial side of the leaves of *J. acuminata* and *J. uxpanapensis*. Cellular structure of the abaxial surface of the *J. hondurensis* not visible because of the dense indumentum.

Anticlinal cell walls are sinuous on the adaxial surface of *J. hondurensis* and *J. acuminata*, straight on the abaxial side of *J. acuminata* and *J. uxpanapensis* and on the adaxial surface of the *J. uxpanapensis*.

Stomata restricted to the abaxial surface at all three species examined and are paracytic with the subsidiary cells equal in size, excluding *J. uxpanapensis* which has hemiparacytic stomata with only one subsidiary cell.

Members of the genus vary widely in their laminar indumentum. All species have unicellular or multicellular nonglandular trichomes on both surfaces. Types of hairs are: acicular, attenuate, aduncate, subulate, peltate, spiral.

J. acuminata has peltate hairs on the adaxial and attenuate hairs on its abaxial surface, this never occurs in any of the other species, this is unique in the genus. Subulate trichomes have restricted occurrence only on *J. hondurensis* on the upper side of the leaves. This species has aduncate types of hairs on the lower side. Acicular trichomes are characteristic only for *J. uxpanapensis* on its abaxial surface. Only one species, *J. uxpanapensis* has the same type of trichomes on both leaf surfaces, spiral hairs are on the adaxial and abaxial surface as well.

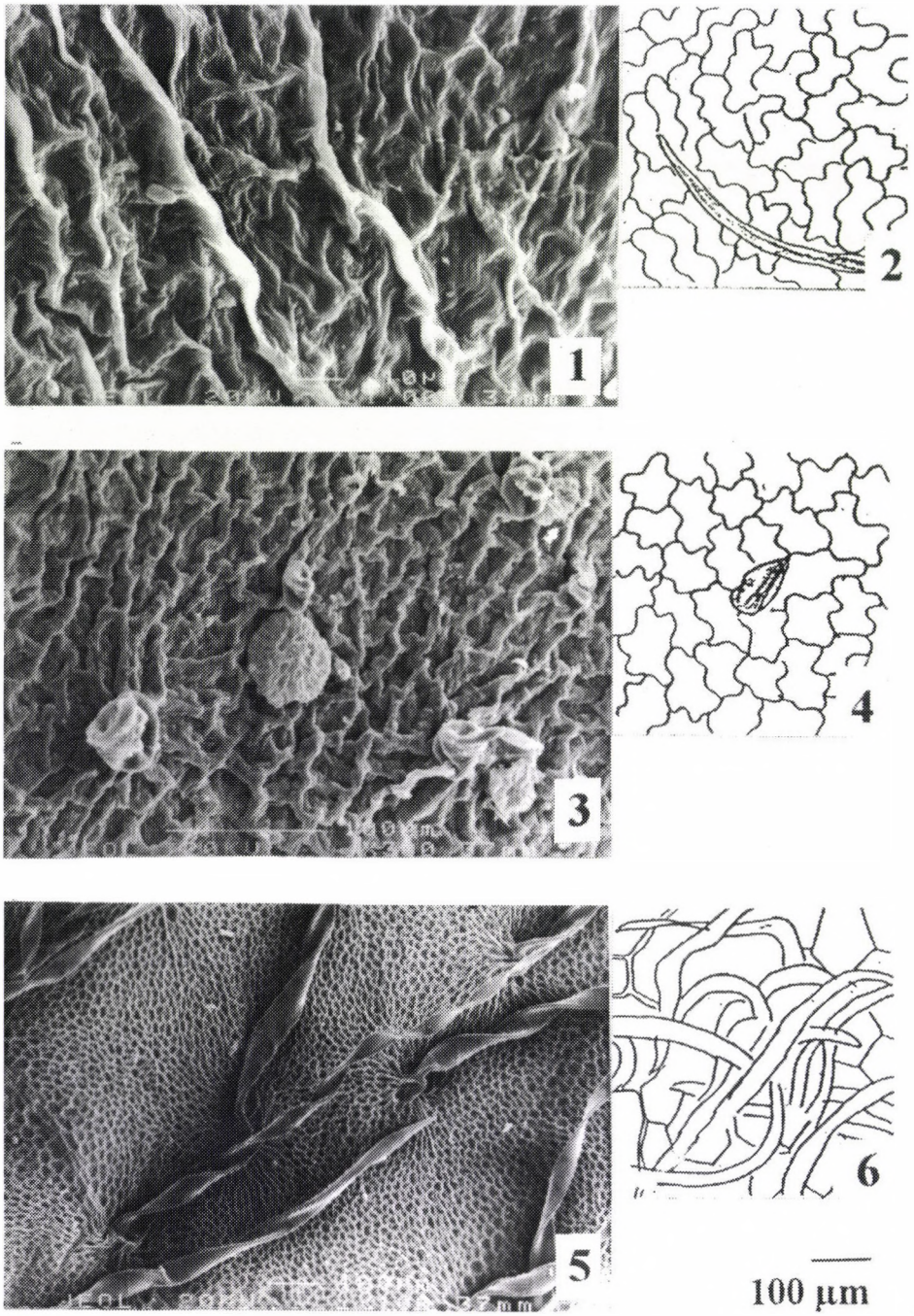
All taxa have cuticular covering with distinctive ornamentations. *J. uxpanapensis* shows primary cuticular ornamentation, reticulate on its adaxial, striate on the abaxial surface. Primary and secondary ornamenta-

Table 1

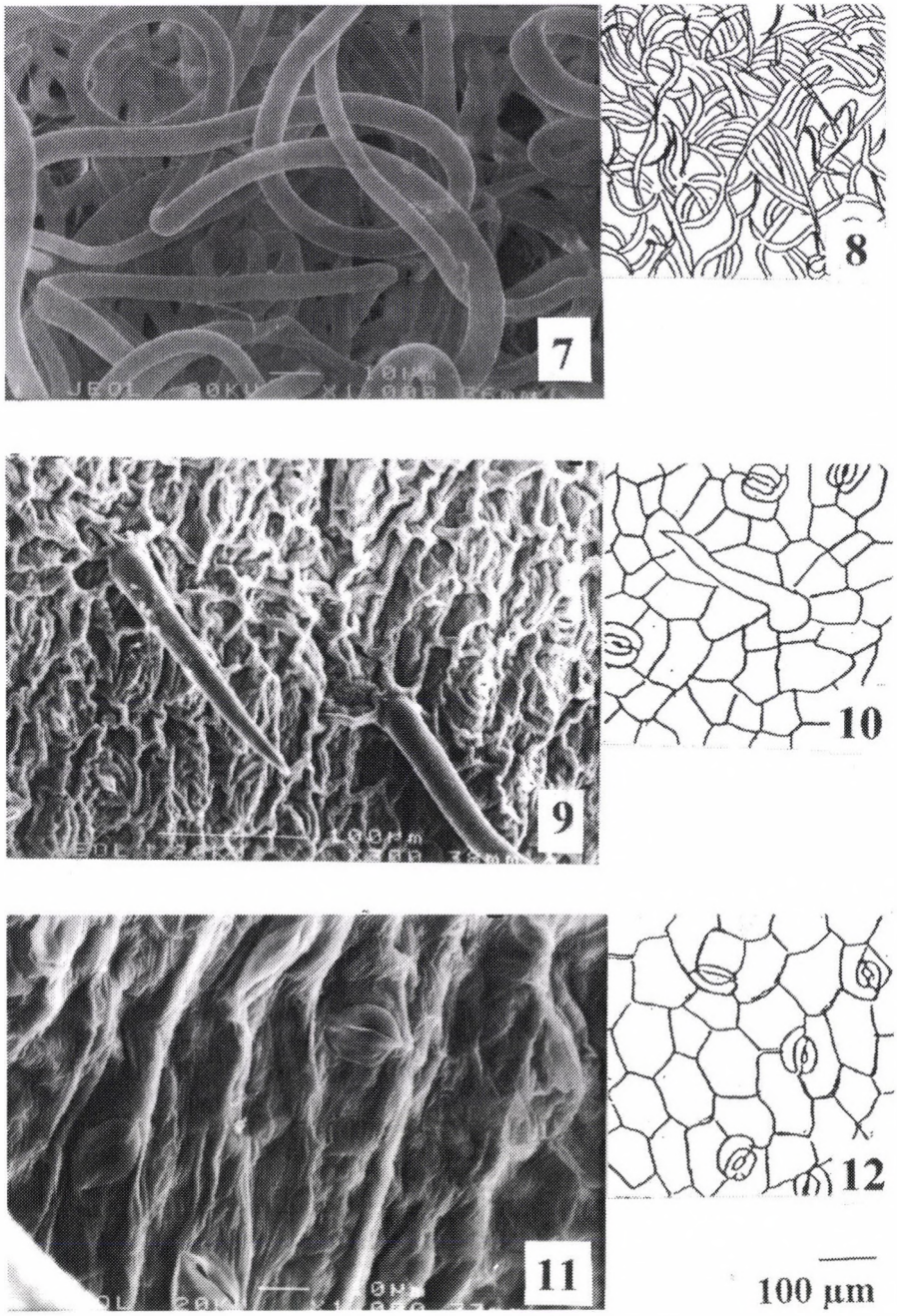
Micromorphological features of the leaf epidermis of *Javorkaea*

Species	Epidermis				Type of stomata	Type of trichomes		Cuticular ornamentation	
	outline of cells		direction of anticlinal walls						
	adaxial	abaxial	adaxial	abaxial		abaxial	adaxial	abaxial	adaxial
J. hondurensis	irregular	–	sinuous	–	paracytic	subulate spiral	aduncate	striate/ corrugate	–
J. acuminata	irregular	polygonal	sinuous	straight	paracytic	peltate	attenuate	striate/ reticulate	striate/ reticulate
J. uxpanapensis	polygonal	polygonal	straight	straight	hemi-paracytic	spiral	acicular spiral	reticulate	striate

Symbols: – no visible



Figs 1–6. Upper leaf surfaces. – *Figs 1–2. Javorkaea hondurensis; Figs 3–4. J. acuminata; Figs 5–6. J. uxpanapensis*



Figs 7–12. Lower leaf surfaces. – *Figs 7–8. Javorkaea hondurensis; Figs 9–10. J. acuminata; Figs 11–12. J. uxpanapensis*

tion are characteristic of *J. acuminata*: striate-reticulate on both leaf surfaces. On the adaxial leaf side of *J. hondurensis* the primary pattern occurs as striae which give rise to prominent corrugate secondary structure. The cuticular ornamentation of the abaxial surface of this species not visible because of the dense indumentum.

Conclusions

Leaves of the species appear to differ in their micromorphological features: in general size and shape of cells, characters of the indumentum, and cuticle ornamentation.

The distribution of these epidermal characters in the genus *Javorkaea* makes it possible to attribute to the micromorphological characters a systematic and phylogenetic value comparable to that shown by the flower and pollen type.

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THE TAXONOMIC IMPORTANCE OF LEAF-SURFACE CHARACTERS IN THE GENUS *EXOSTEMA* (RUBIACEAE)

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Leaf epidermal characters are generally considered as depending mostly of environmental conditions and of less taxonomic value. SEM examinations of the epidermis of mature leaves of 8 species in the 5 sections of *Exostema* (Rubiaceae) have provided information about the similarities and dissimilarities of shape of epidermal cells, direction of anticlinal walls, cuticular ornamentations, type of stomata, type of indumentum. Data obtained in this study turned to be useful for taxonomic differentiation of taxa of different ranks. They may be usually qualified as significant at the species level.

Key words: epidermis, *Exostema*, leaf-microcharacters, taxonomic ranks

Introduction

Floral characters were originally considered the most valuable indicators of taxonomic affinity. Although, this point has stood the test of time, sometimes leaf characters may be also useful in taxonomic studies. Leaves should, however, have a number of theoretical advantages over flowers as taxonomic markers. They are strictly comparable over a wider taxonomic range, and they are generally present on the plant for a much greater part of its life-span (Stace 1984). The past thirty years have witnessed a remarkable increase in the attention paid to leaf characters, partly but not primarily due to the advent of scanning electron microscope (SEM).

Numerous lists of "the taxonomically most useful characters" in a taxon have been drawn up, e.g. that by Hickey and Wolfe (1975) concerning leaf architecture. Barthlott (1981) considers that their high structural diversity renders them most suitable for classification between the levels of family and species, although there is also some evidence for their use above the family level.

One generalisation which can, perhaps, be made is that xeromorphic leaves seem to provide more leaf-surface characters of taxonomic importance than do the mesomorphic leaves. A range of mesomorphic species is

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more likely to present a relatively invariable stereotyped pattern than a range of xeromorphic species, presumably because the latter have responded to the need to tolerate drought various ways. The taxonomy of plants with thick, drought-resistant leaves has thus often been greatly supported by the use of leaf-surface characters, e.g. conifers (Alvin et al. 1982), mangroves (Stace 1966), Magnoliaceae (Baranova 1972), Ericaceae and Epacridaceae (Watson 1964), and various succulents (Cutler and Hartmann 1979, Ihlenfeldt and Hartmann 1982).

Direct observations of a correlation between a particular anatomy and environmental conditions by no means indicate the existence of phenotypic plasticity. For example, Barthlott (1981) got to the conclusion that most herbaceous plants possess sculptured leaf surface, while most tropical plants have leaves of smooth surface. Baas (1975) found in the Aquifoliaceae that high montane species possess thicker cuticles and straight-walled epidermal cells than species from the humid tropics. But in those cases which were further investigated characteristics turned to be genetically controlled adaptations to the environment rather than ecophenetic responses. Although many cases of phenotypic plasticity have been demonstrated, and many epidermal characters do seem genetically to adapt readily to changing conditions, it has often been shown that characters indicating taxonomic relationship equal or outnumber those indicating adaptations to environmental conditions, e.g. in mangroves (Stace 1966) and apophyllous xerophytes (Böcher and Lyshede 1972).

Epidermal characters are only slightly influenced by environmental conditions. Differences in epidermal characters of comparable organs seem always to reflect genetic differences in the plants concerned. Their high structural diversity provides most valuable criteria for the classification between species and family level. There is also some evidence for their systematic applicability above the family level (Barthlott 1981).

Anticlinal undulations of epidermal cells usually are of high taxonomic significance and often characterise taxa between species and genus level; occasionally they even delimit subtribes and tribes (Barthlott and Voit 1979).

The curvature of the outer periclinal wall can serve as a good diagnostic character for the lowest taxonomic categories. Since there is limited micromorphological diversity and as flat or convex epidermal cells occur throughout the plant kingdom there is little systematic impact to be found in these features. However, there are occasionally noteworthy shapes of

cells caused by particular curvature of outer walls, which can be of great systematic interest.

The extent of cutinisation varies greatly, and may itself be a taxonomic character. There are often many intrinsic characters of the cuticular membrane itself which may prove of no less taxonomic value (Stace 1984). Traditionally, these mostly involve the shape and extent of striations, reticulations and micropapillae as observed with the aid of SEM. Cuticular striations and micropapillations seem to be angiosperm characteristics. Cuticular sculptures may serve as excellent diagnostic characters, but their systematic significance for delimitation of categories above the species level is rather limited (Barthlott and Voit 1979).

Ultrastructural, micromorphological, developmental, chemical and taxonomic aspects of epicuticular secretions, waxes and related solid lipophilous substances are discussed by Barthlott and Wollenweber (1981).

In addition to the features mentioned above there are many other epidermal characteristics of systematic significance. As a specialised element, the different stomatal types ought to be mentioned.

Occurrence of stomatal diversity on one and the same surface of any plant part is widespread among the angiosperms (Metcalfe and Chalk 1950, Ramayya and Rao 1968). The shape, size, distribution and orientation of the stomata, and the various thickenings and ornamentations of the guard cell walls, are all characters which are frequently of taxonomic value (Wilkinson 1979). A large number of papers refer to the taxonomic significance of the stomatal type, some of them to the use of it in phylogenetic considerations.

It is relevant that foliar stomata in the subfamily Cinchonoideae (with mostly woody species) are mesogeneous dolabrate, while those in the Rubioideae (comprising mostly herbaceous taxa) are mesogeneous trilabrate (Bahadur et al. 1971).

The principles on which phylogenetic hypotheses have been based are mainly the following: the most primitive species would possess anomocytic stomata and the evolution would have taken place from these species to those with mesogeneous stomata, either bicytic or anisocytic. In this phylogenetic field the species with diversified stomata are particularly important because they show intermediate stages in this evolution. On the contrary, the species with stomata of homogeneous type can be considered as ends of the phylum (Bessis and Guyot 1976).

Material and methods

Fragments of dried leaves were used for SEM. Mature leaves were selected from each of the 9 herbarium specimens of genus *Exostema* used for this study. The material is from Herbario de la Academia de Ciencias de Cuba, Herbario del Jardin Botanico Nacional de Cuba, Rijksmuseum Stockholm. (The locality reference numbers are listed.)

After gold sputtering, the specimens were examined and photographed under JEOL-6300 SEM.

The following specimens were examined

Section Exostema – *E. parviflorum* L. C. Rich., No 3630 (Figs 1–2); *E. selleanum* Urb. et Ekm., Ekman H–7358 (Figs 3–4); *E. velutinum* Standl., HAJB–9761 (Figs 5–6).

Section Floribundae – *E. ellipticum* Griseb., No 4878 (Figs 7–8).

Section Oliganthae – *E. caribaeum* (Jacq.) Schult., Ekman 18660 (Figs 9–10); *E. acuminatum* Urb., Ekman H–3917 (Figs 11–12).

Section Longiflorae – *E. stenophyllum* Britton, Leon HAC–19144 (Figs 13–14).

Section Polyphyllae – *E. polyphyllum* Urb. et Ekm., Ekman H–10148 (Figs 15–16).

Observations and discussion

Section Exostema

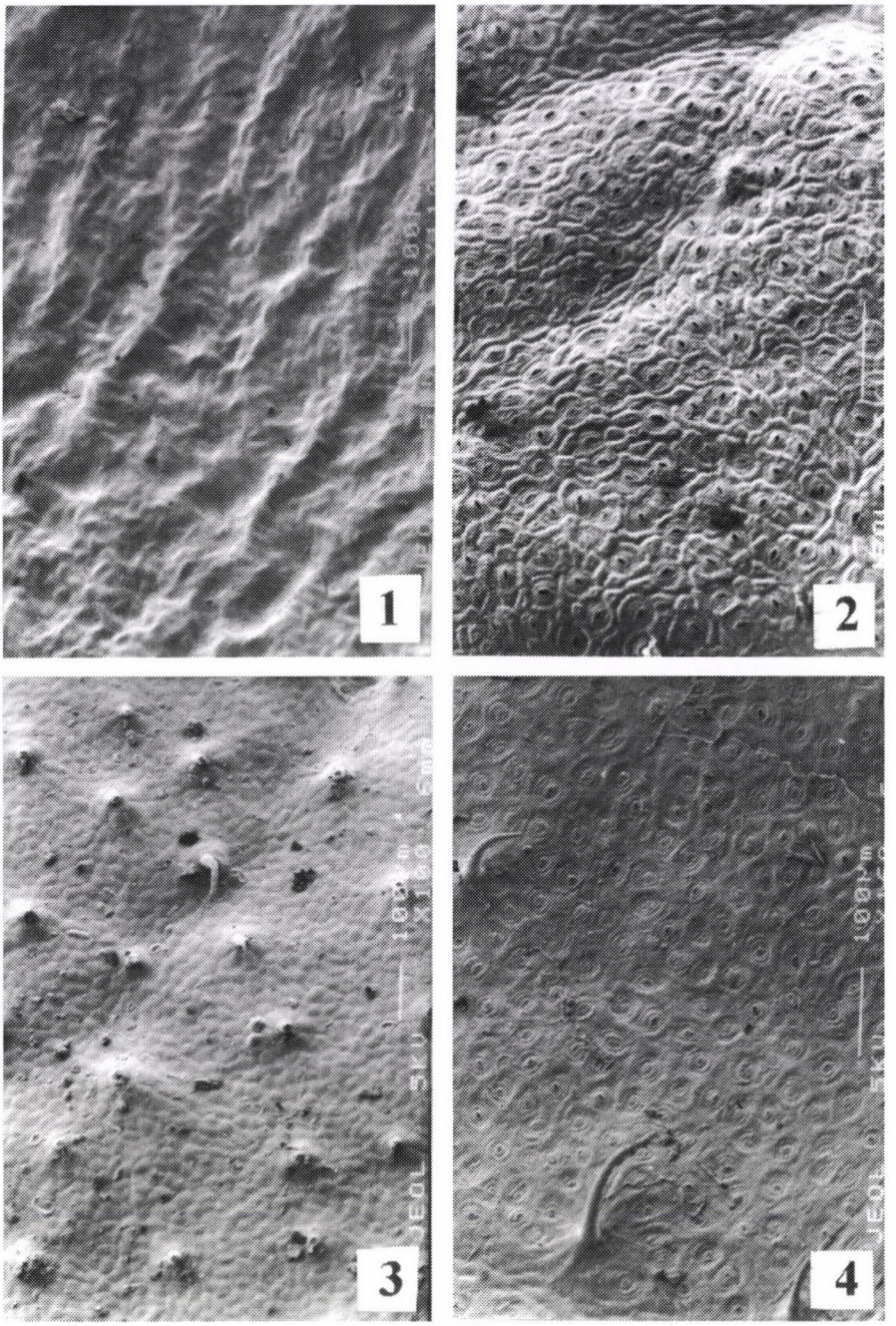
Exostema selleanum

Living on rocks of high montane forest belt in humid macroclimate but open insolated wind blown habitat.

Among the three species examined belonging to the section Exostema *Exostema selleanum* is the most mesophilous one. The cell structure on the adaxial surface is well recognisable because of the thin cuticle. The cuticle on the abaxial cells has short fine striae confined within each cell boundary. Stomata raised from the surface.

Exostema parviflorum

Living in seasonal tropical climate at the semideciduous forest belt, on rocky shrublands. The habitat is dry at least seasonally.



Figs 1–2. E. parviflorum L. C. Rich., No 3630
Figs 3–4. E. selleanum Urb. et Ekm., Ekman H–7358

On the upper leaf surface the cuticle is thick. There are long, continuous striae which pass over several cells. The lower leaf surface has high density of slightly sunken stomata.

Exostema velutinum

Living in dry tropical deciduous forests and seaside sclerophyllous thickets. Taxonomically it is closely related to *Exostema parviflorum*. The adaxial surface is provided with attenuate trichomes and conspicuous development of cuticle is to be observed on it. Features of the abaxial surface are referred to as a strong ecological adaptation. Cell walls have papillae, stomata are deeply embedded. Acicular trichomes cover this surface of which basis are projecting from the neighbouring cell level.

Section Oliganthae

The common features regarding the leaf structure on both surfaces are the corrugated cuticle, the density and arrangement of which can be of characteristic value in the study of the species.

Exostema caribaeum

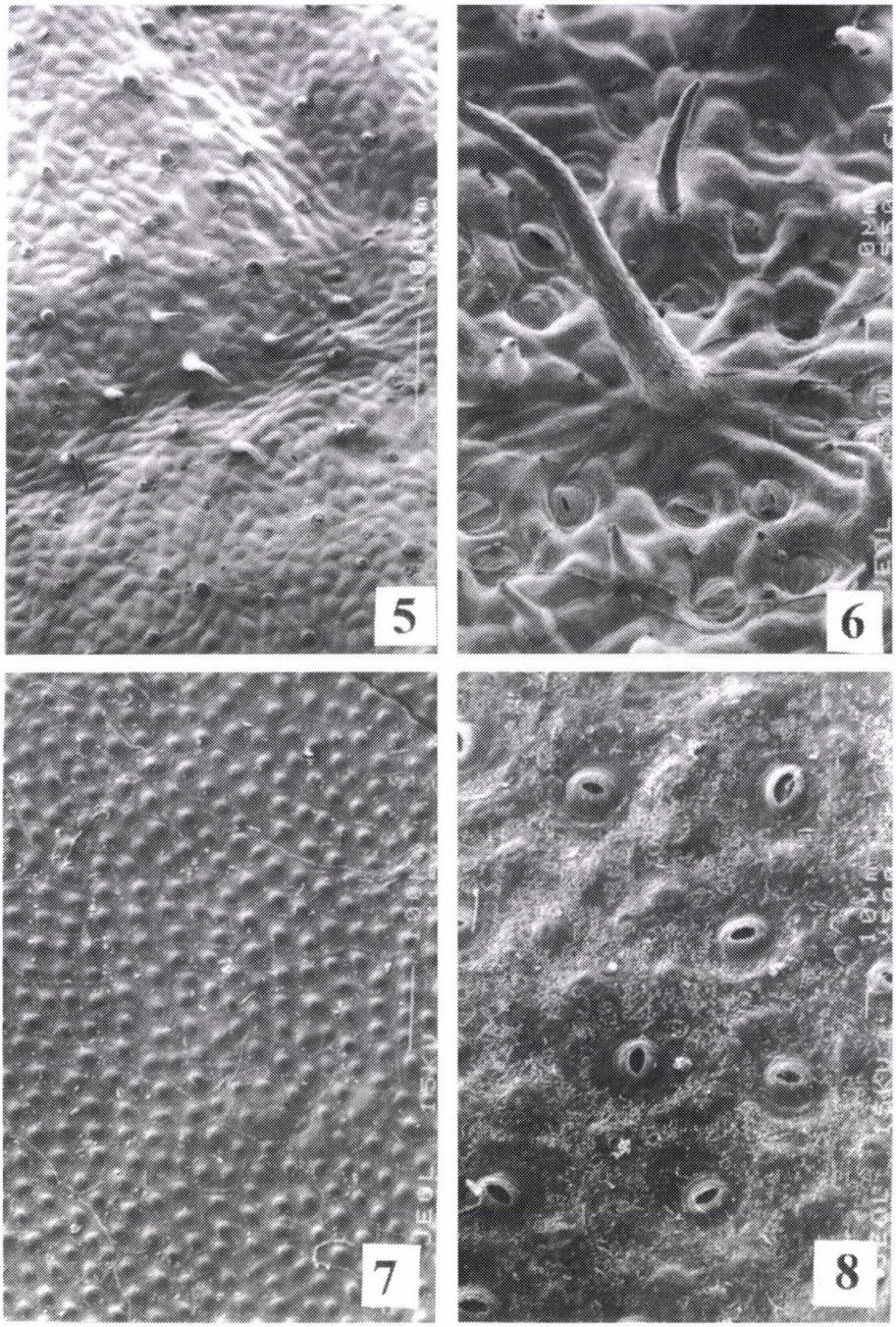
The most widespread species of the genus from the extremely dry littoral thickets to the shrub layer of the semideciduous forests.

Raised cell boundaries longitudinal striae on them and randomly scattered aduncate trichomes are characteristic of this species. The abaxial surface is covered by an extremely thick cuticular striation. The radial walls are deeply sunken. The striation of guard cells is parallel to stomatal pore. The acicular trichomes are thick with a similar striations on them. Some leaves are completely hairy on the lower surface, others were glabrous while all possible transitional forms are to be found. The latters are belonging to *Exostema caribaeum* var. *velutinum*.

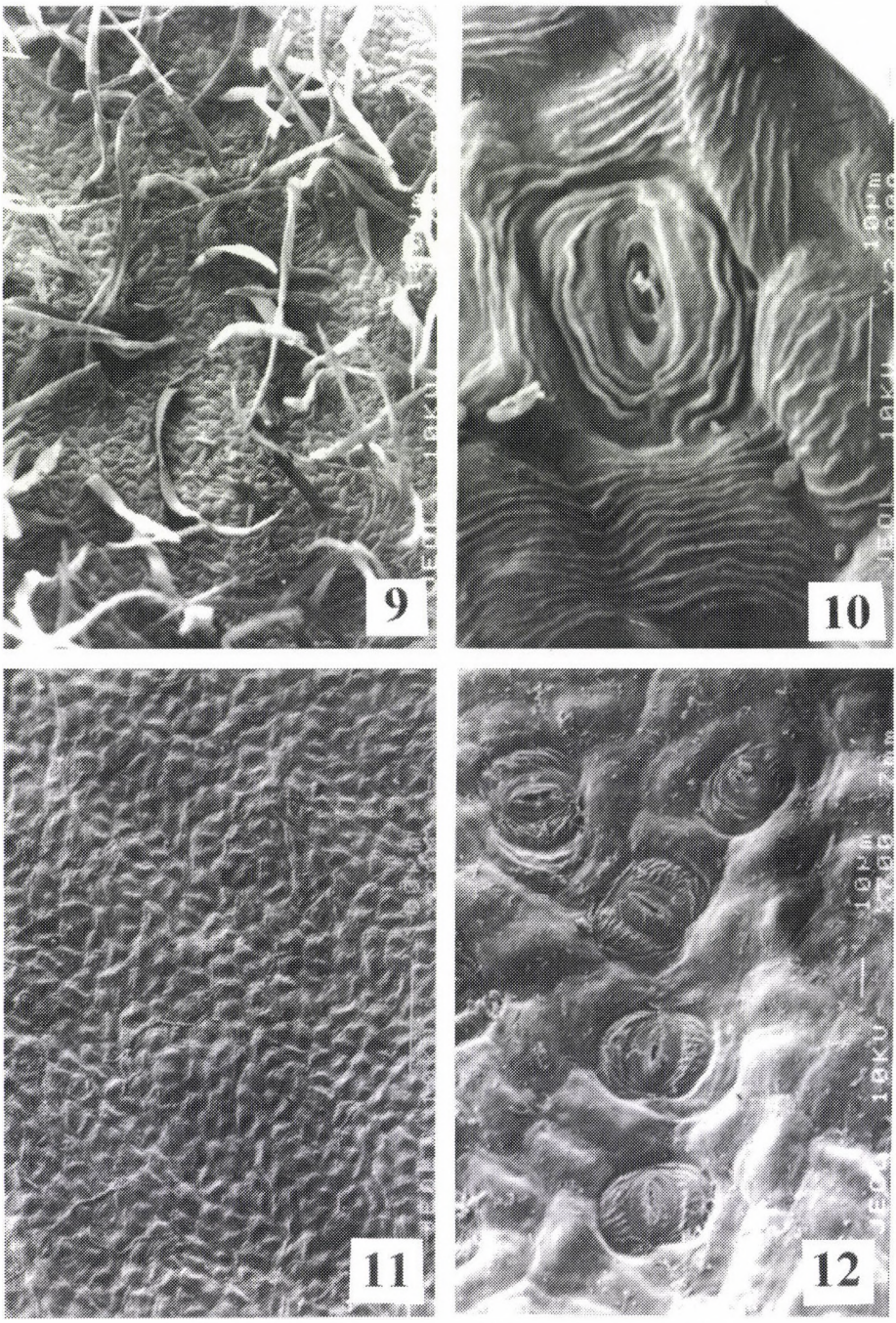
Exostema acuminatum

Living in montane rocky shrublands of Hispaniola. The *Exostema acuminatum* has coriaceous leaves covered by cuticle on both surfaces.

The tangential walls of the adaxial surface are protuberant while the radial walls are sunken. The cells on the abaxial surface are covered by cuticle whereby the cell structure is hidden. On the subsidiary cells of the paracytic stomata peculiar transversal striae are found.



Figs 5–6. E. velutinum Standl., HAJB–9761
Figs 7–8. E. ellipticum Griseb., No 4878



Figs 9–10. E. caribaeum (Jacq.) Schult., Ekman 18660
Figs 11–12. E. acuminatum Urb., Ekman H–3917

Section Floribundae

Exostema ellipticum

It occurs in Cuba and Hispaniola and replaces the typical species of the section: *Exostema sancta-luciae*. It is a medium sized tree living in tropical seasonal evergreen forests.

The leaves are subcoriaceous, shiny and of mesophilous character. The adaxial surface is covered with a thick cuticle. The cellular structure is not recognisable. In contrast the randomly arranged semiorbicular papillas are characteristic throughout the whole surface. The abaxial surface is also covered by a thick cuticle and epicuticular wax scales are also to be observed. The cell structure is not visible here either. The stomata seem to be anomocytic, arising from the surface. The features show a general mesomorphic character.

Section Longiflorae

Exostema stenophyllum

Living on montane riverside wet rocky places on ultramafic (serpentine) bedrock.

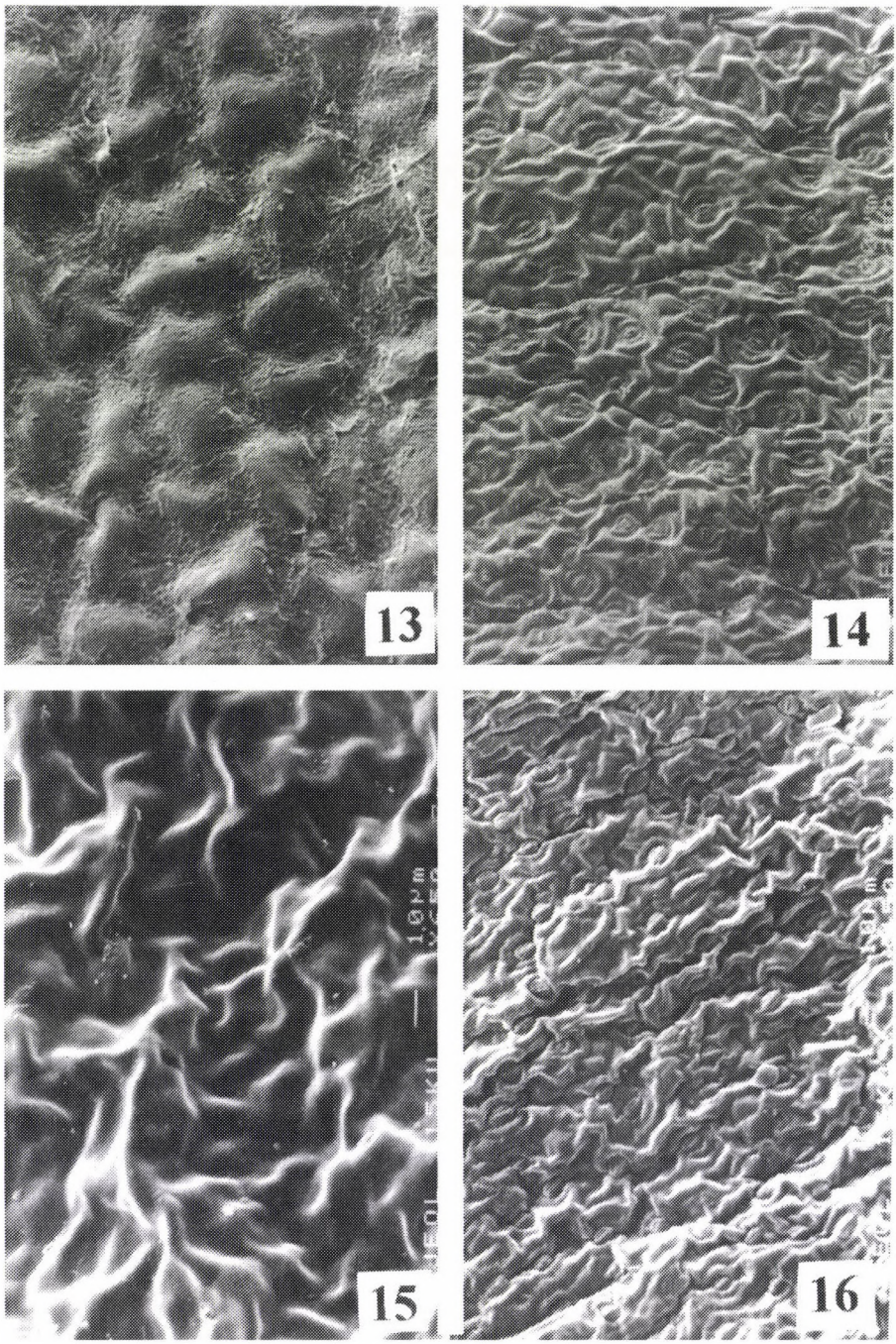
Exostema stenophyllum and *Exostema longiflorum* are close relatives. The latter has soft leaves poor in supporting elements. Because of the general nature of the serpentine adaptation it shows certain pseudo-xeromorphic characters: existing in coriaceous structure of the leaves, whereas on the adaxial surface is the cell structure well recognisable. The cuticle is rather thin but the surface is covered by scale-like filiform wax layer. The radial cell walls of the abaxial surface and the numerous tiny stomata are raised showing markedly mesomorphic character.

Section Polyphyllae

Exostema polyphyllum

The only species of the section is specialised to intensive transpiration although it lives in rocky streambeds exposed to strong insolation and wind effect.

Leaves are dense and scale-like. The features of the epidermis confirm the macromorphological characters: the adaxial surface is covered by thick striated cuticle. The striations are so strong as to obscure the cell outlines. The abaxial surface is rather articulated as well with lengthwise folded



Figs 13–14. E. stenophyllum Britton, Leon HAC–19144
Figs 15–16. E. polyphyllum Urb. et Ekm., Ekman H–10148

protuberant striae. The stomata which are tiny, sunken and generally a lot in number appear more crowded between the striae.

The epidermal structure regarding the cuticular pattern, the shape and arrangement of stomata has more general features characterising the genus while within the sections there are recognisable marks of adaptations to the conditions of the habitat.

The changes of the ecological adaptation showing similar tendency in several sections usually do not eliminate the characters of ground structures of the categories namely species of several sections living in similar habitat have produced different form of drought-resistance.

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QUANTITATIVE CHARACTERISTICS OF STOMATA AND EPIDERMAL CELLS OF LEAVES IN THE GENUS *EXOSTEMA* (RUBIACEAE)

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Seventeen *Exostema* species of the Caribbean region belonging to 5 different sections were studied for quantitative cell characters of the leaf epidermis. The studied features turned to be characteristic on section level, but unuseful for differentiate species within the frame of one section. Cell patterns of the upper epidermal surface seem to be more conservative, while those of the lower surface turned to be more variable and environment-dependent.

Key words: *Exostema*, epidermis, quantitative characters, stomata, epidermal cells

Introduction

It seemed desirable to investigate the reliability of the quantitative micromorphological data investigated in the genus *Exostema* as criteria for the characterisation of species belonging to different sections.

Although the actual characters of the upper or lower epidermis of a leaf varies so largely that such figures are useless for differentiating between closely related species, but in the light of the similarity or differences of the quantitative data regarding the sections it appeared that these figures might be of some use in answering questions emerged during the revision of the genus *Exostema*.

Relatively numerous workers have already studied some of the quantitative characteristics of the leaf of higher plants, particularly of leaf epidermis, mostly for taxonomic reasons. Among studies in comparative anatomy, those by Timmermann (1927) studying in the *Datura* genus the stomatal numbers and their value for distinguishing species should be mentioned, because he was of the opinion that these data were unsuitable for use as a diagnostic character. Gupta (1961) assumed that the relative proportions between different tissue systems within the lamina may be constant, and because the absolute numbers are fixed in a leaf, a definite amount of tissue is produced. The results regarding the *Magnolia* and

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Ligustrum leaves obtained by Kutík (1973) confirmed the existence of correlative relationships between quantitative anatomical characteristics of leaf epidermis.

There is a lack of similar approach on the family Rubiaceae.

The aim of this paper was to evaluate the quantitative data investigated in 5 sections of the genus *Exostema*.

Material and methods

The following characteristics were investigated: number of epidermal cells, length and width of epidermal cells, quotion of the length and width values, the number of stomata, stoma index, the size of stomata.

The data given represent the averages of 50 counts were made in comparable regions and area (0.1 sq mm) of the middle part of the leaves.

Results

The epidermal cells per area unit on the adaxial surface are few in number in the section Oliganthae – except of *Exostema caribaeum*, a lot in section Longiflorae and ranges from the lower values to the higher in the

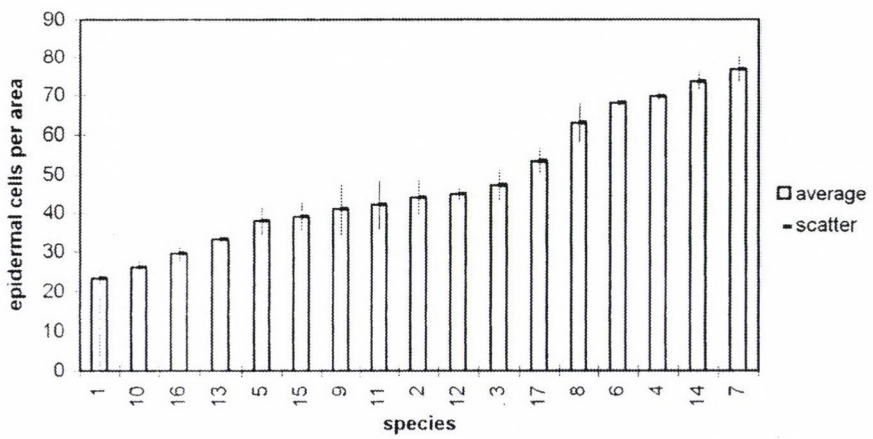

Fig. 1. Epidermal cells per area on adaxial side. 1 = *Exostema purpureum albiflora*, 2 = *E. cordata*, 3 = *E. polyphyllum*, 4 = *E. velutinum*, 5 = *E. parviflorum*, 6 = *E. caribaeum*, 7 = *E. obovatum*, 8 = *E. stenophyllum*, 9 = *E. spinosum*, 10 = *E. purpureum*, 11 = *E. lineatum*, 12 = *E. ellipticum*, 13 = *E. purpureum*, 14 = *E. obovatum*, 15 = *E. angustifolium*, 16 = *E. floribundum nervi*, 17 = *E. longiflorum*

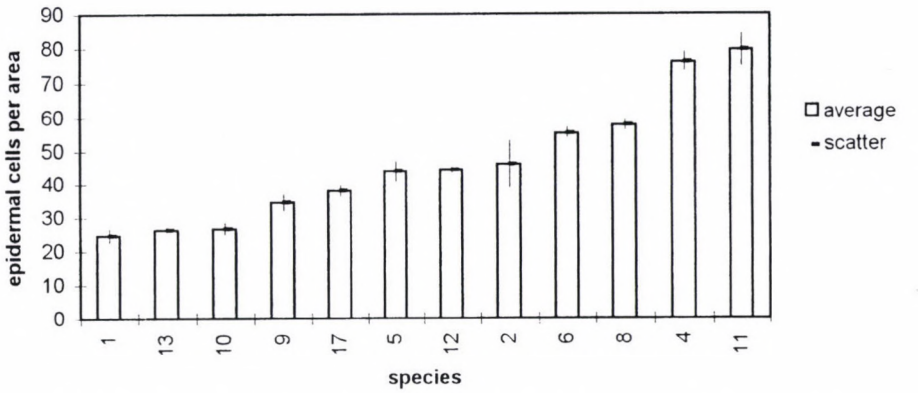

Fig. 2. Epidermal cells per area on abaxial side. (see legends at Fig. 1)

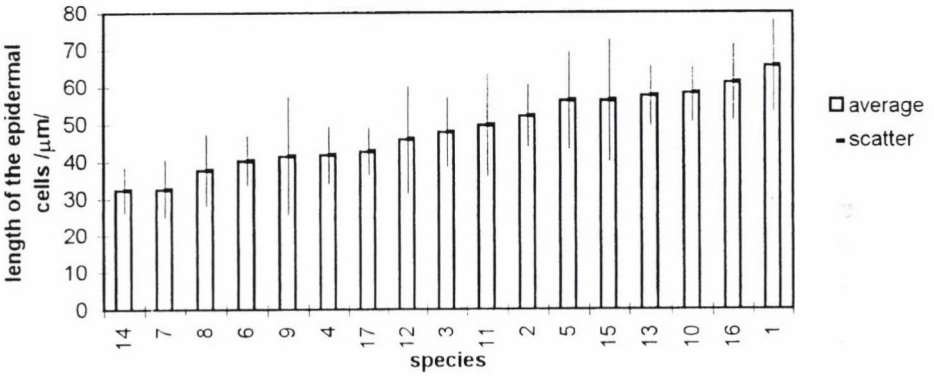

Fig. 3. The lenght of epidermal cells on adaxial side. (see legends at Fig. 1)

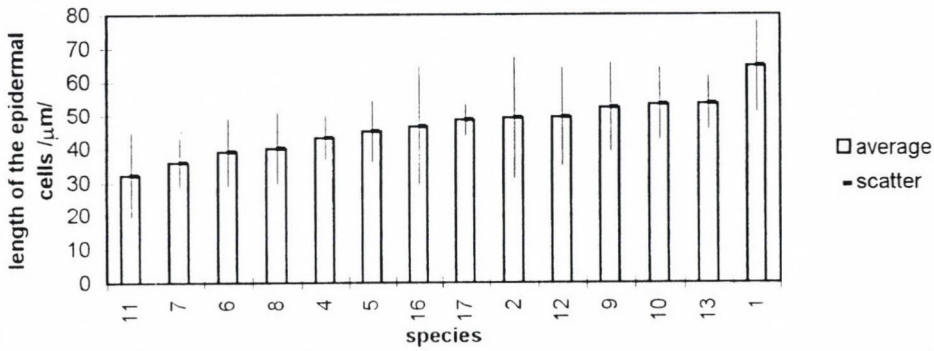

Fig. 4. The length of epidermal cells on abaxial side. (see legends at Fig. 1)

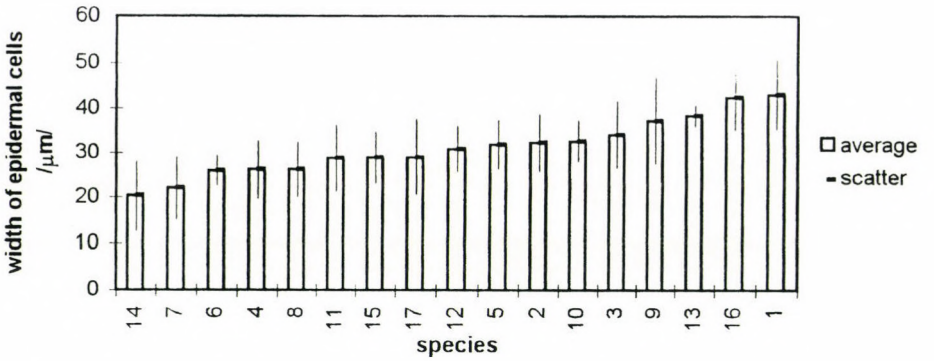

Fig. 5. Width of epidermal cells on adaxial side. (see legends at Fig. 1)

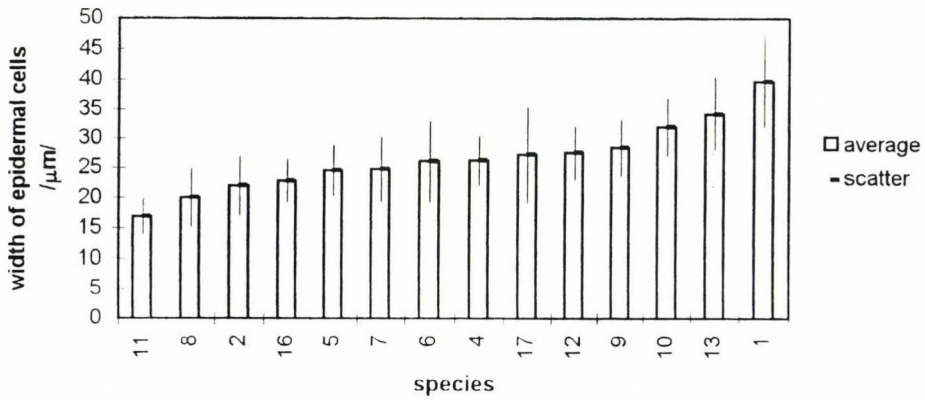

Fig. 6. Width of epidermal cells on abaxial side. (see legends at Fig. 1)

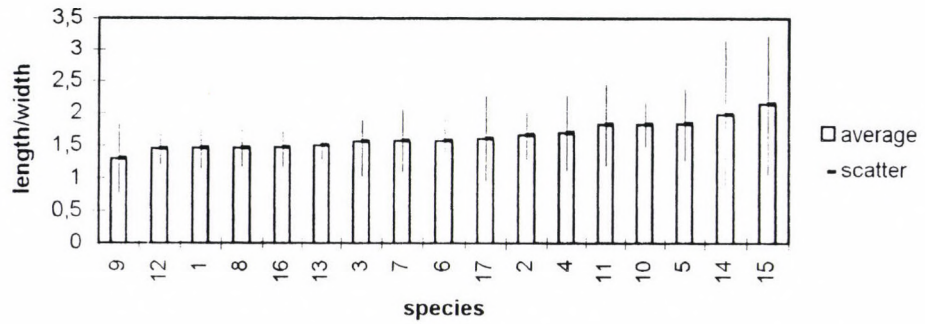

Fig. 7. Ratio of cells length to width on adaxial side. (see legends at Fig. 1)

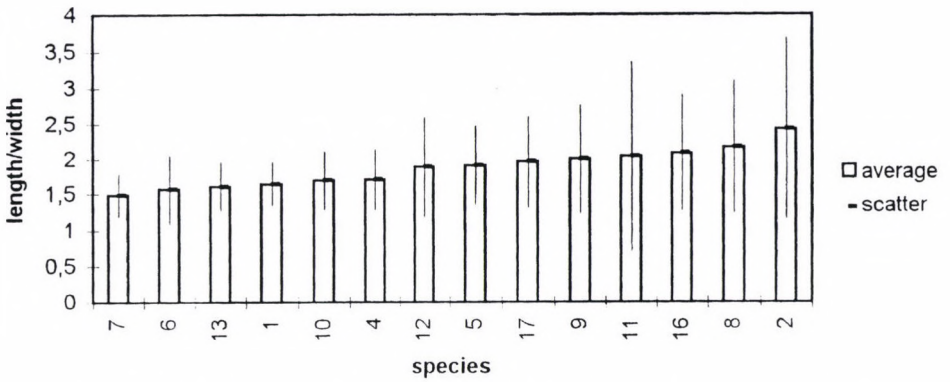

Fig. 8. Ratio of cell length to width on abaxial side. (see legends at Fig. 1)

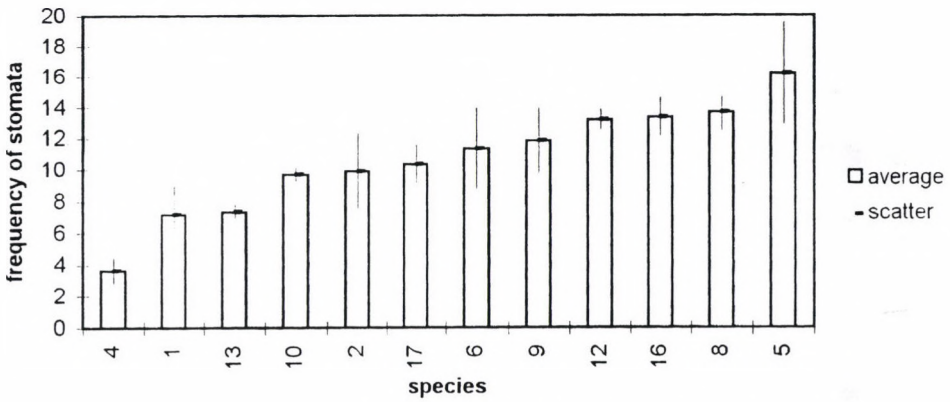

Fig. 9. Frequency of stomata. (see legends at Fig. 1)

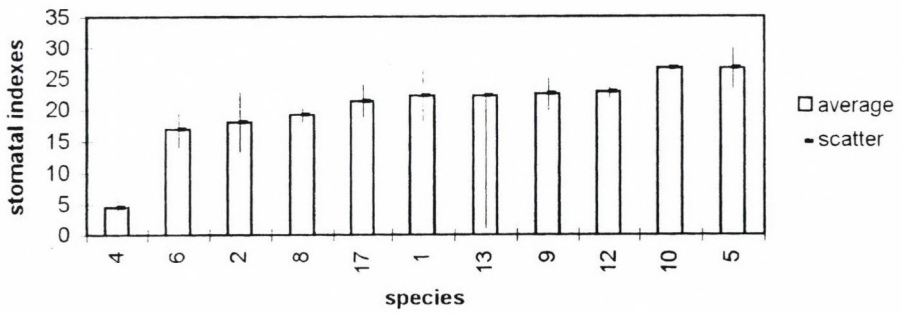

Fig. 10. Stomatal indexes. (see legends at Fig. 1)

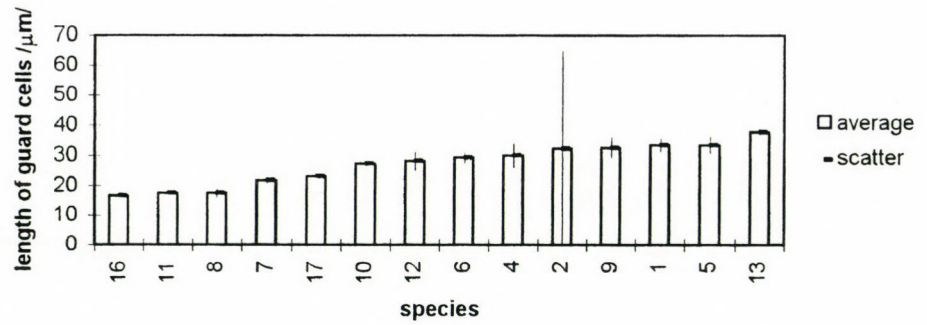

Fig. 11. Length of guard cells. (see legends at Fig. 1)

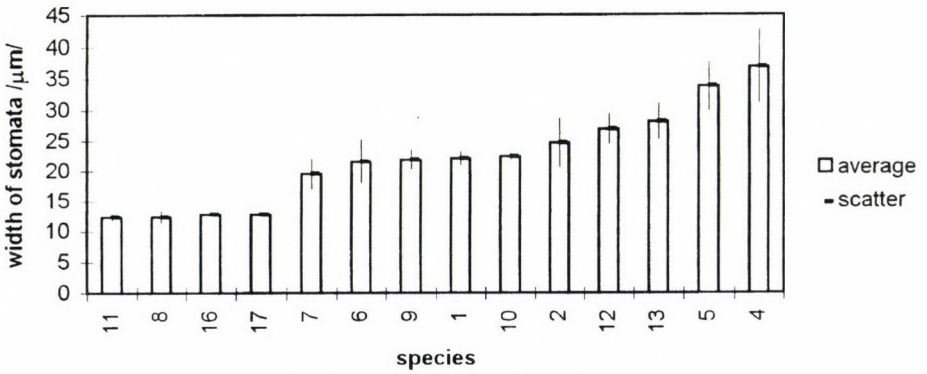

Fig. 12. Width of stomata. (see legends at Fig. 1)

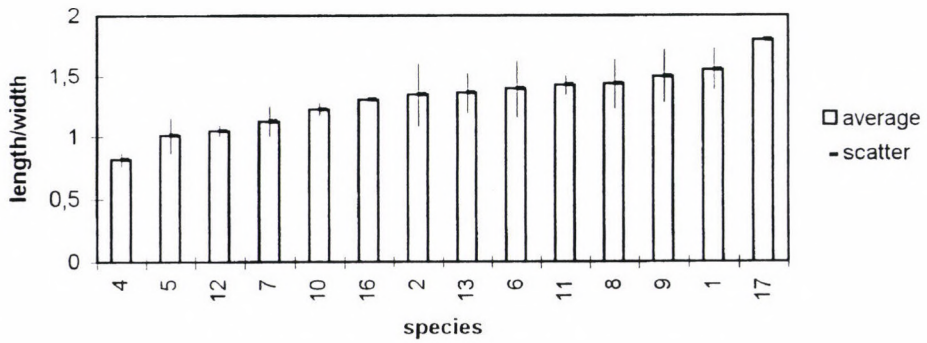

Fig. 13. Ratio of stomatal length to width. (see legends at Fig. 1)

sections Exostema and Floribundae (Fig. 1). This character of the latters and in the section Oliganthae is the same on the abaxial side except of *Exostema caribaeum* again (Fig. 2).

The length of the epidermal cells on both surfaces is different within every but section Oliganthae. All species belonging here have uniformly long cells except of *Exostema caribaeum* (Figs 3–4).

Also the width of cells varies on both surfaces. Only the section Oliganthae proved stable, but *Exostema caribaeum* was divergent again (Figs 5–6). The ratio of the cell length to the width is very different in the sections except of the section Oliganthae whereof species regarding this character on the abaxial surface are very similar (Figs 7–8).

All the leaves are hypostomatic. Regarding the frequency of stomata the section Exostema is the most heterogeneous, section Floribundae the most uniform (Fig. 9). In the light of the latter facts it is obvious that the stomatal indexes show similar differences (Fig. 10). The length and width of stomata are uniformly little in sections Longiflorae and Floribundae whereas section Exostema has species width rather wide and long stomata. Regarding the length of guard cells – section Oliganthae has species with stomata of different length but much more similar width (Figs 11–12). The ratio of the stomatal length to the width – actually the shape of the stoma – shows marked fluctuation in the several sections (Fig. 13).

Discussion

The results reveal that the quantitative data of the leaf epidermis do not correlate exactly with delimitation of species defined on macromorphological features. All the data but the size of stomata show a wide range of fluctuation.

The data regarded the section Oliganthae differ from each other so this section needs revision. Particularly *Exostema caribaeum* does not conform to the earlier arrangement.

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ISOLATION OF CAPSANTHIN 5,6-EPOXIDE FROM *LILIUM TIGRINUM*

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(3S,5S,6R)-capsanthin 5,6-epoxide was isolated from petals of *Lilium tigrinum*, and characterized by its UV/VIS, mass, ^{1}H-NMR and CD spectra.

Key words: capsanthin 5,6-epoxide, carotenoids, isolation, *Lilium tigrinum*, structure elucidation

Introduction

Capsanthin (Fig. 1) and capsorubin (Fig. 2) are typical carotenoids of red pepper (Cholnoky et al. 1956, Curl 1962, Davies et al. 1970). These compounds with one and two acyl-cyclo-pentanol rings respectively are rare in nature and besides their appearance in red peppers have only been identified in anthers and petals of various lilies (Karrer et al. 1950). In the past fifteen years, some novel carotenoids with κ-end group have been isolated from red pepper and characterized, such as capsanthin 5,6-epoxide (Fig. 3), capsanthin 3,6-epoxide (Fig. 4), and 5,6-diepicapsokarpoxanthin (Fig. 5) (Parkes et al. 1986, Deli et al. 1996, 1998a). While the occurrence of capsanthin 5,6-epoxide in red pepper and lilies have been published in many cases (Curl 1962, Valadon and Mummery 1977, Candela et al. 1984), it was isolated from red pepper for the first time only twelve years ago. In different lilium hybrids, capsanthin 5,6-epoxide was detected by Valadon and Mummery (1977), and a biosynthetic pathway was depicted also. An investigation on carotenoids of anthers and petals of *Lilium tigrinum* cv. 'Red Night' were performed in 1985 and 29 carotenoids were separated and characterized (Märki-Fischer and Eugster 1985). Although the main components in the petals were capsanthin and capsorubin, the capsanthin 5,6-epoxide could not be detected. Among the minor components karpoxanthin (Fig. 6) (with *(3S,5R,6R)*-3,5,6-trihydroxy end group), and 6-epikarpoxanthin (Fig. 7) (with *(3S,5R,6S)*-3,5,6-trihydroxy end group) were isolated. Since the main component of carotenoids occurring in the

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red pepper as well as in the petals and anthers of *Lilium tigrinum* is capsanthin (Fig. 1), we assumed that the biosynthesis of carotenoids occurring in small quantities in these plants proceeds in a similar way. Therefore we reinvestigated the occurrence of the minor carotenoids having a 3,5,6-trihydroxy-5,6-dihydro-β end group in the petals of *Lilium tigrinum* (Deli et al. 1998b). In the course of the isolation procedure, we could isolate capsanthin 5,6-epoxide (Fig. 3) from the extract of petals. In this paper, we describe the isolation and identification of this carotenoid.

Materials and methods

Instruments

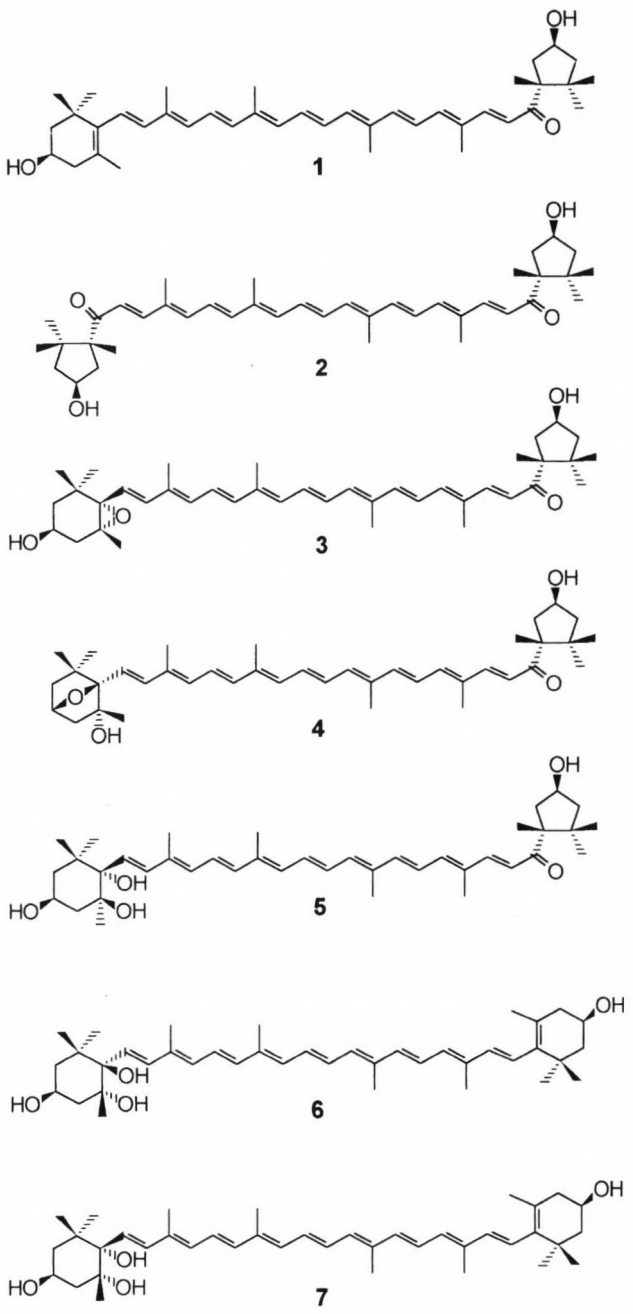
UV/VIS: Beckman DU-65. CD: Jobin-Yvon Dichrograph-6. NMR: Bruker DRX 400 (^{1}H: 400.13 MHz). Solvent: $CDCl_3$, 20 °C. Chemical shifts (δ) in ppm (relative to the solvent signal). MS: Varian MA-CH 7A; m/z (rel. intensity in %).

Plant material

The *Lilium tigrinum* were obtained from a commercial garden in Pécs (southern Hungary) in July 1995.

Extraction

For extraction, 1100 g of petals of lilium (*Lilium tigrinum*) were used. The petals were blended with MeOH, and the suspension was allowed to stand in MeOH. After 20 hrs, the mixture was filtered and the filter cake extracted with MeOH. The extraction was repeated with MeOH and finally with Et_2O. The two MeOH extracts were combined, transferred to a separatory funnel and diluted with Et_2O. The ethereal phase was washed free from methanol with water, and dried over anhydrous Na_2SO_4. The ethereal extract was washed free from methanol, and combined with the ethereal MeOH extract. This solution was saponified with 30% KOH-MeOH at room temperature for 18 hrs. After saponification the ethereal solution was washed free from alkali, evaporated to dryness in vacuum, the residue was distributed between hexane and MeOH/H_2O 9:1, and the hypophasic pigments were precipitated with benzene-hexane (172 mg). The mother liquor was evaporated in vacuum to dryness and dissolved in benzene, and the pigments were precipitated with hexane again (72 mg).



Figs 1–7. 1 = capsanthin, 2 = capsorubin, 3 = capsanthin 5,6-epoxide, 4 = capsanthin 3,6-epoxide, 5 = 5,6-diepicapsokarpoxanthin, 6 = karpoxanthin, 7 = 6-epikarpoxanthin

Column chromatography

The 172 mg hypophasic pigments were submitted to CC: 10 column 6 × 30 cm, $CaCO_3$ (Biogal, Hungary), benzene. Picture after development: Fraction 1: 10 mm brick red (mixture of Z-isomers and 5,6-diepi-capsokarpoxanthin); Fraction 2: 10 mm violet (Fig. 2); Fraction 3: 8 mm yellow (9Z-antheraxanthin and 5,6-diepikarpoxanthin); Fraction 4: 7 mm pink ((Fig. 3)); Fraction 5: 40 mm red (Fig. 1); Fraction 6: 15 mm pale yellow (antheraxanthin and zeaxanthin).

After the processing (cutting the column into pieces and extracting), the Fractions 2, 4, 5, and 6 were crystallized from benzene by addition of hexane; yield: 12 mg of capsorubin (Fig. 2) (m. p.: 195 °C; UV/VIS (benzene): 521, 487, 460 nm), 5 mg of capsanthin 5,6-epoxide (Fig. 3) (m. p.: 169–172 °C; UV/VIS (benzene): 507, 478, nm, after acid treatment: 488, 464 nm), 83 mg of capsanthin (Fig. 1) (UV/VIS (benzene): 485 nm), and 21 mg mixture of antheraxanthin and zeaxanthin respectively. The identification of Fractions 1 and 3 see in Deli et al. (1998b).

*(3S,5R,6S,3'S,5'R)-capsanthin 5,6-epoxide ((all-E, 3S,5R,6S,3'S,5'R)-5,6-epoxy-5,6-dihydro-3,3'-dihydroxy-*β,κ-carotene-6'-one) (Fig. 3): 5 mg; m. p.: 169–172 °C; VIS: λ_{max} in benzene: 507, 478 nm, after acid treatment: 486, 464 nm. CD (EPA, RT): 215 (–4.60), 242 (+3.08), 284 (–7.62), 348 (+2.70); CD (EPA, –180°): 217 (–20.07), 244 (+0.04), 265 (–7.22), 285 (–15.97), 344 (+0.56), 361 (+2.51). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.84 (3H, *s*, H-16'); 0.98 (3H, *s*, H-17); 1.15 (3H, *s*, H-16); 1.19 (3H, *s*, H-18); 1.20 (3H, *s*, H-17'); 1.25 (1H, dd, H_{ax}-2); 1.36 (3H, *s*, H-18'); 1.48 (1H, *dd*, J_{gem} = 14.5, $J_{4'ax,3'}$ = 3.3, H_{ax}-4'); 1.63 (1H, *ddd*, J_{gem} = 14.7, $J_{2eq,3}$ = 3.6, $J_{2eq,4}$ = 1.7, H_{eq}-2); 1.63 (1H, *dd*, J_{gem} = 14.2, $J_{4ax,3}$ = 8.8, H_{ax}-4); 1.71 (1H, *dd*, J_{gem} = 13.7, $J_{2'ax,3}$' = 4.7, H_{ax}-2'); 1.93 (3H, *s*, H-19); 1.96 (3H, *s*, H-19'); 1.98 (6H, *s*, H-20,20'); 2.00 (1H, *dd*, J_{gem} = 13.9, $J_{2'eq,3'}$ = 7.8, H_{eq}-2'); 2.39 (1H, *ddd*, J_{gem} = 14.3, $J_{4eq,3}$ = 5.0, $J_{4eq,2}$ = 1.6, H_{eq}-4); 2.96 (1H, *dd*, J_{gem} = 14.4, $J_{4'eq,3'}$ = 8.2, H_{eq}-C(4')); 3.90 (1H, *m*, H-3); 4.51 (1H, *m*, H-3'); 5.90 (1H, *d*, $J_{7,8}$ = 15.4, H-7); 6.20 (1H, *d*, $J_{10,11}$ = 11.3, H-10); 6.27 (1H, *m*, H-14); 6.29 (1H, *d*, $J_{8,7}$ = 15.5, H-8); 6.34 (1H, *m*, H-14'); 6.37 (1H, *d*, $J_{12,11}$ = 11.4, H-12); 6.44 (1H, d, $J_{7',8'}$ = 14.9, H-7'); 6.51 (1H, *d*, $J_{12',11'}$ = 14.5, H-12'); 6.57 (1H, *d*, $J_{10',11'}$ = 11.4, H-10'); 6.61 (1H, *dd*, $J_{11',10'}$ = 11.4, $J_{11',12'}$ = 14.6, H-11'); 6.63 (1H, *dd*, $J_{11,10}$ = 11.6, $J_{11,12}$ = 13.0, H-11); 6.64 (1H, *m*, H-15); 6.69 (1H, *m*, H-15'); 7.32 (1H, *d*, $J_{8',7'}$ = 15.0, H-C8').

^{13}C-NMR data see in Deli et al. (1998c).

EI-MS: 600 $[M]^+$ (5), 582 $[M-H_2O]^+$ (3), 520 (1), 508 (1), 492 (3), 221 (37), 181 (18), 109 (96), 91 (100).

Results and discussion

The petals of lilium were first extracted with MeOH and then diethylether. After the saponification, the hypophasic pigments were precipitated from benzene/hexane. After column chromatography (see Materials and methods) and crystallization capsanthin (Fig. 1), capsorubin (Fig. 2), capsanthin 5,6-epoxide (Fig. 3) and a mixture of zeaxanthin and antheraxanthin were isolated. The structure elucidation of capsanthin, capsorubin, antheraxanthin and zeaxanthin was based on their UV/VIS-spectra and the chromatography with the authentic samples. The capsanthin 5,6-epoxide (Fig. 3) was characterized by its mass, CD and ^{1}H-NMR spectra.

In the UV/VIS spectra the maxima for **3** (507 and 481 nm in benzene) is in accordance with the data previously reported (Deli et al. 1998c). In addition, also the product from the furanoid rearrangement of **3** (486, 464 nm in benzene) exhibited the expected absorption maxima. The MS spectra of **3** exhibited the same molecular ion (m/e = 600) and fragments at m/e = 582 $[M\text{-}H_2O]^+$, 221, 181) indicating a 3-hydroxy-5,6-epoxy β-end group, and at m/e = 109 characteristic of a κ-end group.

The NMR data of **3** was completely in accordance with its presented constituent and configuration. ^{1}H-NMR experiments allowed complete ^{1}H-signal assignment and the δ(H) and J(H,H) values of the end groups were identical with the semisynthetic *anti*-capsanthin 5,6-epoxide (Deli et al. 1998c) and the corresponding data from the literature (Englert 1995). In the 3-hydroxy-5,6-epoxy β-end group, the δ(H) of CH_2(4) (1.63 and 2.39 ppm) and H-7 (5.90 ppm) differ significantly the *cis*- and *trans*-epoxide (rel. to OH) and therefore prove the *(3S,5R,6S)*-configuration of **3**. In the enantiomeric *(3S,5S,6R)*-epoxy end group these signals appear at 1.89, 2.20 and 5.84 ppm respectively (Deli et al. 1998c).

The structure elucidation of **3** was completed and confirmed by CD spectra. The capsanthin 5,6-epoxide (Fig. 3) exhibits strongly conservative CD spectra. The influence of additional κ-end group is rather small, therefore from the CD spectra of **3**, conclusion can be drawn about the absolute configuration of the epoxy group. The CD spectra of **3** confirmed the *(3S,5R,6S)* configuration (Buchecker and Noack 1995).

The present report represents the first isolation and structure elucidation of capsanthin 5,6-epoxide from *Lilium tigrinum*. Our result demonstrates that the biosynthesis of capsorubin is always the same in the plants. In the biosynthetic route for the formation of the capsorubin from violaxanthin, the capsanthin 5,6-epoxide occurs as intermediate.

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EFFECT OF SALINITY ON CARBOHYDRATE STATUS OF THREE *AMARANTHUS* SPECIES

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Three species of *Amaranthus* (*A. caudatus* L., *A. hypochondriacus* L. and *A. paniculatus* L.) were subjected to increasing levels of salinity (NaCl + $CaCl_2$) in field plots. The higher doses of salinity were found to lower the level of major photosynthetic product starch in different plant parts of all the three species at vegetative as well as flowering stage. The reducing sugar contents recorded increase due to lower salt doses in different parts of the three species. At vegetative stage salinity caused a decline in non-reducing sugars. Thus the carbohydrate metabolism is profoundly altered by salinity in all the three *Amaranthus* species.

Key words: *Amaranthus*, carbohydrate status, flowering stage, salinity, vegetative stage

Introduction

We have already reported influence of salinity on antinutritional factors oxalate and nitrate (Gaikwad and Chavan 1995). The present study covers the effect of salt stress on the important nutritional factor – carbohydrates – in three *Amaranthus* species (*A. caudatus*, *A. hypochondriacus* and *A. paniculatus*). Among these three species *A. hypochondriacus* and *A. paniculatus* yield food grains while *A. caudatus* is a leafy vegetable. Further all the three species are utilised as fodder.

Material and methods

The seeds of *A. paniculatus* and *A. caudatus* were obtained from local stock, while the seeds of *A. hypochondriacus* (AG-114) NBRI evolved were obtained through courtesy of NBRI, Lucknow. The plants were raised in the field plots and subjected to increasing salinity treatments (NaCl + $CaCl_2$) ranging from 4 to 16 ECe (mS cm^{-1}) as described by Gaikwad and Chavan (1995). The carbohydrates were estimated from oven dried root, stem,

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leaves and panicle of *Amaranthus* plants. The soluble sugars, extracted with neutral ethanol and the starch (insoluble residue), were hydrolysed with *cca.* HCl under pressure. Reducing sugars from the neutral hydroxylates and non-hydroxylated ethanol fraction were determined by reading the intensity of colour complex formed after reaction with alkaline copper tartarate and arsenomolybdate reagents, at 560 nm on a spectrophotometer (Nelson 1944). The values depicted in figures represent means of three determinations.

Results and discussion

Influence of NaCl salinity on the level of various carbohydrate fractions in different parts of three *Amaranthus* species at vegetative and flowering stages are recorded in Figs 1, 2, 3 and 4. It is evident from Figure 1 that at vegetative stage reducing sugars are increased at lower concentrations of salinity treatment in root tissue of *A. hypochondriacus* and *A. paniculatus*, and then decreased considerably with increasing salinity doses. In roots of *A. caudatus* reducing sugar content is increased at lower and medium doses

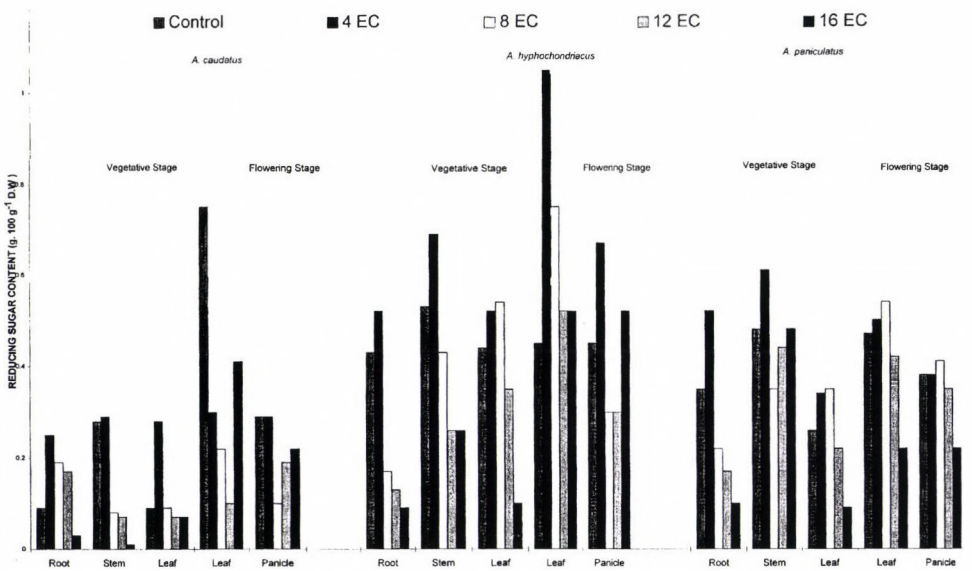

Fig. 1. Effect of salinity on reducing sugar content in different parts of *Amaranthus* species at vegetative and flowering stages

of salinity treatment and decreased considerably only at the highest salinity treatment employed. In stem tissue of all three *Amaranthus* species the content of reducing sugar is increased at lower doses of salinity and decreased at medium and higher dose of salinity treatments. But this pattern is not uniform in stem tissue of *A. paniculatus*. In leaf tissue reducing sugars increased at lower and medium level of salinity and decreased at higher salinity doses in *A. hypochondriacus* and *A. paniculatus* while it increased only at lower salinity treatment and decreased with increasing salinity treatments in *A. caudatus* leaves. Non-reducing sugar content (Fig. 2) at vegetative stage in root tissue of *A. hypochondriacus*, *A. caudatus* and *A. paniculatus* is declined with increasing salinity treatments. While in stem tissue it is increased only at medium salinity doses. Non-reducing sugars in leaf tissue of *A. hypochondriacus* and *A. paniculatus* increased at lower salinity treatment and showed almost inhibitory with increasing salinity treatments. Whereas, it showed decrease and almost inhibitory with increasing salinity treatments in leaf tissue of *A. caudatus*. It is evident from Figure 3 that total sugar content at vegetative stage in root tissue of the three *Amaranthus* species is decreased considerably with increasing salinity treatments. In stem tissue of *A. hypochondriacus* and *A. caudatus* also it is decreased with

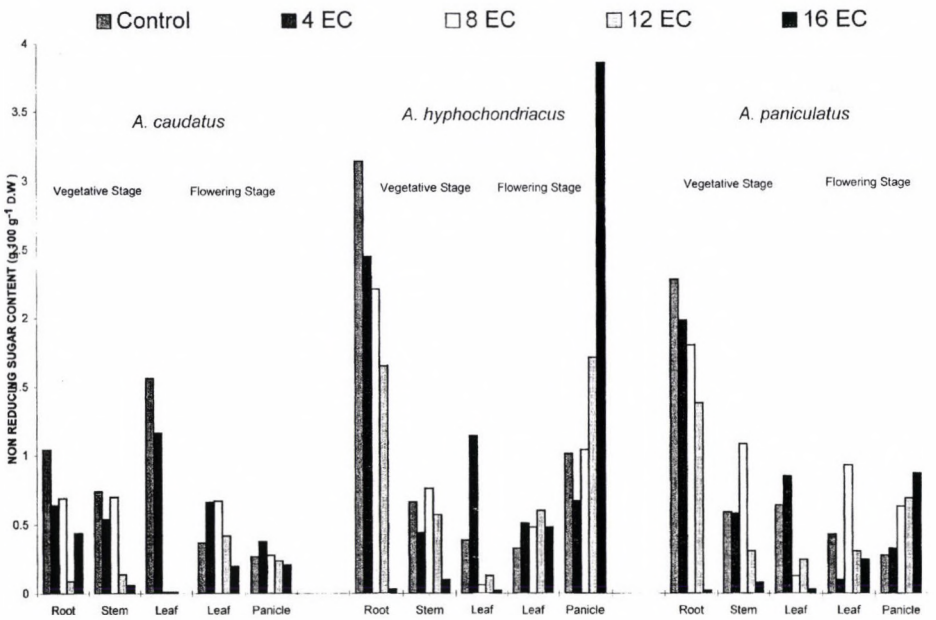

Fig. 2. Effect of salinity on non-reducing sugar content in different parts of *Amaranthus* species at vegetative and flowering stages

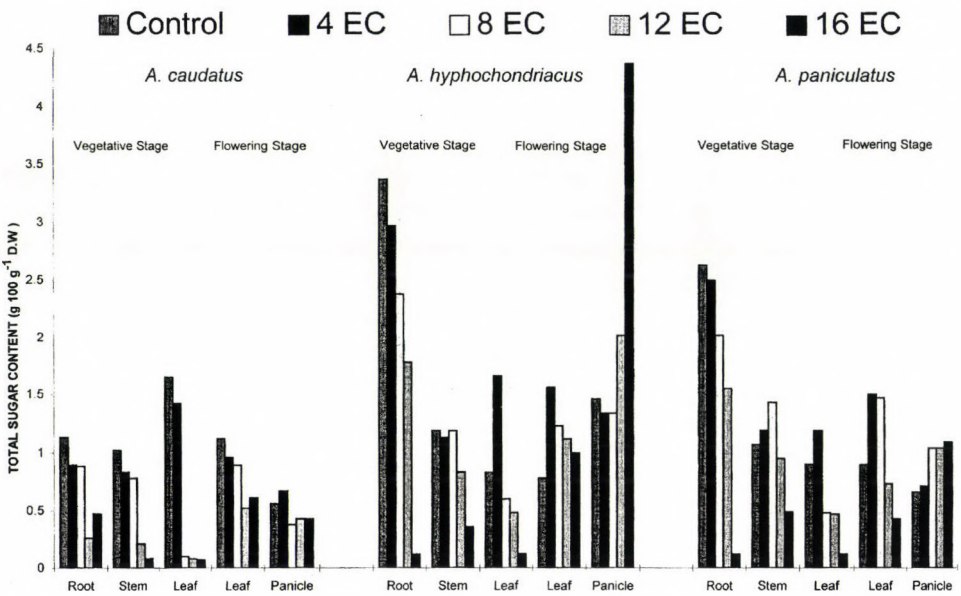

Fig. 3. Effect of salinity on total sugar content in different parts of *Amaranthus* species at vegetative and flowering stages

increasing salinity treatments and in stem tissue of *A. paniculatus* the content increased at medium salinity dose and decreased at higher salinity treatment. In leaf tissue of *A. hypochondriacus* and *A. paniculatus* total sugar content increased at lower level of salinity treatment and decreased at higher doses of treatment and showed almost inhibitory with increasing salinity treatments in leaf tissue of *A. caudatus*. At vegetative stage starch content (Fig. 4) in root and stem tissue of the three *Amaranthus* species is not greatly affected by salinity doses and is slightly decreased due to higher salinity levels. Medium doses of salinity have favoured accumulation of starch in leaf tissue of three *Amaranthus* species. A slight decrease in starch level at higher salinity treatments is evident in *A. caudatus* and *A. paniculatus*. These observations clearly indicate that pattern of carbohydrate accumulation is disturbed to various degrees in different parts of *Amaranthus* due to salt stress.

In plants subjected to salt stress there are several related processes (growth, photosynthesis, respiration and transpiration) which may be the first to affected and changes in any one of these will have far reaching effects on other metabolic activities in general and on the levels of various carbohydrate fractions in particular. Sugar nucleotides play a key role in

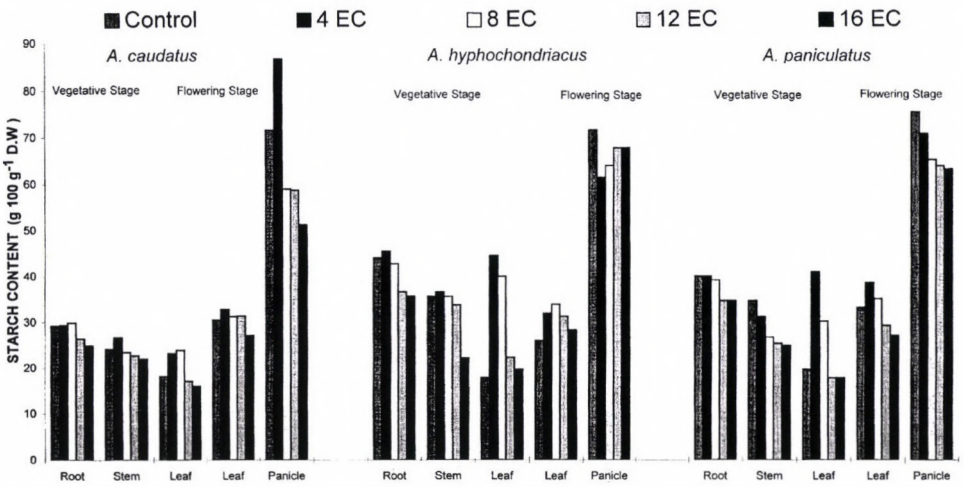

Fig. 4. Effect of salinity on starch content in different parts of *Amaranthus* species at vegetative and flowering stages

the carbohydrate metabolism. Nieman et al. (1988) noticed that in salt tolerant safflower the UDPG pool was not affected while in salt sensitive pepper there was large reduction in this pool. According to Chesseman (1988) allocation or diversion of carbon to storage is an important device to the understanding of salinity responses. Starch and sucrose are the predominant carbohydrates affected by salinity, while soluble sugars are more sensitive to salt stress than starch (Rathert 1983). Matar et al. (1975) noticed that in salt sensitive lettuce sucrose content decreased in the leaves and root considerably due to salinity. On the contrary, in salt tolerant spinach such decrease was there only in roots, while in leaves sucrose content increased along with salinity. Gorham et al. (1981) made an extensive study and found the accumulation of fructose and glucose in old leaves of *Aster tripolium, Daucus carota* and *Honkenya peploides* due to salt stress. Doering and Luedders (1986) noticed in pomegranate that in all salt variations the amount of reducing sugars in the leaves was increased whereas the roots showed a reduced concentrations of the fraction. They concluded that enrichment of reducing sugars in the leaves may be caused either by a salt initiated stimulation of discharging enzymes or by an inhibition of supporting enzymes. Starch is the main form in which the photosynthetically assimilated energy rich carbon is stored in the plants. Although in majority of higher plants the principal end products of leaf photosynthesis are starch and sucrose. Species differ in the partitioning of photosynthetically fixed

carbon between starch and sucrose. Thus, Huber (1989) noticed that the leaves of broad bean, pea and spinach accumulated more sucrose than starch. On the other hand in the leaf tissue of soybean, cucumber, Swiss chard, sunflower, sugarbeet and maize considerably higher starch content was noticed. He has attributed this difference to the differential behaviour of a sucrose metabolizing enzyme invertase. Although the exact level of sucrose is not determined in the present investigation, it can be assumed that the principal non-reducing sugar is sucrose. Hence, it is evident from the analysis of carbohydrate fraction that in all the three *Amaranthus* species the level of starch is considerably more than that of sucrose in different plant parts. According to Matar et al. (1975) even a low sodium can cause profound changes in carbohydrate metabolism of the plants. Our observations indicate similar situation prevailing in salt stressed *Amaranthus* species.

In order to have further insight about the fate of photoassimilates at reproductive stage analysis of panicle and leaf tissue at flowering stage was made. Effect of salinity on the level of different carbohydrate fractions in leaves and panicle of *Amaranthus* species is recorded in Figures 1–4. It is evident from the figures that *A. paniculatus* and *A. hypochondriacus* show more or less similar trend, while *A. caudatus* shows somewhat different trend. Thus, in panicle of *A. hypochondriacus* and *A. paniculatus* the starch level is decreased and it is accompanied by increase in level of non-reducing sugars. In leaves of *A. hypochondriacus* different carbohydrate fractions record an increase up to higher levels of salt treatment (ECe 16 mS cm^{-1}). While in *A. paniculatus* such increase is noticeable at medium doses of salinity (up to ECe 8). In case of *A. caudatus* panicles all the three carbohydrate fractions record a decrease in salinized plants (except at the lower dose, i.e. ECe 4). Adverse effects of salinity on translocation of photosynthate to the developing grains in crops like bean have been evident in some experiments (Yang et al. 1990). This is one of the probable reasons for decline in level of starch in the reproductive structures like panicle. It is evident from our observations that such effects are more pronounced in *A. hypochondriacus*, and *A. paniculatus* rather than *A. caudatus*. A decrease in starch in panicle of the salt stressed plants would undoubtedly affect the quality of grains in all the three species.

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A REVIEW OF THE MENYANTHACEAE DUMORTIER IN INDIA

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The present work, new of its kind, records one species of *Menyanthes* (*M. trifoliata*) and nine species of *Nymphoides* (*N. aurantiaca, N. hydrophylla, N. indica, N. krishnakesara, N. macrosperma, N. minor, N. parviflora, N. peltata* and *N. sivarajani*) from India, while *M. trifoliata* is distributed in Himachal Pradesh and Kashmir within an altitude ranging from 1800–2000 m. Among *Nymphoides* only *N. minor* is Himalayan, both being quite rare. Key to the identification, brief description, flowering and fruiting periods, distribution illustrations and uses of the concerned taxa are given.

Key words: distribution, flowering, fruiting, India, *Menyanthes*, taxonomy

Introduction

The family Menyanthaceae, named after the monotypic genus *Menyanthes* (the only species being *M. trifoliata* L.), is represented in the world by 5 genera and 33 species. The family is cosmopolitan being distributed in the tropical, subtropical, temperate and alpine regions of the world. The rich biological heritage of India has afforded accommodation to this taxon. Bentham and Hooker considered this taxon a part of the family Gentianaceae. Hutchinson, Takhtajan, Dahlgren and Thorme have treated it as a member of the order Gentianales. However, Cronquist (1981) gave it the status of an independent family within Solanales. The Magnoliopsid family draws ones attention by a unique set of attributes like aquatic or marshy habit, absence of intraxyllary phloem, alternate leaves, valvate corolla with fimbriate or crested lobes, hypogynous nectariferous glands, evolvular capsule with irregular dehiscence, cellular endosperm in the seeds. The sap of leaves and rhizome contains a bitter principle of alkaloids in varying quantities with an enrichment by sugars (Pollard and Amuti 1981). Endeavours of Nair and Ornduff (1966), and Nilsson and Ordnuff (1973) have added much to the knowledge concerning this family in form of morphology and palynology, respectively.

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They are also used as tonic, source of vitamins and as remedy for various diseases.

Since taxonomic review on this family in India has remained unfulfilled the present work was undertaken.

Material and methods

This work has emanated from synthesis of imperical and interpretative approaches involving through scrutiny and examination of literature and preserved specimens in the Central National Herbarium (CAL), Herbarium, B. S. I., Southern Circle (MH), Herbarium, Divisions of Genetics and Tree breeding (FRC), Herbarium, B. S. I., Northern Circle (BSD), Herbarium, Forest Research Institute (DD), National Botanical Research Institute, Lucknow (NBRI) and those collected during field survey. Specimens were worked out for preparing their description and referred to pertinent literature for correct identification. A key to the concerned taxa, enumeration of species in alphabetic order under each genus, author citations, basionym if existent, etymology, vernacular name, flowering and fruiting period and specimen examined are furnished as components of the taxonomic discourse. Moreover distribution and uses are also scored on the basis of information availed from literature and collectors experience penned on herbarium slips. Glimpses of the distribution of this taxon is presented in a map of India (Fig. 1).

Taxonomic discourse

Key to the genera

Leaves floating, simple, orbicular-ovate *Nymphoides*
Leaves aerial, trifoliate compound, flowers in a raceme, corolla lobes densely long bearded on the inner surface, ecarinate *Menyanthes*

Menyanthes L.

Menyanthes trifoliata L., Sp. Pl. 145, 1753

Rhizomatous perennial herbs, 13–60 cm high. Rhizome root-stock like, long inclined, creeping or floating, articulate. Leaves palmately compound, trifoliate; leaflets elliptic or obovate-ovate, 2.5–13 cm × 1.2–3.9 cm,

Fig. 1. Map of India showing distribution of Menyanthaceae. *Menyanthes trifoliata* (⊕), *Nymphoides aurantiaca* (O), *N. hydrophylla* (△), *N. indica* (▲), *N. krishnakesara* (●), *N. macrosperma* (+), *N. minor* (□), *N. parvifolia* (*), *N. peltata* (~), *N. sivarajani* (÷)

entire, obtuse at apex and base, petiolate. Scape leafless and fleshy. Flowers in raceme, pentamerous, white, pedicellate, bracteate; corolla lobes fringed internally. Capsule subspherical-ovoid, 7–8 mm long; seeds numerous, brownish, lenticular.

Nativity: Europe.

Etymology: The name *Menyanthes* is derived from "Men" = month, moon and "anthos" = flowers indicating blooming in presence of moon. The specific epithet is based on its trifoliate leaves.

Common name: Bogbean.

Flowering and fruiting: April–August.

Distribution: Himachal Pradesh, Kashmir (alt. 1800–2000 m).

Uses: Decoction of the root, leaves, rhizomes is used as tonic, febrifuge and purgative. It is also used in treatment of rheumatism, gout, dropsy, skin affections, scurvy and worms and as a substitute for tea and hop in beer industry (Kirtikar and Basu 1918, Anon 1962).

Specimens examined: Jammu and Kashmir: Shopiyan, 1800 m, 9 June 1959; T. A. Rao, 9372 (CAL). Himachal Pradesh: Khajiar lake, 6500 ft. (±1950 m), 2 November 1969, G. Singh and C. Sharma, H. P. 279 (DD). (Fig. 2).

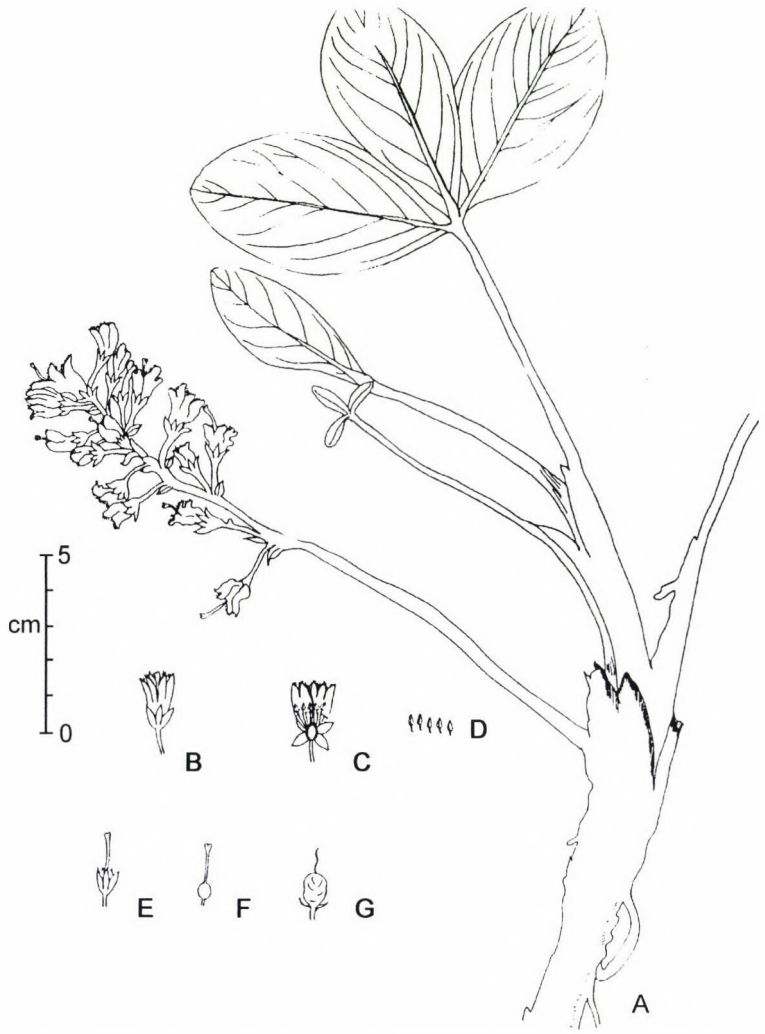

Fig. 2. Menyanthes trifoliata L. – A = habit, B = flower, C = floral parts, D = stamen, E = calyx with style, F = pistil, G = fruit

Nymphoides Sequier
Key to the species

1 Plant dioecious 2
1 Plant monoecious 3

2 Anthers blue, ovary 3 mm long, bilocular, capsule 1 cm long *N. krishnakesara*
2 Anthers pale purplish or pale yellowish, ovary relatively smaller, 1–1.8 mm long, unilocular, capsule 0.6 cm long *N. macrosperma*

3 Both radical and floating leaves present 4
3 Only floating leaves present 5

4 Corolla throat with yellow dense clusters of long, white hairs, corolla lobes with densely fimbriate wings along the margins, floating leaves 2–4.5 × 1.5–4.0 cm *N. sivarajani*
4 Corolla throat glabrous, corolla lobes without densely fimbriated wings along the margins, floating leaves smaller, 0.8–2.3 × 0.6–1.8 cm *N. parvifolia*

5 Corolla yellow 6
5 Corolla white 7

6 Capsule 0.5 cm long; seeds globose, densely puberulous, not winged *N. aurantiaca*
6 Capsule relatively larger, 1.5–2.6 cm long; seeds broadly ellipsoid, margin ciliated, fringed, winged *N. peltata*

7 Corolla lobes with a median longitudinal crest on inner surface, petiole slender, seeds 5–10 in a capsule, tuberculate *N. hydrophylla*
7 Corolla lobes fimbriated, densely papillose on inner surface, petiole thick, seeds 25–more in a capsule, smooth *N. indica*

Nymphoides aurantiaca (Dalz.) Kuntze, Rev. Gen. Pl. 2: 429, 1891

(Syn.: *Limnanthemum aurantiacum* Dalz., in Hook. Kew Journ. 2: 136, 1850)

Rhizomatous, glabrescent perennial, 50.5–90 cm high. Root-stock long, often purplish red. Leaves alternate, orbicular-ovate, 1–5 cm × 1.5–4.5 cm,

fleshy, thick, glabrous, slightly crenate, rounded at apex, indistinctly palmately nerved on the upper side, pale green above, purplish green beneath and dotted with dark purplish gland, deeply cordate at base with a narrow triangular sinus; petiole 2.5–15 cm long, purplish red. Flowers pentamerous, deep yellow-orange, 0.8–1.5 cm long, presence of hypogynous nectariferous gland. Capsule subglobose, apiculate, 5.5–6.5 mm × 3.5–4.5 mm. Seeds globose-lenticular, densely puberulous, not winged, 2 mm in diam.

Nativity: Bombay in India.

Etymology: Name is based on "aurantiacus" (means orange colour) corolla lobe.

Flowering and fruiting: May–December.

Distribution: Maharastra, Rajasthan, South India.

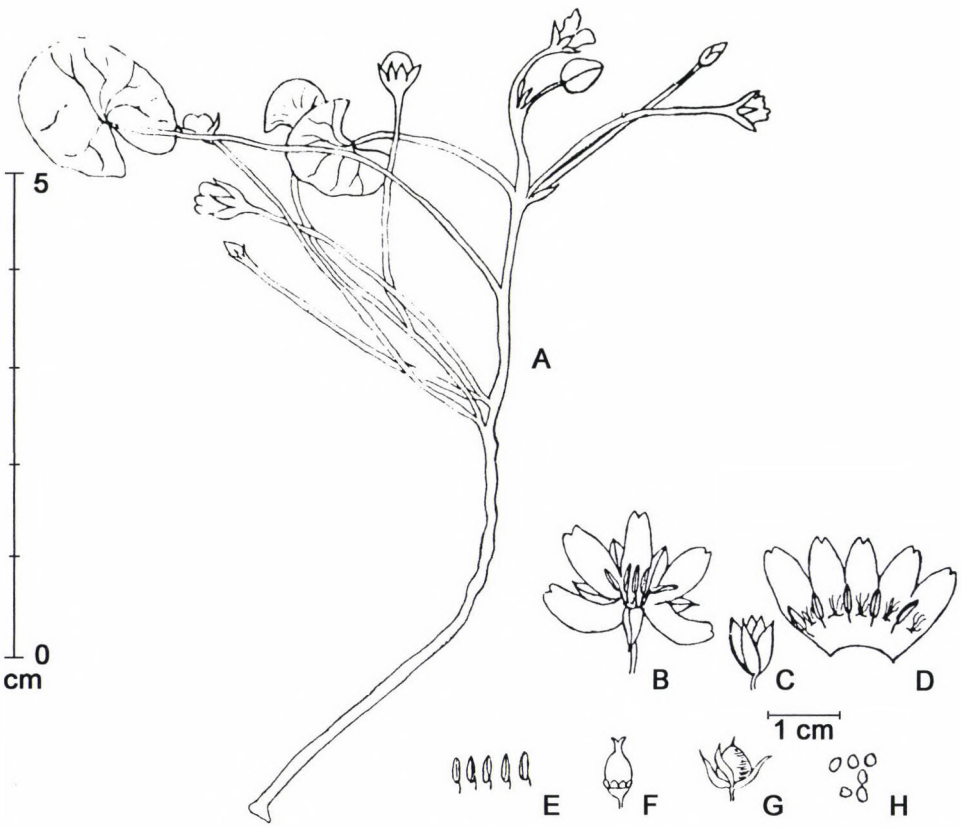

Fig. 3. Nymphoides aurantiaca (Dalz.) Kuntze. – A = habit, B = flower, C = calyx, D = corolla split open, E = stamen, F = pistil, G = fruit, H = seed

Specimens examined: Ceylon: s. loc. Thwaites, 1869 (CAL). Kerala: Travancore, M. A. Lawson, Acc. no. 336684 (MH). Kerala: Trichur, Kodungallur to Iriijalakuda through Chavakad, 300 m, 25 September 1982, K. Ramamurthy, 74859 (MH). Tamilnadu: Malabar, Kannoth, 28 October 1945, J. Gopal Rao, 88259 (MH). Rajasthan: Udaipur, October 1963, K. D. Ramdeo, 33440 (BSD). (Fig. 3).

Nymphoides hydrophylla (Lour.) Kuntze, Rev. Gen. Pl. 2: 429, 1891

(Syn.: *Menyanthes hydrophylla* Lour., Fl. Cochinah 1: 129, 1790)

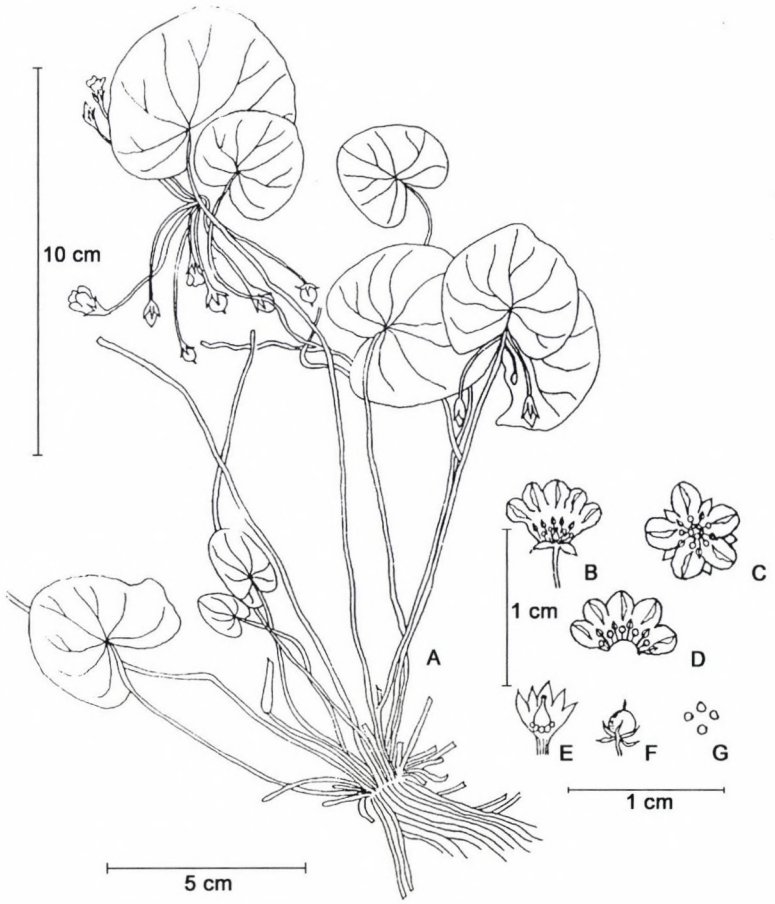

Fig. 4. Nymphoides hydrophylla (Lour.) Kuntze. – A = habit, B = flower, C = floral parts, D = corolla split open, E = calyx with pistil and nectariferous glands, F = fruit, G = seed

Rhizomatous, perennial, glabrescent herbs, 12–55 cm high. Root-stock short, erect or oblique, bearing scale leaves from axils of which long petiole arise and come above the water surface. Floating leaves petiolate; petiole long, varies in length, 5.5–50 cm long, green. Flowers pentamerous, white, yellow inside the throat of the corolla tube, 0.8–1.2 cm, pedicellate, bracteolate; corolla lobes crested along middle on upper surface, presence of hypogynous nectariferous glands. Capsule broadly ovoid-oblong, 5–7.5 mm × 3.5–5 mm. Seeds 5–10, spherical, occasionally lenticular, tuberculate, yellowish brown.

Nativity: Cochin China.

Etymology: The epithet "hydrophylla" or "water leaves", i.e. derived from its foliage in water.

Vernacular name: Beng.: Chuli; Mah.: Kumudini; Sans.: Kumudati; Teling: Antara-tamara; U. P.: Chuli.

Flowering and fruiting: April–December.

Distribution: Andhra Pradesh, Assam, Bihar, Karnataka, Kerala, Madhya Pradesh, Maharastra, Manipur, Orissa, Rajasthan, Sikkim, Tamilnadu, Tripura, U. P., West Bengal or throughout India.

Uses: The plant is commonly used as chiretta. Its leaf extract is used as tonic and in curing stomach disorders. Stem, fruit and leaves are eaten in certain localities through curried or boiled process. This is also used in the preparation of "Darbhyadi" for fever and jaundice (N. N. Sen 1914).

Specimens examined: West Bengal: Midnapur, 12 June 1975, S. Maji, 1616 (CAL). Rajasthan: Asnawar, 19 September 1964, B. M. Wadhwa, 7534 (CAL). Tamilnadu: Madras, 10 August 1957, N. P. Balakrishnan, 299 (MK). Kerala: Cannanore dist., Kuthuparam, 100 m, 20 February 1978, V. S. Ramachandran, 54093 (MH). Uttar Pradesh: Dudhwa National Park, 28 October 1979, Y. Shukla, 69957 (BSD). (Fig. 4).

Nymphoides indica (L.) Kuntze, Rev. Gen. Pl. 2: 429, 1891

(Syn.: *Menyanthes indica* L., Sp. Pl. 145, 1753)

Rhizomatous, perennial, glabrescent herbs, 18–160 cm high. Root-stock or rhizome horizontal or oblique, thick, bearing numerous scale leaves. Floating leaves subsessile-petiolate, ovate-orbicular to orbicular up to 35 cm in diameter, fleshy and thick, coarsely crenate, rounded to obtuse, deeply cordate at base. Flower 5–7-merous, white with yellow centre, 1–2.5 cm long, pedicellate, bracteate; corolla lobes fimbriated, inner surface

densely papillose, with hypogynous nectariferous glands. Capsule suboblong-subglobose, ca 8 mm × 7 mm; seeds numerous, 25–more in a capsule, globose-obovate, sometimes lenticular, smooth, yellowish brown.

Nativity: Sri Lanka.

Etymology: The epithet "indica" is based on "India" – its native place.

Vernacular name: Mah.: Kumud; Mal.: Chinnambal; U. P.: Barachuli.

Flowering and fruiting: April–January.

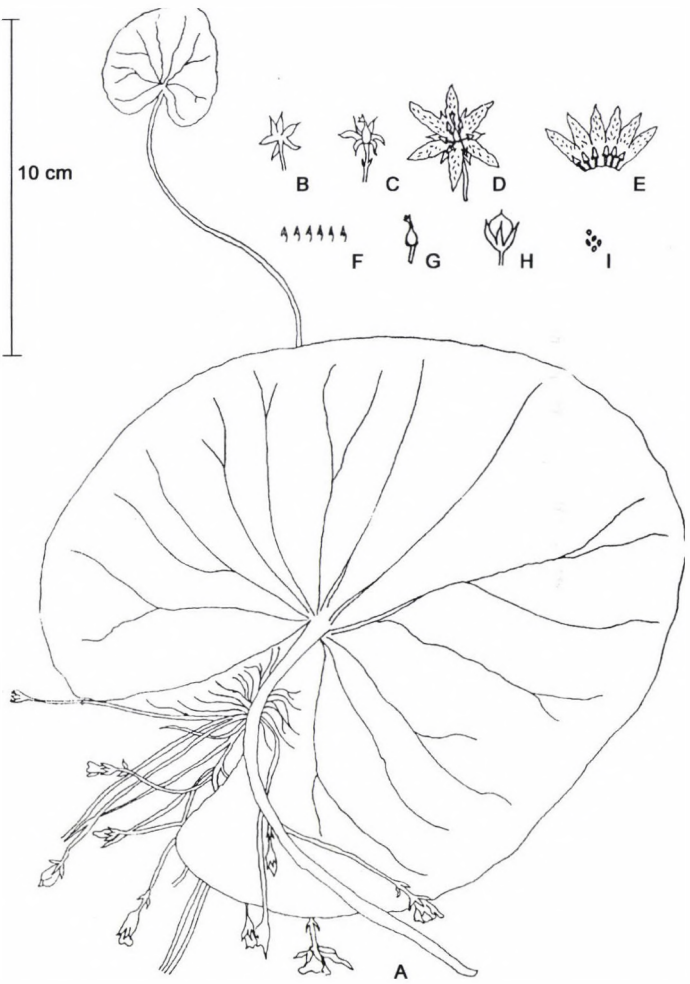

Fig. 5. Nymphoides indica (L.) Kuntze. – A = habit, B = calyx, C = calyx with pistil, D = flower, E = corolla split open, F = stamen, G = pistil, H = fruit, I = seed

Distribution: Assam, Bihar, Karnataka, Kerala, Madhya Pradesh, Maharastra, Rajasthan, Tamilnadu, West Bengal or throughout India.

Uses: Plant decoction is used in fever and jaundice and also as a substitute for chiretta.

Specimens examined: West Bengal: Hooghly dist., Tarakeshwar near Shiva temple, 28 December 1964, M. K. Ghosh, 2674 (CAL). Assam: Monier Khal on the Sonai river, August 1903, A. T. Gage, s. n. (CAL). Manipur: Manipur tanks, 600–900 m, 21 March 1987, George Watt, 6239 (MH). Maharastra: Munara lake, 267 m, 21 November 1957, s. leg., 4732 (MH). Rajasthan: Kota, 2 February 1969, V. Singh, 90892. (Fig. 5).

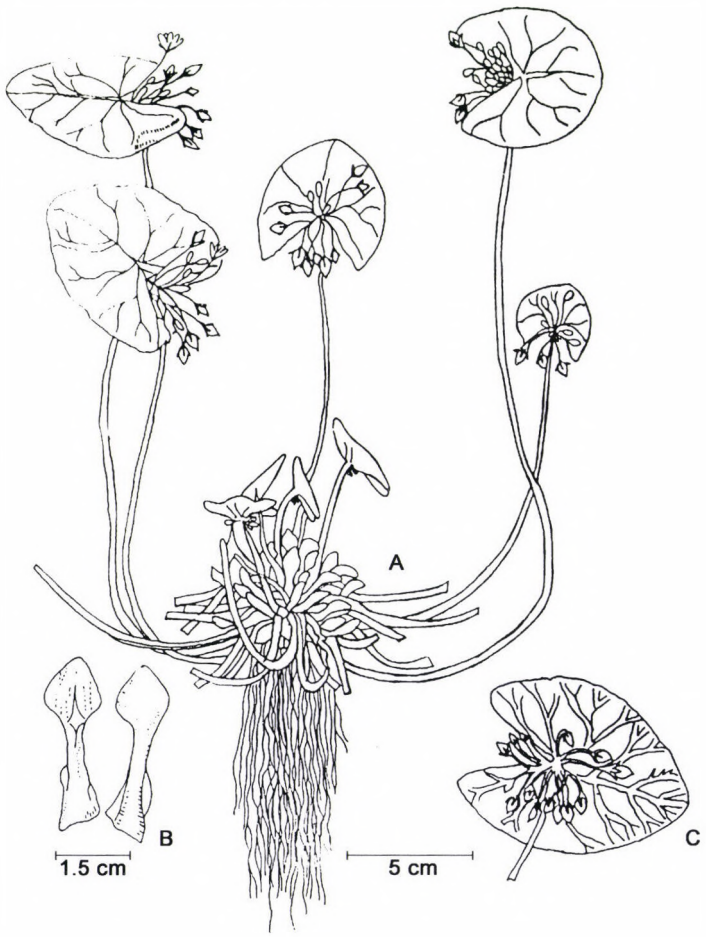

Fig. 6a. Nymphoides krishnakesara Joseph et Sivarajan. – A = habit, B = sterile basal leaf showing winged petiole, C = a single fertile leaf showing floral cluster

Nymphoides krishnakesara K. T. Joseph et V. V. Sivarajan
Nordic J. Bot. 10(3): 281, 1990

Rhizomatous, dioecious, perennial herbs. Rhizome stout, vertical up to about 5 cm in length. Leaves dimorphic, basal submerged, numerous in rosette, spathulate; petiole winged at base; floating leaves subsessile, ovate to orbicular, 9 cm × 6 cm, entire, glabrous, rounded at apex, deeply cordate at base, fertile branches axillary to basal leaves, many, up to 40 cm long.

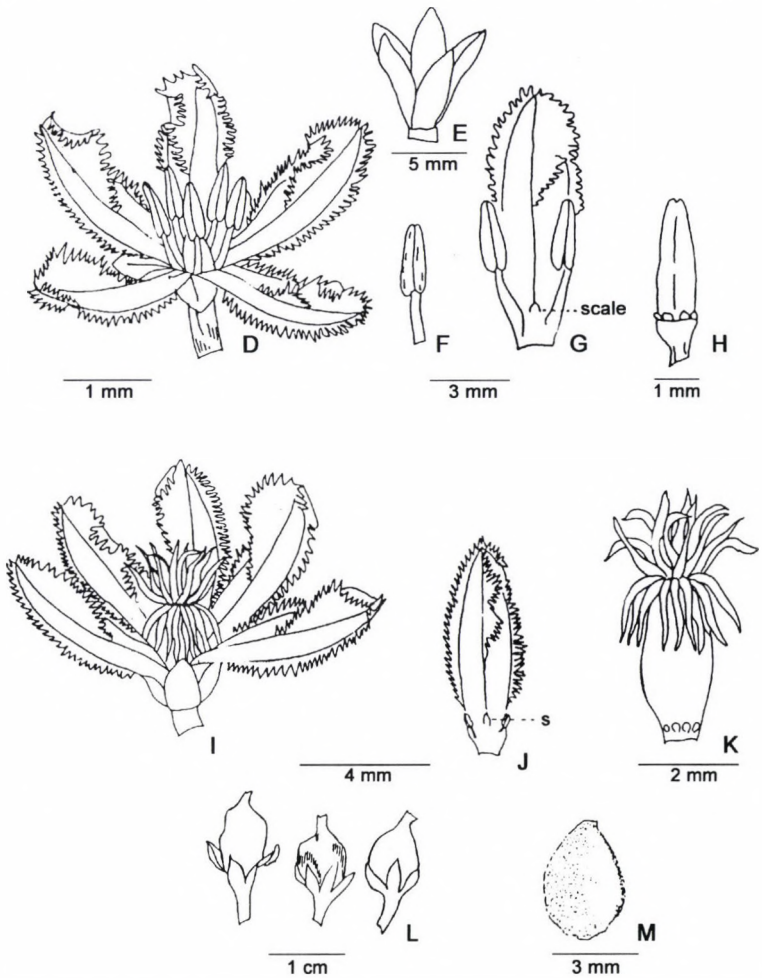

Fig. 6b. Nymphoides krishnakesara Joseph et Sivarajan. (contd.) – D = male flower, E = calyx, F = a single stamen, G = a single petal showing scale and stamens on either side, H = pistillode showing hypogynous disc glands, I = female flower, J = a single petal showing scale and staminode, K = pistil showing the stigmatic hair, L = fruits, M = seed

Flowers unisexual, pentamerous, white; corolla lobes shallowly fimbriated and with median wings; anthers blue, with hypogynous disc glands. Capsule suboblong-subovoid, anomocidal, 1 cm long. Seeds 5–10, discoid, muricate, 3 mm across.

Nativity: Kerala (India).

Etymology: The specific epithet "krishnakesara" is a Sanskrit word and it refers to the distinctive blue anthers.

Flowering and fruiting: during rainy season.

Distribution: Cannanore district of Kerala.

Specimens examined: Kerala: Cannanore dist., Madai, October 1988, Joseph, 43001 (MH holotype, Z. CALI isotypes) – mentioned from protologue. (Fig. 6).

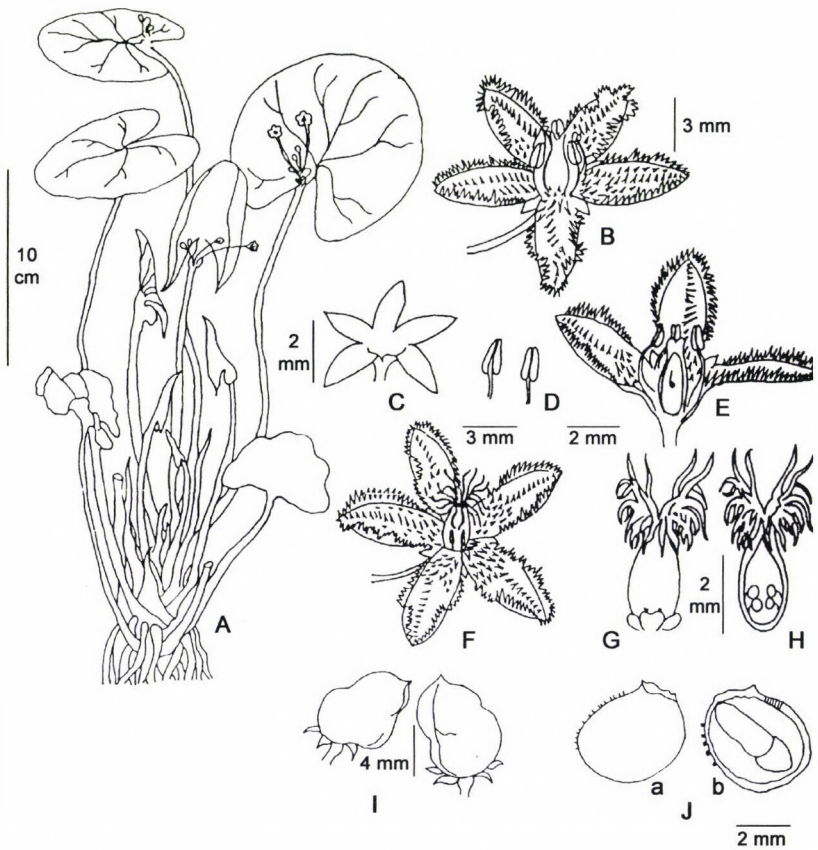

Fig. 7. Nymphoides macrosperma Vasudevan. – A = habit, B = male flower, C = calyx, D = stamen, E = male flower, F = female flower, G & H = gynoecium, I = fruits, J = seed (a) side view, (b) v.s.

Nymphoides macrosperma Vasudevan, Kew Bull. 22: 101–106, 1968

Rhizomatous, dioecious, perennial herbs. Rhizome vertical, monopodial, pale orange-brown. Leaves simple, spiral, semi-circular, apex rounded, subcrenulate wavy margin, pale purplish, lower surface purplish green, with minute brown dots, base cordate. Flower unisexual, bracteate, pedicellate, pure white, 1–1.5 cm in diam.; corolla lobes fimbriated with shorter hairs at the middle of corolla lobes; anther pale purplish, orange coloured minute hypogynous nectariferous glands present. Capsule subglobose, apiculate, yellowish-greenish purple, 6 mm × 8 mm. Seeds very few, 2–6, large, obovate-lenticular, 3.5–4.5 mm in diam., brownish.

Nativity: Kerala (India).

Etymology: Since "macro" stands for large and "spermum" for seed, the specific epithet is certainly based on the large seeds.

Flowering and fruiting: August–January.

Distribution: Kerala, South India.

Specimens examined: Kerala: Badagara, in paddy fields at about sea level, abundant, in association with species of *Nymphaea* and *Nymphoides*, December 1964, R. Vasudevan. Kerala: Alwaye, in paddy fields at sea level, abundant, in association with species of *Nymphaea*, *Nymphoides* and *Alisma*, September 1965, R. Vasudevan (holotype, K) – mentioned from protologue. (Fig. 7).

Nymphoides minor (D. Don ex G. Don) comb. nov.

(Basionym: *Villarsia minor* D. Don ex G. Don, Gen. Hist. Dich. Pl. 4: 169, 1837)

Rhizomatous, perennial, glabrescent herbs, about one fourth in every part of *Nymphoides indica* (L.) Kuntze, floating leaves subsessile-petiolate, reniform-orbicular, rounded apex, divaricate at base, smooth below. Peduncle short, glabrous. Capsule globose, crustaceous.

Nativity: Himalaya.

Etymology: The epithet "minor" stands for "small" (plant body about quarter times smaller than *N. indica*) and speaks about its small strature.

Distribution: Himalaya.

Nomenclatural notes: The characteristic features are adequately strong to bring the species in judicious alignment with the genus *Nym-*

phoides and not *Villarsia* and this transfer remained undone by any other taxonomist. However, as per Index Kewensis (1203) its transfer to *Limnanthemum* is evident.

Nymphoides parvifolia (Griseb.) Kuntze, Rev. Gen. Pl. 2: 429, 1891

(Syn.: *Limnanthemum parvifolium* Griseb., in DC. Prodr. 9: 141, 1845)

Rhizomatous, perennial, glabrescent herbs. Rhizome small, erect with numerous fibrous roots. Leaves dimorphic; radical leaves petiolate, broadly spathulate-deltoid, obtuse, cuneate, slightly crenulate; floating leaves petiolate, broadly ovate-suborbicular, obtuse, cordate, slightly crenulate,

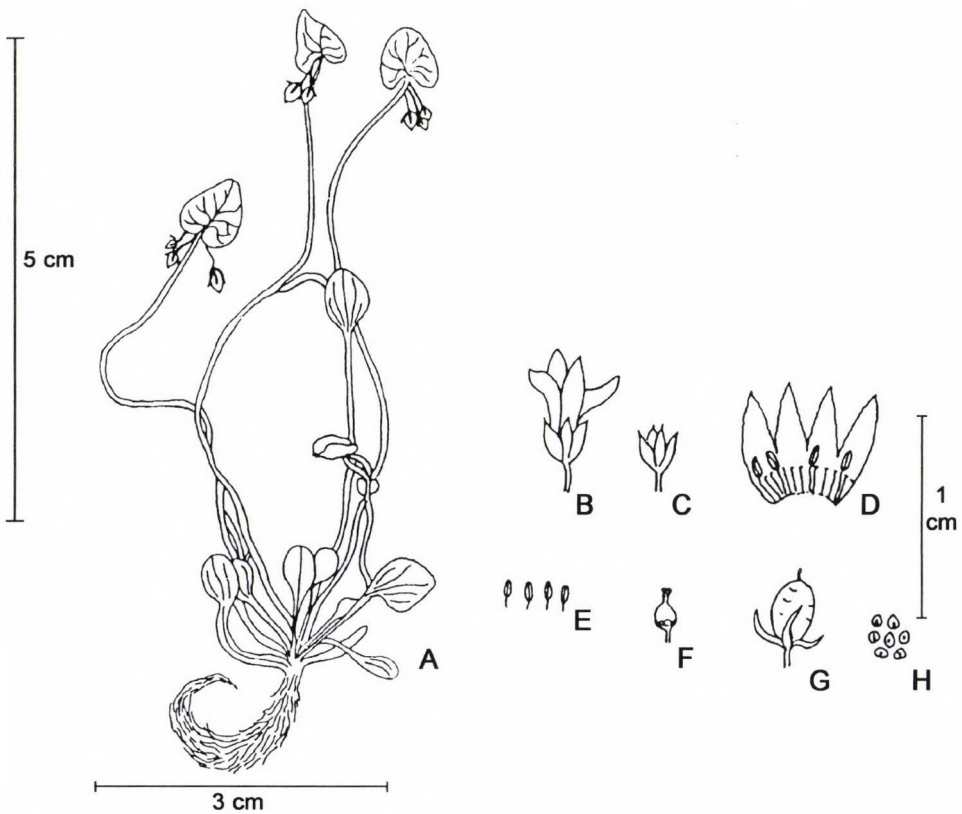

Fig. 8. Nymphoides parvifolia (Griseb.) Kuntze. – A = habit, B = flower, C = calyx, D = corolla split open, E = stamen, F = pistil, G = fruit, H = seed

upper surface glabrous, lower surface glandular and slightly pilose. Flower bisexual, pedicellate, pure white, 1.5–3 mm across; corolla throat glabrous. Capsule oblong, obovoid-turbinate, apiculate, yellowish brown, 1–3.5 mm long. Seeds 10–20, lenticular, pale brown, 1 mm in diam.

Nativity: Burma.

Etymology: The specific epithet owes its origin to small ("parvi") leaves ("folium").

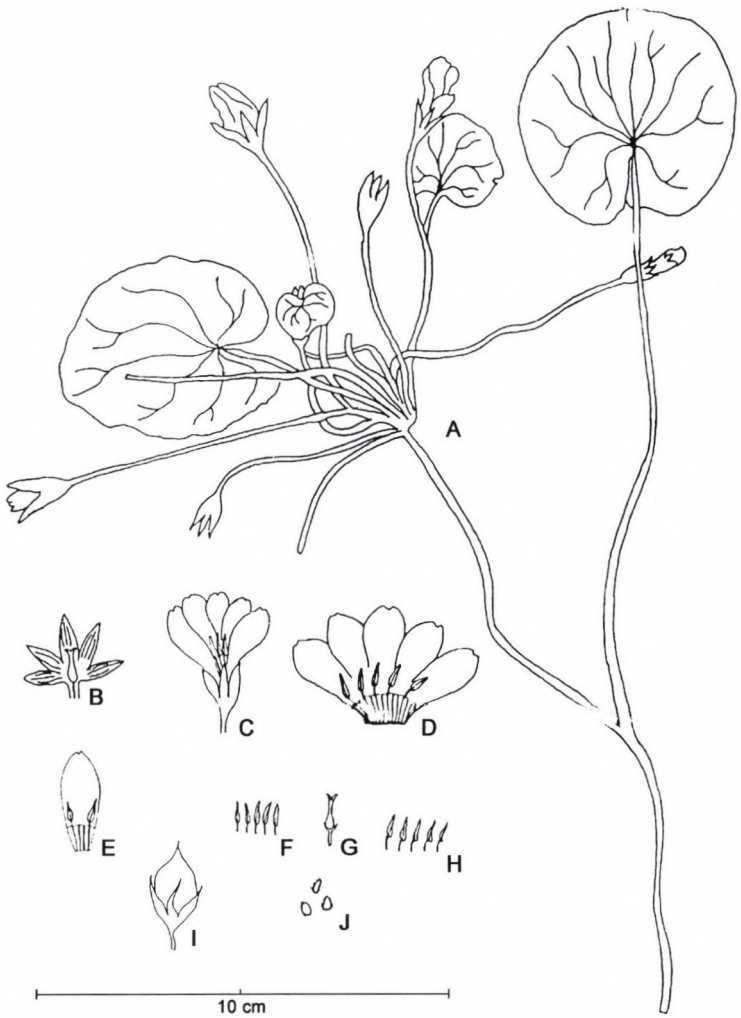

Fig. 9. Nymphoides peltata (Gmel.) Kuntze. – A = habit, B = calyx with pistil, C = flower, D = corolla split open, E = single petal showing stamen, F and H = stamen, G = pistil, I = fruit, J = seed

Flowering and fruiting: May–January.

Distribution: Kerala, Maharastra, Tamilnadu, Karnataka.

Specimens examined: Kerala: Quilon, Oachira, sea level, 4 August 1978, C. N. Mohanan, 58393 (MH). Kerala: Trichur way to Kodungalur, 25 m, 14 July 1976, K. Ramamurthy, 48522 (MH). Karnataka: Karwar (N. Kanara), 15 October 1887, W. A. Talbot, 1577 (DD). (Fig. 8).

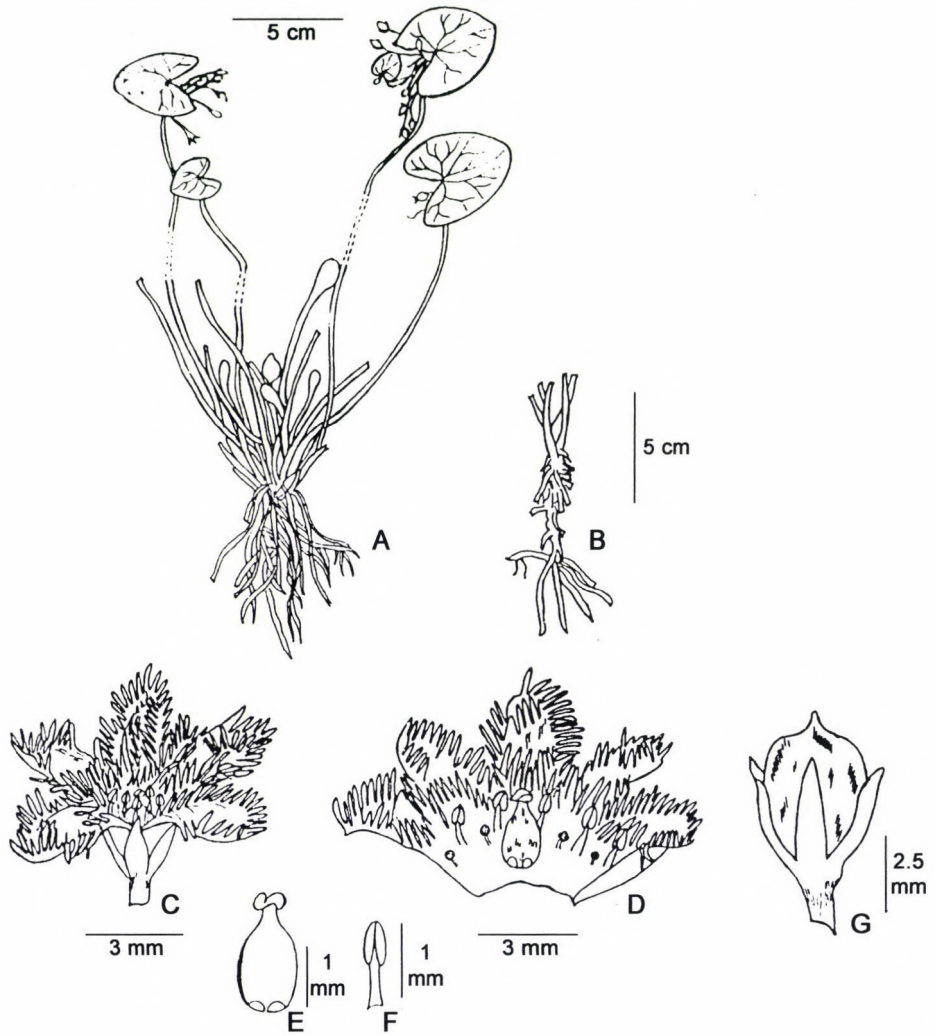

Fig. 10. Nymphoides sivarajani Joseph. – A = habit, B = rhizome with runners, C = flower, D = flower dissected, E = pistil, F = single stamen, G = fruit

***Nymphoides peltata* (Gmel.)** Kuntze, Rev. Gen. Pl. 2: 429, 1891

(Syn.: *Lymnanthemum peltatum* Gmel., Novi-Comm. Acad. Sci. Petrop. 14: 527, 1769–1770)

Rhizomatous, perennial, glabrescent herbs. Rhizome very long, up to 110 cm, creeping. Leaves subopposite, petiolate; floating leaves orbicular, 2–10 cm in diameter, margin somewhat repand, obtuse, cordate-subsagittate, glandular below. Flowers pentamerous, bisexual, 1.5–3 cm long, yellow, pedicellate; corolla tube provided with tufts of long thin hair. Capsule oblong-subovoid, 15–25 mm long, many seeded. Seeds flat, ellipsoid, ca 4 mm × 3 mm brownish.

Nativity: Europe.

Etymology: The specific epithet is based on "peltate" leaf.

Vernacular name: Punjab: Kuru, Khairposh.

Flowering and fruiting: May to January.

Distribution: Kashmir, Punjab, Western Himalaya.

Uses: Decoction of leaves is used in periodic headache (Kirtikar and Basu 1918).

Specimens examined: Jammu and Kashmir: Khambal, 1525 m, 14 September 1962, B. M. Wadhwa and J. N. Vohra, 952 (CAL). Jammu and Kashmir: Srinagar, Nagin Lake, 1525 m, 10 October 1961, B. M. Wadhwa and J. N. Vohra, 10 (CAL). Jammu and Kashmir: Shopiyan, 9 July 1876, C. B. Clarke, 28594 (CAL). Jammu and Kashmir: Wullar Lake, 1675 m, 16 June 1959, T. A. Rao, 9500 (BSD). Jammu and Kashmir: Srinagar, Dal Lake, 6500 ft. (±1950 m), 27 July 1956, J. Ananda Rao, 828 (BSD). (Fig. 9).

Nymphoides sivarajani Joseph, Willdenowia 20: 135–138, 1991

Rhizomatous, perennial herbs. Rhizome robust, about 6 cm long, slant with long runners. Leaves dimorphic; basal leaves submerged in rosette, sterile, spathulate or clavate, petiolate; petiole 8–15 mm long, terete; basal leaves ovate, obovate or rhomboid, rounded. Fertile leaves 2–4.5 cm × 1.5–4 cm, floating, ovate to orbicular, rounded, deeply cordate at base, with a triangular sinus, entire, often pinkish tinged; pedicels 2–5 mm long. Flowers bisexual, 5-merous; calyx 4–5 partite, glabrous, green or pinkish, lobes linear-oblong, 4 mm × 1 mm persistent. Corolla rotate, white, throat yellow with dense cluster of long white hairs, lobes oblong, 8 mm long

with densely fimbriate wings along the margin; presence of hypogynous disc of 4–5 minute glands; pistil battle shaped, capsule oblong or ovoid; seeds 15–20, rounded-lenticular, straw coloured.

Nativity: Kerala.

Etymology: The species is named after Dr V. V. Sivarajan.

Flowering and fruiting: during rainy season.

Distribution: (Malappuram dist.) Kerala.

Specimen examined: Kerala: Malappuram dist., Chettipadi, from flooded paddy fields, Joseph 40243 (holotype MH; isotype B, CALI) – mentioned from protologue. (Fig. 10).

Discussion

The family Menyanthaceae has a couple of genera in India with ten species out of which three occur in North India and five in South India (Fig. 1). The remaining two species, viz. *Nymphoides hydrophylla* and *N. indica* are common to both North and South India. Among the North Indian species *Menyanthes trifoliata* is distributed in Himachal Pradesh and Kashmir within an altitude of 1800–2000 m, *Nymphoides minor* in the Himalaya and *Nymphoides peltata* in Kashmir, Punjab and Western Himalaya. A close view shows *Nymphoides krishnakesara* and *Nymphoides sivarajani* to remain confined to Kerala, *Nymphoides parviflora* to Maharastra, Tamilnadu and Karnataka, and *N. aurantiaca* and *N. macrosperma* to South India. However, the present work records *N. aurantiaca* from Udaipur, Rajasthan for the first time. Because of the peculiar distributional trend to remain confined to or localised within small areas all the Indian species excepting *Nymphoides hydrophylla* and *N. indica* are quite uncommon in general and *Menyanthes trifoliata* and *Nymphoides minor* are very rare in particular.

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PHYSIOLOGICAL RESPONSES INDUCED BY SHOCK AND GRADUAL SALINIZATION IN RICE (*ORYZA SATIVA* L.) SEEDLINGS AND THE POSSIBLE ROLES PLAYED BY GLUTATHIONE TREATMENT

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Rice seedlings (*Oryza sativa* L. convar. Giza 176), 10 days old which were hydroponically grown on half-strength modified Hogland nutrient solution were exposed to different protocols using different concentrations of NaCl. After 15 days, salinity adversely affected the growth of rice seedlings and the highest inhibitory effects were recorded on using 300 mM NaCl. The inhibitory effect was less evident when the seedlings were exposed to gradual salinization than in case of sudden exposure to high salinity "salt shock". In salt shock treatment, the growth parameters were severely inhibited. Also leaf photosynthetic pigments, soluble carbohydrates in shoots and roots declined significantly by increasing the concentration of NaCl particularly at 300 mM in salt shock, while this concentration caused significant increases in soluble carbohydrates of roots of acclimated seedlings. These results are in accordance with arrested growth rate of rice seedlings. In addition, NaCl salinity in both protocols had a stimulatory effect on proline accumulation concurrently with a decrease in soluble protein contents in shoots and roots of rice seedlings. This was more obvious in salt shocked seedlings than in the acclimated ones. Moreover, both shock and gradual salinization protocols induced increases in Na ion contents in shoots and roots of rice seedlings with decreases in K, Mg, Ca, P, Zn, B, Fe and Mn mostly at high concentrations of NaCl (200 and 300 mM). Therefore, salt shocked or salt acclimated rice seedlings appeared to differ in their strategies in accumulating organic compounds and inorganic solutes which might act as osmoregulators increasing the ability of seedlings to cope with salinity stress.

Addition of reduced form of glutathione (GSH) ameliorated the adverse effects of shock salinization on the growth of rice seedlings mainly through decreasing the uptake of Na ions and increasing the photosynthetic pigments, carbohydrate, proline, soluble proteins and ion contents (K, Mg, Ca, P, Zn, B, Fe and Mn) which regulate and restore growth and metabolic activities of rice seedlings.

In conclusion, salt acclimation and GSH are able to improve the vital properties of salt stressed rice seedlings through osmotic adjustment and turgor maintenance. This adds a new and unique information to all efforts in understanding and explaining the physiology of stress tolerance in the rice plant.

Key words: glutathione (GSH), proline, protein, rice, salt acclimation, salt shock

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Introduction

Salinity is one of the major abiotic stresses limiting agricultural production in many areas of the world (Lutts et al. 1996, Rodriguez et al. 1997). Rice, which is adapted to flooded conditions, is very sensitive to salinity; concentrations of NaCl as low as 50 mM applied at the seedling stage caused a range of mortality according to variety (Yeo et al. 1990). It is known that NaCl accumulation in the leaves is correlated with reduced photosynthetic activity and with ultrastructural and metabolic damage (Yeo and Flowers 1986, 1989). The reduction in growth of many crop plants by salinity may result from salt effects on dry matter allocation, ion relations, water status, physiological processes, biochemical reactions or a combination of such factors (Greenway and Munns 1980, Epstein 1983, Shah et al. 1987).

Plants apparently rely on several mechanisms by which they can adapt to salinity stress. These include accumulation of organic molecules like soluble carbohydrates, soluble proteins, proline and possibly other compounds which may act as nontoxic cytoplasmic osmotica in various salt-tolerant plants (Gibson 1988, Shah et al. 1990). The trends and magnitude of the adverse effects of salt stress may vary according to the level and duration of salinization treatment as well as the plant type used (Dell'-Aquila and Spada 1993, Hajar et al. 1993).

Heenan et al. (1988) and Aslam et al. (1993) have already reported changes in salt resistance during rice development and showed that the young seedling and flowering stages are the most sensitive. Therefore, in the present investigation, the effect of salinity was carried out on the seedling stage of rice. Reduced glutathione (GSH) has an important protective role in salt tolerance of many plants (Smith et al. 1990). Accordingly, the present research was undertaken to (1) investigate the response of rice seedlings to either a sudden imposition of salt stress (salt shock) or to a gradual increase in salinity (salt acclimation), (2) to use glutathione (GSH) to counteract the detrimental effects of salinity shock on growth parameters, photosynthetic pigments, carbohydrates, proline, soluble protein, and (3) to search for some possible defense mechanisms of rice seedlings under the suggested different salinity protocols.

Materials and methods

This experiment was carried out during two successive seasons in 1996 and 1997. The experimental plant used in this investigation was rice (*Oryza sativa* L. convar. Giza 176). Pure strains of seeds were obtained from the Egyptian Ministry of Agriculture. Rice seeds were sterilized with 2.5% sodium hypochlorite for 15 minutes and washed thoroughly with distilled water. These seeds were then germinated in Petri dishes (20 cm) containing distilled water at 37 °C under dark condition. After 2 days incubation, uniformly germinated seeds were selected and transferred to plastic containers containing 200 ml of half strength modified Hogland nutrient solution (Epstein 1972). After 10 days from transplantation in the hydroponic solution, the plastic containers containing rice seedlings were divided into 4 groups. The seedlings of the 1st group received no NaCl to serve as control, the seedlings of the 2nd group were divided into 3 sets that received NaCl in one step (salt shock) to maintain the final concentrations at 100, 200 and 300 mM NaCl. The plants of the 3rd group received NaCl gradually by daily increments with 10, 20 or 30 mM from 100, 200 or 300 mM of NaCl. To the seedlings of the 4th group, reduced form of glutathione at 1.0 mM was applied to the shock NaCl concentrations (100, 200 or 300 mM). After 15 days exposure to the nutrient solution with different salinization protocols, the seedlings were harvested at random and divided into roots and shoots to measure the growth parameters (height of shoot, length of root, their fresh and dry weights per plant) and to analyse some metabolic activities such as photosynthetic pigments (Metzner et al. 1965), free proline (Bates et al. 1973), total soluble sugars in the ethanolic extracts and the acid hydrolysed polysaccharides in the dry residue by anthrone-sulphuric acid method (Whistler et al. 1962). For the extraction of mineral ions, the wet ashing procedure was applied following the method described by Chapman and Pratt (1978). Phosphorus, calcium, magnesium, iron, manganese, zinc and boron were determined simultaneously by ICP spectroscopy according to the method of Soltanapour (1985). Sodium and potassium were estimated by the flame emission technique adopted by Irri (1976). Soluble proteins were determined in fresh tissues of both shoots and roots of rice seedlings according to the method described by Bradford (1976).

The experiment described above was repeated at the same condition at least once and the mean values of the 2 experiments were recorded. Statistical analysis of growth parameters (10 samples) and the metabolic activ-

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Table 1

Effect of shock and gradual salinization on growth parameters of rice seedlings and the possible role of reduced glutathione (GSH) on salt shocked seedlings. (F.wt = fresh weight, D.wt = dry weight)

Growth parameter		Height of shoot (cm)	F.wt of shoot/ plant (g)	D.wt of shoot/ plant (g)	Root length (cm)	F.wt of root/ plant (g)	D.wt of root/ plant (g)	F.wt of whole plant (g)	D.wt of whole plant (g)	Ratio of shoot/root	
Treat-ment	NaCl (mM)									Fresh	Dry
Control	–	11.98	0.0352	0.006	5.36	0.0238	0.005	0.063	0.011	1.478	1.2
	100	9.91**	0.0316	0.005	5.83	0.0238	0.003*	0.059	0.007**	1.32	1.66**
Shock	200	8.7**	0.0267**	0.0035**	5.43	0.0212	0.0031*	0.049**	0.0064**	1.26*	1.12
	300	6.8**	0.0250**	0.0030**	5.21	0.0143**	0.002**	0.038**	0.0059**	1.75*	1.5**
	100	11.95	0.0394	0.005	7.49**	0.0412**	0.004	0.076**	0.009**	0.96**	1.25
Gradual	200	10.83*	0.0345	0.0045**	6.95**	0.0229	0.0034*	0.066	0.008**	1.5	1.32
	300	9.26**	0.0270**	0.003**	6.57**	0.0212	0.003*	0.049**	0.0076**	1.27	1.0
	100 + GSH	14.7**	0.047**	0.009**	8.62**	0.0383**	0.0045	0.09**	0.0133**	1.23*	2.0**
Shock + Gluta-thione	200 + GSH	12.64	0.0412**	0.007	7.9**	0.0293	0.004	0.075**	0.011	1.41	1.75**
	300 + GSH	11.98	0.0397**	0.005	7.63**	0.0280	0.003*	0.0709	0.007**	1.41	1.66**
L.S.D. at 5%		0.942	0.006	0.0015	0.795	0.0069	0.0017	0.0083	0.0015	–	–
L.S.D. at 1%		1.249	0.0079	0.002	1.053	0.0091	0.0023	0.011	0.002	–	–

* = significant differences
** = highly significant differences

ities (3 samples) of rice seedlings were analysed using L.S.D. at 5% and 1% levels of probability according to SAS program (1982).

Results and discussion

Growth parameters

Data presented in Table 1 show the mean changes in growth parameters of rice seedlings during the two successive seasons in response to different salinization protocols; salt shock, gradual salinization and salt shock in combination with glutathione. Most growth parameters as height of shoot, fresh and dry weights of shoots, roots and whole seedlings were significantly reduced when the rice seedlings were salt shocked. The highest inhibitory effects of salinity on all the growth parameters was recorded on using 300 mM NaCl. In salt acclimated seedlings less adverse changes were observed in most of the growth parameters of rice seedlings compared to unsalinized control seedlings.

Root growth under 100 or 200 mM shock or gradual salinization was stimulated or unaffected as compared with shoot growth (Table 1). This was reflected in the decrease in shoot/root ratio which presumably improves water balance by maintaining the potential for water absorption and reducing the transpiration rate. These results support those of Lyengar and Reddy (1994). On the other hand, the highest concentration of NaCl (300 mM) in both shock and gradual protocols severely inhibited the growth of rice root as indicated by decreases in their fresh and dry weights more than in the shoots, and this appeared evident from the increased shoot/root ratio.

The inhibitory effects of water stress induced by salinity on the growth parameters of the rice seedlings are in accordance with the results obtained by Lutts et al. (1995), Moons et al. (1995), Lutts et al. (1996) working on different cultivars of rice and by Ortiz et al. (1994), Hernandez et al. (1995) and Lin and Kao (1996) on other plants. These inhibitory effects may be attributed to the fact that salt stress is known to retard plant growth through several facets of plant activities such as osmotic adjustment (Lutts et al. 1996), protein and nucleic acid synthesis (Bejaoui 1985), ion uptake (Rodriguez et al. 1997), hormonal balance (Foda et al. 1991), photosynthesis (Singh and Dubey 1995) or more likely, a combination of these with other facets of metabolism may be involved. In addition, George et al. (1988) and Alislail and

Table 2

Effect of shock and gradual salinization on the photosynthetic pigments of rice leaves, and the possible role of reduced glutathione (GSH) on salt/shocked seedlings

Pigments		Chl (a)	Chl (b)	Chl (a+b)	Carotenoid	Total pigments
Treatment	NaCl (mM)					
Control	–	246.73	160	406.73	111.6	518.33
Shock	100	274.9**	154.0	428.0**	64.0**	492.9**
	200	170.0**	81.0**	251.0**	54.0**	305.0**
	300	154.0**	56.0**	210.0**	47.0**	257.0**
Gradual	100	271.0**	217.6**	488.6*	108.9	597.5**
	200	227.0**	105.0**	332.0**	103.4*	435.4**
	300	209.0**	81.0**	290.0**	74.7**	364.7**
Shock + Glutathione	100 + GSH	297.0**	246.0**	543.0**	129.0**	672.0**
	200 + GSH	275.0**	149.0**	454.0**	183.0**	637.0**
	300 + GSH	256.0*	135.0**	391.0*	84.0**	475.0**
L.S.D. at 5%		6.73	6.73	14.7	6.47	14.17
L.S.D. at 1%		9.07	9.01	19.7	8.72	19.12

* = significant differences
** = highly significant differences
Results are expressed as μg g^{-1} fresh weight

Bartels (1990) reported that the retardation of plant growth by salinity is due to osmotic and specific ion effects. Other authors concluded that reduction of dry weight may be due to a turgor limitation (Mengel and Arneke 1982) or cell wall hardening by limited extension growth (Van Volkenburgh and Boyer 1985, Chazen and Neumann 1994). Furthermore, Sweet et al. (1990) concluded that the reduced growth under NaCl could be attributed to increasing stiffness of the cell wall probably due to altered wall structure induced by salinity.

Glutathione administered to salt shocked seedlings not only has partially or completely alleviated the inhibitory effect of salt shocked stress, but also, in most cases induced marked significant increases in most of the growth parameters of rice seedling. The stimulatory effects of glutathione were more obvious in case of using 100 and 200 mM NaCl as compared to those of non-salinized rice seedlings and those of salt shocked controls. The magnitude of increase in dry weight of rice seedlings in response to

adding glutathione to the different levels of shock treatments were 90%, 71.8% and 18.6% as compared with those of shock salinized seedlings using 100, 200 and 300 mM NaCl, respectively. It appears probable from the response of salt shocked rice seedlings to glutathione treatment that it may act as a growth stimulus through playing a role in reversing the effect of NaCl on metabolic activities relevant to growth through enhancing cell division and/or cell enlargement. This enhanced cell division and cell enlargement would result in longer shoots and roots which consequently increase the fresh and dry matter of roots and shoots presumably as a result of larger surface area available for anabolic activities. These were further corroborated by the significantly higher endogenous phytohormone levels (Hassanein 1998) concomitantly with elevated carbohydrate and protein concentration which were noted generally in the roots and shoots of the tested rice shocked salinized plants treated with glutathione. These results may also indicate that glutathione treatments had increased the plant efficiency of water uptake, conservation and utilization. Also it may play a protective role in salt tolerance of the plants and/or acts as an antioxidant (De Kok and Oosterhuis 1983, Smith et al. 1990, El-Enany 1997).

Photosynthetic pigments

It is clearly demonstrated from Table 2 that chlorophyll a, chlorophyll b, carotenoids and total pigment contents of rice leaves were substantially affected by the different salinity levels and the type of salinization protocol applied. The higher concentrations of NaCl (200 and 300 mM) severely reduced chlorophyll a, chlorophyll b and carotenoid contents in both salt shocked rice seedlings and salt acclimated ones as being compared with the control values. The salt shock induced much higher adverse effect on photosynthetic pigments than the salt acclimation, since the reductions in total chlorophylls were 38.3% and 48.4% in response to 200 and 300 mM in salt shock protocol and 18.4% and 28.7% in salt acclimation protocol below the control value. On the other hand, the low concentration of NaCl (100 mM) in both shock and gradual salinization treatments, induced marked increases in chlorophyll a, chlorophyll b and a non-significant change in carotenoid contents compared to the control values. These results are in good agreement with those of Morales et al. (1992) in barley leaves, Chundra et al. (1993) in sugar cane, Rais et al. (1993) in jojoba, Singh et al. (1994) in *Vigna radiata*, and Singh and Dubey (1995) in rice seedlings.

The severe reduction in the photosynthetic pigments in rice leaves in response to salinity treatment, particularly the salt shock, might be ascribed to the toxic action of NaCl on the biosynthesis of pigments, increasing their degradation and/or maintaining damage of the chloroplast thylakoid. The observed severe reduction in Fe and Mg, which are needed for chlorophyll synthesis, in the shocked and acclimated rice leaves, in the present work, reinforced the view that salinity decreased chlorophyll biosynthesis. Moreover, the decrease in chlorophyll contents in stressed rice plant, in the present investigation, concurrently with the increase in the proline level led to the suggestion that nitrogen may be shifted to the synthesis of proline instead of chlorophylls. De La Rosa-Ibarra and Maiti (1995) recorded a similar conclusion and the destruction of chlorophyll in response to salinity stress was reported by Reddy and Vora (1986). Moreover, the decrease in chlorophyll concentration of salinized plants could be attributed to the increased activity of chlorophyll degrading enzyme chlorophyllase (Rao and Rao 1981). However, Quartacci and Navari-Izzo (1992) mentioned that the decrease in chlorophyll content may be due to changes in the integrity and structure of chloroplast membrane.

Additional increases in chlorophyll a, chlorophyll b, carotenoids and consequently in the total photosynthetic pigment contents of salt shocked rice seedlings were observed when reduced glutathione was added to 100 mM of NaCl, while partial recovery of the adverse effect of 200 mM or 300 mM NaCl on shocked rice seedlings was recorded. The values of recovery in the total photosynthetic pigments in shocked rice seedlings were 108.8% and 84.8% for 200 mM NaCl + GSH and 300 mM NaCl + GSH, respectively, as compared with shock concentrations of NaCl alone. These results indicate that GSH may stimulate the biosynthesis of chlorophylls and carotenoids and/or may retard their degradation. Also GSH may interfere with the protection of chloroplasts and their membranes against NaCl toxicity and thus maintaining their integrity. The effect of glutathione in protecting the chloroplast membrane against salinity stress was reported by Smith et al. (1990). This is due to its characteristics of being an antioxidant (Chen and Kao 1995).

Carbohydrate contents

It is of interest to mention here that there is a close correlation between the total carbohydrate contents detected in shocked and acclimated rice

Table 3

Effect of shock and gradual salinization on carbohydrate contents of rice seedlings and the possible role of reduced glutathione (GSH) on salt shocked seedlings

Carbohydrate fractions		Shoot			Root		
		Soluble sugars	Polysaccha-rides	Total carbohydrates	Soluble sugars	Polysaccha-rides	Total carbohydrates
Treatment	NaCl (mM)						
Control	–	6451.6	21098.5	27550.1	2387.0	34796.8	37183.8
Shock	100	6451.6	20967.9	27419.5	2967.7**	33209.7**	36177.4**
	200	5483.9**	14739.8**	20223.7**	2387.0	27719.9**	30106.9**
	300	2354.8**	12564.0**	14918.8**	2354.8	21189.7**	23544.5**
Gradual	100	2483.9**	25875.2**	28359.1**	3548.4**	36209.7**	39758.1**
	200	1645.6**	23730.7**	25376.3**	3451.6**	33187.1**	36638.7*
	300	1864.6**	18621.3**	20485.9**	2516.1**	26967.7**	29483.8**
Shock + Glutathione	100 + GSH	4993.5**	32283.1**	37276.6**	3935.5**	36967.7**	40903.2**
	200 + GSH	4516.1**	27909.1**	32425.3**	2516.1**	32830.6**	35346.7**
	300 + GSH	4299.9**	20351.2*	24651.1**	2903.2**	31987.0**	34890.2**
L.S.D. at 5%		138.81	580.62	616.59	38.802	405.26	418.51
L.S.D. at 1%		183.22	766.42	813.89	51.21	534.94	552.43

* = significant differences
** = highly significant differences
Results are expressed as mg glucose equivalent 100 g^{-1} dry weight

seedlings (Table 3) and the total chlorophyll contents of the leaves of the same seedlings (Table 2).

When rice seedlings were salt shocked by exposure to 100, 200 or 300 mM of NaCl the low concentration induced slight changes in the carbohydrate contents of their shoots and significant increases of soluble sugars of their roots, concurrently with significant reductions in polysaccharides and total carbohydrates as being compared with the control values. The higher concentrations induced significant reductions in soluble sugars, polysaccharides and total carbohydrates of both shoots and roots of the same plant (Table 3).

These results indicated that salt shock treatments particularly with the higher concentrations (200 and 300 mM NaCl) inhibited the biosynthesis of carbohydrates and/or increased their degradation. This inhibition is directly proportional to NaCl concentration. The increase of soluble sugars in roots exposed to 100 mM NaCl concurrently with the significant reduction in polysaccharides and total carbohydrates could be attributed to the hydrolysis of polysaccharides to soluble sugars as affected by NaCl treatment. These results are in accordance with those obtained by Kolupaev and Trunova (1994) who reported that in wheat coleoptile, moderate salt stress (0.5% NaCl) caused a sharp increase in invertase activity and an accumulation of reducing sugars most likely due to the enhanced oligosaccharides hydrolysis. Therefore, one can conclude that increases in soluble sugars particularly in roots of stressed rice plants are thought to be a protective and adaptive response against physiological drought induced by salinity.

In the salt acclimation experiment, the low concentration of NaCl (100 mM) induced significant increases in soluble sugars, polysaccharides and total carbohydrates of shoots and roots of NaCl acclimated rice seedlings, while the higher concentrations (200 and 300 mM) caused significant increases in soluble sugars of roots of acclimated seedlings concurrently with significant reductions in this fraction in shoots, polysaccharides and total carbohydrates in shoots and roots of the same seedlings (Table 3). The magnitude of reduction in carbohydrate contents in salt acclimated rice seedlings is much lower than that of salt shocked ones. This indicated that the reduction in carbohydrate contents is proportional to salt concentration and is dependent on the type of protocol applied, the level of salinity and plant species. Of interest in this connection Dunn and Neales (1993), Dubey (1994) recorded similar conclusions.

The reductions in total carbohydrate contents of salt shocked rice seedlings and acclimated ones concomitantly with arrested growth rate (Table 1)

and reduction in the leaf photosynthetic pigments led to the conclusion that sodium chloride inhibited the photosynthetic activity and/or increased partial utilization of carbohydrates into other metabolic pathways. In this respect, Plaut et al. (1990) using cow pea leaves found that the decrease in net CO_2 assimilation was attributed to NaCl effect on plant water status. Moreover, Brugnoli and Lauteri (1991), Singh and Dubey (1995) authenticated that the overall reduction of growth parameters is probably due to higher sensitivity of photosystem II and Hill reaction activity to salinity stress which resulted in reduction in photosynthetic capacity in saline stressed plants.

The significant increase in carbohydrate fractions in both roots and shoots of salt acclimated rice seedlings (100 mM) concomitantly with the increased growth rate (Table 1) led to the conclusion that the photosynthetic efficiency was increased in response to gradual increments of NaCl concentration (100 mM) and thus led to enhanced biosynthesis of carbohydrates which are utilized in growth of rice seedlings. In this respect, Patricia et al. (1992) found that in *Mesembryanthemum crystallinum*, salt treatment enhances accumulation of putative osmoprotective sugars. Also, Knight et al. (1992) using *Lycopersicum esculentum* found that NaCl enhanced carbohydrate partitioning towards shoots growth.

Adding GSH to the different concentrations of NaCl (100, 200 and 300 mM) applied in the salt shock protocol significantly increased the polysaccharides and the total carbohydrate contents of rice shoots and roots as being compared with those levels observed in NaCl shocked seedlings without GSH. The increase in total carbohydrates in shocked rice seedlings as a result of adding glutathione were shown to be 35.9%, 60.3% and 65.2% for shoots and 13.1%, 17.4% and 48.2% for roots above the reference controls exposed to 100, 200 and 300 mM shock NaCl, respectively.

Therefore, the increases in total carbohydrate contents in shocked rice seedlings treated with GSH appeared to coincide with increases in all the growth parameters of the same plant. This led to the conclusion that GSH might compensate the sulfhydryl groups which were oxidized as a result of shock salinization, since chloride salinization caused a major shift in the SH $\leftrightarrow$ S-S equilibrium compared to control plants (Strogonov 1973). The accessibility of GSH in shocked rice medium might protect the photosynthetic apparatus against NaCl toxicity.

Table 4

Effect of shock and gradual salinization on the soluble protein and proline contents of rice seedlings and the possible role of reduced glutathione (GSH) on shocked rice seedlings

Treatments	NaCl (mM)	Shoot		Root	
		Protein mg $100\ g^{-1}$ F.wt	Proline mg $100\ g^{-1}$ D.wt	Protein mg $100\ g^{-1}$ F.wt	Proline mg $100\ g^{-1}$ D.wt
Control	–	1383.0	30.1	322.0	4.76
Shock	100	1009.0**	35.7	345.0**	14.8**
	200	643.0**	65.4**	318.0	20.3**
	300	438.0**	85.2**	285.0**	33.0**
Gradual	100	3931.0**	25.0	354.0**	13.0**
	200	1402.0	38.0*	345.0**	14.2**
	300	1233.0**	56.0**	309.0	29.8**
Shock + Glutathione	100 + GSH	3199.0**	20.0**	422.0**	11.9**
	200 + GSH	1999.0**	33.0	393.0**	11.9**
	300 + GSH	1490.0*	44.0**	372.00**	13.0**
L.S.D. at 5%		93.43	7.12	14.49	3.65
L.S.D. at 1%		123.3	9.39	19.12	4.81

* = significant differences
** = highly significant differences

Protein and proline contents

The present work (Table 4) provides evidence that NaCl salinity affects protein and proline contents in 2 opposite directions. Therefore, the increase in NaCl concentration had a stimulatory effect on proline accumulation concurrently with a decrease in soluble protein content in shoots and roots of rice seedlings treated with NaCl in the two applied protocols. This indicated that salinity might have promoted hydrolysis of protein resulting in an accumulation of proline particularly at high concentration of NaCl and/or inhibited protein synthesis. These conclusions are in a good agreement with those obtained by Rais et al. (1993) on jojoba, by Singh et al. (1994) in *Vigna radiata* and by Mahraj and Sudhansha (1995) in peas.

The reductions in soluble protein contents in NaCl shocked rice seedlings were 68.3% and 11.5% for shoots and roots, respectively, while in acclimated rice seedlings these reductions were 10.8% and 4.0% for shoots

Table 5

Effect of shock and gradual salinization on the ion contents of rice seedlings and the possible roles of reduced glutathione (GSH) on salt shocked seedlings

		Shoot								
Treatment	NaCl (mM)	Na	K	Mg	Ca	P	Zn	B	Fe	Mn
Control	–	4.5	6.5	141	2596	1127.7	62.3	25.8	7.9	14.7
Shock	100	6.5	5	127.5**	3002.5**	1156.1	52.4	22.8	6.5	13.4
	200	8.5**	4.2**	97.3**	2422.5**	816.0**	34.7**	18.9**	4.7**	12.5*
	300	14.2**	3.7**	63.7**	718.7**	799.5**	10.7**	13.8**	2.9**	11.0**
Gradual	100	6.3	7.2	148.3	3745**	1024.1**	79.7	21.6*	8.7	12.3**
	200	7.8*	5.6	108.7**	3063.7**	845.7**	67.3	8.9**	6.4	8.6**
	300	11.2**	4.2**	85.6**	2578.7	725.2**	12.7**	5.4**	4.8**	6.9**
Shock + Glutathione	100 + GSH	3.3	7.7	161.0**	4501.2**	2377.4**	83.6**	17.8**	19.2**	20.8**
	200 + GSH	6.8*	8.5*	156.5*	3291.2**	13144.7	69.9	7.4**	16.8**	16.4**
	300 + GSH	7.7**	10.0**	146.4	3095.0**	1170.7**	17.3**	5.4**	10.9**	13.7**
L.S.D. at 5%		2.06	1.59	15.08	69.47	34.4	19.85	3.58	1.33	1.73
L.S.D. at 1%		3.58	2.09	19.9	91.7	45.4	26.2	4.72	1.75	2.28

Table 5 (continued)

Treatment	NaCl (mM)	Root								
		Na	K	Mg	Ca	P	Zn	B	Fe	Mn
Control	–	5.8	7.2	59	1951.2	871	95.7	4.8	10.1	92.0
Shock	100	7.3	3.5	37.2**	1346.6**	820.7	48.8**	2.4	14.7**	81.5
	200	12.7**	4.2	29.0**	819.1**	706.6**	24.2**	1.37*	14.4**	79.8
	300	29.0**	5.0	20.7**	302.9**	431.6**	16.1**	0.51**	7.8	68.5**
Gradual	100	6.5	4**	47.4**	1494.5**	930	230.9**	8.7*	11.5	80.7
	200	9.2**	4.5*	36.0**	851.6**	730.9**	97.4	7.5	9.9	80.6
	300	12.0**	7.0	32.1**	521.2**	605.7**	12.3**	7.1	9.5	70.1**
Shock + Glutathione	100 + GSH	4.2	9.5	48.2*	1951.2**	1014**	85.3**	29.8**	19.2**	208.2**
	200 + GSH	7.7	9.7*	30.5**	1706.6**	927	73.7**	4.7	16.8**	109.1*
	300 + GSH	8.8*	13.2**	28.4**	690**	733.9**	18.4**	4.5	10.9	101.8
L.S.D. at 5%		2.42	2.31	8.13	37.98	61.28	4.6	2.96	2.57	14.51
L.S.D. at 1%		3.19	3.04	10.73	50.13	80.89	6.07	3.9	3.39	19.15

* = significant differences
** = highly significant differences
Results are expressed as mg 100 g^{-1} dry weight

and roots, respectively, in response to treatment with 300 mM NaCl below the control value. These results indicated that the adverse effect of salinity was much more pronounced in shock treatment than in gradual NaCl treatment, and in shoots than in roots in both protocols.

On the other hand, the low concentration of NaCl (100 mM) increased the protein contents of both shoots and roots of salt acclimated rice seedlings by 184.2% and 9.9% above the control value, respectively (Table 4). This increase in protein content concurrently with increase in growth of rice seedlings (Table 1) led to the conclusion that the gradual salinization (100 mM) may enhance protein synthesis which increases the ability of rice seedlings to cope with salinity (Mosallam 1993).

Addition of GSH to 100, 200 or 300 mM NaCl in the salt shock protocol, increased the protein content of shoots of rice seedlings by 3.17, 3.1 and 3.4% and of roots by 1.2, 1.2 and 1.3% above those of shocked seedlings without GSH. These results indicated that GSH could ameliorate the adverse effect of NaCl on protein synthesis and/or protein degradation. Reduced glutathione appeared to increase protein synthesis and/or to retard protein degradation. In this respect, Chen and Kao (1995) found that the positive effect of GSH on rice root growth is mediated through the synthesis of low molecular weight protein, phytochelatin.

The accumulation of proline in plant organs and tissues is one of the most characteristic metabolic consequences of salinity stress (Lutts et al. 1996). The increase in proline of shoots and roots of salt stressed rice seedlings was positively correlated to salt concentration in salt shock and salt acclimation protocols. Moreover, the proline contents were more obvious in shoots than in roots, and in salt shocked seedlings than in salt acclimated ones. These results are in agreement with those obtained by Singh et al. (1994), De La Rosa-Ibarra and Maiti (1995), Lin and Kao (1996), Lutts et al. (1996). Thus the accumulation of proline in salt shocked rice seedlings in higher amounts than in salt acclimated ones substantiates the fact that proline accumulation is considered as one of the major physiological defence mechanisms of salt stressed rice plant.

Proline is now known to act as a compatible solute, it accumulates in the cytoplasm without having any detrimental effects on cytosolic enzyme activities and it is the least inhibitory of cell growth among all amino acids (Palfi et al. 1974, Lutts et al. 1996). Also, the proline has been reported to play an important role in osmoregulation as a buffer against osmotic imbalance caused by high vacuolar ion concentration (Laliberte and Hellebust 1989, Serrano and Gaxiola 1994). The proline might, however, play other

roles in relation to the salt stress effect; Smirnoff and Cumbes (1989) suggested that proline could act as a free radical scavenger, while Venekamp (1989) assumed that an increased proline synthesis could be an attempt to limit cytoplasm acidification in salt stress condition.

The lower proline accumulation in rice seedlings treated with 100, 200 or 300 mM of NaCl in the shock protocol in combination with GSH as compared with the contents of rice seedlings subjected to shock salinization alone could be ascribed to the effect of GSH in partially alleviating the adverse effects of salinity. Therefore, it could be concluded that shoots and roots of salt shocked or salt acclimated rice seedlings differ in their strategy towards proline accumulation under salinity condition or even under the interactive effect of GSH and shock salinization.

Ion contents

Both shock and gradual salinization protocols induced increases in Na ion contents in shoots and roots of rice seedlings concurrently with decreases in Mg, Ca, P, Zn, B, Fe and Mn mostly at the high concentrations of NaCl (200 and 300 mM). This exerted disturbances in growth of rice seedlings which could be regarded as one aspect of one or more of these ions in regulating cellular activities in plant. The retarded growth of salt shocked rice seedlings in the present work (Table 1) particularly by applying 300 mM of NaCl might be attributed to the increase in the osmotic potential of the saline solution, the toxic effects caused by the accumulation of excessive amounts of Na (Table 5) and/or to the deficiency symptoms of K, Mg, Ca, P, Zn, B, Fe and Mn in both shoots and roots of salinized rice seedlings (Table 5). Therefore, salinity may interfere with uptake and functions of elements caused by ionic balance. In this respect, George et al. (1988), Alislail and Bartels (1990) reported that retardation of plant growth by salinity are osmotic and specific ion effects.

In the present investigation, the decrease in K content was compensated by an increase in Na ion as previously shown by Lutts et al. (1996). However, the drastic reduction in Ca contents of shoots and roots of rice seedlings in response to high concentrations of NaCl particularly in shock salinization protocols (Table 5) was previously observed in rice (Grieve and Fujiyama 1987) and in alfalfa (Ashraf and O'Leary 1994). This reduction could impair important metabolic functions or alter membrane permeability (Rengel 1992). Rapid deterioration of salt shocked rice seedlings (Tables 1–4) was a reflection of these effects.

In addition, the low concentration of NaCl (100 mM) increased the Ca and P contents in shoots of salt shocked rice seedlings and Ca, Mg and Zn contents in shoots of salt acclimated ones compared with the values of the control. The increase in these elements in the salinized rice plants is one of the adaptive responses against salinity stress.

Supplemental addition of GSH to 100, 200 and 300 mM of NaCl in salt shock protocol resulted in 49.2%, 20.0% and 45.8% decreases in Na ion contents of shoots and 42.4%, 39.3% and 69.7% decreases in Na ion contents of roots as compared with the shock concentrations alone, respectively. Also, GSH in combination with salt shock treatments mostly increased K, Mg, Ca, P, Zn, Fe, B and Mn contens in shoots and roots of salt shocked rice seedling as compared with those detected in the reference controls.

It is worthy to mention here that GSH ameliorates the adverse effects of shock salinization on the growth of rice seedlings (Table 1) through decreasing the absorption and translocation of Na ions and increasing K, Mg, Ca, P, Zn, Fe, B and Mn contents which regulate the metabolic activities of rice plant.

In conclusion, salt acclimation and GSH are able to improve the vital properties of salt stressed rice seedlings; osmotic adjustment and turgor maintenance through increasing the photosynthetic pigments, carbohydrates, proline and ion contents particularly in roots as compared with salt shock. This adds a new and unique information to all efforts in understanding and explaining the physiology of stress tolerance in rice plant.

Further studies are needed to investigate the salt responsive proteins, genes and endogenous phytohormones in salt shocked and acclimated rice seedlings and the role of GSH in improving their tolerance to salinity shock.

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NUTRIENT-INFLUENCED GROWTH RESPONSE OF *MESOTAENIUM CALDARIORUM* (LAGERHEIM) HANSGIRG TO BREWERY EFFLUENT

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The effect of nutrient enrichment on the growth of *Mesotaenium caldariorum* in brewery effluent was studied for 14 days. Three different sites namely "before" discharge of effluent (BD), point of discharge of effluent (PD) and "after" point of discharge of effluent (AD) as well as the effluent itself were used for the study.

Nutrient spiking was done using 50 g l^{-1} NO_3 + 100 g l^{-1} PO_4. Growth was stimulated by 35.5% in BD+N, 55.4% in AD+N, 40.4% in AD and only 4.8% in PD+N. On the other hand, growth was suppressed by 36.1% in BD, 6.6% in PD and 35.5% each in effluent with or without nutrient.

Key words: *Mesotaenium caldariorum*, brewery effluent

Introduction

Water bodies generally undergo eutrophication with time, either naturally or culturally. Cultural eutrophication results from both point and non-point source addition of nutrients from municipal sewage, septic tank effluents, agricultural and urban run-offs and industrial effluents. Such discharges into water bodies confer changes on the physical and chemical as well as biological (flora and fauna) characteristics of such water bodies (Sankaranarayanan et al. 1986, Joy et al. 1990, Teltsch et al. 1989, 1992). The resultant effect is deteriorating water quality, fish kills, oxygen deficiency and sedimentation. More specifically for algae are changes in biomass (phytoplankton bloom), species composition and primary production rates (Walsh and Merrill 1984). Some pollutants stimulate growth while others suppress it, yet others are both stimulatory and inhibitory depending on the concentration (Walsh et al. 1980). Joy et al. (1990) observed that enhanced phytoplankton crop in Edeyar Eloor stretch of River Periyar (India) was partly attributed to effluent discharged from factories.

Such alterations in water quality can be simulated in the lab using algal cultures to measure growth response to such changes (Forsberg and

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Forsberg 1972, Miller et al. 1978, Bolier 1985). Bioassay using microorganisms have been considered useful as it reveals the relative sensitivity of the test organism to the different pollutant as well as provide information on the chemical pollutants. Genjatulin (1990) studied the chemical and biological water pollution using quantitative bioassay employing *Escherichia coli* phage and virus as test organisms. Dodds (1991) reported that excessive growth of *Cladophora glomerata* resulted from the nutrient enrichment of municipal waste water and secondary sewage effluent. Teltsch et al. (1992) studied community response to addition of treated effluent and observed changes from addition of treated effluent and observed a change from phytoplankton and zooplankton communities dominated by small species, e.g. *Chlorella* sp., *Melosira* sp., *Spirulina platensis* and *Mesocyclops oqunnus* to communities dominated by larger (> 200 μm) species, e.g. *Oscillatoria* sp. and *Epiphanes macrourus*. Mingazzini (1993) in an assay study with Po river, Italy, found that the toxicity of *Selenastrum capricornutum* was influenced by nutrient enrichment. Some other authors have established that high nutrient content and subsequent high algal biomass could ameliorate copper contamination effect to a certain degree that would otherwise ordinarily be highly toxic to a water system with a low density of algae (Swartzman et al. 1990, Mingazzini 1993). Some cases of toxicity would manifest in a temporary inhibition effect, slowing down the initial growth, allowing the algal cells to neutralize the chemical toxicity (Adams et al. 1985, Mingazzini 1993). Mayo and Noiki (1994) studied the effect of glucose on the growth of *Chlorella vulgaris* and observed an increase in biomass with increase in glucose concentration from 25 to 150 mg l^{-1} d^{-1}, when anaerobic conditions resulted in growth inhibition.

Algae are considered useful in bioassay studies because they represent the first link in the food chain of any aquatic ecosystem. They have thus been extremely important exclusively in domestic and industrial waste water treatment. Their role in such systems is photosynthesis and consequent release of oxygen which is supplied to bacteria whose oxidative activity is more efficient (Martinez et al. 1987). Additionally, algae possess the potential to assimilate fertilizing nutrients, such as nitrogen and phosphorus normally implicated for eutrophication of receiving waters. Some algae can also absorb some heavy metals from polluted waters (Vymazal 1985, 1987, 1990, Whitton et al. 1989, Kelly and Whitton 1989).

It is known that lack of growth in effluents is as a result of inavailability of nutrients or toxicity. Several authors are of the opinion that phosphate and nitrate are the most important primary limiting nutrients in tem-

perate waters, undisturbed waters, warm lakes and reservoirs (Lund et al. 1975, Moss 1959, Schindler et al. 1973, Roberts and Southall 1977, Thornton 1986, Lean et al. 1989). Others believe that phosphorus limits algal growth in aquatic environments generally (Schindler 1971, Reynolds 1978, Jansson 1993). Others hold the view that phosphorus is a crucial nutrient for algal productivity in man-made lakes (Schindler 1971, Toerien and Steyn 1975, Beadle 1981, Pollingher et al. 1988).

The objective of this study is to investigate the interactive influence of nutrients on the growth of *Mesotaenium caldariorum*, a desmid, in brewery effluent. It was intended to establish whether effect on growth was due to nutrients deficiency or toxicity. The brewery effluent used is discharged by Guinness brewery which makes Harp Larger beer, Malta guinness and Stout. The effluents produced are released into the Ikpoba river.

The Ikpoba river is the major river located in Benin City, Edo State of Nigeria (Lat. 6.5° N, Long. 5.8° E). It is a fourth order stream which is dendritic in its upper reaches.

Materials and methods

Three sampling points were chosen for study. These were the point before the discharge of effluents (BD), point of discharge of effluents (PD) and point after discharge of effluents (AD). The point of discharge was 50 m equidistant from point before discharge and point after discharge.

Water samples were collected from these three sites as well as the concentrated effluent itself. All samples were sterilized by filtration through 0.45 μm pore size. The basic procedures of Lund et al. (1971, 1975) were used. The experiment was set up in triplicates of two sets for each sample. Another set of triplicate was set up for control. The control flasks contained 50 ml of JM medium. Into both sets of replicate excluding control was measured 50 ml of sample, i.e. BD, PD, AD and effluent. One set of the replicates was spiked with 50 $\mu g\ l^{-1}\ NO_3 + 100\ \mu g\ l^{-1}\ PO_4$. This N/P ratio was chosen based on another study in which this gave the optimum growth. All flasks were inoculated with *Mesotaenium caldariorum* which was obtained from the Culture Collection Centre of the Institute of Freshwater Ecology, U.K. The cultures were maintained at room temperature of 26 °C ±2 °C and continuous light intensity supplied by day light fluorescent tube of 40 W. All flasks were placed near a north-facing window. The cultures were shaken once daily to ensure adequate supply of nutrients and to prevent

clumping of algal cells. The cell number was determined using Lund's counting chamber.

Measurements were taken every other day for 14 days. The composition of the JM used in the experiment is as follows:

Results and discussion

Constituent	Concentration (mg^{-1})
$Ca(NO_3)_2 \cdot 4\ H_2O$	20.00
KH_2PO_4	6.20
$NaHCO_3$	16.00
$MgSO_4 \cdot 7\ H_2O$	25.00
$MnCl_2 \cdot 4\ H_2O$	1.40
$(NH_4)_6Mo_7O_{24} \cdot H_2O$	1.00
Cyanocobalamine	0.04
Thiamine	0.04
Biotin	0.04
EDTA-Na_2-Salt	2.25
EDTA-Fe-Na-Salt	2.25

The response of *Mesotaenium caldariorum* at the three sites as well as the effluent is represented in Figure 1. The growth was maximum for AD+N. Next to this was AD which was followed closely by BD+N and BD. The least was effluent and effluent+N. The growth was maximum on day 6 for most treatments. There was an increase in growth from day 0 to day 6 in AD+N, AD and BD+N. For BD, growth attained peak on day 8 while control and PD+N increased from day 0 to day 4. The only increase in effluent whether with or without nutrient was from day 0 to day 2 after which growth decreased until the termination of the experiment. There was, however, no difference in growth between effluent alone and effluent plus nutrient.

The decrease in growth is attributed to bottle effects such as carbon dioxide limitation, nutrient exhaustion, self-shading and death of some algae. The onset of decline in growth generally occurred early probably owing to a large inoculum relative to the available nutrients.

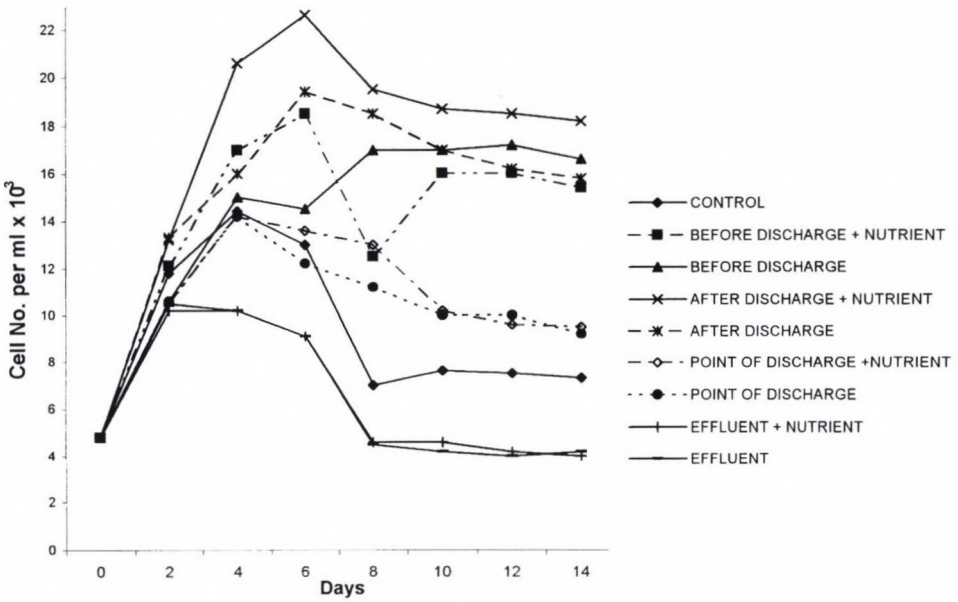

Fig. 1. Growth response of *Mesotaenium caldariorum* to nutrient enrichment in test waters

The 96 hrs yield of the test alga in the various test waters is shown in Figure 2. Addition of nutrients improved growth at BD and remarkably so in AD. However, in the case of PD and effluent, nutrient spiking made no difference to growth.

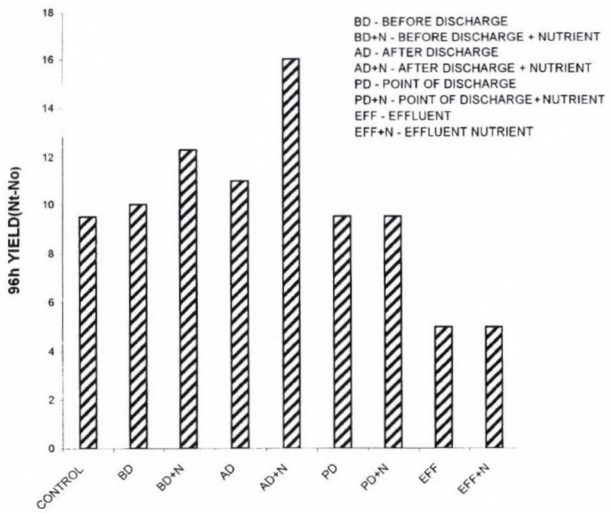

Fig. 2. 96 hrs yield of *Mesotaenium caldariorum* in test waters

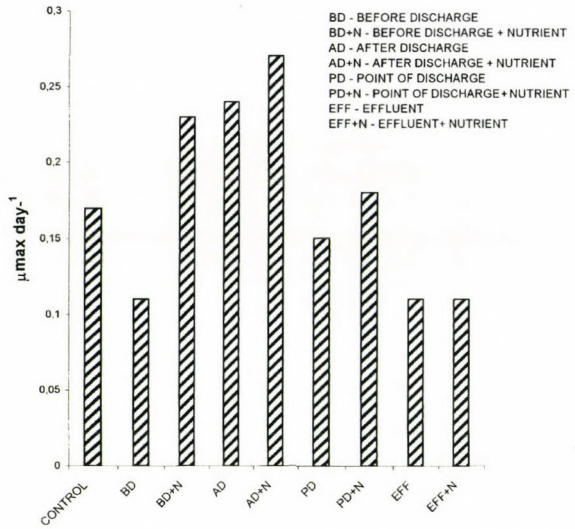

Fig. 3. Maximum specific growth rate of *Mesotaenium caldariorum* in different test waters

The maximum specific growth rate at the different treatment is shown in Figure 3. The response in BD without nutrient was least followed by effluent with or without nutrient. The addition of nutrient to the effluent did not make any remarkable difference in growth. The response of BD to nutrient addition was appreciable. There was also response to nutrient spiking in AD and PD.

Relative to control, there was stimulation in growth by 35.5% in BD+N, 55.4% in AD+N, 40.4% in AD, 4.8% in PD+N and suppression by 36.1% in BD, 6.6% in PD and 35.5% each in effluent and effluent+N. The suppression of growth in BD is attributed to nutrient deficiency or limitation. The background nutrient level in the natural river water, i.e. BD is low. Thus the marked increase in growth in BD+N is presumably a response to nutrient enrichment. In PD, the suppression was minimal (6.6%) because though there was sufficient nutrient, there was probable toxicity owing to effluent as well as to turbidity which could lead to light exclusion. It was high (35.5%) in effluent and effluent+N, because of concentrated toxic component of the effluent as well as increased turbidity.

Although the effluent is richer in nutrient than the other test waters, growth in it was least due to probable growth retardants or toxicants. Young and Barber (1978) in a study of the effect of waste dumping on the growth of natural phytoplankton population attested that the inhibition in

growth was probably as a result of toxic organic materials. At PD and AD, the effluent and consequently the toxicants are diluted hence algal growth potential is more for these test waters. Comparatively, the growth response in AD is, however, greater than that at PD because AD is more dilute of the effluent than PD. Consequently, there is less growth retardant or toxicant at AD than PD. Additionally, PD is more turbid than AD. At AD there is adequate supply of nutrient, so addition of nutrient resulted in only a slight enhancement of growth. Generally, the effluent is very turbid, hence its effect would be partially due to insufficient penetration of light intensity in addition to other factors such as toxicity or eutrophication. PD represents the sites at which the effluent mixes with the natural river water so that the effect here is similar to that of the effluent except that there is an ameliorative effect.

The result obtained in this is corroborated by the report of Bowker and Muir (1981) for rivers Lugg and Ely in the U.K. For river Lugg, nutrient addition did not significantly stimulate algal growth owing to input of metalliferous effluents from industrial and mine workings. Likewise in river Ely addition of nutrient resulted in little or no difference in growth in the polluted section of the river, whereas in the unpolluted reaches growth was markedly improved as a result of nutrient addition.

Generally, in conclusion, the growth of *M. caldariorum* in BD was seriously limited by nutrient deficiency but that at AD and PD was not limited by nutrient judging from the minimal increases owing to nutrient enrichment. This indicates that at BD, there was no pollution but AD and PD were subjected to (cultural) eutrophication. Studies of this nature are important in lake restoration programmes (Does and Klapwijk 1987). Specifically, if the biological effluent treatment technology is to be feasible in the river studied, the effluent requires dilution before application or better still used at the point of AD (after discharge of effluent).

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SPATIAL PROFILE OF PHYTOPLANKTON OF THE LOWER RIVER NIGER, NIGERIA

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Horizontal variation in the phytoplankton of River Niger was examined along some chemical characteristics. Results reveal that the chemical composition is generally poor (with a mean conductivity of only 56.4 μS cm^{-1}) particularly PO_4 and NO_3. Floristically, the phytoplankton is diverse with a total taxa of 121 dominated, qualitatively by Chlorophyta or green algae, in particular Desmidiaceae, Scenedesmaceae and Hydrodictyaceae, and quantitatively, by *Aulacosira* spp.

Key words: heterogeneity, horizontal, phytoplankton, River Niger, Nigeria

Introduction

Reports show that River Niger though a large and important river in Nigeria has been grossly understudied (Brook et al. 1957). Previous phytoplankton studies in similar Nigerian freshwater ecosystems include, in the northern part, those of Khan (1987) on fish farms, Khan (1984), Khan and Ejike (1984a, b) and Khan et al. (1983) on Liberty and Lamingo reservoirs, Khan and Agugo (1990) on Kongingiri dam, Anadu et al. (1990) in mine lakes, all in Jos; Holden and Green (1960) on River Sokoto. Similar studies in the eastern region are those of Biswas (1984) in Opi lake, Biswas (1992), Biswas and Nweze (1990) in Ogelube lake, Nwadiaro (1989), Nwadiaro and Idabor (1990) on Oguta lake, Nwadiaro and Ezefili (1986), Erondu and Chindah (1991) on new Calabar River, Nwankwo (1996) on freshwater swamps in eastern Niger Delta. In the south, are studies of Kadiri (1988, 1993a, b, 1996), Kadiri and Opute (1989) on Ikpoba reservoir as well as Opute (1991, 1992) on Warri/Forcados estuary. Studies of Egborge (1973, 1974, 1979) on River Oshun, Imevbore (1965, 1967, 1968) on Eleiyele reservoir and Egborge and Sagay (1979) on Ibadan freshwater ecosystem are notable for the western region of the country.

The array of studies though fairly impressive does not include any from River Niger, a further corroboration of the evidence of Brook et al.

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(1957) which indicates that River Niger has not been given the attention it deserves in terms of phytoplankton studies. Also the algal flora of many African water bodies have been studied. However, only a few of such studies specifically address total biomass and quantitative distribution of component species (Kadiri 1993c, Kebede and Belay 1994).

River Niger though a large and one of the most important rivers in Nigeria, is grossly understudied. Available studies include Imevbore (1970) on its water chemistry. There is a dearth of studies on phytoplankton in the river in the lower reaches. Earlier reports on the river are concerned principally with the fauna with some information provided on the water chemistry (Daget 1954, 1957, Blanc et al. 1955, Dumont et al. 1981). Some other studies carried out on the river and its adjacent flood plains as well as parts of the smaller tributaries include those of Cooke (1968), Imevbore (1970), Imevbore and Bakare (1974), Imevbore and Visser (1969). Other reports include those of Grove (1972) on the dissolved solids and Rzoska (1985) on a review of the hydrobiology and water quality. Available record of algal flora in River Niger and its vicinity are those of Bourelly (1957), Couté and Rousselin (1975) in Mali and Macina regions and by Serpette (1955) around Bamako. From the foregoing it is obvious that most of the research, especially on algal flora was concentrated either mainly outside Nigeria or the few within Nigeria in the upper stretch of the river. Therefore, this paper is a contribution to knowledge in phytoplankton studies in Nigeria in particular and Africa in general, River Niger being one of the major rivers in the continent.

Study area

River Niger is the third longest river in Africa. Its watershed covers an area of about 1,250,000 km^2. It flows through many countries in the subregion. It takes its source from Guinea highlands, collects its main headwaters, the Milo and Tin Kisso in Guinea and flows northeastward through Mali, then southernly through the southwestern end of Niger, finally crossing Nigeria from northwest to south (Iloeje 1991). In Nigeria it connects its main tributary, River Benue and enters the Atlantic by a delta. River Niger is the longest river in West Africa with a total length of 4,200 km and within Nigeria only 1,200–1,300 km. In the lower section it is fed by rivers Benue, Sokoto and Kaduna. It is divided into five sections (Fig. 1): a) Upper Niger, from its source to Segou, b) Upper Middle Niger, from Seguo to Bourem, east of Timbuktu, c) Lower Middle Niger, from Bourem to the

beginning of Kainji lake, d) Lower Niger, from Kainji lake to the head of the delta, e) the delta.

Reports of Imevbore (1970) show that hydrographically, the river is regulated by two annual floods. The major flood called the white flood occurs during the months of July to September with an average maximum discharge of 6,000–8,000 $m^3 s^{-1}$. The second flood is termed black flood and it occurs between December and February with an average maximum discharge of 1,750 $m^3 s^{-1}$. The river attains a high peak of 3 m during the white flood and a lower peak of 0.6 m during the black flood. The two floods also differ in origin as well as in chemical composition. The white flood originates from drainage basin within Nigeria while the black floor originates in the headwaters of the river outside Nigeria. While the white flood result in an enrichment of chloride, phosphates and sodium ions, black flood causes an enrichment of calcium, potassium, silica and iron (Imevbore 1970). Both floods are weakly alkaline although the pH of black flood is slightly higher (Imevbore 1970).

Materials and methods

Samples were collected during the wet period from six stations (Fig. 1). Stations 1 to 4 were located on the river, Station 6 is a creek which flows into the river while Station 5 is the confluence between the River Niger and the creek. Three sets of samples were collected, one for chemical parameters and the other two for phytoplankton (qualitative and quantitative).

pH and conductivity were measured in situ with digital HACH pH meter and conductivity meter, respectively. Dissolved oxygen was also fixed in situ and later estimated in the laboratory with the Winkler's technique. Total alkalinity was determined titrimetrically with 0.1 N H_2SO_4. Total hardness, calcium and magnesium were also analysed by titrimetry using EDTA. Chloride was measured with mercuric nitrate titrimetric method. Sodium and potassium were estimated by flame photometry (Corning 400). The determinations of nitrate and phosphate were made with the respective spectrophotometric method of cadmium reduction and ascorbic acid with HAGH DR 2000 spectrophotometer.

For phytoplankton studies, a 55 μm mesh net was towed to obtain qualitative samples while for quantitative samples, a 50 dm^3 of water was concentrated to 25 cm^3 and counted with the aid of a haemocytometer. Identification was done using several text among which are Couté and

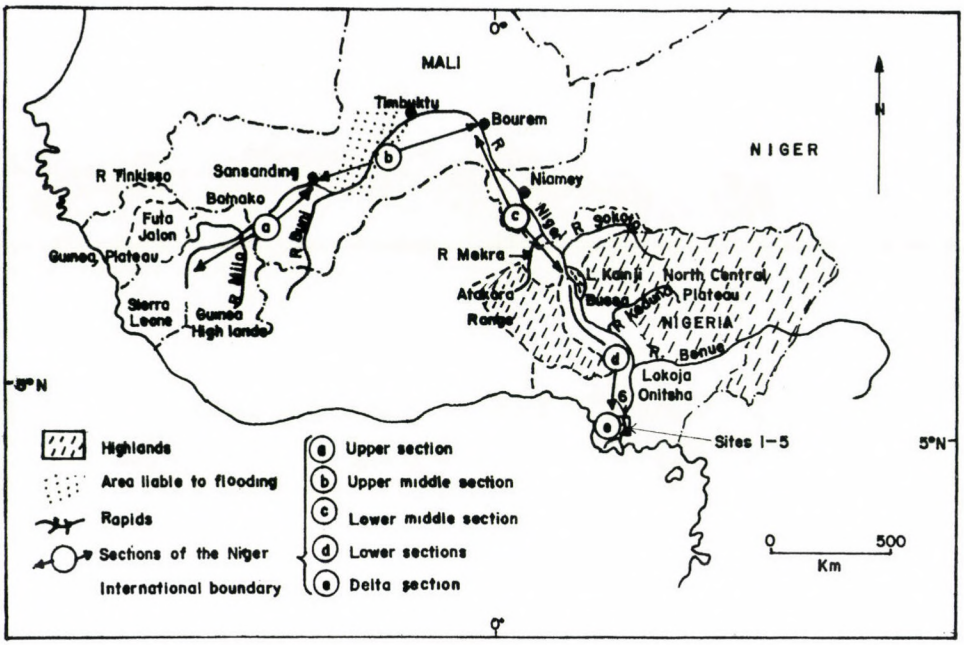

Fig. 1. The course of the River Niger

Rousselin (1975), (Kadiri 1988, 1993a, b, 1996). The species indices (evenness, diversity and richness were calculated using the computer basic program Spdivers.bas of Ludwig and Reynolds (1988).

Results

Chemical characteristics

Table 1 shows data on the chemical variables of the River Niger. The pH of the river is circumneutral ranging from 7.1 to 7.9, that of the creek was 6.9. The conductivity was generally low and conservative in the River Niger not exceeding 70 μS cm^{-1}, it was slightly different at the creek, i.e. Station 6. Dissolved oxygen values had a range of 4.43–4.75 mg l^{-1}. Total alkalinity and total hardness were moderate with respective range of 28.00–39.00 and 21.00–48.00 mg l^{-1} $CaCO_3$. These two variables decrease somewhat from Station 1 to 4 and an increase at Station 5 followed by a slight decrease at Station 6. The exchangeable cations were generally low with a divalent to monovalent cation ratios of between 0.78 to 2.06. There

Table 1

Chemical characteristics of lower River Niger

	Stations					
	1	2	3	4	5	6
Conductivity ($\mu S\ cm^{-1}$)	53.90	53.60	55.40	54.70	64.60	66.80
Dissolved oxygen ($mg\ l^{-1}$)	4.75	3.93	4.65	4.95	4.60	4.43
Alkalinity ($mg\ l^{-1}$)	28.00	32.00	30.00	29.00	39.00	37.00
Total hardness ($mg\ l^{-1}\ CaCO_3$)	21.00	28.00	21.00	22.00	48.00	36.00
Cl ($mg\ l^{-1}$)	10.00	14.00	12.00	11.00	7.00	6.00
pH	7.50	7.90	7.50	7.10	7.10	6.90
Ca ($mg\ l^{-1}$)	5.20	4.40	4.80	4.80	7.60	6.40
Mg ($mg\ l^{-1}$)	1.95	1.71	2.20	2.44	7.08	4.88
Na ($mg\ l^{-1}$)	4.44	3.33	3.33	3.55	3.77	3.55
K ($mg\ l^{-1}$)	2.64	2.23	2.23	2.23	2.44	2.23
Ca + Mg/Na + K (meq^{-1})	0.78	0.85	1.01	1.01	2.06	1.65
NO_3 ($mg\ l^{-1}$)	0.62	0.53	0.58	0.49	0.27	0.18
SO_4 ($mg\ l^{-1}$)	0.15	0.00	0.00	0.02	0.00	0.00
PO_4 ($mg\ l^{-1}$)	0.01	0.06	0.06	0.04	0.01	0.05

was a gradual increased in this ratio from Station 1 through to 5, followed by a decrease at Station 6. It was less than unity at Station 1 and 2, about unity at Stations 3 and 4, and greater than unity at Stations 5 and 6. Chloride was fairly low decreasing from Station 2 through to 6. The essential primary limiting plant nutrients, phosphate and nitrate were extremely low and also with a decrease from Station 1 through to 6.

Phytoplankton composition and distribution

Table 2 shows the composition and distribution of phytoplankton along the River Niger. A total of 121 taxa of phytoplankton were found in the river. These are classified into six divisions, namely Bacillariophyta, Chlorophyta, Chrysophyta, Cyanophyta, Dinophyta and Euglenophyta. The qualitative composition of the phytoplankton flora shows that Chlorophyta or green algae constituted the bulk of flora making up 48.8%,

Table 2

Composition and distribution of phytoplankton in River Niger

	Stations					
	1	2	3	4	5	6
Division Bacillariophyta						
Achnanthes affinis A. G. C.				+		+
Amphora sp.	+					+
Aulacosira granulata (Ehr.) Ralfs	+	+	+	+	+	+
A. granulata var. *curvata*	+	+	+	+	+	+
A. granulata var. *angustissima* Muller	+	+	+	+	+	+
A. granulata var. *angustissima* f. *spiralis*	+	+	+	+	+	+
A. moniliformis Muller	+					
Caloneis sp.		+				
Caloneis klamathesensis Patrick et Reimer			+			
Cyclotella meneghiniana Kutz.					+	
Cymbella affinis Kutz.		+				
Cymbella sp.		+	+			
Leptocylindrus danicus Cl.	+	+				+
Navicula sp.		+	+	+	+	+
Nedium sp.					+	
Nitzschia palea (Kutz.) W. M. Smith	+					+
Nitzschia sp.	+		+			+
Pinnularia sp.					+	
Pleurosigma australe Grun.	+	+				
Stauroneis phoenicentron (Ehr.) Hust.	+					
Surirella elegans Ehr.		+		+		
S. celebasiana	+					
S. muelleri Muller	+					
S. ovalis Breb.	+	+	+	+	+	
S. robusta Ehr.	+		+	+		
S. tenera Greg.				+		
Synedra acus Kutz.	+			+		
S. delicatissima W. Smith		+	+	+	+	+
S. gallonii (Bory) Ehr.		+				
S. ulna var. *biceps* W. Smith		+				
S. filiformis W. Smith		+				
Tabellaria fenestra (Rothe.) Kutz.					+	
Division Chlorophyta						
Actinastrum hantzschi Lag.		+			+	+
Ankistrodesmus convolutus (Corda) Ralfs				+		
Asterococcus limneticus G. M. Smith	+	+				
Closterium pseudolunula Borge	+				+	
C. setaceum Ehr.			+			
C. turgidum Ehr.					+	
Coelastrum microspora Naeg.		+		+	+	+
C. tetrapedia (Kirch.) West et West						+
Cosmarium askenayi Schmidle				+		
C. circulare Reinsch					+	+

Table 2 (continued)

	Stations					
	1	2	3	4	5	6
C. contractum var. *incrassatum* (Scott et Gronbl.) West et West					+	
C. monodii Bourelly		+	+	+		
C. raciborskii Lag.					+	
C. subtumidum Nordst.						+
Cosmarium sp.		+	+			
Golenkinia radiata Chod.					+	
Eudorina elegans Ehr.	+	+	+	+	+	+
Kirchnerella obesa (W. West) Schmidle					+	
Micrasterias echinata Brandham	+	+	+	+		
Oedogonium crassum (Hass.) Wittr.			+			
Pediastrum clathratum (Schroter) Lemm.		+	+		+	+
P. duplex Meyen		+	+	+	+	
P. duplex var. *subgranulatum* Racib.	+	+	+	+		
P. gracillimum (West et West) Thunmark	+	+	+	+	+	+
P. simplex (Meyen) Lemm.		+	+	+	+	
P. simplex var. *echinulatum*					+	
P. sturmii Reinsch.	+		+			
P. tetras (Ehr.) Ralfs					+	
Scenedesmus aldvei Hew et Schrep	+					
S. dimorphis (Turp) Kutz.					+	
S. ecornis (Ehr.) Chod.						+
S. quadricauda (Printz.) Breb.	+	+	+			+
Scenedesmus sp.	+	+			+	
Sphaerocystis schroeteri Chod.	+		+	+	+	+
Sphaerozosma granulatum Roy et Biss		+		+		+
Spirogyra dubai Kutz.	+					
S. insignis (Hass.) Kutz.			+			
S. majuscular Kutz.	+		+			
Spirogyra sp.	+	+		+		
Staurastrum asterias Nygard in Krieg		+		+		+
S. gladiosum Turn.		+	+	+		
S. irvesenni Nygard				+	+	+
S. leptocladum Nordst.	+			+	+	
S. leptocladum var. *cornutum* Wille f. *crassius* Gronbl.	+	+	+			
S. lezae Thom.	+	+	+	+	+	
S. octoverrucosum Scott et Gronbl.	+					
S. sagitarium Nordst.					+	
S. sebaldi Reinsch.				+		
Staurastrum sp.	+		+		+	
Staurodesmus convergens Ehr.					+	
S. subpygmaeus (West et West) Croasd. var. *spiniferous* (Scott et Gronbl.) Teil.					+	
Staurodesmus sp.						+

Table 2 (continued)

	Stations					
	1	2	3	4	5	6
Division Chrysophyta						
Dinobryon cylindricum Imof.					+	+
Division Cyanophyta						
Anabaena cylindrica Lemm.					+	+
A. scheremetivei Elenk.						+
A. solitare KIeb.			+		+	+
A. spiroidis Kleb.					+	
Chroococcus limneticum Lemm.		+				
Lyngbya sp.	+				+	
Merismopedia elegans A. Braun	+			+	+	+
Microcystis aeruginosa Kutz.					+	
M. flos-aquae (Wittr.) Kitch					+	
Oscillatoria sp.	+	+		+		+
Trichodesmium lacustre Kleb.				+		
Division Dinophyta						
Peridinium cinctum (Mull.) Ehr.				+		+
Division Euglenophyta						
Euglena deses (O. F. M.) Ehr.					+	
E. oxyuris Schmarda		+				
E. rubra Hardy					+	
Lepocinclis playfairiana Defl.					+	+
Phacus longicauda Duj.	+			+	+	+
P. orbicularis Hubn.					+	
P. tortus (Lemm.) Skvort.					+	
Strombomonas limnonensis Yacubson					+	
Trachelomonas caudata Stein			+		+	
T. echlora (Ehr.) Lemm.					+	
T. eurystoma Stein ex Ralfs					+	
T. superba (Swir.) Defl.					+	
T. sydneyensis Playf.					+	

followed by Bacillariophyta or diatoms with 28.1%, Cyanophyta or blue-green algae and Euglenophyta equitably represented by 10.7% each and the least were Chrysophyta and Dinophyta insignificantly constituting 0.8% each.

The green algae were represented by mainly Zygnematales (Desmidiaceae), Chlorococcales particularly Scenedesmaceae (mainly *Scenedesmus* genus), a few Oocystaceae and Hydrodictyaceae (notably *Pediastrum* spp.). Remarkably, the desmids comprised about 24% of the total phyto-

plankton flora and 49.2% of the Chlorophyta division. The diatoms were represented by mainly Pennales notably Naviculaceae and Surirellaceae. The few Centrales were *Aulacosira* spp., *Cyclotella*, and *Leptocylindrus*. Cyanophyta was represented by mainly Nostocales (Nostocaceae, especially *Anabaena* spp., a few Oscillatoriales (Oscillatoriaceae, notably *Oscillatoria* and *Lyngbya*). Euglenophyta was represented particularly by *Trachelomonas*, *Phacus* and *Euglena* spp. Chrysophyta and Dinophyta were variously inconsequentially and respectively represented by *Dinobryon cylindricum* and *Peridinium cinctum*.

The taxa which had a wide distribution, i.e. > 60% were, among the diatoms, *Aulacosira*, *Navicula*, *Surirella ovalis*, *Synedra delicatissima* and *S. ulna*, among the Chlorophyta were *Botryococcus braunii*, *Coelastrum microsporum*, *Eudorina elegans*, *Micrasterias echinata*, *Pediastrum* spp., *Scenedesmus quadricauda*, *Sphaerocystis schroeteri* and *Staurastrum lezae*. Among the blue-green algae were *Lyngbya* and *Oscillatoria* spp.

Some of the taxa were rare and these include the euglenoids, most of which were restricted only to the creek, some desmids such as *Staurodesmus convergens* also present only in the creek, *Staurastrum sagitarium*, *Staurastrum sebaldi*, *Spirogyra* spp., *Pediastrum simplex* var. *echinulatum*, *Scenedesmus* spp. (except *S. quadricauda*), *Golenkinia radiata*, *Kirchnerella obesa*, desmids such as *Cosmarium* spp., *Coelastrum tetrapedia*, *Closterium kuetzingi*, *Centritractus rotundata* and *Ankistrodesmus falcatus*. The rare diatoms were *Tabellaria floculosa*, *Synedra* spp. (except *S. ulna* and *S. delicatissima*), *Surirella muelleri*, *Stauroneis phoenicentron*, *Cymbella affinis*, *Cyclotella meneghiniana*, species of *Cocconeis* and *Caloneis*. The dinoflagellate *Peridinium cinctum* and the chrysophyte *Dinobryon cylindricum* were restricted to the creek and the confluence.

Phytoplankton biomass and diversity

The biomass and indices of diversity of the phytoplankton of River Niger is depicted in Figure 2. Biomass represented by cell number is highest in Station 5, generally decreasing from Station 2 through to 4. The richness of species is equally maximum at Station 5 with a similar trend of decrease from Station 2 to 4. The Shannon–Weaver diversity index reached a peak also in Station 5 and a decrease from Station 2 to 4. On the other hand evenness or equitability species was highest in Station 4 and least in Station 2.

The phytoplankton density was dominated by *Aulacosira* spp. The variation pattern in these dominant forms is represented in Figure 3. *A.*

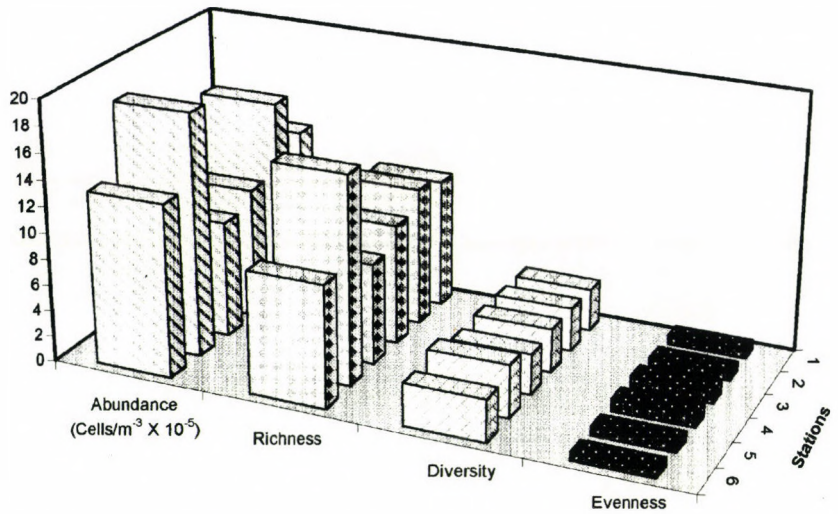

Fig. 2. Phytoplankton biomass and diversity indices of lower River Niger

granulata was maximum at Station 1 with density of 270 × 103 cells m^{-3}. For *A. granulata* var. *curvata*, there was not much discernible spatial variation between the Stations, though it attained a peak of 133 × 103 cells m^{-3} at Station 4. *A. granulata* var. *angustissima* represented the dominant taxon among the *Aulacosira* species in all the stations. Station 2 recorded the maximum, in this taxon with a value of 324 × 103 cells m^{-3}. Interestingly, there was a decrease in this taxon from this Station through to 4 and increased again at Stations 5 and 6. Of all the *Aulacosira* species, *A. granulata* var. *angustissima* f. *spiralis* was the least represented by a density of not more than 38 × 103 cells m^{-3} in any station.

Discussion

The ionic content of River Niger is considered low with a mean conductivity value of 56.4 μS cm^{-1}. It generally falls within the Class I of the popular classification of African waters by Talling and Talling (1965). In this typology, Class I waters are those with conductivity < 600 μS cm^{-1}, while Classes II and III water types have conductivity and alkalinity values of 600–6,000 μS cm^{-1}, 6–60 meq^{-1} and 6,000–160,000 μS cm^{-1}, 60–1,500 meq^{-1}. Class III waters are usually very salinic. River Niger is particularly poor in nitrate, sulphate and phosphate. In comparison with other large African

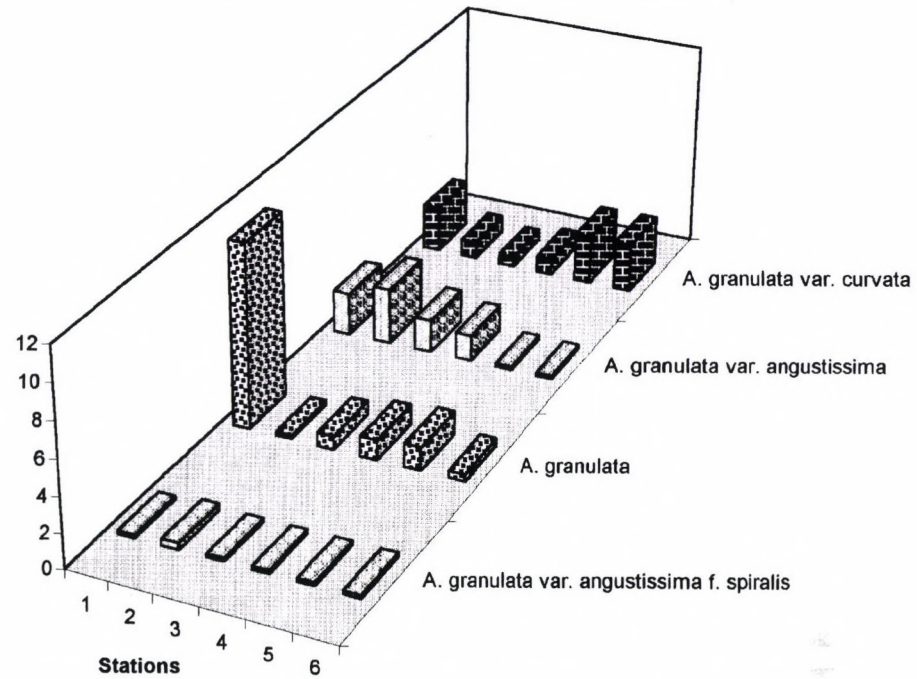

Fig. 3. Amplitudinal variation in the dominant phytoplankton of lower River Niger. (Values are cells $m^{-3} \times 10^5$)

rivers like Sokoto (Holden and Green 1960), Blue and White Nile (Talling and Rzoska 1967), River Niger can be regarded generally as rather dilute except for chloride. This low solute content was also observed elsewhere in the stretch of the river (Daget 1957, Imevbore 1970, Grove 1972). In fact conductivity values as low as 30 μS cm^{-1} were obtained at Diafarabe during the flood season (Daget 1957) and less than between 30 μS cm^{-1} in River Mopti and a rise to 50 μS cm^{-1} below Niamey (Grove 1972).

The order of dominance of cations is Ca > Na > K > Mg and anion as HCO_3 (alkalinity) > Cl^{-1} > NO_3. These orders agree with the report of Imevbore (1970) for the northern area of the river. This trend is however at variance with the commonest order of cation dominance in Africa in which Na is considered to be most important (Talling and Rzoska 1967). Similarly, in terms of actual values there is a lot of agreement in the parameters determined in this study with that of Imevbore in the Kainji area in the northern area of the river except Cl^{-1} in which higher values were recorded in this study.

The phytoplankton of River Niger is characterised by a large variety of taxa. Qualitatively, the taxa were dominated by green algae. This agrees perfectly with earlier studies on algae in the river, though in the stretch outside Nigeria by Bourelly (1957) in which 53% of the total flora were Chlorophyta, mainly desmids. The findings of the present report also accord well with that of Couté and Rousselin (1975) with majority (85.4%) being Chlorophyta, dominated by desmids. The dominance of the phytoplankton species composition by Chlorophyta is typical for most of African waters (Kalff and Watson 1986, Kebede and Belay 1994). According to Wetzel (1983), Chlorococcales species inhabit waters of differing salinity and alkalinity. The occurrence of high percentage of desmids is an attestation of the low nutrient status of the river. Desmids are characteristic of freshwater environments with poor ionic composition (Kadiri 1988, 1993a, b, 1996, Nwankwo 1996).

The euglenoids and blue-green algae were qualitatively less numerous accounting for only 10.7% each. These two groups of algae are characteristic of eutrophic or nutrient-rich water bodies (Gibson and Smith 1982, Caljon 1987, Comforti 1991). Therefore their poor representation is a further corroboration of the poor nutrient status of the river. The fair representation of diatoms could be attributable to alkalinity of the river (Talling and Talling 1965, Kebede and Belay 1994). In a comprehensive survey of African water bodies, the former authors recognised alkalinity as a crucial factor which determines diatom communities. This was further corroborated in a survey of diatoms of East African water bodies where it was found that diatom assemblages in water with such level of alkalinity as in the River Niger were dominated by *Aulacosira* spp. Qualitatively, the phytoplankton flora of River Niger was dominated by *Aulacosira* spp. This is corroborated by the report of Eaton (1965) in the Kainji area. The dominance of *Aulacosira granulata* has been reported in similar water bodies and in fact is considered cosmopolitan. Some of this water bodies include River Oshum (Egborge 1973, 1974), Warri River (Opute 1990), River Nile (Prowse and Talling 1958, Rzoska and Talling 1966).

Spectacularly, Station 5 is very unique in the sense that it represents a confluence of two water bodies – a creek and the River Niger itself. This is reflected in the parameters studied as values obtained here are outstanding between the two ecosystems. For phytoplankton, diversity and biomass were the highest at this station.

The reason being that it represents a more heterogeneous system with varying conditions and thus avail opportunities which permit the develop-

ment of a wide array of phytoplankton flora. According to Pieterse and Zyl (1988), Proulx et al. (1996), Washington (1984), an alteration in environmental factors consequently causes a change in diversity. Report of Watson et al. (1997) stated that differences obtained in terms of biomass, taxonomic diversity could also be attributed specifically to morphological diversity, differential herbivory and mixing regime.

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PLANT SPECIES SUITABLE FOR ESTABLISHING LIVING ROOFS IN HUNGARY I. *SEDUM* SPP.

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Among CAM (crassulacean acid metabolism) plants *Sedum* species are especially suitable as pioneers for establishing living roofs. Under the continental, extreme climatic conditions of Hungary, among the *Sedum* taxa having been planted and observed in Budapest, Mosonmagyaróvár and Pécs for 10 years, the different varieties and forms of *S. acre*, *S. album*, *S. ellacombianum*, *S. floriferum*, *S. hispanicum*, *S. hybridum*, *S. kamtschaticum*, *S. neglectum*, *S. ochroleucum*, *S. reflexum*, *S. rupestre*, *S. sexangulare*, *S. spurium* and *S. urvillei* excel in their adaptation ability, especially in their drought and frost resistance.

Key words: carbon fixation types, drought tolerance, leaf-succulents, roof plants, *Sedum*

Introduction

Succulent plants often exhibit a mode of carbon fixation called crassulacean acid metabolism (CAM). CAM plants under conditions of drought, short hot days and long cool nights, will fix carbon dioxide at night via PEP (phosphoenol pyruvate) carboxylase and store it as malic acid in the vacuole. During the day the malic acid is decarboxylated and the carbon dioxide fixed via rubisco and the Calvin cycle. Some CAM plants are obligate and can only fix carbon in this way. Others are facultative and only use the CAM mode under conditions of water stress.

Water availability must have been the selective factor for the evolution of CAM in land plants, where nocturnal carbon dioxide fixation saves loss of water by transpiration and increases water-use efficiency (Lüttge 1993). Under favourable conditions they can utilise the C_3 mode of photosynthesis. Although certain superficial similarities do exist between C_4 and CAM, the differences are fundamental enough that they probably had a separate origin (Dietz and Keller 1997, Orsenigo et al. 1997).

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The CAM pathway has now been identified in 27 families (Smith 1997). Some families contain all three photosynthetic types. CAM plants appear to be more united by adaptations to xeric environments than by phylogeny.

The roof as a special habitat creates unusual demands for plants, as well, including of drought, UV-B radiation, frost, anthropogenic pollution (smoke, soot, dust), wind and the need for a root system penetrating down to a small depth.

Plants on the roof are rather exposed to high temperatures and direct solar radiation, consequently they have to endure both long-lasting dry periods and short-term abudence of water. The appropriate vegetative or generative reproduction ability of plants which are suitable for planting a living roof is another important characteristic since the equable and rich covering of the surface can be attained only in this way.

In Hungary under the extreme continental climate relatively fewer taxa can be counted on than under humid conditions. Nowadays the interest for living roofs is increasing here, too, since the special technical and architectural conditions are known and the building materials are available (Kerner 1997, Prekuta 1997).

First our 10-year-long experiences gained with *Sedum* taxa belonging to the Crassulaceae family will be summarised. *Sedum* species can be characterised not only by CAM but also by other advantageous features (Praeger 1921, Stephenson 1994). They are not deeply rooted, adaptable species. Richness in forms and colours allows them to be the pioneer plants of the living roof taking into consideration that according to the strategy type classification, most involved species are ruderal, natural pioneers (Borhidi 1995, Priszter 1993).

Regular evaluations concerning drought resistance, good reproduction ability and an attracting, spreading habit have been carried out for 10 years in Budapest (in a collection planted in the garden of a detached house next to Kamara-erdő), in Mosonmagyaróvár (in the representative roof terrace collection of the Department of Botany at the Agricultural University) and for 2 years in Pécs (in the Botanical Garden of Janus Pannonius University).

Results

On the basis of a practical classification (Priszter 1995, Soó 1966, Webb et al. 1993) the taxa considered to have advantageous characteristics are divided into two groups (*Sedum* taxa with cylindrical leaves and *Sedum* taxa with flattened leaves).

Sedum taxa with cylindrical leaves

Sedum acre L. – (SBT = ruderal, R, natural pioneer, NP, Val.: 3)

Together with *S. album* it is widespread in Europe with a highly variable shape. Its densely sessile leaves are small, egg-shaped. Its vegetative reproduction is very fast: its violent spreading exceeds even that of *S. album*. Every small, broken piece of the shoot develops quickly. From the many different forms the dwarf f. *microphyllum* Stefanoff with leaves of only 1 mm diameter seems to be the most suitable.

Sedum neglectum Ten. – (SBT = stress tolerant, ST, specialist, S, Val.: 6)

Reminding of *S. acre*, slightly bigger and has bigger flowers. It is widespread at the coast of the Adriatic Sea, the subsp. *sopianae* Priszter found in the Mecsek and Villány Mts is of a protected value. Its slightly more elongated leaves are archedly leaning out and the dry leaves remain on the stem.

Sedum rupestre L. (= *S. reflexum* L.) – (SBT = ruderal, R, anthropogenic, adventitious element, A, Val.: –1)

It is actually a group of species including several kinds of taxa. The common characteristic of its evergreen leaves is their tip ending in a tiny, translucid spikelet. The narrow-cylindrical, cone- or spindle-shaped, usually greyish green leaves are sessile and they cover the stem densely. Its bright yellow flowers often have 6–7 petals. It is a very diversified species. The typical *S. reflexum* L. var. *glaucum* (Lej.) Janchen has hoary-grey leaves and its young, not open inflorescence is bowing. It is widely distributed in Central and Western Europe.

Another taxon *S. reflexum* L. var. *viride* Koch is a cultivated variety with a similar habit, but with green leaves. It is planted in gardens, but it runs wild very easily everywhere. It naturalised in Hungary, too, and occurs frequently. The f. *cristatum* Praeger is also a promising one, its special feature is the characteristic ribbon-like appearance: its shoot tips look like a cockscomb.

Sedum ochroleucum Chaix is in close relationship with this group of species (*S. rupestre*). This taxon occurs mainly in the Mediterraneum. Its flower is cream-coloured and leaves are greyish green. Another species, *S. sediforme* (Jacq.) Pau is similar to *S. rupestre* or *S. ochroleucum*, but has stronger habit. Its leaves are grey and flat at the top, its length approaches even 2 cm. The height of the inflorescence can reach 50 cm. The thick stem is slightly woody. It is frost-hardy, can be recommended mainly for bigger roof terraces.

Sedum album L. – (SBT = ruderal, R, natural pioneer, NP, Val.: 3)

It occurs throughout Europe except for northern Scandinavia. It can also be found in North Africa, in the Caucasus and Asia Minor.

Its leaves are usually short or elongated cylindrical, their surface is sometimes flattened. From Central Europe towards the south the number of species or subspecies with different leaf shapes is increasing. There are some having ruddy leaves, others are covered by glandular trichomes, others have small flowers and totally globular leaves creeping close to the soil surface. Two taxa of small habit: f. *murale* Praeger and the cultivar 'Coral Carpet' prooved best in our experiments. The subsp. *clusianum* Gussone is a colourful hardy form from Iberia.

Sedum sexangulare L. – (SBT = ruderal, R, natural pioneer, NP, Val.: 3)

It is an excellent carpet plant, it does not spread violently. Its very tiny leaves are very narrow, in top view they are characteristically arranged in 6 rows often brownish red. Its flowers are tiny, lemon yellow. The subsp. *boloniense* (Lois.) Huber originates from the surroundings of the Mediterranean Sea, it is slightly higher, its leaves are always green and are not arranged in 6 rows.

The *S. urvillei* DC. (= *S. sartorianum* Boiss., *S. hillebrandtii* Fenzl) belonging also to here can be planted successfully, too. It is distributed on the Balkan Peninsula and Asia Minor, it is a small plant with very fragile shoots, greyish, elongated leaves having a bit granulated surface, wearing a small spur at their base and remaining on the stem after drying.

Sedum hispanicum Jusl. – (SBT = ruderal, R, natural pioneer, NP, Val.: 3)

The var. *bithynicum* (Boiss.) Sch. et K. originates from the surroundings of the Black Sea and it is not annual like the base species but perennial. It forms a greenish blue, thick and low carpet. Its white flowers are generally sterile.

Sedum taxa with flattened leaves

In contrast with the above main group the leaves of *Sedum* taxa belonging here are succulent but laminar. When winter is approaching, most of them loose their leaves, their shoots above soil level dry. Soon, mainly in autumn but often already at the end of summer new leafy shoots appear out of their usually woody, often nodulous rhizome. They originate from Central and East Asia.

Sedum spurium M. B. – (SBT = ruderal, R, anthropogenic, introduced element running wild, I, Val.: –1)

It is rich in colours and forms, it was introduced to Europe from the Caucasus. It is small-built, with stolons or sarmentums, a hardy species which has mostly been used for planting burial hills. Its opposite leaves are several cm long usually lobed. Its selected cultivars with pink or white flowers are popular and can be applied for establishing living roofs very well. The leaf of cv. 'Tricolor' is varicoloured (with three colours), cv. 'Atropurpureum' has scarlet flowers and red leaves. Among well proved cultivars are cv. 'Roseolum', cv. 'Schorbuser' and cv. 'Salmoneum'.

The leaves of *S. ellacombianum* Praeger which originates from Japan are bright light green, its yellow flowers are filled. Its characteristic feature is that its shoots above soil level totally disappear in winter.

S. kamtschaticum Fisch. et Mey. has also proved excellent. Its orange flowers are bright red in bud stage, its fruit groups are of the same colour. The cv. 'Variegatum' with yellow-multicoloured leaves is worth planting above all.

S. hybridum L. is distributed from the Ural Mts to Mongolia. It has excellent characteristics. (Really it is not a hybrid!) Its leaves are cold tolerant in winter, the plant can be considered as "evergreen" species in Hungary. The intense yellow flowers often appear again in autumn.

S. floriferum Praeger is closely related to *S. hybridum*. It is originated from China. The flowers are yellow, the narrow leaves become red coloured in autumn.

By planting the listed *Sedum* taxa considered the most important by us beautiful and lasting living "roof carpets" with varied colours can be attained under Hungarian conditions. They can also be planted together with taxa from other genera. An account above these will be given later.

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CONTRIBUTIONS TO THE BRYOFLORA OF THE BODROGKÖZ (NE HUNGARY)

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A list of 61 bryophyte species so far recorded in the Bodrogköz (NE Hungary) is presented. Of the 61 species nine are liverworts from eight genera and six families, and 52 are mosses from 33 genera and 18 families. Three species are rare and six are very rare in Hungary as a whole, and two are rare in the Hungarian lowlands.

Key words: liverworts, lowland, mosses, NE Hungary

Introduction

The area called Bodrogköz is the alluvial plain between the rivers Bodrog and Tisza. Holocene alluvial sand, silt and clay cover the plain with Pleistocene sand islands at Tiszakarád, Cigánd, Révleányvár and Zemplénagárd. For details of the geography see Borsy (1969). The climate is moderately warm and dry with cold winters (Péczely 1969).

The Bodrogköz lies in the Samicum phytogeographical district of the Pannonicum flora province. The first overview of the vascular flora and vegetation of the Bodrogköz was given by Tuba (1995). However, there has been no detailed data available regarding its cryptogamic flora. Peciar (1968) summarized the bryophyte flora in the Slovakian part of the Bodrogköz. Boros (1968) mentioned only three bryophyte species: *Neckera besseri* Lob. (an Eurasian-Mediterranean element; rare on the Great Hungarian Plain) from the Long-erdő, *Riccia fluitans* L. and *Ricciocarpus natans* (L.) Corda (cosmopolitan elements) from Pallagcsa-láp near Cigánd. Furthermore, *Pleurozium schreberi* (Brid.) Mitt. (a circumboreal element) is known from Long-erdő (Hargitai 1938).

Our own survey of the moss flora of the Bodrogköz started in 1979. A particular attention was given to the forest fragments. Of course there are species yet to be expected, e.g. *Phascum* and other annual shuttle species.

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The full list of species hitherto collected and identified so far is presented together with their localities, floral element and life strategy.

Species list

The list contains all bryophyte species collected between 1979 and 1984. The nomenclature is according to Frey et al. (1995). The species name is followed by the locality(ies) (for abbreviations see List of localities), specimen number(s) (in brackets), then the flora element categories, and finally the life strategies (During 1979, Orbán 1984) are described. The distribution of the species in Hungary is given after Orbán and Vajda (1983), Boros (1968), and Soó (1973). The specimens are deposited in the Herbarium of the Department of Botany, Eszterházy Károly College of Education, Eger (EGR).

List of localities

Bh = Budahomok: grazed dry sandy grassland (originally *Festucetum vaginatae*)

Bzug1 = Bodrogzug, Tokaj, Kis Bodrog: willow-poplar gallery forest at old earth fort (*Salicetum albae-fragilis*)

Bzug2 = Bodrogzug, between Tokaj and Timár, "Gelin-erdő" (right bank of Tisza): willow-poplar gallery forest at old earth fort (*Salicetum albae-fragilis*)

Dc = Dámóc: alder fen

Dc-Lcs = between Dámóc and Lácacséke: willow fen and tufted sedge (*Caricetum elatae*)

Klő-Zd = between Kenézlő and Zalkod, Gice: on littoral mud

Klő = Kenézlő-Tisza-part: oak-poplar grove on side of embankment and flood area of river Tisza

Long = Sárospatak-Vajdácska, Long-erdő: hornbeam-oak wood (*Querco petraeae-Carpinetum*)

Nh1 = Nagyhomok, Hatház-tanya: wet meadow (*Agrostetum albae*)

Nh2 = Nagyhomok, Detka-dűlő: drying out willow-poplar gallery forest (*Salicetum albae-fragilis*)

Nr = Nagyrozvágy (Sasó-hegy): degraded sand dune

Őrh = Őr-hegy: swamp-mire (*Caricetum acutiformis-ripariae*)

Őrh-Mh = between Őr-hegy and Monyha: dried up alder fen

Ri-Rvl = between Ricse and Révleányvár: ash-elm forest (*Fraxino pannonicae-Ulmetum*)

Ri1 = Ricse: ash-elm forest (*Fraxino pannonicae-Ulmetum*)

Ri2 = Ricse: on stone fence

Ri3 = Ricse: Semjén-erdő (forest ranger lodge), ash-elm forest (*Fraxino pannonicae-Ulmetum*)

Ri4 = Ricse: Pap-erdő, in (regenerating) hornbeam-oak wood (*Querco petraeae-Carpinetum*)

Rvl = Révleányvár: ash-elm forest (*Fraxino pannonicae-Ulmetum*)

Sp = "Ó-Bodrog" at Sárospatak (Füzesér junction): willow-poplar gallery forest (*Salicetum albae-fragilis*)

Tcs = Tiszacsermely: ash-elm forest (*Fraxino pannonicae-Ulmetum*)

Vajd = northwest of Vajdácska: on mud at bottom of drying out backwater bed of river Bodrog

Zard1 = Zemplénagárd: embankment and flood area of river Tisza

Zard2 = Zemplénagárd: clear-felled dried up alder fen

Hepaticae

Ricciaceae

Riccia fluitans L. emend. Lorbeer – Őrh-Mh (81030/A); Nh2 (81025/K); Klő-Zd (81026/B, 81026/C). – Cosmopolitan element. (AS).

R. huebeneriana Lindenb. – Klő-Zd (81026/A). – European element with atlantic character. (AS). Rare in Hungary: Őrség, Vasi-hegyhát, Belső-Somogy. (Nanocyperion).

R. rhenana Lorbeer – Őrh (81024/A); Nh (81025/I). – European-North American element. (AS). Rare in Hungary. On the Great Hungarian Plain (Alföld) only at Tőserdő, Folyás; Penyige and recently was found in Nyírség: Bátorliget (Standovár et al. 1991).

Metzgeriaceae

Metzgeria furcata (L.) Dum. var. *ulvula* Nees – Ri1 (83045/H). – Circumboreal species. (C). Rare in the Great Hungarian Plain (Alföld): Vácrátót, Tatárszentgyörgy, Tőserdő, Nyírség, É-Alföld. (Fagetalia).

Geocalycaceae

Lophocolea heterophylla (Schrad.) Dum. – Ri1 (80010/B, 83045/O, 83045/Q); Ri3 (81029/C, 82026/F); Rvl (80014/E); Ri-Rvl (80015/A, 80021/C); Dc (81022/L). – Circumboreal element. (P).

Radulaceae

Radula complanata (L.) Dum. – Ri1 (83045/E, 83045/S); Tcs (80019/A, 80019/B); Ri-Rvl (80021/E); Ri3 (81029/A). – Circumboreal element. (LS).

Porellaceae

Porella platyphylla (L.) Pfeiff. – Ri3 (82026/H). – Circumboreal element. (LS).

Jubulaceae

Frullania dilatata (L.) Dum. – Tcs (80019/B); Nh2 (81025/C); Ri3 (81029/B, 82026/A); Ri1 (83045/G). – Eurasian element. (C).

Musci

Polytrichaceae

Atrichum undulatum (Hedw.) P. Beauv. – Ri-Rvl (80015/C); Dc (81022/H, 81022/N), Klő-Zd (81026/D). – Circumboreal element. (P).

Polytrichum juniperinum Hedw. – Rvl (80014/B); Zard (80016/C). – Cosmopolitan element. (P).

Ditrichaceae

Ceratodon purpureus (Hedw.) Brid. – Rvl (80014/F); Zard (80016/A); Nh (80017/A); Bh (80018/A); Nr (80020/A); Bh (83043/A). – Cosmopolitan element. (C).

Dicranaceae

Dicranella heteromalla (Hedw.) Schimp. – Dc (81022/B); Klő (81027/D); Bzug1 (82024/B). – Circumboreal element. (C).

Dicranum scoparium Hedw. – Ri1 (83045/D). – Circumboreal element. (P). Rare on the Great Hungarian Plain (Alföld). (Pino-Quercetalia).

Orthodicranum montanum (Hedw.) Loeske – Ri3 (81029/F). – Montane, circumboreal element. (C). Rare in Hungary. On the Great Hungarian Plain (Alföld) occurs only in the forest of Újszentmargita and recently reported from several forests of Nyírség (Jakab 1997, p. 50) (Pino-Quercetalia).

Fissidentaceae

Fissidens taxifolius Hedw. – Ri1 (80010/C, 83045/C); Ri3 (81029/E, 81029/F, 82026/K); Klő (81027/E); Long (81028/A, 82033/C). – Circumboreal element. (C).

Pottiaceae

Barbula unguiculata Hedw. – Ri1 (80010/E); Bzug1 (82024/E, 82024/G). – Circumboreal element. (C).

Pottia truncata (Hedw.) B., S. et G. – Nh (80017/A; Bh (83043/A). – Circumboreal, disjunct element with sub-Mediterranean character, also in South America. (SL).

Tortula muralis Hedw. – Ri2 (81030/B). – Circumboreal element. (C).

T. papillosa Wils. – Nh2 (81025/G). – European-American, disjunct element with atlantic character (also in South America, Australia). (C).

T. ruralis (Hedw.) Gaertn. et al. – Bh (83043/B). – Circumboreal element. (C).

Weissia brachycarpa (Nees et Hornsch.) Jur. – Bzug2 (82025/E). – Circumboreal with Mediterranean character.

Funariaceae

Funaria hygrometrica Hedw. – Rvl (80014/A). – Cosmopolitan element. (F).

Physcomitrium pyriforme (Hedw.) Hampe – Vajd (82032/A). – European-Mediterranean, disjunct element (also in Australia and in Mexico). (AS).

Bryaceae

Bryum argenteum Hedw. – Ri-Rvl (80015/G); Ri2 (81030/A); Bh (83043/A). – Cosmopolitan species. (C).

Bryum caespiticium Hedw. – Zard (80016/B). – Circumboreal element. (C). On the Great Hungarian Plain (Alföld) grows only near forests. (Quercetea pubescentis, Festucetalia val.)

Bryum capillare Hedw. – Ri-Rvl (80015/D). – Circumboreal, disjunct element. (C).

Bryum flaccidum Brid. – Ri1 (80010/D, 83045/I, 83045/N); Ri3 (81029/G, 82026/B); Rvl (80014/D, 83044/A); Ri-Rvl (80021/A); Nh2 (81025/F); Klő (81027/C); Bzug1 (82024/C). – Circumboreal element. (C).

Pohlia nutans (Hedw.) Lindb. – Dc (81022/G); Long (81028/D); Bzug1 (82024/B). – Cosmopolitan element. (C).

Mniaceae

Plagiomnium cuspidatum (Hedw.) Kop. – Ri1 (80010/A, 80010/F, 83045/B, 83045/M); Ri3 (81029/I); Ri-Rvl (80015/K); Tcs (80019/C); Dc (81022/K), Klő-Zd (81026/E); Klő (81027/K); Long (81028/F). – Cosmopolitan element. (LS).

Plagiomnium ellipticum (Brid.) Kop. – Dc (81022/O). – (LS).

Plagiomnium undulatum (Hedw.) Kop. – Dc (81022/M); Klő (81027/H). – Circumboreal with Mediterranean-atlantic character. (LS).

Orthotrichaceae

Orthotrichum affine Schrad. – Ri4 (83046/A). – Circumboreal with Mediterranean character. (C).

Orthotrichum lyellii Hook. et Tayl. – Bzug1 (82024/H); Bzug2 (82025/B). – Circumboreal with subatlantic character. (LS).

Orthotrichum obtusifolium Schrad. – Nh2 (81025/E). – Circumboreal element. (C).

Orthotrichum speciosum Nees – Zard2 (81023/A). – Circumboreal element. (LS).

Orthotrichum sp. – Ri-Rvl (80015/E).

Neckeraceae

Homalia trichomanoides (Hedw.) B., S. et G. – Ri1 (83045/R, 83045/F); Ri3 (81029/H); Ri-Rvl (80021/D); Long (82033/H). – Eurasian element with subatlantic character. (P). Rare on the Great Hungarian Plain. (Fagetalia).

Leskeaceae

Leskea polycarpa Ehrh. ex Hedw. – Ri-Rvl (80015/G); Nh (81025/H); Klő (81027/A); Bzug1 (82024/A); Bzug2 (82025/A, 82025/G); Dc-Lcs (82030/C); Ri4 (83046/B). – Circumboreal element. (P).

Amblystegiaceae

Amblystegium serpens (Hedw.) B., S. et G. – Ri1 (80010/G, 83045/L, 83045/P); Sp (80011/A); Nh2 (81025/C, 81025/D); Bzug1 (82024/F); Ri3 (82026/B, 82026/E, 82026/G); Dc (82029/C); Long (82033/A). – Cosmopolitan element. (P).

Calliergonella cuspidata (Hedw.) Loeske – Zard2 (81023/F); Klő (81027/B). – Cosmopolitan element. (P).

Campylium polygamum (B., S. et G.) J. Lange et C. Jens. – Dc-Lcs (82030/A). – Circumboreal with atlantic character. (P). Not frequent (Molinio-Juncetea).

Drepanocladus aduncus (Hedw.) Warnst. – Zard2 (81023/C); Dc-Lcs (82030/D). – Subcosmopolitan element (P).

Leptodictyum riparium (Hedw.) Warnst. – Bzug2 (82025/C); Dc (82029/A). – Circumboreal element (P).

Brachytheciaceae

Brachythecium albicans (Hedw.) B., S. et G. – Bh (83043/C). – European-North-American element with atlantic-Mediterranean character. (P).

Brachythecium glareosum (Spruce) B., S. et G. – Rvl (80014/H). – Circumboreal element. (P). It occurs everywhere in Hungary, but is not common (Quercetalia pubescentis).

Brachythecium mildeanum (Schimp.) Milde – Nh (80017/A); Bzug2 (82025/F). – Circumboreal element. (P). Not frequent in Hungary. On the Hungarian lowlands: Duna–Tisza köze, Tiszántúl, É-Alföld, Győri-sík.

Brachythecium populeum (Hedw.) B., S. et G. – Tcs (80019/E). – Circumboreal element. (P).

Brachythecium rutabulum (Hedw.) B., S. et G. – Ri1 (80010/I, 83045/R); Dc (81022/C, 81022/F); Long (82033/B). – Circumboreal, disjunct (also in South America) element. (P).

Brachythecium salebrosum (Web. et Mohr) B., S. et G. – Ri-Rvl (80021/B); Ri3 (82026/I); Long (82033/E). – Circumboreal, disjunct element (also in Australia). (P).

Brachythecium velutinum (Hedw.) B., S. et G. – Bzug1 (82024/C); Ri1 (83045/R); Ri3 (82026/B); Dc-Lcs (82030/B); Long (82033/F). – Subcosmopolitan element. (P).

Eurhynchium schleicheri (Hedw. f.) Jur. – Ri1 (80010/N); Ri-Rvl (80016/H); Zard (80016/D). – (P).

Eurhynchium hians (Hedw.) Lac. var. *swartzii* – Ri1 (80010/K, 83045/A, 83045/C); Ri-Rvl (80015/I); Zard2 (81023/E); Nh (81025/A); Long (81028/C, 82033/D). – Circumboreal element. (P).

Pterigynandraceae

Pterygynandrum filiforme Hedw. – Klő (81027/C). – Circumboreal element. (P). Very rare in the lowland forests: Vácrátót, Tőserdő, Újszentmargita, É-Alföld. (Fagetalia).

Plagiotheciaceae

Plagiothecium cavifolium (Brid.) Iwats. – Dc (81022/E); Ri3 (81029/D). – (P).

Plagiothecium denticulatum (Hedw.) B., S. et G. – Rvl (80014/C); Ri1 (83045/K, 83045/T). – Cosmopolitan species. (P).

Hypnaceae

Hypnum cupressiforme Hedw. – Ri1 (80010/M); Ri-Rvl (80015/F); Rvl (80014/G); Tcs (80019/D); Dc (81022/A); Ri3 (82026/D). – Cosmopolitan species. (P).

Platygyrium repens (Brid.) B., S. et G. – Zard2 (81023/B); Bzug1 (82024/I); Ri3 (82026/L); Long (82033/G). – Circumboreal element with subatlantic character. (C).

Pylaisia polyantha (Schreb.) B., S. et G. – Ri3 (2026/D, 82026/C). – Circumboreal element. (P).

Table 1

Phytogeographical composition of the bryophytes from the Bodrogköz (NE Hungary)

Flora element	No of species	Percentage (%)
Circumboreal	39	63
Cosmopolitan	14	22
European	1	2
European and North American	2	3
European–Mediterranean	1	2
European–American–Australian	1	2
Eurasian	1	2
Eurasian and North African	1	2
Eurasian–Mediterranean	1	2

Most of the bryophytes of the Bodrogköz are of circumboreal (63%) and cosmopolitan (22%) distribution. The number of other categories is low.

Table 2

Life strategy spectrum of the bryophyte species of the Bodrogköz (NE Hungary)

Type of life strategy	No of species	Percentage (%)
Fugitive (F)	1	2
Colonists (C)	19	30
Annual shuttle species (AS)	6	9
Short-lived shuttle species (SL)	2	3
Long-lived shuttle species (LS)	7	11
Perennials (P)	29	45

Most species are perennial (45%) which can be attributed to the remnant forests providing ensuring relatively permanent environment for the bryophytes. Colonists make up the second highest proportion of the species in the strategy classes. A possible reason for this is that most of the habitats are periodically inundated by water. Other strategy types are scarcely represented.

Discussion

The Bodrogköz which lies in NE Hungary seems to be poorer in mosses than that of the northern Slovakian part where there are even five *Sphagnum* species present in the peat bogs. However, thirteen bryophytes recorded here are new for the whole area of the Bodrogköz (*Riccia huebeneriana, Porella platyphylla, Weissia brachycarpa, Plagiomnium ellipticum, Orthotrichum lyellii, Neckera besseri, Campylium polygamum, Brachythecium glareosum, B. mildeanum, B. rutabulum, Eurhynchium praelongum, E. schleicheri, Plagiothecium cavifolium*).

There is no data from the neighbouring Rétköz.

The Szatmár-Bereg Plain (also in the Samicum area) has a richer bryophyte flora (Fintha 1994) owing to its *Sphagnum* bogs and also some montane-type forest. Twenty-four woodland moss species are common to the Szatmár-Bereg Plain and the Bodrogköz.

Although the species list of the Bodrogköz is not at all complete, making a comparison with the bryophyte flora of the Nyírség – which was published by Jakab (1997, 1998) – the Bodrogköz seems to be poorer. However, the species that occur in both regions and are rare on the Alföld (Hungarian lowland), show a bryogeographical relationship between the Bodrogköz and the Nyírség and a connection with the mountains of the Carpathians. Such species are: *Metzgeria furcata, Riccia rhenana, Lophocolea heterophylla, Orthodicranum montanum, Bryum caespiticium, Pohlia nutans, Plagiomnium ellipticum, Homalia trichomanoides, Eurhynchium schleicheri, Pterygynandrum filiforme, Plagiothecium denticulatum.*

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ASSOCIATUM CAN BE GREATER THAN FLORULA DIVERSITY

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It is shown by a theoretical and a practical example that associatum can be greater than florula diversity, in contrary to the widely accepted views. In general, a sufficient condition, which in the special case when all florula probabilities are equal is also necessary, is given.

Key words: associatum, distinctiveness, floral diversity, information theory, interdependency, microtopography, scale-interval

Information theory methods for vegetation research were proposed by Juhász-Nagy (1967, 1976, 1984) and proved to be very fruitful in the study of spatial processes and succession (Juhász-Nagy and Podani 1983, Bartha 1992, Podani et al. 1993). Therefore, it is important to understand the correct relationship between the functions included in the above-mentioned methodology.

Juhász-Nagy (1984) stated that floral (or florula as named by Juhász-Nagy and Podani) diversity "is served as an upper bound for associatum estimates, or even, for associatum". His Fig. 3 shows that "Associatum (mIj(λ)) is a measurable subset of the intersection in a Venn-diagram, whose bound is mHj(Φ), floral diversity". However, the *elements* of any set appearing in his Venn-diagram were never specified, which is thus a nonsense.

These methods were used by me for microtopographic research of some fen communities in Hungary and some interesting results are obtained, which will be published later. One of the spatial processes investigated shows clearly that associatum is greater than florula diversity on an interval of sample areas (Fig. 1).

In order to make this problem clear, let us consider the following, very simple elementary table (ET):

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	$q1$	$q2$	$q3$	$q4$	...	and so on,
a	1	0	1	0	...	
b	1	0	1	0	...	
c	0	1	0	1	...	
d	0	1	0	1	...	(ET1),

where a, b, c, d are the species occurring in the samples (s = 4). For the sake of simplicity and brevity, suppose that m, the number of sample sites is even (this assumption is not important). For m = 8, this elementary table is essentially the same as (V; 2) of Juhász-Nagy (1976).

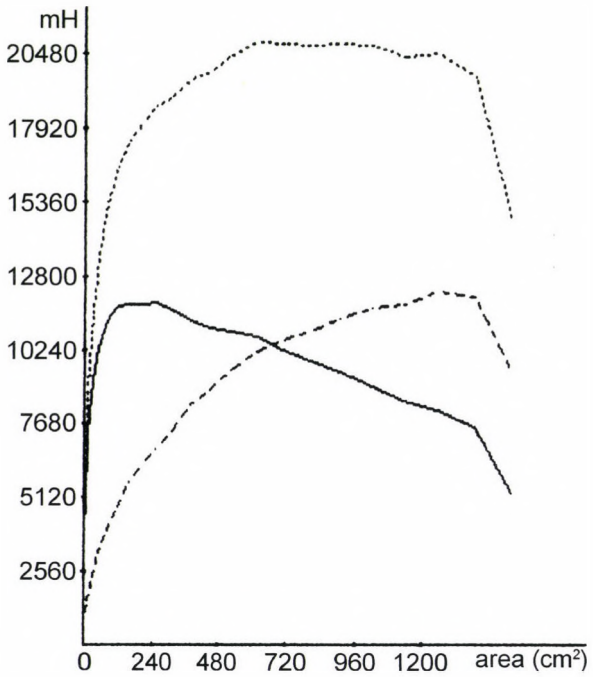

Fig. 1. Local distinctiveness (upper dotted line), florula diversity (solid line), and associatum (lower dotted line) as functions of sample area in a stand of *Caricetum rostratae* Osvald 1923 emend. Dierßen 1982 by Szőce (West Hungary), when herbs and mosses (10 and 5 species, resp.) are investigated together. Sampling was based on a point map surveyed beforehand. Sampling sites were circles with radii of 0...22 cm (23 radii in all, with a step of 1 cm. There was no sense in inserting additional radii because of the deficiences in measuring). Each sampling circle was applied m = 2000 times. Information were counted in bits. It is worth mentioning that without mosses or without herbs no intersection occurs (florula diversity is higher than associatum at all scales except when each value is zero). This indicates very strong interdependences between mosses and herbs in some interval of scales

Let us notice that there are only 2 species combinations (florulas) in (ET1), namely {a,b} and {c,d}, each of them possessing a frequency of m/2 (f(1) = f(2) = m/2). The local valence of each species is n(i) = m/2, so m–n(i) = m/2. With these values, we obtain for associatum (the base of logarithm is 2 everywhere in the following):

$$\mathrm{ASSOC} = (s-1)\, m \log(m) + \sum_k f(k) \log(f(k)) -$$

$$- \sum_i (n(i) \log(n(i)) + (m - n(i)) \log(m - n(i)) =$$

$$= 3\, m \log(m) + 2\, ((m/2) \log(m/2) - 4\, ((m/2) \log(m/2) +$$
$$+ (m/2) \log(m/2)) = 3m$$

and for florula diversity,

$$\mathrm{FLORDIV} = m \log(m) - \sum_k f(k) \log(f(k)) = m \log(m) - 2\, ((m/2) \log(m/2)) = m$$

Because m is a positive number, 3m is always greater than m, therefore associatum is greater (3 times) than florula diversity. Of course, (ET1) was chosen so that there are very strong positive and negative associations, on the other hand, few florulas in it. Nevertheless, some communities consisting of hummocks and hollows can show (at some scales) a similar structure. This is interpretable by a self-organising process treated in detail for tussock communities very recently (Lájer 2000).

In general, we can formulate the following:

Theorem. – Let us consider an arbitrary elementary table (ET), with s occurring species (or other attributes) and m sample sites, in the Juhász-Nagy's sense. Let P(f) be the probability of florula f in (ET) and p(i) the probability (in ET) of the i-th species state (presence or absence of the i-th species) appearing in f. If

$$P(f)^2 > \prod_{i=1}^{s} p(i) \qquad \textbf{(1)}$$

for all f occurring in (ET), associatum mI(ET) is greater than florula diversity mH(ET). In the special case when P(f) is the same for all occurring f, this condition is also necessary.

Proof. – First of all, associatum and florula diversity will be written in more compact forms.

$$mH(ET) = m \log(m) - \sum_f x(f) \log(x(f)) =$$

$$= m \log(m) - m \sum_f P(f) \log(mP(f)),$$

where x(f) is the frequency of f in (ET). This expression gives

$$mH(ET) = -m \sum_f P(f) \log(P(f)) \qquad \textbf{(2)}$$

$$mI(ET) = ms \log(m) - \sum_i (n(i) \log(n(i)) + (m - n(i)) \log(m - n(i))) +$$

$$m \sum_f P(f) \log(P(f)) =$$

$$ms \log(m) - m \sum_i \sum_p p(i) \log(mp(i)) + m \sum_f P(f) \log (P(f)) =$$

$$- m \sum_i \sum_p p(i) \log(p(i)) + m \sum_f P(f) \log(P(f)) = - m \sum_1 .. \sum_3 P(f) \log(p(i)) +$$

$$m \sum_f P(f) \log(P(f)),$$

where p below Σ means that summation is performed over presence and absence of species i, and $\Sigma .. \Sigma$, 1..s denotes that s such summations are performed (for each species occurring). We obtain

$$mI(ET) = m \sum_f P(f) \log(P(f) / \prod_{i=1}^{s} p(i)) \qquad \textbf{(3)}$$

(this is Kullback's discrimination information in favour of dependence against independence).

Suppose, that $P(f)^2 > \Pi p(i)$ for all f with positive P(f) in **(3)**. Then

$$\log(P(f)^2) > \log (\Pi p(i)),$$

because logarithm is a strictly monotone growing function. Thus,

$$\log(P(f) / \Pi p(i)) > \log(P(f) / P(f)^2) = -\log(P(f)),$$

and $mI(ET) = m \Pi P(f) \log(P(f) / \Pi p(i)) > -m \Pi P(f) \log(P(f)) = mH(ET)$.

If $mI(ET) > mH(ET)$ and, for all florulas occurring, P(f) = P does not depend on f,

$$m \omega P \log(P / \Pi p(i)) > - m \omega P \log(P),$$

where ω is the number of florulas occurring in (ET). After reducing the latter inequality we obtain

$$P(f)^2 > \Pi p(i).$$

Q.E.D. – It is worth mentioning that if P(f)-s are different, associatum can be greater than florula diversity even when **(1)** is fulfilled only for the more frequent florulas. Of course, their number depends on the distribution of florulas.

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SELF-ORGANISING STRUCTURE IN TUSSOCK PATTERNS

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A model based on a scalar reaction-diffusion equation is proposed to explain tussock development in wetlands. It involves positive and negative feedbacks as well as an interaction of autogenic mechanisms and allogenic causes. The model exhibits self-organisation and a phase transition like phenomenon: under certain definable circumstances tussocks can develop, otherwise they cannot. The transitional state provides a microtopographical diversity. The conditions for different outcomes of the model are discussed. The treated process is considered as an example of ecosystem engineering.

Key words: ecosystem engineers, phase transition, reaction-diffusion equation, self-organising, tussock, wetland

Introduction

Tussock sedge beds (*Caricetum elatae* Koch 1926) and related communities are very characteristic of the Hungarian mires. Their past century investigator, Anton Kerner introduced the Hungarian word "zsombék" into the international scientific usage (German: "Zsombék-Formation"). This type of vegetation with its pole- or bell-shaped mounds has a striking appearance. Its structure is interpreted conventionally as resulting from the growth habit of the dominant species (Koch 1926), which is rather a circular argument (i.e. tussocks appear because that species forms tussocks). Actually such species with creeping rhizomes as *Carex nigra, C. gracilis* (see also Hejný 1960), *C. acutiformis, C. buekii*, that usually constitute horizontally extended clones, under particular circumstances can also form tussocks. On the other hand *Carex elata, C. appropinquata, C. davalliana* and other tussock-forming species sometimes appear in meadow-like stands (Kovács 1957, Lájer 1998). Such communities are described also by Klötzli (1969) from Switzerland. He states the following: "Trockenere Ausbildungen des *Caricetum elatae* sowie diejenigen nährstoffärmerer und durch nur schwache Spiegelschwankungen ausgezeichneter Muldenlagen bilden einen dicht geschlossenen Rasen aus" (i.e. drier formations of *Caricetum*

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elatae as well as which are in hollows poor in nutrients, and with only weak fluctuations of water level form tight closed grasses). In this respect see also Krausch (1964) who informs about non-tussock like *Carex appropinquata* stands, or Passarge (1955) etc.

It was earlier suggested (Lájer 1998) that tussock formation presumably involves a positive feedback process. Indeed, permanently stagnant water is unfavourable for plant life due to anoxia and toxic compounds developing in such a medium. Prolonged submergence on organic, highly reductive substrates causes a growth reduction also in wetland species (van den Brink et al. 1995). Absorptive roots entirely deprived of oxygen cannot survive a long time (Crawford 1996). Vartapetian and Andreeva (1986) showed that 24 hours of anoxia was sufficient to cause irreversible destruction of mitochondrial membranes in some wetland species. Under such circumstances each process (starting from random fluctuations) that results in emerging over the permanent water level surfaces is to a greater extent favourable for plant life. The plants growing wild contribute further to the rising of surface so this process is self-reinforcing.

The concept of reaction, i.e. vegetation modifies its environment, was introduced by Clements (1916). This concept was rather neglected in vegetation science for a long time because of such fashionable trends as environmental determinism, interpretations by cyclic successions, disturbances, etc. Nevertheless, recently Roberts (1987) emphasised again the importance of reactions in vegetation dynamics and recognised the organisation of these into positive and negative feedback loops. He states: "Two similar environments which become part of different feedback loops may diverge." This phenomenon is essentially the same as that was later named "vegetation switches" (Wilson and Agnew 1992), i.e. a vegetation state modifies its environment so that it will be more favourable to itself. Such a process can produce sharp ecotones. Agnew et al. (1993) explained so a sharp ecotone between beech-podocarp forest and mire vegetation in New Zealand.

In the followings I propose a model based on a scalar reaction-diffusion equation to explain the formation of tussock patterns. This model involves positive and negative feedback loops that lead necessarily to sharp ecotones at small scales. A good introduction to the applications of partial differential equations in ecology is given by Holmes et al. (1994). Reaction-diffusion equations are treated thoroughly by Britton (1986) and, at an intermediate level, by Murray (1989). These methods seem very poorly represented in vegetation science, while they are increasingly used in other fields of ecology.

Results

Let N(i, x, y, t) be the tussock height of the i-th clone ($N \geq 0$). This is assumed to depend on the horizontal coordinates (x, y) and time (t). The partial differential equation governing the tussock development:

$$\partial N/\partial t = a \cdot N \cdot (1 - N/K) + D \cdot (\partial^2 N/\partial x^2 + \partial^2 N/\partial y^2) \qquad \textbf{(1)}$$

where a is the vertical growth rate of tussocks. The first term on the right hand side is familiar in population dynamics as logistic growth but here figures in a different sense, namely that of vertical growth of rhizomes. This gives an expression to the assumption (mentioned in the introduction) that, while small relative to K, the higher the tussock, the stronger is its growth (because the conditions for plant life are better due to keeping away of anoxia). However, when a tussock grows too high ($N \geq K$), the moisture and nutrients will be insufficient to ensure further growth.

The second term on the right-hand side describes the horizontal growth of rhizomes.

This means a two-dimensional diffusion assuming that growth of rhizomes takes place in every direction with equal probabilities. Moreover, it is supposed that the time and length scale of rhizome growth is much smaller than that of observation (Okubo 1980). Cain (1990) used successfully a diffusion model for long-term clonal growth of *Solidago altissima*. D is the coefficient of diffusion and assumed to be the greater, the stronger is the effort to avoid competition within the tussock. a, D, K are supposed to be constant.

When two different clones touch (say i and j) equation **(1)** alters as follows:

$$\partial N(i)/\partial t = a \cdot N(i)\,(1 - N(i)/K) + D \cdot (\partial^2(N(i) - b \cdot N(j))/$$
$$/\partial x^2 + \partial^2(N(i) - b \cdot N(j))/\partial y^2 \qquad \textbf{(2)}$$

where b is a constant expressing the competition between tussocks. In the present investigations usually $b = 1$, but the results with $b = 0$ are very similar. If $b < 0$, then the tussocks tend to fuse.

There are some constraints that restrict tussock size also horizontally. Indeed, we can assume that nutrient uptake extends over an area while nutrient supply occurs through the outline of this area. Therefore, when the area of tussock exceeds a threshold value, the nutrient supply can no longer meet its consumption. Let now s be the shoot density and suppose that

this is constant. If c is the concentration of the limiting nutrient (e.g. absorbable nitrogen), then (taking the starting point of coordinate system to the centre of tussock's base) the equation describing concentration changes within cylindrically symmetric tussocks:

$$[2 \cdot \pi \cdot (r + \Delta r) \cdot f - 2 \cdot \pi \cdot r \cdot f] \; \Delta c/\Delta r = 2 \cdot \pi \cdot r \cdot \Delta r \cdot s \cdot B \qquad \textbf{(3)}$$

where B and f is the specific uptake rate of nutrient and its diffusion rate, respectively. r is the distance from origo. Reducing the equation we obtain

$$dc/dr = r \cdot s \cdot B/f \qquad \textbf{(4)}$$

The solution of this ordinary differential equation gives an expression for c at tussock boundary:

$$c = s \cdot B \cdot A/(2 \cdot f \cdot \pi) + c^\circ \qquad \textbf{(5)}$$

where c° is the nutrient concentration at the centre of tussock and A is the tussock's basal area. Because of $c^\circ \geq 0$,

$$A \leq 2 \cdot \pi \cdot f \cdot c/(B \cdot s) = cmx \qquad \textbf{(6)}$$

i.e. there is a maximal area (clone size) of tussocks. This will be indicated by cmx in the followings. A consequence of **(6)** is that, all other things being equal, the maximal clone size (cmx) is proportional to the nutrient concentration (c).

Now the problem is to solve **(1)** or **(2)** with the boundary conditions following from **(6)** and initial conditions taking as random fluctuations.

We search for solutions of **(1–2)** on a square of size L × L, so that

$$N(0, y, t) = 0,$$

$$N(L, y, t) = 0,$$

$$N(x, 0, t) = 0,$$

$$N(x, L, t) = 0$$

and

$$\iint \chi(0, N(i, x, y, t)\, dx\, dy \leq cmx \text{ for each i,} \qquad \textbf{(7)}$$

where χ (ε, N) is the characteristic function of the set $\{N \mid N > \varepsilon\}$. As initial conditions, point-like random excitations are applied, each of them corresponds to different clones.

A solution of **(1)** with boundary conditions **(7)** is N(i, x, y, t) = 0. This means a state devoid of tussocks. Now the question arises that under

which circumstances is this state stable. Starting the system with a single excitation, we can investigate the possibility of tussock development. The horizontal projection most favourable for tussock formation is circle, so **(7)** may be replaced with $N(x, y, t) = 0$ at a circle of radius $R = (\sqrt{}cmx)/\pi$. The centre of circle is at the point of initial excitation. The solution of **(1)** with such conditions is

$$N(r, t) = \sum_k c(k) \cdot \exp(\lambda(k) \cdot t) \; J°(k \cdot r) \qquad \textbf{(8)}$$

where $J°$ is the 0-th order Bessel function, $r = \sqrt{(x^2 + y^2)}$ is the distance from origo. $J°(k\ r)$ is a solution of the eigenvalue-problem:

$$\partial^2\varphi/\partial x^2 + \partial^2\varphi/\partial y^2 + k^2 \cdot \varphi = 0 \qquad \textbf{(9)}$$

with the given boundary and initial conditions (in respect of Bessel functions, see Bateman and Erdélyi 1953). This can be seen clearly by writing (9) in polar coordinates. In **(8)**,

$$\lambda(k) = a - D \cdot k^2 \qquad \textbf{(10)}$$

and

$$k = \eta/R$$

where η is a zero of $J°$ (i.e. $J°(\eta) = 0$. This is realised by several η-s, each of them is a mathematical constant). Let now η' be the first zero of $J°$. The eigenvalue λ' corresponding to this is the maximal one:

$$\lambda' = a - D \cdot \eta'^2/R^2 = a - D \cdot \eta'^2 \cdot \pi/cmx \qquad \textbf{(11)}$$

If $\lambda > 0$, i.e. $a/D > \eta'^2 \cdot \pi/cmx$, then the tussock height grows exponentially at first. As N approaches K, the growth slows down so that the stationary tussock height is $N = K$.

If $\lambda < 0$, i.e. $a/D < \eta'^2 \cdot \pi/cmx$, then no tussock can endure permanently. The initial excitation drops exponentially so that $N \to 0$ as $t \to \infty$.

If $\lambda = 0$, i.e. $a/D = \eta'^2 \cdot \pi/cmx$, then asymptotically ($t \to \infty$):

$$N(r, t) = s \cdot J°(\eta' \cdot\ r/R)/(cmx \cdot J'^2(\eta')) \qquad \textbf{(12)}$$

where s is the height of initial excitation, J' is the 1st order Bessel function. This solution does not change in time and is proportional to s.

Summing up the foregoing, if a/D is greater than a critical value, then the state $N = 0$ is not stable, tussock beds arise even due to smallest excitations. This process is a self-organisation in the sense of Haken (1978), i.e. a uniform structure develops from random initial state (the height of tussock

is independent of the initial excitation). If a/D is smaller than the critical value, however large initial excitation drops exponentially, i.e. the N = 0 (devoid of tussocks) state is asymptotically stable. If $a/D = \eta'^2 \cdot \pi/cmx$, the final height of tussock depends on the initial excitation. If by this condition initial excitations appear with random heights, the developing tussocks take random sizes (Fig. 1).

This phenomenon is analogous with phase transitions. Actually, we can consider the presence or absence of tussocks as an order parameter. Moreover,

$$\tau = 1/\lambda'$$

can be regarded as the characteristic time of the system. τ diverges near the transition point: if $a/D \to \eta'^2 \cdot \pi/cmx$, then $\tau \to \infty$. The spatial correlation and covariance functions of stationary states were calculated from $\chi(\varepsilon, N)$ at $a/D = \eta'^2 \cdot \pi/cmx$ and $a/D = 10 \cdot \eta'^2 \cdot \pi/cmx$ on a 200 × 200 square grid, where the distance between grid points was 0.1 (in any units) and the maximal clone size was $cmx = 400\ \pi$ (Fig. 2). Each function is depicted by double logarithmic representation. Only the first positive half-wave is retained (each of the functions has several half-waves because of some periodicity connected with the maximal clone size in the system). Either function is in-

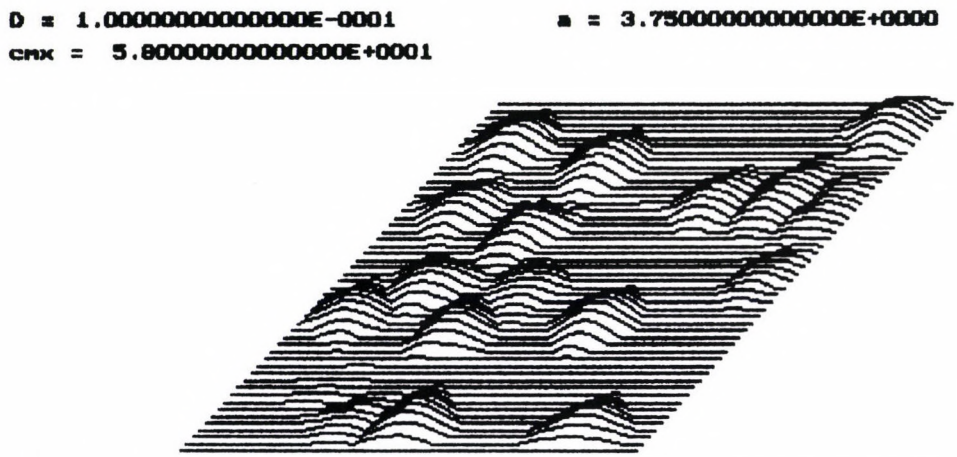

Fig. 1. A stationary state at the transition point. The lower picture is a top-view, where the points of N > 0.01 are dark ($\chi(0.01, N)$). Each of the initial excitations differed from zero only in one point. Their heights were sampled from a normal distribution with 0 mean, and $\sigma = 0.034$ dispersion. For more details, see in text. It is worth mentioning that a similar state occurs in the course of development far from the transition point, but in that case the system changes continuously towards a more uniform state

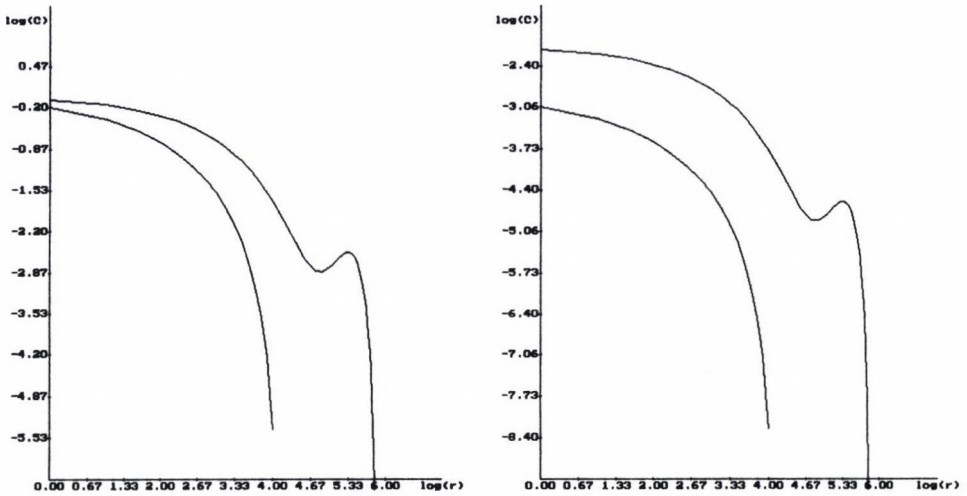

Fig. 2. Averaged (over 50 realisations) correlation (above) and covariance (below) functions of the characteristic functions in stationary states at the transition point (upper curve) and far from it (lower curve). The definition of covariance function: $\mathrm{Cov}(r) = \iint(\chi(r + r') - \langle\chi\rangle)(\chi(r') - \langle\chi\rangle)dr'$, where $\langle\ldots\rangle$ means averaging in space. The correlation function is $c(r) = \mathrm{Cov}(r)/\langle(\chi - \langle\chi\rangle)\rangle$. The functions are depicted, by double logarithmic representation, until the first point where they become negative or zero. The heights of initial excitations were sampled from a normal distribution with 0 mean, and $\sigma = 0.034$ dispersion. For more details, see in text

creased and widened at the transition point. Moreover, the first two half-waves have fused into a joint one. The increase is more explicit in the case of covariance function, indicating that variance is also greater at the transition point than far from it. Considering only one tussock, the correlation function differs from zero only at the transition point.

Equations **(1–2)** were solved numerically with various parameters ($a/D \geq \eta'^2 \cdot \pi/cmx$) on a 50×50 square grid. If the initial excitations are well spaced, the arising structure consists of uniform, regular tussocks (Fig. 3). Otherwise, the developing tussocks influence one another (see eqn. **2**) and their shapes will be more irregular (Fig. 4). Near the transition point, the development is very slow and the tussocks tend to be bell-shaped, rather regular and well spaced, even if the initial excitations were close one to another (Fig. 5). Far from the transition point, the shape of tussocks will be pole-like and the stationary state is achieved much quicker. The tussocks are more irregular than near the transition point because they have not enough time to move one from another. The stationary state is very stable with respect to proportional changes, e.g. after scaling down the height of

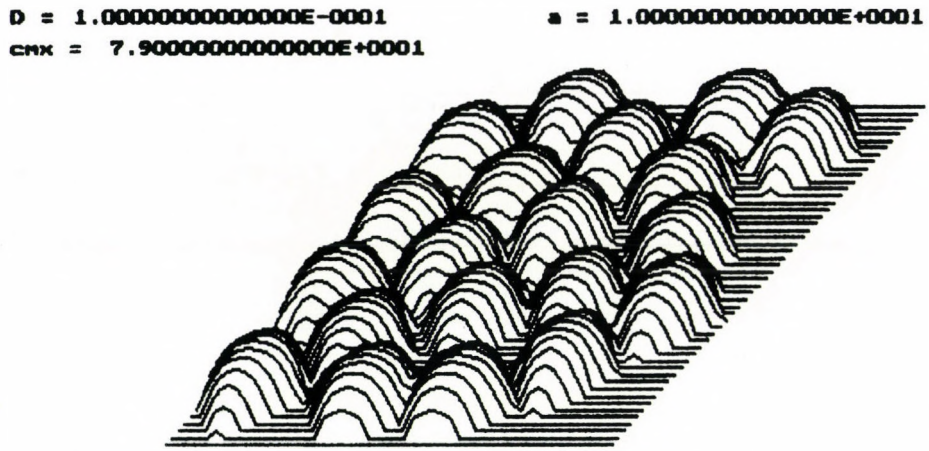

Fig. 3. A stationary state of tussock pattern far (but to a lesser degree as in the case of Fig. 4) from transition point. D = 0.1, a = 10, cmx = 76, K = 0.5. The initial excitations were the same as in the case of Fig. 1

tussocks by 90%, the original structure will be restored. Nevertheless, stability can become damaged if the disturbance is not proportional. Thus, if tussocks are diminished in a part of the system, the neighbouring tussocks, maybe, somewhat extend to a part of this area.

A system with irregular tussocks can alter irreversibly after parameter changes. Thus, if a/D is diminished in a stationary state, the tussocks with smaller basal areas can disappear, and the remaining tussocks become

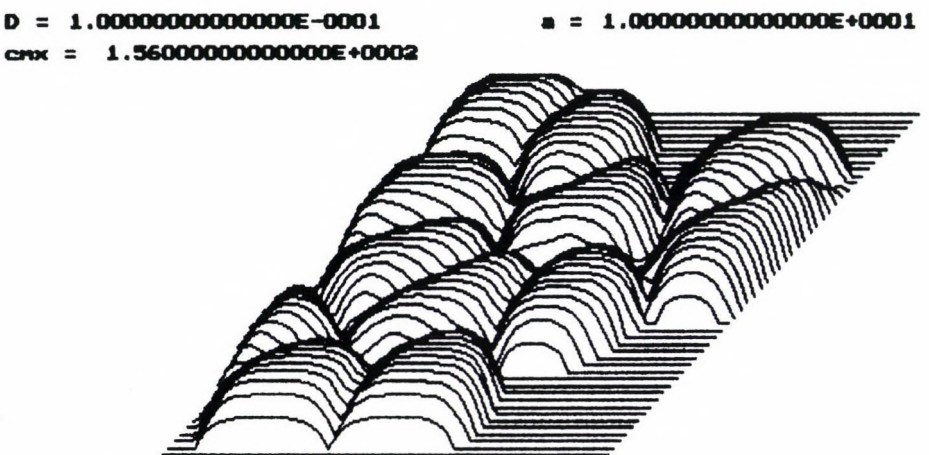

Fig. 4. A stationary state of tussock pattern far from transition point, when initial excitations were in part close one to another. D = 0.1, a = 10, cmx = 156, K = 0.5

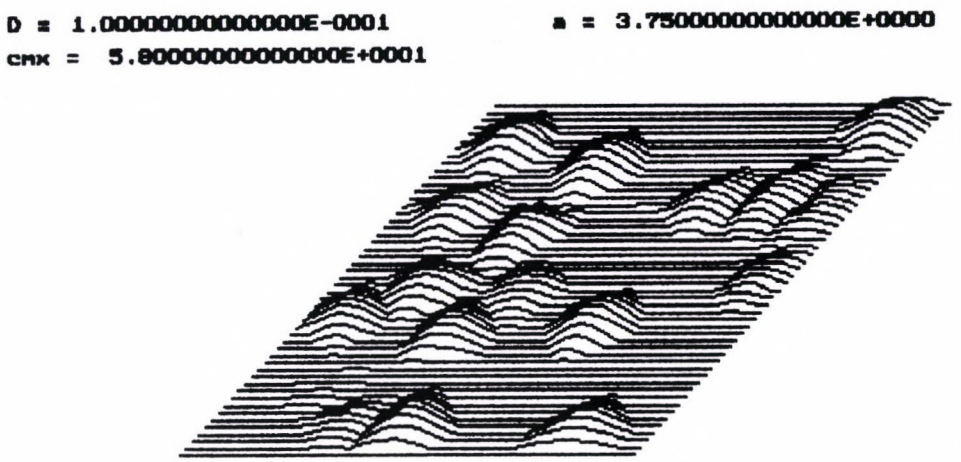

Fig. 5. A tussock pattern near the transition point, after 33,000 time steps. Stationary state is not achieved yet, but changes are extremely slow. Initial excitations were the same as in the case of Fig. 4. D = 0.1, a = 3.75, cmx = 58, K = 0.5

more regular. If in the new stationary state a/D will be increased, the original state will not be recovered, even if the original excitations are applied.

In general, the original state can only be recovered by driving the system through the transition point.

Discussion

In vegetation science, it is important to reveal mechanisms responsible for spatial structures. Single species cellular automaton models have shown, that sharp discontinuities in population density can arise along smooth environmental stress gradients (Wilson et al. 1996, Wilson and Nisbet 1997). While cellular automaton models (as in statistical physics) are important in exploring new general ideas, partial differential equations are more suitable to approximate a concrete spatial problem. Moreover, plants can move (van der Maarel 1996) and do not always constitute such a rather rigid arrangement as suggested by Czárán and Bartha (1992). Nevertheless, the relevance of reaction-diffusion equations is admitted also by the latter authors. The aim of the model proposed here is to understand the developing of tussock patterns. According to my knowledge, this is the first one, based on a reaction-diffusion equation, that exhibits a self-organisation of vegetation. Moreover, it has a phase transition: under circum-

stances tussocks can form, otherwise they cannot. At the transition point, a variety of tussock heights can arise.

Vegetation is treated generally as a self-organising system by Pignatti (1996). However, his conclusion, that "It appears impossible to characterise this system with mathematical expressions, because most of the basic processes are non-linear" seems to be false in the light of quick development in this area. The latter manifests itself also by the fact, that already such particular mathematical journals exist as "Nonlinearity".

There are some experiences supporting the assumption that nutrient supply is an important factor influencing the development of tussocks. Really, increased nitrogen supply can stimulate shoot biomass production in wetlands (Solander 1982, 1983, Verhoeven et al. 1983). According to **(6)**, tussocks of a particular species (with given a and D) cannot arise by too low nutrient concentrations. I examined 5 regular (columns 7–9, 13, and 14 of Table 31 in Lájer 1998), and 5 meadow-like stands (columns 1–6, ibid.) of *Caricetum appropinquatae* in order to compare them with respect to N supply. This was based on ecological indicator values established for the Hungarian flora by Borhidi (1995). Each quadrat had a size of 25 m^2. The meadow-like stands consisted of flat tussocks of variable size, so these can be regarded as being approximately transitional. The distribution of indicator values (Fig. 6) shows a clear shift to low N-values in the case of transitional relative to the regular stands. The difference between the two distributions is highly significant (according to χ^2-test the null-hypothesis of being identical can be rejected with a probability, $p > 99.95\%$). This is in accordance with the model. It is worth mentioning that there is a difference between the two types of stands also in water level, albeit not so significant than in N values. The water supply is somewhat richer in the case of regular stands. Because the higher water level (and higher fluctuations of it) can enlarge a/D, it acts in the same direction as an enhanced N supply. In this regard, see also the passage quoted from Klötzli in the introduction.

Similar conclusions can be drawn from the results obtained by Tomaszewska (1993). She investigated shapes and stratigraphy of *Carex appropinquata* tussocks in Poland. Rather oligotrophic species as *Carex chordorrhiza, Menyanthes trifoliata* were found only in the stand of hemispherical tussocks while *Lycopus europaeus, Cirsium palustre, Peucedanum palustre* were present only in that of pole-like tussocks.

As a result of the model was stated above, that near the transition point the tussocks are bell-shaped. This was experienced by Tsuyuzaki and Tsuji (1992), who investigated *Carex meyeriana* tussocks in China. The

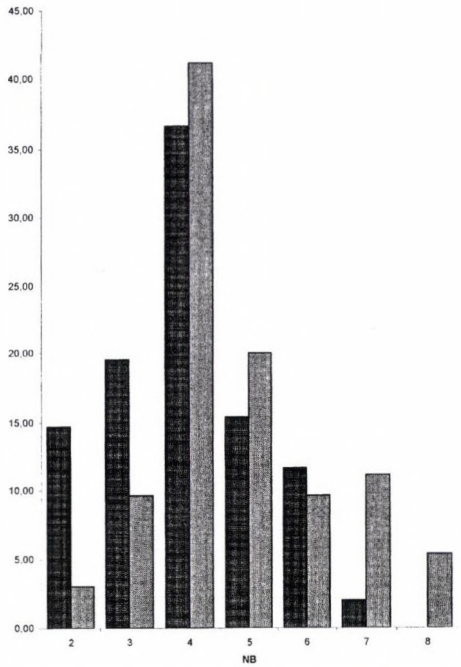

Fig. 6. Distribution of the relative ecological indicator values of nitrogen supply (NB) in transitional (the left, darker columns) and in regular (tussocky) stands (the right, lighter columns) of *Caricetum appropinquatae* in Hungary. For more details, see in text

site they worked on was an alpine wetland, so it can be supposed that nutrient supply and/or a/D ratio were rather low due to low temperature and short vegetation period. This makes it likely that circumstances were close to the transition point.

In a wetland, favourable for stolonous sedges (e.g. because water movements provide enough oxygen) D is great, therefore a/D is too low for developing of tussocks. In this case, typical *Carex* meadows (Caricenion gracilis (Neuhäusl 1959) Oberd. et al. 1967) come into existence.

It was assumed when formulating the model, that the positive feedback loop was mediated by water level although this can be done by other factors as well. Thus, tussocks of *Eriophorum vaginatum* in the Alaskan Arctic tundra have a higher temperature and lower temperature fluctuations than does inter-tussock vegetation (Chapin et al. 1979, Oberbauer and Miller 1979). Therefore, the environmental modification by plants can lead to increased plant growth. This is a positive feedback loop (switch) mediated by temperature (Wilson and Agnew 1992). The model proposed here can essentially be applied to this case as well.

The horizontal extension of tussocks may be restricted also by other factors than nutrient supply. So, Dawkins (1939) reported that the peat level between *Schoenus nigricans* hummocks had been eroded by water. Martin (1960) informs about a sedge fen (South Africa) becoming broken up into discrete tussocks by trampling, "the spaces between tussocks being paths made by grazing animals". This can be observed also in Hungary, but the formations arising in such a way appear as very irregular pseudo-tussocks, e.g. in reeds or related communities (Phragmition australis Koch 1926).

Enhanced carbon dioxide concentration in tussocks can also be imagined to restrict horizontal growth. As Končalová (1988) has shown experimentally in *Carex gracilis*, when the pressure of carbon dioxide is higher in the root medium than in the internal gas spaces of roots, the oxygen supply is depressed by the mass flow of air driven by the pressure difference of carbon dioxide.

Because the discussed model is based on the interaction of autogenic mechanisms (e.g. eqns **1–2**) and allogenic causes (e.g. eqn. **3**), it gives an example of "ecosystem mechanisms of succession" in the sense of Van Andel et al. (1993). However, already Tansley (1935) thought, that "autogenic and allogenic factors are present in all successions", albeit seemingly without distinguishing causes and mechanisms.

Near the transition point a variety of tussock sizes occur, moreover, their shapes are bell-like or hemispherical. This offers diverse microhabitats for numerous species. Vivian-Smith (1997) showed experimentally, that small-scale variability in microtopography, on the order of 1–3 cm, enhanced floristical diversity in wetland communities. Tomaszewska (1993) observed that plants could grow only at the top of pole-like tussocks, while they had established on the entire surface of hemispherical tussocks. Therefore, transitional communities are very important for nature conservation, as "diversity hotspots" (Myers 1990). The result described above, that they react very slowly on small environmental changes, makes their conservation rather difficult because the changes are recognised too late. On the other hand, they seem to be less sensitive to small environmental fluctuations.

Van Andel et al. (1993) stated that positive feedback switches in plant communities represented inhibition, "at least the opposite of facilitation". In turn, tussock development seems to be also facilitation, because emerging surfaces become favourable for additional species. Tsuyuzaki and Tsuji (1992) found positive correlation between tussock height and number of species. Tolerance may be also present (occurrence of *Carex lasiocarpa* on high tussocks of *Carex elata* may be, to some degree, considered as an exam-

ple). Already Connell and Slatyer (1977) recognised, that all three processes occur during most successions.

Because the tussock-forming organisms (mainly several sedges) create habitats and therefore control the availability of resources to other organisms by causing physical state changes, they are "ecosystem engineers" in the sense of Jones et al. (1997). I hope, the model proposed here help us to understand better, how these engineers work.

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ÜBER DIE MOORE DER UMGEBUNG VON PIEKIELNIK

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Ein pflanzensoziologisches Bild in erster Linie über das Hochmoor Puścizna Wielka und andere Moore in seiner Umgebung wird dargestellt. Die charakteristischen Pflanzengesellschaften werden (auch durch Aufnahmen) geschildert. Das gemeinsame Vorkommen von ozeanischen, montanen und boreo-kontinentalen Arten (wie *Calluna vulgaris, Pinus mugo* und *Ledum palustre*) in der Hochmoorvegetation ist bemerkenswert. Naturschutzmassnahmen werden empfohlen.

Key words: Hochmoor, Pflanzengesellschaften, boreo-kontinental, ozeanisch, West-Karpaten

Einleitung

Piekielnik ist eine Ortschaft in Süd-Polen, im Gebiet Orawa (slowakisch: Orava), westlich von Nowy Targ. Orawa ist ein Becken der nördlichen Karpaten, durch das sich die Staatsgrenze von Polen und der Slowakei zieht. Es ist eine Senkung in der Flysch-Zone der Karpaten. Die vorherrschenden karpatischen Sandsteine wechseln hier mit schieferigen und tonigen Gesteinen. Das auf den wasserdichten Schichten heraustretende beziehungsweise sich anhäufende Wasser wurde zum Ausgangspunkt der Moorbildung. Das Klima des von der West-Tatra nördlich gelegenen, 680–700 m hohen, schwach bevölkerten Beckens ist ziemlich extrem, mit beträchtlichen Schwankungen der Temperatur. Der Winter des stark durch die Temperatur-Inversion geprägten Beckens ist rauh. Das Gebiet ist wegen des angrenzenden Grossgebirges (West-Tatra, Babia-góra) reich an Niederschläge. Das Klimadiagramm für das Hochmoor Puścizna Wielka ist auf Abb. 1 zu sehen. Die dazu verwendeten Angaben wurden aus den von Nowy Targ und Ustie n. O. (Temperatur), beziehungsweise Nowy Targ und Oravská Polhora (Niederschlag) in umgekehrtem Verhältnis zur jeweiligen Distanz interpoliert. In den breiten Flusstälern, quelligen Hängen, auf den abfliessengehemmten Wasserscheiden konnten viele Moore, darunter auch echte Hochmoore zustande kommen (László 1915).

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Methode

Das Untersuchungsgebiet wurde in den Jahren 1994–1996 mehrmals aufgesucht und in annähernd typischen Beständen der Moore pflanzensoziologische Aufnahmen nach der Braun-Blanquet-Methode gemacht. Die Aufnahmefläche war in der Regel rechteckig und von 2–10 m^2 Grösse, den erfahrensgemässen Empfehlungen entsprechend (Dierschke 1994). Die verwendete Abundanz-Dominanz-Skala:

5: mehr als 75% der Fläche deckend, Individuenzahl beliebig,
4: 50–75% der Fläche deckend, Individuenzahl beliebig,
3: 25–50% der Fläche deckend, Individuenzahl beliebig,
2b: Deckung 15–25%, Individuenzahl beliebig,
2a: Deckung 5–15%, Individuenzahl beliebig,
2m: Deckung < 5%, Individuenzahl > 50,

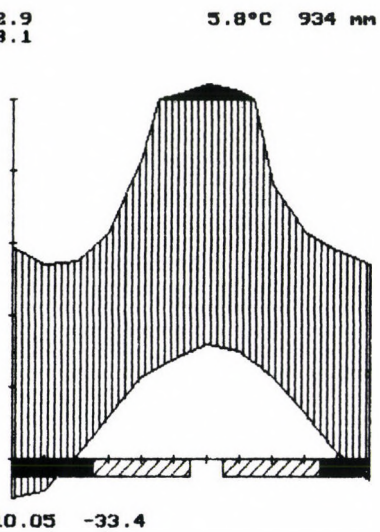

Abb. 1. Klimadiagramm für Puścizna Wielka auf Grund von interpolierten Angaben. Abszisse: Monate, Ordinate: ein Teilstrich = 10 °C beziehungsweise 20 mm Niederschlag. Obere Kurve: mittlere monatliche Niederschläge. Untere Kurve: mittlere Monatstemperaturen. Mittlere monatliche Niederschläge über 100 mm (schwarz) wurden in Massstab 1/10 dargestellt, in diesem Fall entspricht also ein Teilstrich 200 mm Niederschlag. Unter der Abszisse schwarz: Monate mit mittlerem Tagesminimum unter 0 °C (Kalte Jahreszeit). Schräg schraffiert: Monate mit absolutem Minimum unter 0 °C (Spät- oder Frühfröste kommen vor). Oben rechts: mittlere Jahrestemperatur in °C und mittlerer Jahresniederschlag in mm. Oben links: mittleres tägliches Maximum des wärmsten Monats und höchste gemessene Temperatur. Unten links: mittleres tägliches Minimum des kältesten Monats und tiefste gemessene Temperatur (letztere aus Ustie n. O.)

1: Deckung < 5%, Individuenzahl 6–50,
+: Deckung < 5%, Individuenzahl 2–5,
r: Deckung < 1%, Individuenzahl 1.

Die Abstufung von Soziabilität (Willmanns 1989):
5: in grossen Herden wachsend,
4: in kleinen Kolonien wachsend oder grössere Flecken oder Teppiche bildend,
3: truppweise wachsend (kleine Polster oder Flecken bildend),
2: gruppen- oder horstweise wachsend,
1: einzeln wachsend.

Die Untersuchung der Hochmoore bedarf besonderer Aufmerksamkeit, weil ihre Oberfläche in kleinen Distanzen heterogen und strukturiert

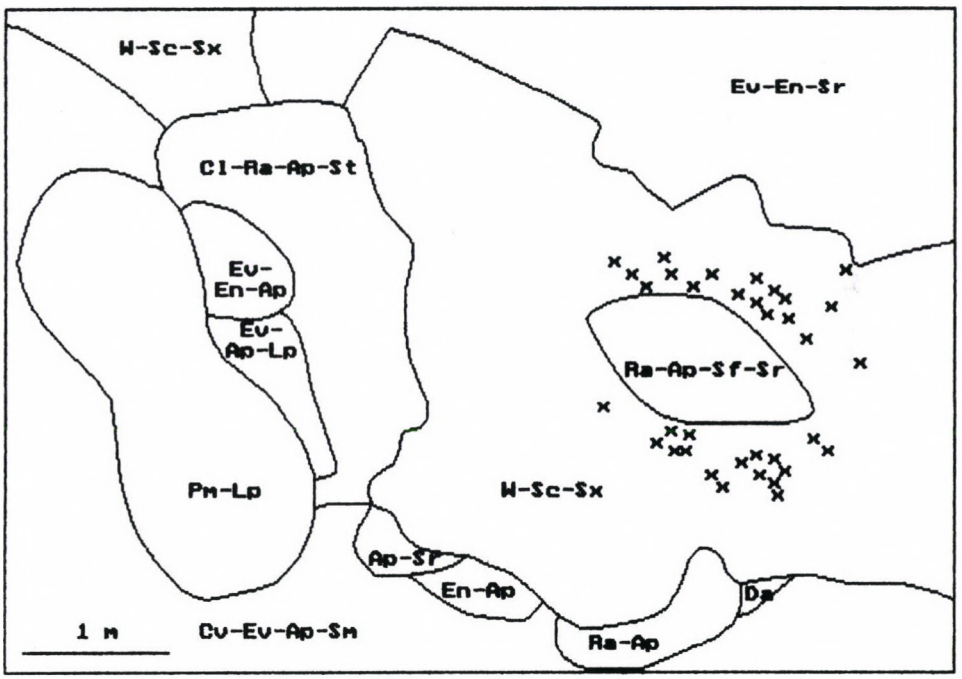

Abb. 2. Teilkarte der Vegetation auf der Hochmorweite von Puścizna Wielka, mit Andeutung der dominanten Pflanzensippen. Abkürzungen: Ap: *Andromeda polyfolia*, Cl: *Carex limosa*, Da: *Drosera anglica*, En: *Empetrum nigrum*, Ev: *Eriophorum vaginatum*, Lp: *Ledum palustre*, Pm: *Pinus mugo*, Ra: *Rhynchospora alba*, Sc: *Sphagnum cuspidatum*, Sf: *Sphagnum fuscum*, Sm: *Sphagnum magellanicum*, Sr: *Sphagnum rubellum*, St: *Sphagnum tenellum*, Sx: *Sphagnum fallax*, W: offenes Wasser, x: Einzelsprosse von *Rhynchospora alba*

ist, was zum Wesen dieser Gebilde gehört. Weil die Vegetationstypen der Bulten beziehungsweise Schlenken syntaxonomisch verschiedene Assoziationen, sogar verschiedene Klassen darstellen, wurden diese getrennt aufgenommen. Die Aufnahmefläche war meist nicht rechteckig. Trotzdem blieben die Probeflächen der Bulten recht heterogen, was vielleicht auf Bedarf an Verfeinerung der Aufnahmemethode (und der syntaxonomischen Gliederung) hinweist. Es kann aber auch so argumentiert werden, dass man in diesem Fall eine gewisse Heterogenität in Kauf nehmen muss, sonst kann man den betrachteten Vegetationstyp nicht in seiner Vollentwicklung studieren. Zusätzlich wurden auch pflanzensoziologische Detailkarten und Transekt-Profile zubereitet. Die verwendete taxonomische Nomenklatur ist nach Szafer et al. (1988), ausgenommen das streitige Formenkreis von Bergkiefer (*Pinus mugo* s.l.). Matuszkiewicz (1984b) hat angedeutet, dass in dieser Hinsicht das Bestimmungsbuch "Rośliny polskie" nicht befriedigend ist. Hier wird etwa dem Standpunkt von Hejný und Slavík (1988) gefolgt.

Die untersuchten Pflanzengesellschaften

Das bedeutendste Hochmoor des untersuchten Gebietes ist das Puścizna Wielka genannte. Es ist ein gewölbtes Moor mit einer Grundfläche von etwa 225 ha (ursprünglich noch mehr), das sich auf einer Wasserscheide entwickelte. Einige Teile im Randbereich wurden durch Torfstich beseitigt. Trotzdem zeigt der Rest des Moores noch ein recht natürliches Bild.

Physiognomisch kann man auf diesem Moor Hochmoorweite, Randgehänge und eine Laggzone unterscheiden, obwohl die Natürlichkeit der letzten ist wegen des Torfstiches fraglich. Der Torfstich hat nämlich senkrechte Wände am Rande gebildet, deshalb ist der Wasserverlust hier viel grösser als in natürlichem Zustand.

Auf der Hochmoorweite findet man Bulte, Bultflächen, (meist kleinflächige) Teppichhorizonte, Torfmoosschlenken, Torfschlammschlenken und Kolke. In den Kolken sind stellenweise Inseln entstanden. Die Bulten sind in Reihen geordnet, die im grossen und ganzen parallel verlaufen. Über die kleinflächige Variabilität der Vegetation gibt Abb. 2 einige Auskunft.

Die Vegetation der Bulten und Bultflächen gehört zur bunten Torfmoosgesellschaft (*Sphagnetum medii* Kästner et Flössner 1933, vor allem zur Subassoziation *Sphagnetosum fusci* (Kästner et Flössner) Dierssen in Oberdorfer et al. 1977). *Sphagnum fuscum* bildet dichte Polster. Es ist interessant,

dass *Ledum palustre* recht oft, zwar mit niedriegen Deckungswerten, in diesem Vegetationstyp vorkommt, obwohl der Sumpfporst nach Neuhäusl (1969) die Kennart der Waldkieferhochmooren (*Ledo-Sphagnetum medii* Sukopp ex Neuhäusl 1969) sei. Trotzdem kann man diese Gesellschaft nicht dem *Ledo-Sphagnetum medii* zuordnen. Die letzterwähnte ist nämlich eine baumreiche Hochmoorgesellschaft, mit einer lockeren bis schwach geschlossenen Baumschicht. Wie Neuhäusl schreibt, "Die Einzelbäume stellen einen homogenen Bestandteil der Gesellschaft dar, sie sind also an keine Sondermikrostandorte (Bulten u. ä.) gebunden". Zwischen den Bulten gibt es keine echte Schlenken, die Bodenvegetation ist eher ausgeglichen (Sukopp 1959, Matuszkiewicz 1984a, Steiner 1992). Die Hochmoorweite des Moores Puścizna Wielka ist dagegen ausdrücklich strukturiert, mit tiefen, dauernd wasserbedeckten Korken. Auf den Bulten kann man zwar verkrüppelte Bergkiefer (*Pinus rotundata*, aber auch *Pinus mugo*) und Fichten (*Picea excelsa*) zerstreut finden, diese aber durchaus keinen homogenen Bestandteil der Gesellschaft bilden. Einige kleine Bäume sinken in das Moor ein. Man kann beobachten, dass die älteren Stämme schon im Wasser stehen. Diese Erscheinung trägt zum Erhalten der Oberflächenstruktur bei.

Über das Eindringen der Fichte in das *Sphagnetum medii* hat auch Steiner (1992) aus Österreich informiert. Es kann übrigens damit in Zusammenhang stehen, dass sich diese Baumsippe ein sehr seichtes Wurzelwerk entwickelt, das die oberflächliche, etwas trockenere Torfschicht nutzen kann. Aber auch die Fichte is hier verkümmert, misswüchsig und bleibt meist unter 1 m Höhe. Ähnliche Gesellschaft studierten Jurko und Peciar (1959) neben Suchá Hora (Rudné), als *Eriophoreto-Sphagnetum*. Dieses Moor wurde aber kurz darauf vernichtet.

Das *Sphagnetum medii* ist nicht auf die Hochmoorweite beschränkt, es befindet sich auch im Randgehänge und im Fussbereich. Seine Aufnahmen sind in Tabelle 4 zu finden. Es kommt aber hier und besonders auf Puścizna Mała auch das *Pino rotundatae-Sphagnetum medii* vor. Eine typische Aufnahme (Puścizna Wielka, 100 m^2): Baumschicht: *Pinus rotundata* 3. Krautschicht: *Empetrum nigrum* 3.3, *Calluna vulgaris* 2a.3, *Eriophorum vaginatum* 2m.2, *Oxycoccus quadripetalus* 2m.1, *Ledum palustre* 1.1, *Andromeda polyfolia* +.1, *Vaccinium uliginosum* +.2.

Moosschicht: *Sphagnum fuscum* 3.3, *Sphagnum magellanicum* 3.3, *Sphagnum fallax* 2m.3, *Aulacomnium palustre* 1.1.

Das *Ledo-Sphagnetum medii* kommt auch im Gegend vor, z. B. an der slowakisch-polnischen Staatsgrenze. In diesem findet man eine lockere Baumschicht aus Waldkiefer (*Pinus sylvestris*) und eine schwache Strauchschicht

von *Ledum palustre* und *Vaccinium uliginosum*. In der Krautschicht auch andere Ericaceaen wie *Vaccinium myrtillus* und *Vaccinium vitis-idaea* kommen zu einer bedeutenderen Rolle. Die Torfmoose sind noch mehr oder minder teppichbildend.

Die Natürlichkeit dieser eher kleinflächigen Bestände ist jedoch fraglich wegen des ausgedehnten, intensiven Torfbaus auf der slowakischen Seite. Nach Neuhäusl (1972) zu dieser Gesellschaft gehören auch die Aufnahmen von Staszkiewicz (1958) aus der Umgebung von Nowy Targ, die ursprünglich als *Sphagnetum medii et rubelli* Schwick. bezeichnet wurden.

Auf die Frage, warum der Sumpfporst in jedem erwähnten Vegetationstyp hier vorkommt, gibt es allerdings eine naheliegende Antwort. Das Klima des Gebietes ist nämlich zugleich ozeanisch (viel Niederschlag), kontinental (grosse Temperaturschwankungen) und montan (z. B. langdauernde Schneedecke) geprägt, was das Zusammentreten der entsprechenden Pflanzensippen (z. B. *Calluna vulgaris, Ledum palustre, Pinus rotundata*) ermöglicht.

In den Schlenken des Hochmoores Puścizna Wielka entwickelten sich die Gesellschaften des Verbandes Rhynchosporion. Sie sind zum Teil am Rande der Korke oder bilden kleine Inseln in denen. Das *Sphagno tenelli-Rhynchosporetum albae* Osvald 1923 emend. Dierssen 1982 *sphagnetosum tenelli* (Osvald 1923) Dierssen ex Dierssen et Reichelt 1988 ist stets sehr kleinflächig gebildet, oft zusammen mit *Carex limosa* und *Andromeda polyfolia*. Es wurde nicht hier, sondern zum Vergleich auf der slowakischen Seite, nördlich von Suchá Hora aufgenommen: (Rudné, 2 m^2): Krautschicht : *Rhynchospora alba* 4.4, *Drosera rotundifolia* 2m.2, *Calluna vulgaris* 1.1, *Oxycoccus quadripetalus* 1.2, *Andromeda polyfolia* +.1, *Betula pubescens* r, *Eriophorum vaginatum* r, *Vaccinium uliginosum* r.

Moosschicht: *Sphagnum tenellum* 4.3, *Sphagnum rubellum* 2a.2, *Gymnocolea inflata* 2m.2.

Auf dem Torfschlamm konnten sich hier auch einige *Oxycocco-Sphagnetea*-Arten ansiedeln. Jurko und Peciar (1959) kartierten grosse Bestände auf dem seither vernichteten Hochmoor Rudné in der Slowakei.

Das *Caricetum limosae* Osvald 1923 emend. Dierssen 1982 findet man etwas öfter. Eine typische Aufnahme davon (Puścizna Wielka, 2 m^2): Krautschicht: *Carex limosa* 2a.1, *Andromeda polyfolia* 1.1, *Drosera anglica* +.2, *Eriophorum vaginatum* r.

Moosschicht: *Sphagnum cuspidatum* 4.4, *Sphagnum fallax* 4.4, *Sphagnum palustre* 2m.2, *Sphagnum majus* 1.1.

Drosera anglica und *Scheuchzeria palustris* kommen an ähnlichen (ungestörten) Stellen vor.

In der Umgebung von Piekielnik sind schöne Übergangsmoore zu finden. Einige davon werden durch das abfliessende Wasser der Puśćizna Wielka versorgt. Im fliessenden Wasser entwickelten sich verhältnismässig ausgedehnte Bestände von *Calletum palustris* (Osvald 1923) Van den Berghen 1952. Die herzblättrige Sumpfkalla (manchmal zusammen mit *Comarum palustre*) bildet Schwingrasen, in denen neben Scheuchzerio-Caricetea- auch einige Phragmitetea-Arten vorkommen. Seine Aufnahmen sind in Tabelle 1 zu finden.

Tabelle 1

Calletum palustris (1–4.: Piekielnik)

Artenzahl	6	4	7	8
Aufnahme	1.	2.	3.	4.
Krautschicht				
Calla palustris	4.5	3.4	4.5	4.5
Carex canescens	–	–	1.2	–
Carex fusca	2m.1	–	+.1	+.1
Carex rostrata	–	–	–	+–1
Comarum palustre	+.1	3.4	–	3.4
Eleocharis uniglumis	–	–	1.2	–
Eriophorum angustifolium	–	2m.1	–	+.1
Equisetum limosum	2m.2	–	2m.2	1.1
Sparganium ramosum	–	–	–	1.1
Agrostis canina	2m.3	–	–	–
Moosschicht				
Drepancladus exannulatus	–	–	3.3	–
Sphagnum cuspidatum	–	–	3.3	–
Sphagnum fallax	5.5	5.5	–	5.5

Das strömende Wasser wird an einigen Stellen durch die Vegetation weitgehend angestaut. Hier findet man unter anderem Ausbildungen von *Amblystegio scorpioidis-Caricetum diandrae* Osvald 1923 (Tabelle 2) und öfter die von *Caricetum rostratae* Osvald 1923 emend. Dierssen 1982 (Tabelle 3). Zwischen den im Boden wurzelnden Seggen bilden die Moose eine schwimmende Schicht, das heisst, eine dysaptische (engl.: dysaptic, Kulczyński 1949) Struktur liegt vor. Bemerkenswert ist das Vorkommen des recht seltenen *Eriophorum gracile*. *Comarum palustre* und *Viola palustris* findet man fast überall, auch *Menyanthes trifoliata* wächst in grossen Men-

gen. *Carex limosa* kommt in beiden Aufnahmen des *Amblystegio scorpioidis-Caricetum diandrae* vor, im *Caricetum rostratae* mit mittlerer Stetigkeit. In der Moosschicht des *Amblystegio scorpioidis-Caricetum diandrae* scheint *Sphagnum subsecundum*, in der von *Caricetum rostratae* meist *Sphagnum flexuosum* eine grössere Rolle zu spielen.

Tabelle 2

Amblystegio scorpioidis-Caricetum diandrae (1–2.: Piekielnik)

Artenzahl	9	17
Aufnahme	1.	2.
Krautschicht		
Scheuchzerio-Caricetea fuscae		
Carex canescens	+.2	–
Carex diandra	3.4	4.4
Carex limosa	1.1	1.1
Carex rostrata	+.1	+.1
Comarum palustre	2m.1	2b.2
Eleocharis uniglumis	–	1.2
Eriophorum gracile	–	1.1
Menyanthes trifoliata	–	4.4
Viola palustris	2m.1	–
Phragmitetea		
Equisetum limosum	–	2m.2
Galium palustre	–	+.1
Molinietalia		
Agrostis canina	+.2	–
Equisetum palustre	–	+.1
Lychnis flos-cuculi	–	r
Valeriana simplicifolia	–	2m.1
Indiff.		
Caltha palustris	–	+.1
Juncus articulatus	+.1	+.1
Moosschicht		
Calliergon stramineum	–	2a.3
Plagiomnium elatum	–	2a.3
Sphagnum subsecundum	3.3	2m.3

Das *Caricetum goodenowii* Braun 1915 ist recht oft zu finden, auch sekundär auf Moorwiesen. Eine annähernd typische Aufnahme von *drepanocladetosum exannulati* (Nordhagen 1942) Dierssen 1982: Krautschicht: *Carex*

fusca 5.5, *Carex canescens* 2b.2, *Galium palustre* 2m.1, *Juncus filiformis* 2m.1, *Agrostis canina* +.2.

Moosschicht: *Drepanocladus exannulatus* 3.3, *Sphagnum fallax* 3.3, *Polytrichum commune* +.1.

In Kiefern- und Fichtenwäldern versteckt, an quelligen Stellen entwickelten sich die kleinflächigen Bestände von *Carici echinatae-Sphagnetum flexuosi* Soó 1954 corr. Lájer hoc loco. Neotypus: Lájer 1998, Tab. 17., Aufnahme 5.

Nomenklatorische Bemerkung: Die erste gültige Mitteilung dieser Assoziation unter dem Namen *Cariceto echinatae-Sphagnetum* vollbrachte Soó in 1954, indem er eine Originaldiagnose durch synthetische Tabellen gab. Er führte nicht Balázs an, die darauf gestützte Typisierung (Borhidi 1996) beziehungsweise Korrektur (Lájer 1998) sind ungültig. Der Neotypus wurde an einem solchen Ort (Farkasfa, Fekete-tó) aufgenommen, den auch Soó in Betracht gezogen hat.

Tabelle 3

Caricetum rostratae (1–3.: Piekielnik)

Artenzahl	9	17	8	10	11	11	10
Aufnahme	1.	2.	3.	4.	5.	6.	7.
Krautschicht							
Scheuchzerio-Caricetea fuscae							
Calla palustris	2b.3	–	1.2	–	–	–	–
Carex canescens	1.2	–	–	1.2	1.2	1.2	2m.2
Carex fusca	–	1.1	–	1.1	–	+.1	–
Carex rostrata	3.4	4.4	4.5	3.4	4.5	3.4	3.4
Carex limosa	–	–	–	1.1	1.1	1.1	1.1
Comarum palustre	–	4.4	+.1	2m.2	1.1	1.1	1.1
Crepis paludosa	–	+.1	–	–	–	–	–
Eriophorum angustifolium	2m.1	–	+.1	1.1	–	+.1	–
Menyanthes trifoliata	–	2m.3	1.2	3.3	4.4	–	3.3
Viola palustris	–	1.1	+.1	1.1	1.1	+.1	1.1
Phragmitetea							
Epilobium palustre	+.1	+.1	–	–	–	–	–
Equisetum limosum	1.1	2m.2	1.1	2m.2	2m.2	2m.2	2m.2
Sparganium ramosum	+.1	–	–	–	–	–	–
Molinietalia							
Agrostis canina	+.2	1.2	–	–	–	+.2	+.2
Galium uliginosum	–	+.1	–	–	–	–	–
Equisetum palustre	–	–	–	–	–	–	–
Valeriana simplicifolia	–	2m.2	–	–	–	–	–

Indiff.							
Angelica syvestris	–	+.1	–	–	–	–	–
Caltha palustris	–	+.1	–	–	–	–	–
Cirsium palustre	–	+.1	–	–	–	–	–
Juncus articulatus	–	–	–	–	–	–	+.1
Moosschicht							
Calliergon cordifolium	–	–	–	–	+.1	–	–
Calliergon stramineum	–	+.1	–	–	+.1	–	–
Plagiomnium elatum	–	+.1	–	–	–	–	–
Polytrichum commune	–	–	–	–	2m.1	1.1	–
Sphagnum flexuosum	5.5	–	5.5	1.2	2b.3	1.2	2b.3
Sphagnum subsecundum	–	5.5	–	–	–	–	–

Tabelle 4

Sphagnetum magellanici (1–3.: Puścizna Wielka, 4.: Puścizna Mala, 5–8.: Puścizna Wielka. Alle 5 m²)

Artenzahl	17	11	12	12	13	7	10	10
Aufnahme	1.	2.	3.	4.	5.	6.	7.	8.
Krautschicht								
Oxycocco-Sphagnetea								
Andromeda polyfolia	3.2	–	1.1	–	2m.1	–	+.1	2m.1
Carex pauciflora	1.1	–	–	–	–	–	–	–
Eriophorum vaginatum	4.4	3.2	–	2b.2	3.2	5.5	2b.2	2b.2
Ledum palustre	+.1	+.1	+.1	1.1	–	–	–	–
Oxycoccus quadripetalus	2m.1	2m.1	2a.2	3.2	2m.1	2m.1	2m.1	–
Scheuchzerio-Caricetea fuscae								
Drosera anglica	–	–	–	–	+.2	–	–	–
Drosera rotundifolia	+.2	–	–	–	1.2	–	+.2	1.2
Eriophorum angustifolium	–	–	2b.2	–	–	–	–	–
Juncus filiformis	–	–	–	–	–	+.1	–	–
Calluno-Ulicetalia								
Calluna vulgaris	2a.2	2m.2	2b.2	2b.2	2a.2	+.1	2b.2	2b.2
Empetrum nigrum	+.2	4.4	3.4	–	–	–	+.2	+.2
Vaccinium uliginosum	+.1	–	–	2b.2	–	–	–	–
Vaccinium vitis-idaea	–	–	–	+.1	–	–	–	–
Moosschicht								
Aulacomnium palustre	1.1	1.1	1.1	1.1	2m.1	–	1.1	1.1
Polytrichum commune	–	–	–	2a.2	–	2m.1	2m.1	2m.1
Polytrichum strictum	2m.1	–	2m.1	–	2m.1	–	–	–

Sphagnum angustifolium	4.4	2m.1	–	–	3.2	–	–	–
Sphagnum fallax	2a.3	1.2	–	–	–	2b.2	–	–
Sphagnum fuscum	–	2b.3	2b.3	2a.3	3.3	–	2b.3	2m.3
Sphagnum magellanicum	4.4	3.4	3.4	1.2	2m.3	2m.3	4.4	2m.3
Sphagnum centrale	2m.3	–	1.2	3.4	1.2	–	–	–
Sphagnum rubellum	4.4	1.2	2a.3	2m.3	3.4	–	–	4.4
Sphagnum subnitens	1.2	–	–	–	–	–	–	–

Dieser Vegetationstyp ist floristisch etwas ähnlich dem *Caricetum goodenowii*, aber die Krautschicht ist viel lockerer, wo *Carex stellulata* in den Vordergrund tritt. Die Moosschicht ist durch einen zusammenhängenden Torfmoos-Teppich gekennzeichnet. Die Assoziation kommt hier vor allem in kleinflächigen Quell- und Überrieselungsmooren vor. Aufnahme (Piekielnik, 10 m^2): Krautschicht: *Carex stellulata* 2a.2, *Carex fusca* 2a.2, *Potentilla erecta* 2a.1, *Equisetum limosum* 1.1, *Equisetum palustre* 1.1, *Festuca capillata* 1.2, *Viola palustris* 1.1, *Agrostis canina* +.2, *Calluna vulgaris* +.1, *Juncus conglomeratus* +.2, *Luzula multiflora* +.2, *Nardus stricta* +.2, *Vaccinium myrtillus* +.1.

Moosschicht: *Polytrichum commune* 2m.1, *Sphagnum flexuosum* 5.5, *Sphagnum centrale* 1.2. In dieser Gesellschaft findet man hier (aber recht selten) auch *Sphagnum warnstorfii*.

Schlussbemerkungen

Die Moore in Orawa werden seit langem genutzt, vor allem durch Torfstich, in der Vergangenheit aber auch durch Rindweide (Nyárády 1911). In der Slowakei stehen die noch zurückgebliebenen, kleinflächigen Moore unter Naturschutz. Sie sind zweifellos wertvoll, aber nur noch ein Bruchteil der einstigen. Manchmal ist fraglich, wie es langfristig funktionieren kann. Neben Suchá Hora, wo ein Fragment des ehemaligen Hochmoores Rudné zum Naturschutzgebiet erklärt und umfriedet wurde, ist z. B. *Drosera anglica* schon vor den Naturschutzmassnahmen verschwunden (Cvachová 1988). Heutzutage wird der Torf weiter noch industriell abgebaut. Es ist zu befürchten, dass auch in der polnischen Orawa das letzte grosse Hochmoor vernichtet wird. Es wäre empfehlenswert, wenigstens Puścizna Wielka und ihre Umgebung dringend zum Naturschutzgebiet zu erklären und auf den Torfstich zu verzichten. Die Torfstichwände sollte man mit einer natürlichen Böschung rekultivieren.

Im ostwärts gelegenen Becken von Nowy Targ ist das langher studierte (Szafer 1928) Hochmoor Bór na Czerwonem auf 49.7 ha geschützt (Pawłowski 1977).

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EINE METHODE ZUR RÄUMLICHEN ABGRENZUNG VON PFLANZENGESELLSCHAFTEN

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Eine Methode wurde zur einheitlichen pflanzensoziologischen Untersuchung der Übergangsbereiche zwischen verschiedenen, durch Pflanzensippen trennbaren Gesellschaften erarbeitet. Sie ist auf die Feststellung der minimalen Flächengrösse für eine bestimmte Artenverbindung um einen gegebenen Punkt begründet. Ein Affinitätsbegriff wurde daraus abgeleitet. Die Methode ermöglicht, jeden Punkt auf dem untersuchten Gelände einem Vegetationstyp oder einer Grenze eindeutig zuzuordnen. Ihre Anwendung wird an einem Beispiel von Szársomlyó-Berg demonstriert.

Key words: Pflanzengesellschaft, Braun-Blanquet-Methode, Übergang, Abgrenzung, Kontinuum, Heterogenität

Einleitung

Eine wichtige Grundbedingung für die Untersuchung der Vegetationskomplexe ist, dass die erkennbaren Vegetationstypen sich voneinander räumlich abgrenzen lassen. In der Praxis (zum Beispiel bei Vegetationskartierung) ist es aber nicht immer einfach, weil die in der Natur vorkommenen Grenzen meist mehr oder weniger breite Übergangsbereiche haben. Der Kontaktbereich wird in anspruchsvollen Arbeiten auf Grund von lokalen Differentialarten oder direkter Gradientenanalyse durch Quadrataufnahmen entlang einem Transekt untersucht (Matuszkiewicz 1972, van der Maarel 1976). Die Feststellung der geeigneten Quadratgrösse und -gestaltung scheint aber in diesem Fall äusserst problematisch zu sein. Die von der Braun-Blanquet-Methode gegebenen Kriterien für die Auswahl und Abgrenzung von Aufnahmeflächen (physiognomisch-strukturelle sowie floristische Homogenität, Minimum-Areal) können nicht einmal annähernd erfüllt werden, wenn sich die Vegetation auf unvorhersehbare Art räumlich ändert (Bagi 1991). Das Minimum-Areal ist in diesem Fall sogar theoretisch undefiniert. Ausserdem ist der Gradientswert (z. B. der H-Wert von van der Maarel) eigentlich nur ein Mass der lokalen Heterogenitäten, welches über das wirkliche Verhältnis von ganzen Pflanzengesellschaften fallweise (wenn überhaupt) nur wenig Aussagekraft hat.

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Jeder Pflanzenbestand verfügt über eine Horizontalstruktur, die sich zwar durch Feinkartierung (Kleinquadrate oder Transekt-Profile) ausführlich wiedergeben lässt, was aber das Zuordnen des jeweiligen Syntaxons nicht erleichtert. Zu grosse Aufnahmeflächen dagegen verwischen den feinen räumlichen Ablauf der Grenze und vortäuschen ein Kontinuum. Tatsächlich können verschiedene Quadratgrössen und -gestaltungen zu recht unterschiedlichen Ergebnissen führen.

Um diese Schwierigkeiten zu überwinden, scheint der folgende Einfall naheliegend zu sein: Statt die in einem vorher festgelegten Quadrat vorkommenen Arten aufzuzeichnen, kann man einigermassen umgekehrt vorgehen, indem man untersucht, welche minimale Flächengrösse für eine bestimmte Artenverbindung um einen gegebenen Punkt benötigt wird.

Die Methode

Zuerst müssen die auf dem untersuchten Gelände vorkommenen floristischen Vegetationstypen samt ihren (lokal gültigen) Differentialarten festgestellt werden. Man kann es auf die herkömmliche Art vornehmen (Dierschke 1994). In dem hier erörterten Verfahren werden die Trennarten möglichst hoher Stetigkeit verwendet. Nehmen wir an, wir haben mit 2 Vegetationstypen, *A* und *B* zu tun. Wir setzen *a(i)* und *b(i)* für die i-te Trennart des Vegetationtyps *A*, beziehungsweise *B*. Sei *m* die Anzahl der Trennarten von *A*, *n* die von *B*.

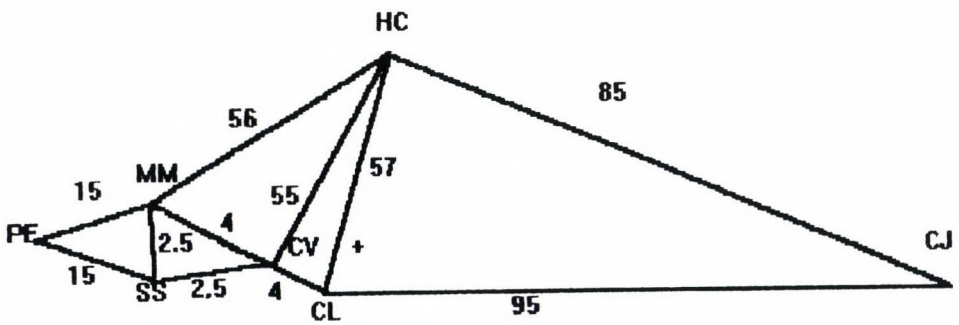

Abb. 1. Die Skizze eines tatsächlich aufgenommenen Minimalpolygons im Felsenrasen des Szársomlyó-Berges (nicht massstäblich). Die Zahlen bedeuten Distanzen in Zentimetern. Abkürzungen: CJ: *Ceterach javorkaeanum*, CL: *Carex liparicarpos*, CV: *Crupina vulgaris*, HC: *Helianthemum canum*, MM: *Medicago minima*, PE: *Potentilla erecta*, SS: *Sedum neglectum* subsp. *sopianae*, +: Lokalität des Punktes. Wenn der Punkt ausser dem Polygon läge, brauchte man ein zusätzliches Dreieck mit dem Punkt auf seiner Spitze

Der Kern der Methode ist die Bestimmung der minimalen Flächengrösse mit der die Trennarten um den untersuchten Punkt herum vorkommen. Das Verfahren wird an dem Beispiel der *a(i)* erklärt: Für den ausgewählten Punkt sucht man die nächeste *a(i)* und markiert man diese auffällig. Es wird für $i = 1$ bis m wiederholt. Man bildet aus den Lokalitäten der *a(i)* Dreiecke, so dass sich jedes Dreieck an eine Seitenlinie des zuletzt bildeten lehnt. Der Punkt selbst soll dabei nur in Betracht gezogen werden, wenn dieser ausser dem durch den Trennarten gebildeten Polygon liegt (Abb. 1). Kann man verschiedene Polygone bilden, muss man das mit der kleinsten Oberfläche wählen. Die Flächengrösse des so gebildeten Polygons wird (zweckmässig durch ein kleines Computerprogramm) ausgerechnet. Dabei hilft die folgende Formel:

$$t = \frac{\sum\sqrt{(x + z + y)(x + z - y)(y + x - z)(y - x + z)}}{4}$$

wo t die Flächengrösse, x, y, z die Seitenlängen der Dreiecke sind. (Um überflüssige Dateneinträge zu vermeiden, ist z. B. die folgende Konvention zweckmässig: x ist immer die Länge derjenigen Seite, die sich an die Seite des vorigen Dreiecks lehnt. Die Seite, auf die das nächste Dreieck stosst, heisst z). Die Summierung soll auf alle Dreiecke vorgenommen werden. Die so erhaltene Quantität werden wir *die minimale Flächengrösse der untersuchten Trennarten bezüglich des betrachteten Punktes* nennen.

In dem typischen Bestand von A wählt man zufälligerweise Punkte aus. Für jeden Punkt wird die minimale Flächengrösse der *a(i)*, $i = 1$ bis m, bestimmt. Der Durchschnitt der Flächengrösse wird ausgerechnet. Diesen betrachtet man als kennzeichnend für den Vegetationstyp A. Das gleiche Verfahren mit *b(i)*, $i = 1$ bis n, wird für den Vegetationstyp B verrichtet.

Jetzt ist man in der Lage, beliebigen Punkt auf dem untersuchten Gelände einem bestimmten Vegetationstyp zuzuordnen. Dazu bestimmt man die *minimale Flächengrösse* der jeweiligen Trennarten bezüglich des Punktes und berechnet man für jeden Vegetationstyp die Quantität

$$k = \langle F \rangle / F$$

wo F die minimale Flächengrösse bezüglich des Punktes und $\langle F \rangle$ die durchschnittliche minimale Flächengrösse bezüglich des jeweiligen Vegetationstyps ist. Man kann k die *Affinität des Punktes zu dem betrachteten Vegetationstyp* nennen. Bei einer pflanzensoziologischer Kartierung ordnet man einen Punkt dem Vegetationstyp zu, zu dem seine Affinität die grösste ist.

Ist die Affinität die grösste zu verschiedenen Vegetationstypen zugleich, so liegt der Punkt an deren Grenze.

Es ist bemerkenswert, dass die oben definierte Affinität eine dimensionslose Quantität ist, aus der sich im Prinzip zahlreiche andere bilden lassen, die aber nur einer Umskalierung entsprechen. Jede solche Quantität ist nämlich eine Funktion von *k*:

$$q = f(k)$$

Solche Umskalierungen können manchmal aus darstellungstechnischen Gründen zweckmässig sein.

Ein Beispiel: Szársomlyó-Berg

Die geschilderte Methode wurde am Beispiel der Abgrenzung von Trockenrasen in einem Bereich des Szársomlyó-Berges (Ungarn, Komität Baranya) erprobt. Hier berühren sich ein Felsenrasen (*Sedo sopianae-Festucetum dalmaticae* Simon 1964) und eine Hangsteppe (*Cleistogeni-Festucetum sulcatae* Zólyomi 1958, aber auch mit *Festuca dalmatica* (Hack.) Richt.) (Lehmann 1975, syntaxonomische Nomenklatur nach Borhidi und Sánta 1999). Ihr Übergangsbereich und die angrenzenden Zonen bildeten den Gegenstand der vorliegenden Untersuchung. Die lokal gültigen Trennarten wurden festgestellt. Sie sind wie folgt:

Felsenrasen:
Carex liparicarpos Gaud.
Ceterach javorkaeanum (Vida) Soó
Crupina vulgaris Pers.
Helianthemum canum (L.) Baumg.
Medicago minima (L.) Grufbg.
Potentilla erecta (L.) Räuschel
Sedum neglectum Ten. subsp. *sopianae* Priszter

Hangsteppe:
Cleistogenes serotina (L.) Keng.
Geranium columbinum L.
Hypericum perforatum L.
Teucrium chamaedrys L.

(Taxonomische Nomenklatur nach Simon 1992).

Diese Arten kommen stets und verhältnismässig einheitlich im entsprechenden Vegetationstyp vor, weisen aber eine deutlich kleinere Stetigkeit, beziehungsweise Artmächtigkeit im anderen auf. Entlang einem Transekt wurden Punkte in Abstand von 5 m ausgewählt und für diese die minimalen Flächengrössen bezüglich beider Vegetationstypen festgestellt. Die durchschnittlichen minimalen Flächengrössen wurden für beide Vegetationstypen auf Grund von je 40 Exemplaren aus den typischen Beständen berechnet. Der geschätzte Mittelwert für den Felsenrasen ist 3409 cm^2 (Standardabweichung: 8.62%), der für die Hangsteppe ist 217 cm^2 (Standardabweichung: 6.17%). Danach konnte in jedem Punkt die Affinität zu beiden Vegetationstypen bestimmt werden.

Die Abb. 2, wo die Affinität in einer logarithmischen Skala aufgenommen wurde, zeigt die Ergebnisse. Diese weisen unter anderem darauf hin, dass der Kontaktbereich kein Kontinuum, sondern vielmehr ein durch Kontinua gesäumter Durchdringungskomplex (Pfeiffer 1958, Dierschke 1994) ist. Die Vegetationstypen wechseln hier in kleinen Mosaiken, wo die Affinität zu beiden recht niedrig (< 0.1) ist.

Nach der Konzeption des Verfassers lässt sich dieser Komplex durch eine Selbstorganisation interpretieren: In einer Grenzlage, wo sich beide Vegetationstypen eigentlich mit der gleichen Chance hätten entwickelt können, kommt die biotische Wirkung der Vegetation auf den Standort (hier vielleicht die Erosionsverminderung) zu einer wichtigen Rolle. Die im Anfang kleinen zufälligen Bodenunterschiede verstärken sich, die positive Rückkoppelung erhält das Vegetationsmosaik.

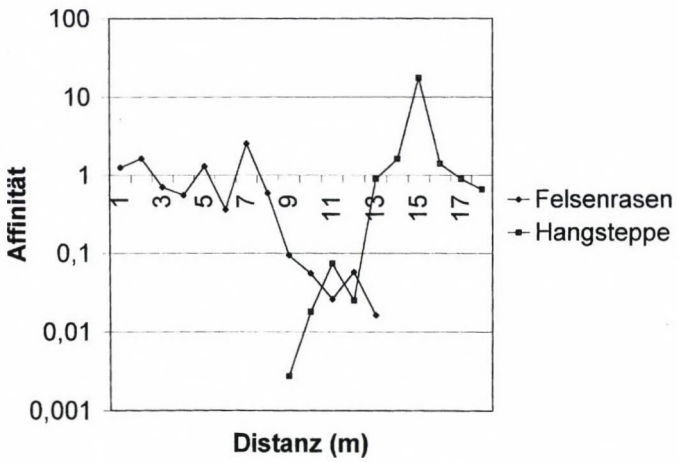

Abb. 2. Die Änderung der Affinitäten entlang einem Transekt auf dem Szársomlyó-Berg. Weitere Erläuterungen im Text

Diskussion

Die vorgeschlagene Methode bietet einen einheitlichen pflanzensoziologischen Grund zur Untersuchung der Übergangsbereiche zwischen verschiedenen, durch Pflanzensippen trennbaren Gesellschaften. Ein weiterer Vorteil ist, dass der statistische Fehler des Verfahrens durch die Berechnung der Standardabweichungen schätzen lässt. (Bei den üblichen Methoden bleibt diese Frage ganz offen). Wenn die Proben annähernd einer Gauss-Verteilung folgen oder die Probenanzahl gross genug ist, kann man sogar Konfidenz-Intervalle aufstellen (z. B. Jánossy 1965). Ein Fehler an dem Mittelwert beeinflusst alle Affinitäten zum entsprechenden Vegetationstyp in der gleichen Richtung. Dabei kann man diesen Fehler durch die Erhöhung der Probenanzahl (d. h. der Anzahl der ausgewählten Punkte in den typischen Beständen) im Prinzip beliebig erniedrigen. Die Standardabweichung des Mittelwertes nimmt nämlich mit der reziproken Quadratwurzel der Probenanzahl ab.

Man kann über die Anzahl der untersuchten Punkte je nach dem erzielten Massstab entscheiden. Die Auflösung der Ergebnisse hängt nur von der Entscheidung des Forschers, nicht aber von unvorhersehbaren inneren Eigenschaften der Methode ab.

Praktisch betrachtet ist es zweckmässig, mit möglichst wenigen, möglichst stetigen Arten zu arbeiten, damit man nicht zu grosse Distanzen messen muss und die Standardabweichung kleiner wird. Ausschlaggebend ist aber, durch welche Arten die Vegetationstypen getrennt werden. Ausserdem benötigt die Anwendung der Methode mindestens 2–3 solche Arten pro Vegetationstyp, sonst können die Dreiecke nicht gebildet werden. Reichen die festgesetzten Trennarten nicht aus, kann man allerdings zusätzliche Arten mit weniger ausdrücklichen Stetigkeitsdifferenzen hinzunehmen. Wichtig ist nur, dass die ausgewählte Artenverbindung die betrachteten Vegetationstypen voneinander trennt.

Dieser Beitrag geht nicht die Klassifizierbarkeit der Vegetation im allgemeinem an. Eine ausreichende Erfassung von Vegetationstypen auf dem untersuchten Gelände ist vorausgesetzt. Die Methode kann aber einigermassen andeuten, wenn diese Erfassung nicht vollkommen ist. Zum Beispiel können in einem weiten Bereich beinahe gleichmässig niedrige Affinitäten zu allen angenommenen Vegetationstypen darauf hinweisen, dass ein Vegetationstyp übersehen wurde.

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THE EFFECT OF PHENOLIC AMINO ACIDS ON DIFFERENTIATION OF DNA, RNA, PROTEINS AND CELL GROWTH IN *CICER ARIETINUM* L.

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The effect of three amino acids (phenylalanine, tyrosine, tryptophan) has been examined on differentiation of DNA, RNA, protein synthesis and cell growth in cell culture for *Cicer arietinum* L. cultivated in Murashige and Skoog medium. The *Cicer arietinum* L. cultures were treated with 20, 40, 60, 80 and 100 µg levels of each phenolic amino acid. Amount of DNA, RNA, proteins and cell growth was determined by the levels in the time intervals of 24 hrs incubation time during 6 days. Obtained data were evaluated statistically.

Consequently, experimental results reveal that excessive amount of phenolic amino acids cause significant inhibition on RNA, protein and cell growth. Phenylalanine has no strong effect on DNA synthesis, or activity of DNA polymerase was unaffected by phenylalanine but tyrosine and tryptophan have significant effect on DNA. On the other hand, it has been supposed that extreme amount of phenolic amino acids may be converted to phenolic compounds by cell regulatory systems.

Key words: cell culture, cell growth, *Cicer arietinum*, in vitro, phenylalanine, tyrosine, tryptophan

Introduction

Phenylalanine (*Phe*), tyrosine (*Tyr*) and tryptophan (*Trp*) are to be formed by combination of phenyl, hydroxyphenyl and indol rings, respectively, to the methyl group of alanine. These amino acids containing aromatic rings are converted into some important compounds in plants. A solid polymer lignin is derived by *Tyr* and *Phe*. *Trp* is precursor molecules for plant growth such as auxins.

Many other phenolics also arise from shikimic acid pathway and subsequent reactions. Among these are the acids cinnamic, *p*-coumaric, caffeic, ferilic, chlorogenic, protocatechuic, and Gallic. They are important not because they are abundant in combined form, but because they are converted into several to alexins, coumarins, lignin and flavonoids such as the anthocyanins (Floss 1986, Jensen 1985, Siehl and Conn 1988).

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Also these derivatives are important phenolic compounds. Protocatechuic and chlorogenic acids may be converted into harmfulness free radicals in plant cells by the effect of polyphenol oxidase enzyme (Vamos-Vigyazo 1981, Mayer 1987).

Gallic acid is important because of its conversion to gallotanins, which are heterogeneous polymers containing numerous gallic acid molecules connected in various ways to one another and to glucose and other sugars. Many gallotanins greatly inhibit plant growth have described some other phenolic compounds originated from aromatic amino acids in plant cell (Hemingway and Karchesy 1989). Growth of plant cells could be inhibited if there are much amount of the compounds named as coumarin in media and also scopletin which is a phenolic compound has an inductive effect on germination and dormancy (Mathew and Parpia 1971).

The studies about effect of amino acids on plants show that there are many advantage of performing them in cell culture media (Torres 1989), and those investigators declared that cell cultures are negatively effected if aromatic amino acids are used lonely.

In this study, the effects of different aromatic amino acids have been examined on differentiation of DNA, RNA, cell growth and protein quantity in cell cultures obtained from *Cicer arietinum*.

Materials and methods

Biological material: Ten days old seedlings obtained from in vitro germination of mature seeds of *Cicer arietinum* L. were used as explants source in this study. The nodal explants, which were obtained from this seedlings, were cultivated in Murashige and Skoog medium (Murashige and Skoog 1962) that contains 2 mgl^{-1} NAA + 1.5 mgl^{-1} BAP to grow callus. After about four weeks later, these callus were transferred into a liquid culture medium that contain MS to produce suspension culture. Twenty gl^{-1} saccharose were added to this medium. The liquid is shaking by horizontal shaker at 100 rpm during a period of 3 days in Erlenmeyers of 150 ml. Then, the cells that removed from callus by this way were washed under sterile conditions, incubated at 25±2 °C, exposed to 16/8 hrs photoperiod. The cell culture was divided into 96 tubes each containing 10 ml medium. Six of 96 tubes were used for controls. Thirty tubes were used for Phe levels so that 20, 40, 60, 80 and 100 µg *Phe* were added to each six tubes of thirty. In this

way, we have six replicates for each level of *Phe*. The same design for levels and replicates were prepared for *Trp* and *Tyr*.

Data collection process: The five variables, amount of DNA, RNA, protein, amino acids and cell growth were determined for one replicate by the level of *Phe*, *Trp*, *Tyr* and control, by the following way to be stated. This process was repeated six times between the time periods of 24 hrs of incubation time successively during 6 days. The data matrix obtained by this process is presented in Table 1.

Table 1

The matrix resulted from experimental study

Treatment	Level	Hour	Cell growth	DNA	RNA	Protein	Amino acid
Control	–	0	0.030	12.00	0.300	26.00	0.0
Control	–	24	0.035	16.00	0.310	33.00	0.0
Control	–	48	0.042	22.00	0.320	38.00	0.0
Control	–	72	0.055	29.00	0.315	43.00	0.0
Control	–	96	0.063	38.00	0.310	45.00	0.0
Control	–	120	0.071	43.00	0.310	46.00	0.0
Phenylalanine	20	0	0.030	12.00	0.300	26.00	20.0
Phenylalanine	20	24	0.037	15.00	0.310	32.00	11.0
Phenylalanine	20	48	0.043	19.00	0.290	35.00	8.0
Phenylalanine	20	72	0.055	21.00	0.285	36.00	3.0
Phenylalanine	20	96	0.063	23.00	0.273	37.00	1.0
Phenylalanine	20	120	0.068	24.00	0.270	38.00	1.0
Phenylalanine	40	0	0.030	12.00	0.300	26.00	40.0
Phenylalanine	40	24	0.033	13.00	0.300	31.00	28.0
Phenylalanine	40	48	0.042	19.00	0.280	33.00	19.0
Phenylalanine	40	72	0.051	24.00	0.270	35.00	11.0
Phenylalanine	40	96	0.063	26.00	0.264	35.00	8.0
Phenylalanine	40	120	0.066	27.00	0.260	36.00	6.0
Phenylalanine	60	0	0.030	12.00	0.300	26.00	60.0
Phenylalanine	60	24	0.033	16.00	0.290	27.00	44.0
Phenylalanine	60	48	0.045	18.00	0.270	32.00	33.0
Phenylalanine	60	72	0.058	24.00	0.265	32.00	24.0
Phenylalanine	60	96	0.061	25.00	0.250	33.00	23.0
Phenylalanine	60	120	0.062	26.00	0.240	34.00	21.0
Phenylalanine	80	0	0.030	12.00	0.300	26.00	80.0
Phenylalanine	80	24	0.034	16.00	0.270	27.00	66.0
Phenylalanine	80	48	0.036	22.00	0.250	28.00	51.0
Phenylalanine	80	72	0.039	23.00	0.230	29.00	34.0
Phenylalanine	80	96	0.046	24.00	0.223	29.00	33.0
Phenylalanine	80	120	0.048	24.00	0.220	30.00	33.0
Phenylalanine	100	0	0.030	12.00	0.300	26.00	100.0
Phenylalanine	100	24	0.038	17.00	0.260	27.00	73.0
Phenylalanine	100	48	0.039	19.00	0.240	27.30	51.0

Treatment	Level	Hour	Cell growth	DNA	RNA	Protein	Amino acid
Phenylalanine	100	72	0.040	20.00	0.220	28.00	47.0
Phenylalanine	100	96	0.040	21.00	0.210	28.40	46.0
Phenylalanine	100	120	0.041	21.00	0.200	29.00	44.0
Tyrosine	20	0	0.030	12.00	0.300	26.00	20.0
Tyrosine	20	24	0.034	13.00	0.320	26.40	16.0
Tyrosine	20	48	0.041	15.00	0.270	27.10	11.0
Tyrosine	20	72	0.046	17.00	0.260	28.00	6.0
Tyrosine	20	96	0.053	19.00	0.250	28.80	2.0
Tyrosine	20	120	0.061	21.00	0.250	29.00	1.0
Tyrosine	40	0	0.030	12.00	0.300	26.00	40.0
Tyrosine	40	24	0.035	14.00	0.290	26.70	31.0
Tyrosine	40	48	0.040	17.00	0.270	26.90	26.0
Tyrosine	40	72	0.046	21.00	0.258	27.50	14.0
Tyrosine	40	96	0.054	23.00	0.244	28.30	8.0
Tyrosine	40	120	0.059	24.00	0.240	28.80	3.0
Tyrosine	60	0	0.030	12.00	0.300	26.00	60.0
Tyrosine	60	24	0.040	14.00	0.260	26.30	42.0
Tyrosine	60	48	0.043	17.00	0.259	26.80	31.0
Tyrosine	60	72	0.051	19.00	0.250	27.20	22.0
Tyrosine	60	96	0.053	21.00	0.230	27.30	16.0
Tyrosine	60	120	0.056	22.00	0.220	27.40	11.0
Tyrosine	80	0	0.030	12.00	0.300	26.00	80.0
Tyrosine	80	24	0.030	13.00	0.270	26.80	69.0
Tyrosine	80	48	0.032	15.00	0.230	27.00	51.0
Tyrosine	80	72	0.034	17.00	0.200	27.10	36.0
Tyrosine	80	96	0.036	17.00	0.170	26.00	29.0
Tyrosine	80	120	0.037	16.00	0.160	25.00	24.0
Tyrosine	100	0	0.030	12.00	0.300	26.00	100.0
Tyrosine	100	24	0.030	11.00	0.250	27.00	77.0
Tyrosine	100	48	0.029	12.00	0.230	27.20	54.0
Tyrosine	100	72	0.029	13.00	0.150	27.00	44.0
Tyrosine	100	96	0.030	12.00	0.147	24.80	40.0
Tyrosine	100	120	0.028	12.00	0.140	23.00	38.0
Tryptophan	20	0	0.030	12.00	0.300	26.00	20.0
Tryptophan	20	24	0.033	12.00	0.280	28.00	10.0
Tryptophan	20	48	0.038	16.00	0.270	30.00	6.0
Tryptophan	20	72	0.049	19.00	0.240	32.00	2.0
Tryptophan	20	96	0.052	19.60	0.236	32.70	0.0
Tryptophan	20	120	0.058	20.00	0.230	33.00	0.0
Tryptophan	40	0	0.030	12.00	0.300	26.00	40.0
Tryptophan	40	24	0.034	13.00	0.280	27.00	23.0
Tryptophan	40	48	0.044	15.00	0.260	29.00	11.0
Tryptophan	40	72	0.049	16.80	0.240	29.40	4.0
Tryptophan	40	96	0.051	17.50	0.223	29.80	3.0
Tryptophan	40	120	0.052	18.00	0.220	30.00	0.0
Tryptophan	60	0	0.030	12.00	0.300	26.00	60.0

Treatment	Level	Hour	Cell growth	DNA	RNA	Protein	Amino acid
Tryptophan	60	24	0.034	13.40	0.260	27.00	23.0
Tryptophan	60	48	0.044	14.40	0.230	27.30	11.0
Tryptophan	60	72	0.049	15.00	0.210	27.00	4.0
Tryptophan	60	96	0.051	15.80	0.205	26.80	3.0
Tryptophan	60	120	0.044	16.00	0.200	26.90	0.0
Tryptophan	80	0	0.030	12.00	0.300	26.00	80.0
Tryptophan	80	24	0.039	12.60	0.250	26.40	48.0
Tryptophan	80	48	0.034	13.20	0.210	26.30	32.0
Tryptophan	80	72	0.035	14.60	0.170	25.80	18.0
Tryptophan	80	96	0.035	14.20	0.150	24.40	12.0
Tryptophan	80	120	0.035	14.00	0.140	24.00	9.0
Tryptophan	100	0	0.030	12.00	0.300	26.00	100.0
Tryptophan	100	24	0.031	12.30	0.250	27.00	59.0
Tryptophan	100	48	0.028	12.50	0.180	26.00	38.0
Tryptophan	100	72	0.026	12.10	0.150	23.30	29.0
Tryptophan	100	96	0.026	11.30	0.130	21.00	26.0
Tryptophan	100	120	0.025	11.00	0.130	20.00	24.0

DNA isolation: 2 ml of cell suspension culture was centrifuged for 15 min at 10,000 g, and supernatant was removed. A tight-fitting pestle until pestle moves freely up and down (8–10 times) homogenised pellet. Through 10 layers of sterile gauze (prewet 5% citric acid) was poured and centrifuged for 5 min at 2,500 g. Centrifuge was repeated twice. Overlay homogenate was spined on a 15 ml tube at 5,000 g for 5 min. Nuclear pellet was resolved in 10 ml of 10 mM tris, 10 mM NaCl, 25 mM EDTA, and centrifuged at 2,500 g. One ml of SDS 1% was added. Then 11 mg proteinase K powders, and extract was subjected to 5 ml phenol-chloroform-isoamil alcohol (10–10–1), and centrifuged at 5,000 g for 15 min. Aqueous extract phase was incubated in 20 µl DN-ase free RN-ase (10 $mgml^{-1}$) for 30 min at 37 °C (Davis 1986).

RNA isolation: 5 ml of cell culture was homogenised then added 1 ml of SDS 1% mix by vortexing. One ml phenol, 1 ml chloroform-isoamil alcohol (24–1) was added, and incubated at 55 °C by 5 min. Then incubated in ice water. Addition of phenol-chloroform-isoamil alcohol and transferring aqueous phase 2.5 volumes prechilled ethanol were repeated. Supernatant was discarded and RNA stored at –70 °C (Davis 1986).

Protein content was determined by Lowry method (Lowry et al. 1951). Nucleic acid content was determined at 260 nm by ultraviolet absorption spectroscopy by Maniatis (Maniatis et al. 1982). Amino acid concentration

Table 2

Paired t-test results between each pair of treatments by levels

	Level	Control-*Phe*	Control-*Tyr*	Control-*Trp*	*Phe-Tyr*	*Phe-Trp*	*Tyr-Trp*
DNA	20	–	+	+	+	+	–
	40	+	+	+	–	+	+
	60	–	+	+	+	+	+
	80	–	+	+	+	+	+
	100	–	+	+	+	+	–
RNA	20	+	+	+	–	+	–
	40	+	+	+	+	+	+
	60	+	+	+	+	+	+
	80	+	+	+	+	+	+
	100	+	+	+	+	+	–
Protein	20	+	+	+	+	+	+
	40	+	+	+	+	+	+
	60	+	+	+	+	+	–
	80	+	+	+	–	+	+
	100	+	+	+	–	–	+
Cell growth	20	–	–	+	+	+	–
	40	–	–	–	–	–	–
	60	–	–	–	–	–	–
	80	+	+	–	+	–	–
	100	–	+	+	+	+	–

+ = $p < 0.05$, – = $p > 0.05$

was measured in optical density by UV spectrophotometer (*Trp* at 280 nm, *Phe* at 260 nm, and *Tyr* at 275 nm). Cell growth was establishment at 440 nm by spectrophotometer and counting in thomalam.

Statistical analysis: The paired t-test was used to determine whether the differences between treatments were statistically significant or not. And levels by treatments were clustered by Ward's Minimum Variance method (Everitt 1974). The statistical analyses were performed by using SYSTAT (version 5.1) (Wilkinson 1990).

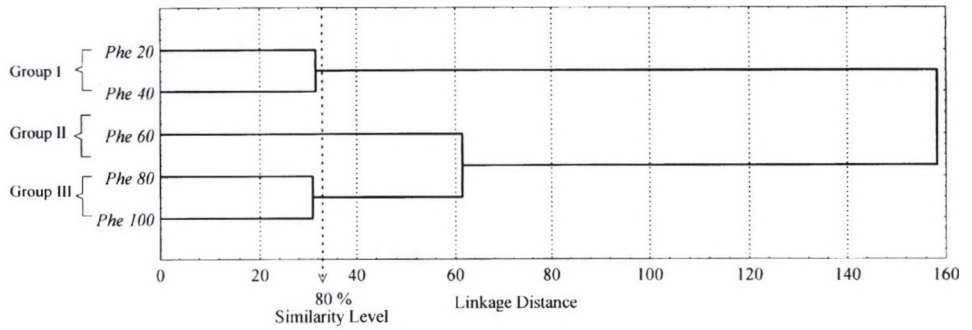

Fig. 1. Dendrogram obtained for levels of *Phe* by using Ward's Minimum Variance method with Euclidean distance

Results

The data matrix resulted from the process stated in material and method is presented in Table 1.

The paired t-test was employed to test differences between each pair of treatment. The results of these tests are summarized in Table 2 and whether these differences are statistically significant or not are marked.

The levels by treatments were clustered by using Ward's Minimum Variance method with Euclidean distance to determine which levels are more similar. Resulted dendrograms from this analysis were presented in Figures 1, 2 and 3.

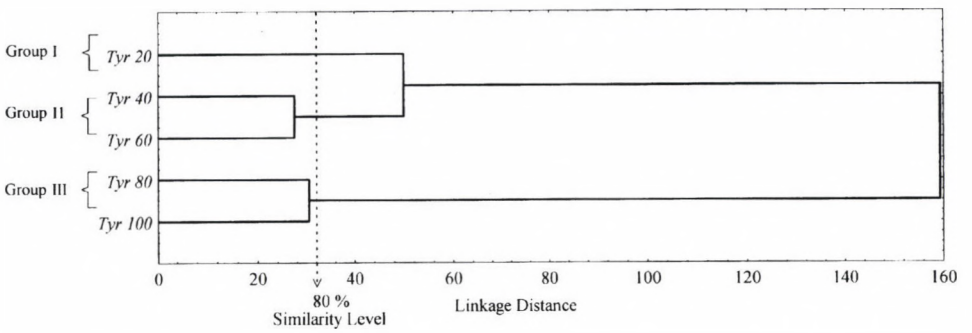

Fig. 2. Dendrogram obtained for levels of *Tyr* by using Ward's Minimum Variance method with Euclidean distance

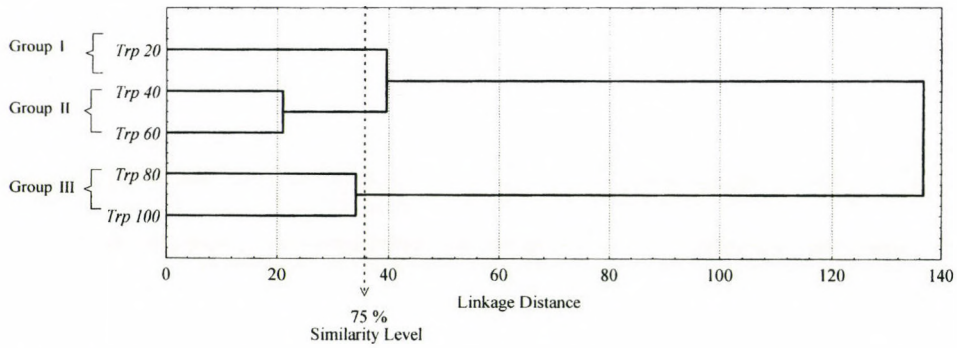

Fig. 3. Dendrogram obtained for levels of *Trp* by using Ward's Minimum Variance method with Euclidean distance

Discussion

There are no statistical differences between control and *Phe* levels with respect to amount of DNA (see Table 2). So, it has been supposed that phenolic compounds produced by *Phe* do not strongly effect on DNA synthesis or activity of DNA polymerase unaffected. A similar study has been carried out by Jacopsen (1977).

Amount of DNA was decreased significantly by effects of *Tyr* and *Trp* at about all levels. This case may be resulted from inhibition of DNA polymerase. Some investigators (Vamos-Vigyazo 1981, Mayer 1987, Hemingway and Karchesy 1989) reported that coumarins have such a similar effect. Scolopoletin that is a phenolic compound and induce dormancy in seeds may cause an effect of this kind on *Cicer* cells (Jensen 1985, Siehl and Conn 1988). On the other hand, *Trp* is more effective than *Tyr* on decrease of DNA amount.

In general, *Phe*, *Tyr* and *Trp* caused significant decreasing on amount of RNA and protein. There could be many reasons of this decreasing such as insufficient RNA synthesis, inhibition in transcription, translation or a stage of protein synthesis, e.g. elongation. It has been stated by Floss (1986), Jensen (1985), Siehl and Conn (1988) and Jacopsen (1977) that some of phytoalexins and coumerins produce phenolic compounds that inhibit activity of RNA polymerase and protein synthesis. By means of the obtained results, effectiveness of these amino acids on amount of RNA and protein can be ordered as *Phe*, *Tyr*, *Trp* in descending order.

Naturally, cell growth is paused due to strong effect of phenolic amino acids on DNA, RNA and protein. Metabolic performance of cell decreases due to inhibition of protein synthesis that effect production of many enzymes, which are necessary for cell metabolism. *Trp* is a precursor substance for IAA but it may be converted to phenolic compounds to regulate cell growth if there are excessive amount in medium. Moreover, this indicates that there is a certain regulatory system for growth of plant cells.

As a result, it has been supposed that excessive amount of phenolic amino acids, which cause inhibition in DNA, RNA and protein synthesis, converted to phenolic compounds by regulatory systems to prevent cell. The studies carried out on gallic acid, phytoalexin, coumerin, scolopectins, protocatechuic acid, and chlorogonic acid are in accordance with this idea (Jensen 1985, Mathew and Parpia 1971, Matheis and Belitz 1975). Also, phenolic acids are converted to free radicals by polyphenol oxidase enzyme and the free radicals especially inhibit enzymatic activity that causes some inhibition in cells (Vamos-Vigyazo 1981, Mayer 1987).

When phenolic amino acids are used merely for resource of N and C, inhibitions occur in cells at some stages. So, it is appropriate to use either inorganic resource of N and C or amino acids that could not been convertible to phenolic compounds.

As it is seen in Figure 1, tree clusters are obtained at about 80% similarity level. Twenty–40, 60, 80–100 μgl^{-1} of *Phe* levels constitute cluster I, II and III respectively. Twenty, 40–60, 80–100 μgl^{-1} of *Tyr* and *Trp*'s levels constitute cluster I, II and III at 80% and 75% similarity levels, respectively (see Figs 2 and 3). These results reveal that effect of levels in the same cluster are similar to each other as much as the similarity level with respect to the determined variables (amount of DNA, RNA, protein and cell growth).

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TAXONOMIC STUDY OF *FESTUCA WAGNERI* (DEGEN, THAISZ ET FLATT) DEGEN, THAISZ ET FLATT IN DEGEN 1905

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This paper reviews the nomenclatural history of an important grass species of the Carpathian Basin, *Festuca wagneri*, clarifies its valid and legitimate name, and provides additional anatomical data for complementing the original description of the species. The legitimate name of the taxon is *Festuca wagneri* (Degen, Thaisz et Flatt) Degen, Thaisz et Flatt in Degen. Based on the original specimens and living individuals collected from the locus classicus and from several areas of the Danube–Tisza Interfluve, the sclerenchyma may form a mostly unequal ring in the transverse sections of old leaves of *Festuca wagneri*. Furthermore, the abaxial epidermis of its leaves wears macro-hairs. These additional features facilitate the identification of *Festuca wagneri* in the field.

Key words: *Festuca*, grass species, taxon, epidermis, hybrid, lectotype

Introduction

The identification of *Festuca* species poses a range of problems. The characteristic features of the species are hard to recognise and the nomenclature of the genus is confusing. Several authors give illegitimate names or quote others imprecisely while plenty of associations are named after these dominant species of the *Festuca* genus.

Festuca wagneri is one of the most important grass species in the Great Hungarian Plain having transitional sclerenchyma (Häckel 1882, Horánszky et al. 1971, Simon 1992). Clarifying its legitimate name, and describing unambiguous features for its identification in the field are essential for plant taxonomy and coenology.

Material and methods

Four specimens from Gramina Hungarica exsiccate (Degen 1905, 1915) collected by János Wagner from Deliblato ("Deliblát") in 1904 and 1905 were examined.

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Thaisz (1905) and Degen (1905, 1915) did not select the holotype specimen, therefore the designation of a lectotype is necessary. The designated specimen was collected by Wagner on 25th of May, 1905 from Deliblato ("Deliblát"). The lectotype indicated by the number 173 in Degen's Gramina Hungarica (1905) is in the Herbarium of the Department of Botany and Plant Physiology, Szent István University, Gödöllő.

The studied living individuals were collected in 1998 from the Danube–Tisza Interfluve (in sites of Csévharaszt, Örkény, Imre-hegy and Pirtó), from the Gödöllői-dombság (in Domony-völgy) and from the locus classicus: Deliblato ("Deliblát"), Yugoslavia.

Transverse sections were made from the lower third part of late and early leaves of both living individuals and herbarium specimens. Leaves at the end of the vegetation period will be treated as "old" or "late", while leaves from the same stocks in the springtime are reported to be "early" or "spring" leaves.

Similar to Horánszky's works (1954, 1955), the epidermis was studied based on the method of Ujhelyi (1954). Thus, the abaxial, adaxial and marginal epidermis were examined at the same time.

Anatomical terms used in this paper follow Metcalfe (1960).

Results

Nomenclature

In 1904 and 1905, the first individuals of *Festuca wagneri* were collected by Wagner on the sand hills near to Deliblato ("Deliblát"; east of Beograd, Yugoslavia) and published by Thaisz (1905) as a new variety of *Festuca sulcata* with original description in Hungarian and Latin. Unfortunately, the Hungarian title "Festuca wagneri Deg. Thsz. et Flatt, a new variety of Festuca sulcata" could be misunderstood since, the first impression suggested that the paper was about a species, and the fact that the new plant is a variety of *F. sulcata* (considered at that time as the subspecies of *F. ovina*) was only explained later in the title. Furthermore, the title in the German translation of the paper "Eine neue Subvarietät der F. sulcata" declared the new taxon as a subvariety. The correct name of the plant considering the full Hungarian title of Thaisz's paper is: *Festuca ovina* L. subsp. *sulcata* Häckel var. *wagneri* Deg., Thsz. et Flatt.

On the labels of Gramina Hungarica exsiccate, Degen (1905) wrote the expression "nobis" after the name, *Festuca wagneri*, and indicated the origi-

nal description, referring to the Thaisz's paper: "MBL 1905 4: 30–31". Thus Degen declared that the plant was regarded as a species whose correct name is as follows: "Festuca wagneri Degen, Thaisz et Flatt". Also this name was indicated on the later specimens of Degen's exsiccate, published in 1915.

Subsequent authors did not consider Degen's combination, they mostly quoted Thaisz's (1905) article in various ways.

Jávorka (1925) regarded the plant as a taxon under the species *Festuca sulcata*, however its precise taxonomic category came to light only in his exsiccate (FHE 1927). Consequently the name given by Jávorka is "Festuca sulcata (Hack.) Beck var. Wagneri Deg., Thsz. et Flatt".

Saint-Yves (1928), used the name "Festuca ovina L. subsp. sulcata Häckel var. Wagneri Thaisz et Flatt" following the Hungarian title of the Thaisz's publication (1905).

Domin (1929) published Krajina's description of *Festuca pseudodalmatica* and quoted Krajina's remark, who also mentioned *Festuca Wagneri* Thaisz among the taxa related to *F. pseudodalmatica*. This remark of Krajina has been considered as the first species combination of *F. wagneri*, and in the Slovak literature (Dostál 1989) the name is used as "Festuca wagneri (Degen, Thaisz et Flatt) Krajina".

Festuca wagneri does not appear in The handbook of Hungarian flora (Jávorka and Soó 1951).

Májovský (1962) treated the taxon as a variety of the species described by himself for the first time as *Festuca javorkae* and published its name as follows: "Festuca javorkae Májovský var. wagneri (Dg. Thsz. Flatt), comb. nov.".

Soó (1963a, b) declared the above-mentioned combination of Májovský to be illegitimate. At the same time Soó was also wrong when arguing that, what Thaisz (1905) has described was a species which cannot be assorted as a variety of a later described species (*F. javorkae*). Therefore the legitimate name given by Soó (1963a, b) is : "Festuca wagneri Degen, Thaisz et Flatt em. Soó".

In the Romanian (Săvulescu 1972) and the Serbian (Josifović 1976) works "Festuca valesiaca Schleich. var. wagneri (Degen, Thaisz et Flatt)" is published, however, neither of them explained why *F. valesiaca* includes the mentioned variety.

The Flora Europaea (Markgraf-Dannenberg 1980) lists the taxon as "Festuca wagneri (Degen, Thaisz et Flatt) Krajina" based on the Slovak usage.

Pils (1985) proposed that in the Carpathian Basin all taxa (including *Festuca wagneri*), which can be characterised by transitional sclerenchyma and occasionally even sclerenchyma rings can be considered as belonging to a single species, *Festuca javorkae* Májovský.

Simon's (1992) Plant identification book of the Hungarian vascular flora lists the taxon as follows: "Festuca x wagneri Degen, Thaisz et Flatt em. Soó".

In accordance with the §29–31 of the International Code of Botanical Nomenclature (Greuter et al. 1994), all corrections that raised a taxon to the level of species and that was indicated before 1953 in the labels of exsiccates published in several copies, provides a legitimate species name. These paragraphs are valid for the case of Degen's combination, therefore the legitimate name of the studied taxon with its real authors is as follows: *Festuca wagneri* (Degen, Thaisz et Flatt) Degen, Thaisz et Flatt in Degen GH, 1905. In this case the combination of Krajina must be considered as a later homonym.

Several authors consider *Festuca wagneri* to be of hybrid origin. In The handbook of the Hungarian Flora of Jávorka and Soó (1951) the taxon name, *Festuca firma* Vetter (1917) appears as a hybrid of *Festuca sulcata* and *F. vaginata*, while later Soó (1973) published *Festuca x wagneri* Deg., Thaisz et Flatt 1905 emend. Soó 1963 as the hybrid of the above–mentioned two species. Horánszky et al. (1971) put forward that *Festuca wagneri* comes from cross-fertilization of *Festuca vaginata* and *Festuca pseudovina*. In his view (Horánszky 1992) other species that may also have participated in the crossing, in addition to *Festuca vaginata*, includes *Festuca sulcata*, *Festuca pseudovina* and *Festuca valesiaca*.

Anatomical studies

Based on the tissue investigations of the original specimens and living individuals collected from the locus classicus, and from several areas of Danube–Tisza Interfluve, *Festuca wagneri* has thin leaves having three more expressed, and one or two less expressed, sclerenchyma-strands in the transverse sections of early (spring) leaves (Fig. 1).

Subsequently, all sclerenchyma-strands become expressed, they may even form mostly irregular ring in the old leaves (Fig. 2).

Figure 3 shows the epidermis of *Festuca wagneri*. During the whole vegetation period the abaxial epidermis of all leaves wears macro-hairs. These macro-hairs are also apparent in the transverse sections (Figs 1–3).

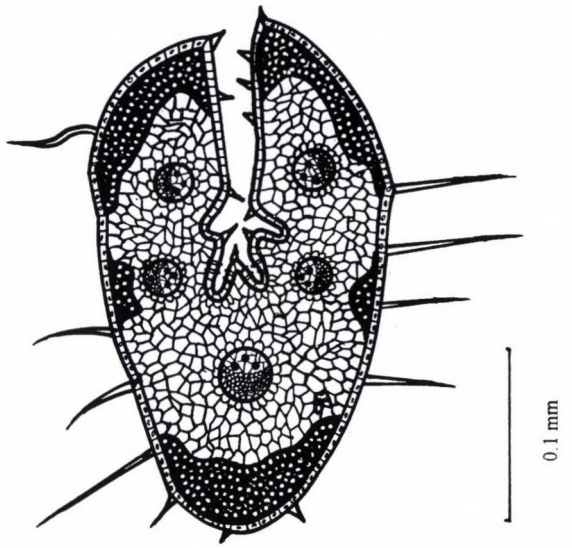

Fig. 1. Transverse section of a spring leaf of *Festuca wagneri*. Three more expressed and one less expressed sclerechyma-strands bundles and macro-hairs on the abaxial epidermis can be seen (K. Penksza)

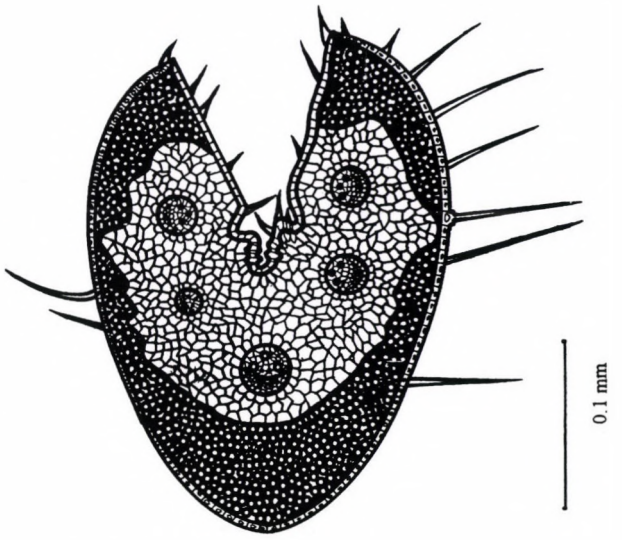

Fig. 2. Transverse section of an old leaf of *Festuca wagneri*. Sclerenchyma-strands form a continuous, irregular ring (K. Penksza)

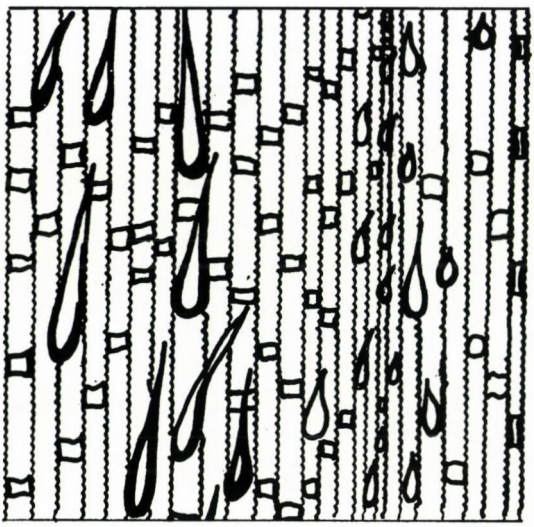

Fig. 3. Abaxial, transitional and adaxial epidermis of *Festuca wagneri*. Abaxial epidermis wears long macro-hairs, adaxial epidermis is sporadically covered by spickle-hairs while transitional epidermis is frequently covered by micro-hairs (L. Bauer)

The adaxial epidermis is sporadically covered by spickle-hairs, and macro-hairs. Marginal epidermis is frequently covered by micro-hairs.

Discussion

The International Code of Botanical Nomenclature regulates unambiguously the names of taxa and their authors. In accordance with the Code, the legitimate name of the investigated taxon with its valid author name is *Festuca wagneri* (Degen, Thaisz et Flatt) Degen, Thaisz et Flatt in Degen GH, 1905. Other names, either related to the taxon or to its author(s), are not legitimate.

Festuca wagneri has well-definable anatomical features which make the identification of the species unambiguous, even in the field. Although it is a species having transitional sclerenchyma with 5 strands most frequently, the sclerenchyma in the transverse sections of old leaves may form a mainly unequal ring. Furthermore, the abaxial epidermis of its leaves wears macro-hairs, which have not been published before.

In the Carpathian Basin *Festuca* species of the group of *F. ovina* (Soó 1955, Horánszky et al. 1971) do not have the above-mentioned macro-hairs on the abaxial epidermis, except *Festuca wagneri*, thus in terms of this feature the investigated taxon can be easily separated from other *Festuca* species.

Presence of abaxial macro-hairs in *Festuca wagneri* also refutes Soó's opinion that *Festuca stricta* living also in sandy habitats and *F. wagneri* are the same species. Soó together with Horánszky (1955, pp. 216–217) published pictures of abaxial epidermis of *F. stricta* with no hairs on, and consequently *F. stricta* and *F. wagneri* cannot be considered as the same species.

In addition, the presence of macro-hairs makes disputable the supposed hybrid origin of *Festuca wagneri*, since none of the possible parents have macro-hairs on its abaxial epidermis.

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LONG-TERM VEGETATION TEXTURAL CHANGES OF THREE FEN COMMUNITIES NEAR CLUJ-NAPOCA (ROMANIA)

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The question discussed in this paper is whether changes of environmental factors can be supported by changes reflected in vegetation texture. A case study is presented on the statistical analysis and interpretation of changes in vegetation texture in some rich fen associations: *Carici flavae-Eriophoretum latifolii*, *Junco obtusiflori-Schoenetum nigricantis* and a *Cladietum marisci* association using the species indicator values for moisture (W), acidity (R) and nutrients (N). For characterising trends of changes the social behaviour types (SBT) and coenological grouping of the species are also used. The study of changing vegetation are evaluated on the basis of relevés made in the beginning of the 1940s, in 1956, 1961 and 1998 on non-permanent plots with varying numbers among the years. Data were collected by different people in the mentioned years in the 7 fen patches in Malom Valley (Romania). The pattern of indicator values was analysed by concentration analysis. Significant correlation was found between the coordinates calculated from indicator values for moisture and acidity. Similar behaviour was found in vegetation textural changes indicated for moisture, acidity and nutrient status of the two rich fen associations, but the rate of coenological groups and social behaviour types changed differently. The *Cladietum marisci* association presented completely different behaviour.

Key words: annual precipitation, calcareous ground-water, chronosequence, indicator values, non-permanent plot

Introduction

Wetlands are interesting from a nature conservation point of view being generally relatively poor in nutrients, but very rich in species. The soil of wetlands of this kind is, however, eutrophic peat. The total amount of nutrients is enormous, but their availability is controlled by certain growth limiting factors maintaining it at a low level by the hydrological conditions. Therefore, the species composition of these mires is mainly determined by the fluctuations and composition of the groundwater table (van Diggelen et al. 1991b).

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In this work for studying the textural changes of three fen communities several characteristics of the species from these vegetation units (their indicator values, social behaviour types, coenological status) were used. The use of indicator values in the vegetation studies is widespread following Ellenberg's activity (Ellenberg 1950, Ellenberg et al. 1991). In Hungary many authors dealt with the classification of species into ecological groups (Zólyomi et al. 1967, Soó 1964–1980, Kárpáti and Kárpáti 1972, Kárpáti et al. 1968, Kárpáti 1978, Simon et al. 1992, Borhidi 1993, 1995). Borhidi's (1995) work contains the classification of the whole Hungarian flora following the system of Ellenberg et al. (1991) and extended it for the SE European species. The former native works did not contain the whole Hungarian flora (except Soó 1964–1980), and used different indicator scales resulting incompatible with groupings of Ellenberg and other authors. In Romania Sanda et al. (1983) classified the species into ecological groups also after Ellenberg (1950). Using indicator values in vegetation research is an aid in those cases where detailed environmental measurements, although wanted, are impractical or impossible to obtain (Persson 1981).

Borhidi (1993, 1995) introduced the term of social behaviour types (SBT) of species as a useful tool to evaluate of the naturalness of plant communities. This classification is based upon how species take part in an association and it refines Grime's (1979) C-S-R strategy classification. Earlier, Simon (1988) made a classification of the native flora for evaluate the nature conservation value of the flora of different areas. In this systematisation he took into consideration the species' rarity and conservation status, too. Borhidi's (1995) systematisation is theoretically more established.

The coenological status of a species suggests its affection for a coenological group. The comparison of the proportion of species with different coenological statuses is a method for evaluating the direction of succession.

Permanent plots are appropriate tools for studying the long-term changes of vegetation (Bakker et al. 1996, Herben 1996). However, we can get information about the trends of vegetation changes without permanent plots, for example by space-for-time substitution (Pickett 1989). The chronosequence may be reconstructed based on hypothetical succession schemes (Précsényi 1981, Zólyomi et al. 1988, Fekete 1992), or on time spent since abandonement by land-history data (Molnár and Botta-Dukát 1998). The other two possibilities for studying succession are repeated mapping (Czenthe 1985, Borhidi et al. 1991, Horváth and Csontos 1992) and repeated sampling method (Molnár 1996, Molnár et al. 1997). We were using the latter approach in our work.

In our study we are searching for the answer to two main questions:

1. How narrow is the correlation between the fluctuation of precipitation quantity (the alternation of wet and dry periods) and the vegetation-textural changes?

2. What is the degree of reliability of comparisons of non-permanent plots recorded by different people with greater time-lag in describing successional processes?

Materials and methods

Description of the study area

The Malom Valley ranges 8 km S of Cluj-Napoca (Romania), southwestward to Feleacu, at 650 m above sea level. After Borza (cit. Pop et al. 1962) this area floristically belongs to the Bihar sector and phytogeographic district of Apuseni Mountains. The Malom brook scoops its bed in Sarmatian sand and sandstone sediments covered by a Miocene clayey-marly strata. On this red clayey stratum a reddish-brown forest-soil had formed. The west–east directed valley is embraced by oak and oak-hornbeam forests on its northern slopes, and by beech and hornbeam-beech forests on its southern ones. On the slopes of the 5 km long valley there are mesophilous and poor meso-xerophilous hayfields and *Nardus stricta* stands, too. These are mowed regularly once a year at the end of July or at the beginning of August, and are grazed moderately by sheep and sometimes by cattle. The brook is accompanied by alders and willows, with marshmeadows and high-sedges in some places.

Where the water of the springs flows together the clayey surface rich fens were forming, which are fed by calcareous ground water. These fens are patchily inserted into the hayfields. There are three associations: *Carici flavae-Eriophoretum latifolii, Junco obtusiflori-Schoenetum nigricantis* and *Cladietum marisci* which are mosaically situated. On the edges of the fens there are haymeadows with little extension in some places. These fens were mown regularly till the beginning of the 1960s which could cause changes in the structure of the vegetation. The continuous forest cover of this valley, the relative integrity and the relatively gentle land use system through centuries could be proved using palinological data, military maps and land descriptions (Ruprecht 1999). That is why the changes in vegetation-texture could be caused mostly by environmental factors.

Description of the studied associations

The *Carici flavae-Eriophoretum latifolii* Soó 1944 association could be found mainly on the surroundings of springs where the soil is saturated with cold water coming from deep strata (Jakucs 1956). It is tightly linked to adequate water-supplied peat soils but from another point of view it has wide ecological spectra and rich species composition. Its drying may cause afforestation or transformation into haymeadows (Borhidi and Sánta 1999).

The *Junco obtusiflori-Schoenetum nigricantis* Allorge 1921 association could be found on strongly humid places, on calcareous soils. This association is considered to be successionally stable, this stability is maintained by adequate moisture conditions and regular mowing and grazing. Drying or stopping the mentioned activities can cause afforestation or transformation into haymeadows (Borhidi and Sánta 1999). These stands are living on the eastern boundary of the habitat of this association, representing the *Schoenetum nigricantis transsilvanicum* geographical variant. These stands are rich in montane-subalpine elements, that is why they are similar to the stands known from the western part of Hungary (Soó 1927, Kovács 1962).

The *Cladietum marisci* Zobrist 1935 association could be found on calcareous, oxygen-rich, oligo-mesotrophic or mesotrophic soils, without considerable water level fluctuation. In the dense stands formed mostly by the dominant species, *Cladium mariscus* the accumulation of the dead phytomass (leaves) is considerable, which permits only few species to inhabit. During succession their stands can transform into *Schoenetum* association (Borhidi and Sánta 1999).

Data collecting and vegetation recording

Former coenological data from our study area (the Malom Valley) were collected in 1940–44 (Soó 1949), 1956 (Soran in Pop et al. 1962) and 1961 (Pop et al. 1962).

Our relevés were made according to the Braun-Blanquet method in the summer of 1998. Stands were identified on the base of floristical composition and their physiognomy. The size of the relevés was 25 m^2 in the case of the *Carici flavae-Eriophoretum latifolii* and *Junco obtusiflori-Schoenetum nigricantis* association and 100 m^2 in the case of the *Cladietum marisci* association adjusting to relevé-sizes of former years. Abundance-dominance (AD) values were noted according to the Braun-Blanquet approach (in order to compare the relevés of different years). Due to the extension-limits of the stands 14 (*Carici flavae-Eriophoretum latifolii*), 8 (*Junco obtusiflori-Schoenetum nigricantis*) and 4 (*Cladietum marisci*) relevés were obtained.

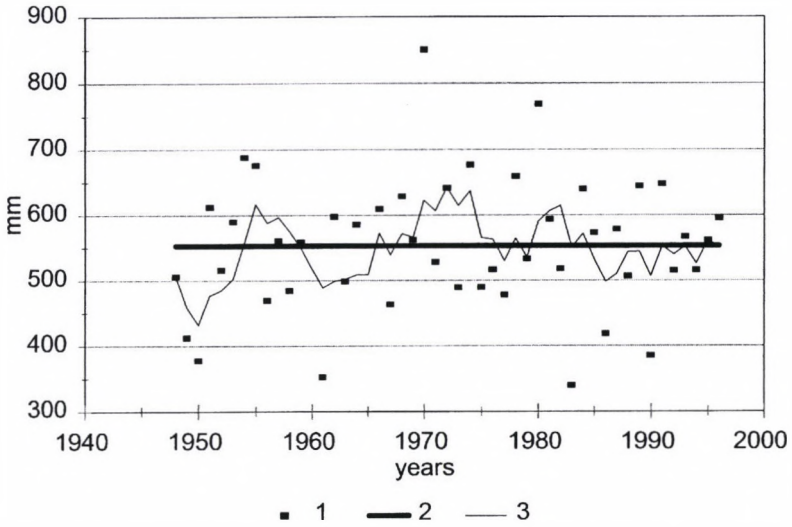

Fig. 1. Annual precipitation of the period 1948–1996 according data of the meteorological station at Cluj-Napoca. Legend: 1. annual precipitation, 2. average annual precipitation in the studied period, 3. annual precipitation smoothed by moving average

The annual precipitation of the period 1948–1996 (according to the data of the meteorological station at Cluj-Napoca) was used. Moving averaging was used for smoothing annual precipitation data (Fig. 1).

Former vegetation relevés were compared with ours by using the species indicator values for moisture (W), acidity (R) and nutrients (N). The W, R and N values were used because we consider them being the most deterministic parameters (factors) of a fen community. In the comparison only vascular plants were taken into consideration. The taxonomic nomenclature follows Borhidi (1995). In the case of *Eriophoretum* association (relevés of the year 1956) two groups were distinguished: one typical (E1956a) and a drier *caricosum paniceae* facies (E1956b) (Table 1).

Table 1

The number of relevés in certain cases with the signs of the associations in the parentheses

Year of sampling	*Carici flavae-Eriophoretum latifolii* (E)	*Junco obtusiflori-Schoenetum nigricantis* (S)	*Cladietum marisci* (C)
1940–44	11	–	–
1956	2(E1956a) + 3(1956b)	5	–
1961	5	2	2
1998	14	8	4

The pH measurements were made with a digital pH-meter in the summer of 1998. The pH of the ground water was similar in the habitats of all the three associations and fluctuated in the 7–8.2 interval.

Data processing

Data transformation. – Braun-Blanquet type data were transformed into cover according to Tüxen and Ellenberg (1937) modified by Soó and Zólyomi (1951). For decreasing the distortion during the transformation the following correction was applied:

$$B_i = \frac{b_i}{\sum_i b_i} \cdot 100$$

where: b_i = uncorrected cover of species i, B_i = corrected cover of species i.

Representative species. – The changes of abundance of some representative species were examined. Species indicating wetness belongs to the created "wet group" (*Equisetum palustre, Scirpus sylvaticus* and *Menyanthes trifoliata*), whilst species indicating dryness belong to the created "dry group" (*Carex panicea, Potentilla erecta, Plantago media, Molinia coerulea, Briza media, Succisa pratensis, Sanguisorba officinalis* and *Cirsium rivulare*). Changes in the average abundance of some specialists (*Parnassia palustris, Pedicularis palustris, Epipactis palustris, Ophioglossum vulgatum* and *Liparis loeselii*) were examined, too.

The pattern analysis of indicator values

Our aim was to describe the changes of indicator value patterns by the least variates possible. For this reason concentration analysis was used (Feoli and Orlóci 1979, Précsényi 1995). This method computes the scores of species-groups and relevé-groups in the same ordination space. Only binary data can be analysed with this method. In our opinion this constrain is not a disadvantage because if weighted data (e.g. cover, number of individuals, etc.) were used, some species would be over-emphasised.

The groups of relevés applied in the analyses are listed above. The grouping of species was based on their indicator values for moisture (WB), acidity (RB) and nutrients (NB) according to Borhidi (1995). In the case of species not living in Hungary the work of Sanda et al. (1983) was used. In our earlier study (Botta-Dukát and Ruprecht 2000) we stated that using

fewer groups is more efficient than Précsényi's (1995) original method. That is why species are concentrated into three groups based on their WB, RB and NB values in the following way:

Moisture:
W1 = xerofrequent group (WB1, WB2, WB3, WB4)
W2 = mesofrequent group (WB5, WB6, WB7)
W3 = hygrofrequent group (WB8, WB9, WB10).
Acidity:
R1 = acidofrequent group (RB3, RB4)
R2 = neutral group (RB5, RB6, RB7)
R3 = basifrequent group (RB8, RB9).
Nutrients:
N1 = oligotrophic group (NB1, NB2, NB3)
N2 = mesotrophic group (NB4, NB5, NB6)
N3 = eutrophic group (NB7, NB8).

It is known from other works (Grootjans et al. 1991, van Diggelen et al. 1991a, b, ter Braak and Wiertz 1994) that there is a significant correlation among some environmental parameters (moisture, acidity, nutrients) measured or indicated by vegetation in rich fens. We are likely to observe the same phenomenon. Thus Spearman's rank correlation coefficients among the scores of relevé-groups for moisture, acidity and nutrients were computed to test this hypothesis. The *Cladietum* relevés were excluded from this analysis, because it was not a rich fen association. There are two possible reasons which can account for the correlation between scores. One is the real correlation between habitat parameters. The other is that different indicator values of the same species are not independent from each other. For example, let us imagine the following situation: a xerofrequent species is probably acidofrequent and a hygrofrequent species is probably basifrequent, that is the two indicator numbers are not independent. In this case the correlation is an artefact. The independence of different indicator values of the same species was tested by G^2 test (Sokal and Rohlf 1981).

Coenological groups

Three groups were made based on the coenological status of species. The first contains the Caricion davallianae elements (characteristic rich fen species), their decrease in number is the first indicating of degradation. The second group contains the other fen species, their indication role is important, too. They are not tightly related to rich fens (their focus of appearance

is not on rich fens). The members of the third group are non-fen species, which would be occasionally present on rich fens of natural conditions but their increase in number and abundance is an indicator of degradation (drying). Species' classification follows Borhidi (1995) and Sanda et al. (1983). The relevés of *Cladietum* association were not used here.

Social behaviour types

In the case of social behaviour types (Borhidi 1993, 1995) the number of specialists and the abundance of disturbance tolerant species were used. In our case, fen species with narrow intervals of tolerance belong to the group of specialists. Only a few weed species and mostly natural disturbance tolerants belong to the group of disturbance tolerants, characteristic of managed fields (e.g. hayfields).

Results

Changes in the average abundance of some representative species

Some species like *Carex panicea, Potentilla erecta, Plantago media* and *Cirsium rivulare* present a great increase in abundance between 1940–1956, whilst the abundance of *Molinia coerulea* presents a little increase. There is a decrease in the abundance of *Equisetum palustre, Scirpus sylvaticus, Briza media, Succisa pratensis* and *Sanguisorba officinalis*. Some rare species, mostly fen specialists like *Parnassia palustris, Pedicularis palustris, Epipactis palustris* are increasing in abundance. *Menyanthes trifoliata* became extinct (Pop et al. 1962).

On the average, an opposite trend can be observed compared to the former between 1956–1961, except that the abundance of *Molinia coerulea* keeps increasing and the abundance of the mentioned rare species keep decreasing (except *Epipactis palustris*). *Scirpus sylvaticus* presents a decrease in abundance. Pop et al. (1962) reported the extinction of *Liparis loeselii* and about the decreasing of the local population of *Ophioglossum vulgatum*.

Between 1961–1998 changes were smaller and the abundance of some species were invaried. It is to be mentioned that there was a prominent increase in the average abundance of *Equisetum palustre* and *Potentilla erecta*. *Liparis loeselii* appeared again and the local population of *Ophioglossum vulgatum* increased.

Changes in indicator values of the relevé-groups

Changes in indicator values for moisture (W) and acidity (R) are represented by the same figure (Figs 2a, b), so it is possible to compare their changes. The *Eriophoretum* relevés from 1956 belong to two groups: a typical (E1956a) and a drier one (E1956b). If they had been amalgamated, the results of the analysis would have been biased. Having these two groups, the recognition of the tendencies of textural changes in this association becomes difficult.

In the *Eriophoretum* association the very high moisture and high pH values (E1940) change to drier ones (as indicated) for both E1956 groups and becomes more acid at the same times (as indicated). In 1961 this association proved to be a bit drier than the typical group from 1956 (E1956a), but considerably wetter than the dry facies from this year (E1956b). If we take into consideration that in 1956 the number of relevés of the dry group is higher, it can be concluded that the *Eriophoretum* association from a dry state (1956) changed into a bit wetter one (1961). Finally, till 1998 it presents higher moisture values and simultaneously much higher pH values (Fig. 2a). The changes of the *Schoenetum* association show a similar tendency as regards both its direction and the rate of changing. It can be observed that the values of *Schoenetum* association are in a higher pH interval compared to those of *Eriophoretum* in each of the dates, but the two communities do not differ considerably in their moisture values (Fig. 2b).

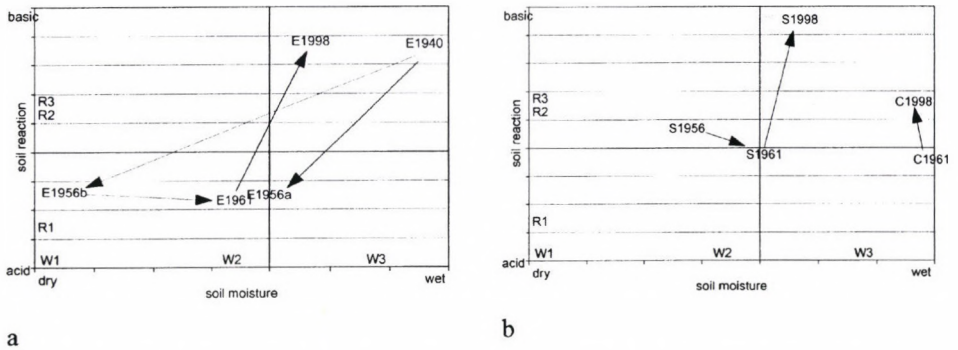

Fig. 2. Changes in moisture and acidity as indicated: scores of relevé-groups along the first ordination axis created by concentration analysis based on indicator values for moisture vs. scores along the first ordination axis created by concentration analysis based on indicator values for acidity. The values are not displayed on the axes, because only the relative position of relevé-groups and species-groups can be interpreted. *Carici flavae-Eriophoretum latifolii* (a) and the other two associations (b) were represented on separate graphs

The *Cladietum* association differs from the two other ones described above in its situation and both in direction and rate of changing (Figs 2b, 3b), except the nutrient values change simultaneously with those of the former associations (Fig. 3b). It indicates a weak decline in wetness and a weak increase in pH values (Fig. 2b).

If the two rich fen associations are considered only, a significant positive correlation can be found between the coordinates calculated for the W and R indicator values ($r = 0.7142$, $p < 5\%$). The W indicator values of species proved to be independent of their R indicator values, in this way the possibility of the artefact was excluded. On the other hand a significant negative correlation ($r = -0.8571$, $p < 1\%$) was found between scores based on R and N indicator values which may be caused by the fact that the R indicator value of a species depends on its N indicator value ($G^2 = 26.248$, $p < 0.1\%$). Significant correlation between scores for W and N indicator values were not found.

The two fen associations (compared to *Cladietum* association) remain all the time in a lower nutrient value interval and show little changes, except E1956b group (Figs 3a, b). A decrease in nutrient values can be observed between 1940–1956 and 1956–1961 and an increase between 1961–1998 in the case of *Eriophoretum* association. A similar trend prevails in the case of *Schoenetum* association as well, but the rate of decrease in nu-

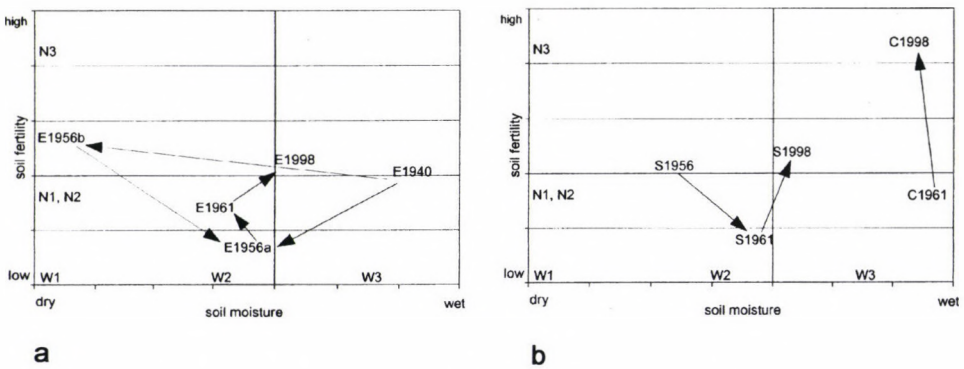

Fig. 3. Changes in moisture and nutrients indicated by plant species: scores of relevé-groups along the first ordination axis created by concentration analysis based on indicator values for moisture vs. scores along the first ordination axis created by concentration analysis based on indicator values for nutrients. The values are not displayed on the axes, because only the relative position of relevé-groups and species-groups can be interpreted. *Carici flavae-Eriophoretum latifolii* (a) and the other two associations (b) were represented on separate graphs

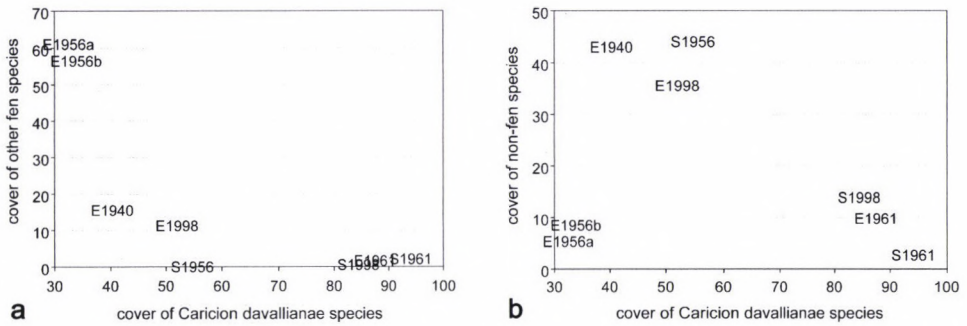

Fig. 4. Coenological composition of relevé-groups. – a = average cover of other fen species vs. average cover of Caricion davallianae species, b = average cover of non-fen species vs. average cover of Caricion davallianae species

trient value was higher between 1956–1961. The two fen associations got closer to each other regarding to their moisture and nutrient values observed in 1998. In comparison, the *Cladietum* association presents a big increase in nutrient values between 1961–1998 (Fig. 3b).

Changes in cover of the coenological groups

An outstandingly high cover (based on combined abundance-dominance values) of Caricion davallianae elements in the *Schoenetum* association can be observed changing considerably between 1956–1961 (Figs 4a, b). In the S1956 group the ratio of non-fen species is high, and decreases to a minimum in 1961, then remains at this level (Fig. 4b). In the *Eriophoretum* association a different trend can be registrated: the rate of the Caricion davallianae elements is relatively constant, it shows higher values only in 1961 (Figs 4a, b). There is a more prominent change in other fen species, their rate shows a sudden increase between 1940–1956 and a slow decrease till 1961 (Fig. 4a). The rate of non-fen species is considerable high in 1940, then decreases till 1956 and increases again until 1998 (Fig. 4b).

Changes in social behaviour types

The number of specialists changed in a narrow interval, only the C1998 relevé-group can be considered being poorer in specialists, whilst the S1998 group was remarkably rich in them. The number of specialists increased gradually from 1956 until 1998. In the S1998 group the cover of dis-

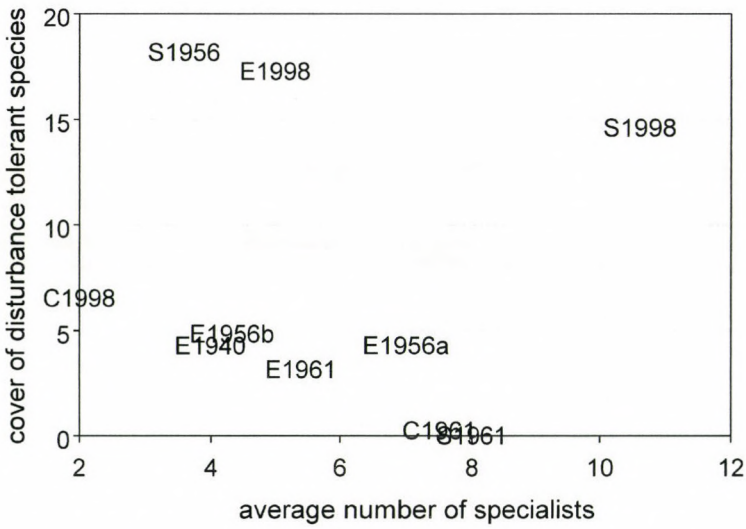

Fig. 5. Naturalness of relevé-groups based on Borhidi's social behaviour types: average cover of disturbance tolerant species vs. average number of specialist

turbance tolerants was high, too. It can be observed that the cover of disturbance tolerants is higher in the S1956 and E1998 group, too (Fig. 5).

Discussion

The land use system took a turn as late as in the beginning of the 1960s when mowing stopped. Except this impact, changes in vegetation texture were caused by changes in environmental factors and it could be regarded as an equilibrium case.

According to the general indication principle in equilibrium communities the appearance and distribution of plant species (their cover cannot be neglected) give information about every habitat parameter important for the life of plant (Juhász-Nagy 1986). In our study information about long-term changes of the site are taken by using indicator values for moisture, acidity and nutrients of species growing in two fen and a *Cladietum marisci* associations.

In the two fen associations – *Carici flavae-Eriophoretum latifolii* and *Junco obtusiflori-Schoenetum nigricantis* – a relatively great fluctuation could be observed caused first of all and almost exclusively by changes in moisture status followed by changes in acidity. A significant positive correla-

tion was found between the coordinates calculated for moisture and acidity of the relevé-groups. The results of many works on wetlands report about a linkage between moisture and acidity in very different ways (Grootjans et al. 1991, Kazda et al. 1991, van Diggelen et al. 1991a, ter Braak and Wiertz 1994), which confirm our results.

Generally, a fen system may react in three different ways to the sinking of the ground water table. First, the system can compensate for a slight lowering of the water table by shrinking. Second, when the fall in water table is bigger, the system can no longer compensate by shrinking; then an alternative water source (rainfall or surface water) can fill this space, at least in the wet season. This may lead to acidification (in the case of rain water) or to eutrophication (in the case of surface water) and also to an increased fluctuation in the water table. Third, when the drop of the water table is even greater, the process of refill cannot compensate for the loss of ground water, consequently desiccation and mineralisation of the peat will proceed (van Diggelen et al. 1991a). Some peats and clays may contain a large amount of Ca attached to the exchange complex, causing neutral to alkaline reactions persisting in the root zone for a long time, even when the supply of Ca-rich ground water has stopped completely (van Diggelen et al. 1991b). Since the changes in pH produced by changes in hydrological conditions are controlled by very complex processes, we can hardly reproduce the changes in environmental conditions using vegetation data. Detailed studies on the correlation between moisture and acidity status in the case of the two fen associations need to be done in the future.

Between 1940–1956 the decrease in moisture values of the *Eriophoretum* association goes parallel with a decrease in reaction values. In the *Eriophoretum* association the cover of the other fen species increased as a response to the decrease in moisture (Fig. 4a). This response indicates a successional process towards haymeadows, while the association resists to the infiltration of the disturbance tolerant species. Drying process causes an increase in the cover (drying) of non-fen and disturbance tolerant species in the *Schoenetum* association, whilst the rate of Caricion davallianae elements remained high all the time. The dominant species, *Schoenus nigricans* of the calciphilous *Schoenetum* association is a very long living one and can survive for many years in obviously unfavourable conditions like sinking of the water table, being a relatively deep-rooting species, but its rejuvenation diminishes as a reaction to decalcification (Ernst and van der Ham 1988).

Changes in the average cover of the representative species support the indicated decrease in moisture between relevé-groups. The species of the

"dry group" (species indicating drying) like: *Carex panicea, Potentilla erecta, Plantago media, Cirsium rivulare* and *Molinia coerulea* increased in cover, whilst the species of the "wet group" like: *Equisetum palustre* and *Scirpus sylvaticus* decreased in cover, and *Menyanthes trifoliata* disappear.

The degree of change was very small between 1956–1961, presumably because of the short period (5 years), in comparison with the degree of changes between 1940–1956 (12–15 years), or 1961–1998 (37 years). In any cases the vegetation indicated a small increase in moisture status during these years, which was followed by the decrease in cover of the species from the "dry group", except for the *Molinia coerulea* whose average cover continued increasing. In the *Eriophoretum* association the cover of other fen species diminished and the rate of Caricion davallianae elements increased considerably in both fen associations. The changes developed in a short period like this, showed us that it was enough for a fen vegetation to react to environmental changes with changing its floristical composition.

From 1961 to 1998 an increase in moisture and an even greater increase in soil reaction values can be read from our results which went parallel in the two fen associations. The increase in soil reaction values of the fen associations corresponds with the results of the pH measurements in 1961 and 1998; soil pH was 5–6 in 1961 (Pop et al. 1962), and 7–8.2 in 1998. The average cover of the *Equisetum palustre* populations increased (indicating wetness), but the cover of *Potentilla erecta* increased (indicating dryness), too. The latter species is sensitive to the long flood during the vegetation period (Grootjans et al. 1991), its increase in cover indicates the absence of inundations. We have to remark that fen vegetation turns to be the most sensitive to maximum height of the level of ground water, which may be considered as a key factor in controlling vegetation composition (Wierda et al. 1997). Recently the cover of disturbance tolerants has increased remarkably, as an indirect consequence of the ceased mowing. This change in the land use system caused the invasion of *Phragmites australis* and its increase in cover. This negative influence of the reed caused the disturbance of the fen communities giving way to the settling of the disturbance tolerants.

Changes indicated by the vegetation correspond with changes of the annual precipitation values (Fig. 1) taking into consideration the delayed reaction of vegetation (changes in composition proceed very slowly). These fens are supplied by ground water (springs) which follows with delay the changes of precipitation.

The period between 1948–1952 was a dry one and its effect on the vegetation had been manifested four years later. Similarly, the wet period of 1952–1955 was detectable only few years later in the composition of vegetation. The period till 1998 was predominantly wet, from the beginning of 1970s and 1980s precipitation could fill up the ground water reserves to an extent, that the shorter dry periods did not influence the composition of the vegetation.

The mean cover of the rich fen specialists decreased gradually till 1961, when the process stopped and the situation stabilised. During this period stressed species (dry group, wet group) showed little change. The increasing number of specialists and disturbance tolerants in the *Schoenetum* association led to the lower dominance of *Schoenus nigricans*.

The relevés of the *Schoenetum* show a higher soil reaction interval by their composition compared with those of *Eriophoretum* at the same moisture values. This indicates the affinity of *Schoenetum* to more calcareous soils (Borhidi and Sánta 1999). The fen associations did not present great changes in nutrient status, while *Cladietum* reflected in a completely different way between 1961–1998. Its nutrient status has increased considerably. Probably, this association reacted more sensitively to a real increase in nutrients compared to the fen communities by its lower buffering capacity. This increase in nutrient status could be caused by the ceased mowing in fen areas at the beginning of 1960s. As its consequence, dead phytomass was not removed and its decomposition might promote this process. Moisture changes were indicated by small changes in vegetation composition and showed an opposite trend compared with those of the fen associations. Data in our disposition from the two studied years are not enough to make far reaching conclusions. The indicated drying and eutrophication could cause the observed degradation in the *Cladietum* stands. A lessening in species number could be observed compared to the relevés from 1961 and *Cladium mariscus* did not flower in 1997 and 1998. The supposed degradation until 1998 is justified by the high cover of disturbance tolerants in these years.

Many observed changes can be related to the invasion of *Phragmites australis* which causes negative processes on fens. It means the faster drying of the fen (due to the intensive evapotranspiration of the reed), the extinction of the specialists, causing the degradation of the habitat. This process is fastened by nutrient-infiltration, mineralisation of the peat caused by drying, and the ceased of mowing (Haslam 1971, Seregélyes and Csomós

1995). Unfortunately, we do not have comprehensive quantitative information about the changes in cover of *Phragmites australis*, although it would have been very important. The objective estimation of its cover according to the Braun-Blanquet method is difficult (it would be more correct to give the number of stems per unit area, but this method needs time and intensive labour). We did not observe a clear increase in the cover of weeds, except *Eupatorium cannabinum* whose mean cover and constancy have increased.

Generally, non-permanent plots are not quite suitable tools in vegetation dynamic studies. Fortunately, in our case there were no signs of a transformation of these fen associations into others (more drier), however in such a situation the dominant species of the former associations would survive and demonstrate this process. Therefore, the association borders did not move and in the floristical composition of the vegetation only small or medium sized changes have happened. So it can be stated that the relevés from the same associations, but different years are the representative samples of the same stands.

Conclusion

Changes were similar only in the case of the two fen associations, the *Cladietum* association responded in a different way. The *Eriophoretum* association proved to be the most resistant against drying. It prevented the settling of new disturbance tolerants and non-fen species, however the number of specialists decreased. In the case of the *Schoenetum* association only the dominant species, *Schoenus nigricans* maintained its position, however the floristical composition of the community changed remarkably: the cover of the disturbance tolerants and non-fen species increased. Symptoms of degradation became conspicuous earlier, compared to the *Eriophoretum* association, that is why *Schoenetum* is a better indicator of the sinking water table. The *Cladietum* association resists better to hydrological changes due to the competitive property of the dominant species, but our data was not enough to come to far reaching conclusions.

There was a significant positive correlation between the coordinates calculated for moisture and acidity. Our results agree with other references about wetlands.

Changes in annual precipitation data are correlated with the changes reflected by indicator values. The fen vegetation reacts with a delay of some years to a change in amount of precipitation which can be explained by the slow compositional changes and the compensating effect of the ground water reserves.

As changes indicated by vegetation using indicator values are in accordance with data of precipitation ("real changes") we can receive valid results about the trends in fen associations using non-permanent plots. These studies are necessarily limited for detecting the coarse-scale changes in vegetation texture, but they cannot be used for revealing the fine-scale or spatial changes in vegetation texture and their mechanisms. There are other methods wich can be used for the elimination of the mentioned deficiency: vegetation mapping (for spatial changes in vegetation texture), permanent plots (for fine-scale changes) and experiments (for studying mechanisms). The advantage of the used method is to revitalise past samples for including them into quantitative comparing analysis with recent and more sophisticated studies.

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MORPHOLOGICAL NATURE AND TRENDS OF EVOLUTION IN THE PAPPUS OF THE ASTERACEAE

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In the present work the author has studied the vascular anatomy of the flower of 100 species of the family Asteraceae. The vascular pappus occurs in *Adenostemma lavenia, Ageratum conyzoides, Amberboa ramosa, Bidens biternata, Cymbia occidentalis, Dicoma tomentosa, Eclipta prostrata, Saussurea candicans, Sclerocarpus africanus, Tagetes erecta, Tricholepis radicans, Tridax procumbens* and *Verbesina oncophora*. In all such cases, each member of the pappus receives a single trace from the compound vascular supply of the floral leaves in the top of the inferior ovary. The presence of vascular supply to the pappus scales shows that it is a phyllome structure. Once the pappus is considered as a phyllome structure, three major lines of specialisation can be established in the family from a primitive type of pappus consisting of five vascular scales, a condition seen in *Ageratum conyzoides*. In one line of evolution, represented by taxa like *Adenostemma, Bidens, Eclipta, Sclerocarpus, Verbesina*, etc., there has been a reduction in the number of pappus scales from five to two accompanied by the suppression of vascular supply, such as is seen in *Helianthus annuus* and finally total suppression of the scales themselves, such as is seen in *Cyathocline purpurea*. This is a reduction series.

In second line of evolution exemplified by taxa like *Cymbia, Eupatorium, Saussurea, Tridax*, etc., there has been an increase in the number of pappus scales from five to as many as twenty or even more arranged in one whorl. Initially the pappus retain their vascular supply but finally it is totally suppressed. This is an amplification cum-reduction series.

In third line of evolution starting from *Amberboa, Dicoma, Emilia, Tricholepis*, etc., there has been an increase in the number of pappus from ten to indefinite arranged in many whorls. Initially chorisis (longitudinal splitting) of sepal-like members of the outer whorl and further splitting of the inner whorl and assumption of setose form, accompanied by total loss of vascular supply would give rise to the condition seen in *Centratherum anthelminticum*. This also represents an amplification series.

Key words: evolution, morphology, pappus, phyllome, sepals, trichome

Introduction

The Asteraceae comprising some 25,000 species is one of the largest families of flowering plants. The family is a natural one and all the species are characterised by the possession of a capitulum inflorescence. The pres-

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ence of a pappus is yet another unique feature of the Asteraceae, unmatched by any other angiosperm. However, there is extreme diversity in the details of the structure of the pappus in the different taxa of this family. This has led to an acrimonius debate regarding its morphological nature. Some consider it as a more or less modified calyx and others as of the nature of trichomes. The present work was undertaken with a view to investigate vascularisation morphological nature and trends of evolution in the pappus of the Asteraceae. Literature on the vascular anatomy of the flower of the Asteraceae has been thoroughly reviewed by Tiagi and Singh (1972), and Singh (1973).

Material and methods

The present investigation is based on the study of the vascular anatomy of the flower of more than 100 species of the Asteraceae, but a few selected ones, with their localities of collection, which have been described are as follows:

Taxon	Source of collection
Tribe: Vernonieae	
Centratherum anthelminticum (Willd.) Kuntze	Sagar
Elephantopus scaber L.	Jaipur
Vernonia cinerea (L.) Less.	Gwalior
Tribe: Eupatorieae	
Adenostemma lavenia (L.) Kuntze	Mussoorie
Ageratum conyzoides L.	Ganganagar
Tribe: Asteroideae	
Cyathocline purpurea (Don.) Kuntze	Ujjain
Tribe: Helianthoideae	
Bidens biternata (Lour.) Merr. et Sherff.	Ganganagar
Chrysanthellum indicum DC.	Ganganagar
Eclipta prostrata (L.) L.	Ganganagar
Glossocardia bosvallea (L. f.) DC.	Khetri
Helianthus annuus L.	Ganganagar
Lagascea mollis Cac.	Gwalior
Sclerocarpus africans Jacq.	Gwalior
Siegesbeckia orientalis L.	Jaipur
Tridax procumbens L.	Ganganagar
Verbesina enceliodes (Cav.) Benth. et Hook. f.	Ganganagar
Verbesina oncophora Robinson et Seaton	Dehradun

Taxon	Source of collection
Tribe: Helenieae	
Tagetes erecta L.	Mussoorie
Tribe: Anthemideae	
Artemisia scoparia Waldst. et Kit.	Ganganagar
Artemisia vulgaris L.	Mussoorie
Tribe: Senecioideae	
Emilia sonchifolia (L.) DC.	Gwalior
Tribe: Cynaroideae	
Amberboa ramosa (Roxb.) Jafri	Gwalior
Cirsium wallichii DC.	Ganganagar
Saussurea candicans Clarke	Mussoorie
Tricholepis radicans DC.	Ganganagar
Tribe: Mutisieae	
Dicoma tomentosa Cass.	Jaipur

Customary methods of microtechnique were employed. Serial transverse sections of the floral buds were cut at thickness ranging from 8–12 μm. Double staining with crystal violet-erythrosin combination gave satisfactory results. In most cases, the floral buds were also made transparent by heating in 10% KOH for some time and subsequently in Lactic acid. The cleared buds were dissected under a stereoscope. This was found to be very useful in understanding the 3-dimensional picture of the vascular skeleton.

Observation and discussion

The pappus is an important characteristic structural feature of the family Asteraceae. Its morphological nature has remained an enigma for quite a long time. The term is used collectively for epigynous scales, hairs, spines, setae or bristles borne on the top of the inferior ovary around the base of the corolla tube. There are two schools of thought regarding the morphological nature of the pappus. According to one school of thought, they have been considered as a more or less modified calyx (Bentham 1873, Coulter 1883, Martin 1892, Ridley 1930, Good 1931, Rendle 1938, Lawrence 1951, Cronquist 1955, Ramayya and Sayeeduddin 1958, Carlquist 1961, Singh 1973). This is also sometimes known as the phyllome theory of the morphological nature of pappus. According to the second school of thought, the pappus does not represent a modified calyx but is of the nature of trichomes (McNab 1873, Masters 1878, Small 1919). Manilal (1963)

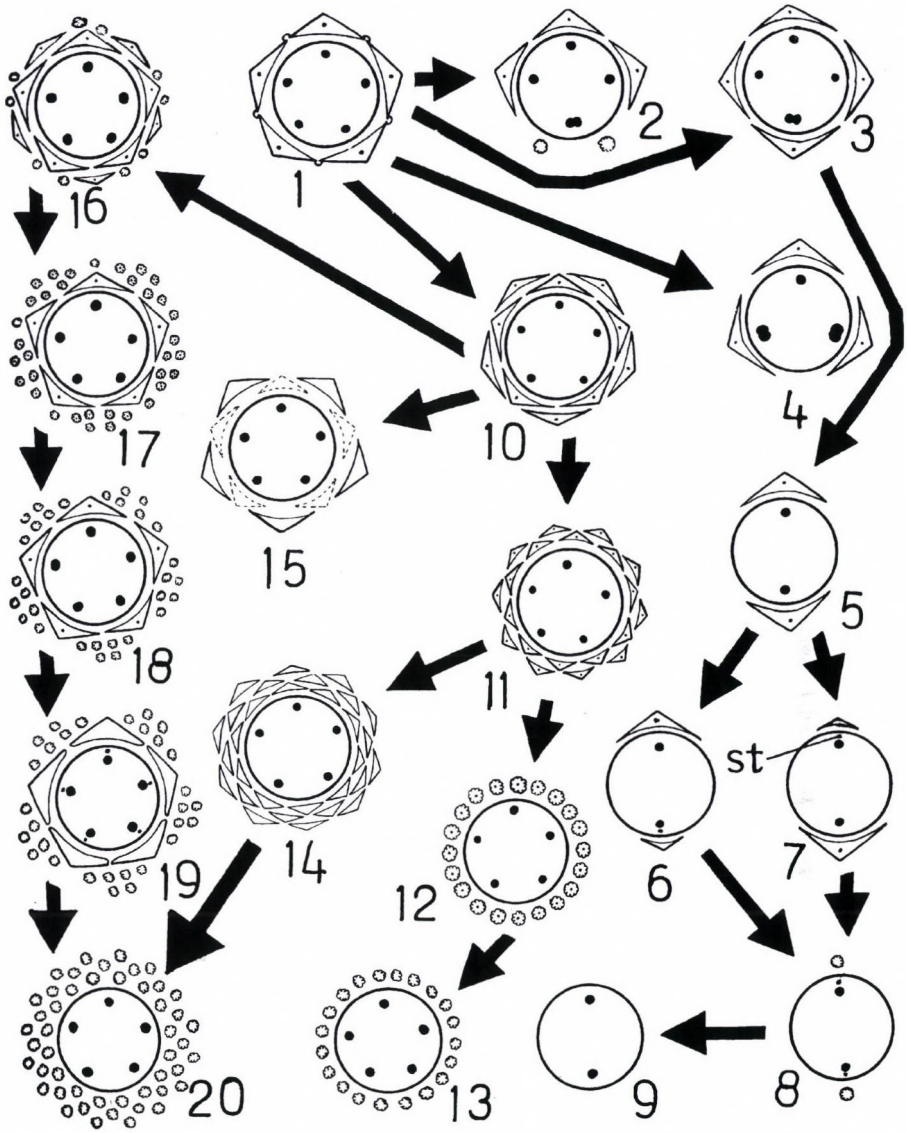
regarded all the cases in which vascular supply is present in the pappus as of the nature of calyx but among those cases which had a setose pappus, the lower tubular portion was regarded as calycine in nature and setae borne upon its rim as of the nature of trichomes. The present study which is based on careful analysis of the structure, vasculature and trends of evolution in the pappus serves to clear the confusion to a large extent. The author believes that the pappus is a calyx which has undergone modification in several ways. But the course of this evolutionary modification is still preserved in the extant species. The following stages and lines of evolution in the pappus can be recognised in this family (Figs 1–20).

Primitive types: In *Adenostemma lavenia, Ageratum conyzoides, Bidens biternata* and wild form of *Tagetes erecta* (Fig. 1) the pappus represents the least modified calyx and is characterised by the following features: (a) It consists of a definite number of members, namely five with the odd number on the posterior side; (b) The aestivation is valvate or imbricate; (c) The general appearance of the pappus members is sepal-like; (d) The fact that they are adnate to the ovary making it inferior is evident from the longitudinally ridged surface of the inferior ovary; (e) Each member possesses a well-developed vascular supply whose mode of origin has already been described in detail (Tiagi and Singh 1972, Singh 1973).

As far as the pappus of this type is concerned, the question is not about its phyllome versus trichome nature but whether we should continue to call it a pappus or should use the term calyx for it. In my opinion to call such a structure as pappus only serve to create confusion. The only uncommon thing about the sepals (pappus) of this type is that are one traced. This is understandable in view of the reduction, the calyx in the Asteraceae has undergone. However, members of Asteraceae are not only the exception in having one traced sepals. One traced sepals occur in *Colpoon, Echinocystis, Lycopersicum, Menispermum, Olea* and *Primula*. The literature on this topic has been comprehensively reviewed by Puri (1951).

From the type of calyx seen in *Ageratum conyzoides*, the calyx has undergone modification in several ways and some of the derived types are given below. They can be classified into two categories, one is the result of reduction and suppression and the other of amplification. The evolutionary modifications are superimposed upon a similar modification of the vascular ground plan inside the wall of the inferior ovary.

Reduction series: In *Bidens biternata*, in the wall of inferior ovary two anterior bundles are fused, quite often the two anterior sepals are also



Figs 1–20. Evolution of pappus (T. S. top of inferior ovary with calyx) (showing author's view on the morphological nature and trends of evolution in the pappus of the Asteraceae). – Reduction series: 1 = *Ageratum conyzoides*. 2 = *Bidens biternata*. 3 = *Bidens biternata*. 4 = *Adenostemma lavenia*. 5 = *Eclipta prostrata*. 6 = *Sclerocarpus africans*. 7 = *Verbesina oncophora*. 8 = *Helianthus annuus*. 9 = *Cyathocline purpurea*. – Amplification series: 10 = *Cymbia occidentalis* (Schaffner 1934). 11 = *Tridax procumbens*. 12 = *Saussurea candicans*. 13 = *Eupatorium triplinerve*. 14 = *Cirsium wallichii*. 15 = *Elephantopus scaber*. 16 = *Dicoma tomentosa*. 17 = *Amberboa ramosa*. 18 = *Tricholepis radicans*. 19 = *Tricholepis radicans* (occasional). 20 = *Emilia sonchifolia*. (st = stub)

fused together so that the calyx consists of only four members (Fig. 3). This type can be derived from *Ageratum* type by fusion of two anterior members of the calyx. In some cases the two anterior members are extremely reduced and fail to receive vascular supply (Fig. 2).

In *Adenostemma lavenia*, the pappus consists of only three sepal-like structures, a median and two in the antero-lateral positions (Fig. 4). Vascular traces inside the wall of inferior ovary in similar positions are double. It can, therefore, be safely presumed that this type has originated from the *Ageratum* type by fusion on either side between a lateral and an anterior members on each side to form a single structure. This type is also found in *Blainvillea acmella* and *Calendula officinalis* (Singh 1973).

In *Chrysanthellum indicum*, *Eclipta prostrata* and *Glossocardia bosvallea* the pappus scales, though sepal-like, are reduced to only two members, medianly placed in *Eclipta prostrata* and laterally in *Chrysanthellum indicum* and *Glossocardia bosvallea*. This type can be visualised to have originated from the *Bidens biternata* having four members by suppression of the lateral members (Figs 3 and 5) or antero-posterior members. Further reduction in size of the anterior or the posterior pappus member gives rise to the condition, respectively seen in *Sclerocarpus africanus* (Fig. 6), and *Verbesina enceliodes* and *V. oncophora* (Fig. 7). The larger pappus member has a well-developed vascular supply, whereas the vascular supply of the other member is represented only by a stub (st).

The pappus type of *Helianthus annuus* can be easily derived from that seen in *Sclerocarpus* and *Verbesina* by reduction of the size of the larger of the two pappus members, though the vascular supply is still present in the form of vascular stubs (Fig. 8). Total loss of both the pappus members, as well as their vascular stubs gives rise to the condition seen in taxa like *Artemisia scoparia*, *A. vulgaris*, *Cyathocline purpurea* and *Siegesbeckia orientalis* (Fig. 9).

Amplification series: From the condition seen in *Ageratum conyzoides* and other similar types, we can visualise the origin of a two whorled calyx by what Schaffner (1934) called as "duplicate-evolution", or by the common process known as doubling. Cronquist (1955) believes that the pappus members have become augmented, both as far as the number of members in the same whorl is concerned, as well as the number of whorls. A pappus consisting of two whorls of members is known to occur in *Cymbia occidentalis*, in which the odd number of the inner whorl is posterior and that of the outer whorl is anterior in position (Fig. 10).

From the *Cymbia* type of pappus, further amplication of members of the same whorl would give rise to the condition of pappus seen in *Tridax procumbens*, where there are ten members in each whorl with well-developed vascular supply (Fig. 11). Loss of dorsiventral form and assumption of setose form would give to the condition of *Saussurea candicans* (Fig. 12). Further loss of vascular supply then would give rise to the condition of pappus seen in *Eupatorium triplinerve* (Fig. 13). In this line of evolution setose pappus are arranged in one whorl and is common in all the tribes except the Cichorieae.

There are secondary reduction types from the type of biseriate pappus seen in *Cymbia occidentalis*. Loss of three of the members of the outer whorl would give rise to the condition seen in *Lasthenia glabrata* where the pappus consist of an inner whorl of five and an outer whorl of two members, and in which the vascular supply of the members is represented only by vascular stubs (Singh 1973). Complete loss of the inner whorl would give rise to the condition seen in *Elephantopus scaber* where the five pappus members become antipetalous in position (Figs 10 and 15). Increase in the number of whorls but accompanied by the loss of vascular supply would give rise to the condition seen in *Cirsium wallichii* (Figs 10–11 and 14).

From a pappus consisting of two whorls of sepal-like members, such as is seen in *Cymbia occidentalis* with five members in each whorl, further amplification by way of addition would give rise to the condition of *Dicoma tomentosa* (Figs 10 and 16). In the other line of evolution starting from *Dicoma tomentosa*, chorisis of the sepal-like members of the outer whorl gives rise to the condition seen in *Amberboa ramosa* (Figs 16–17) where all members of the pappus, whether flat and sepal-like or setose, have got a well-developed vascular supply. The condition in *Tricholepis radicans* can be easily derived from that seen in *Amberboa ramosa* by loss of vascular traces as well as bundles in setose members of the pappus (Fig. 18). In certain varieties of *Tricholepis radicans*, vascular bundles may also be lost from the inner sepal-like members but their vascular supply is still present in the form of vascular stubs (Fig. 19). Further splitting of the inner sepal-like members and assumption of setose form, accompanied by total loss of vascular supply would give rise to the condition seen in *Centratherum anthelminticum*, *Emilia sonchifolia*, *Guizotia abyssinica*, *Lagascea mollis* and many species of the Senecioideae and Cichorieae where the pappus consists of two or more whorls of setose structures without any vascular supply (Fig. 20). This type can also be derived from *Cirsium* type by loss of dorsiventral form and assumption of setose structure (Figs 14 and 20).

To conclude, therefore, we can say that the pappus in the ancestral Asteraceae consisted of five sepal-like members. In one line of evolution (Figs 1–9), there has been reduction in number of pappus members, either by loss or by cohesion, finally accompanied by loss of its vascular supply. This line starts with *Ageratum* and ends up with taxa like *Artemisia*, *Cyathocline*, *Siegesbeckia*, etc., where the pappus is totally lacking. In the other line of evolution (Figs 10–20) also starting from *Ageratum*, there has occurred an amplification of the number of whorls and numbers of members in a whorl of the pappus and which assumed a setose form. On purely morphological grounds, Cronquist (1955) believed that the pappus in several genera of Helianthoideae like *Dysodia*, has originated by longitudinal splitting of sepal-like members. It is true that in its most highly modified form, the pappus segments cannot be always homologised with sepals, but this cannot negate their evolutionary origin from sepals. The evolutionary history of the pappus as outlined above on the basis of factual evidence is an elegant testimony to the evolution of the setose pappus from an ordinary calyx. It may be pointed out here that the evolutionary process which has operated upon the calyx to produce the setose pappus has even effected the corolla of the ray-floret of certain taxa like *Conyza japonica*. In this species, after furnishing the vascular supply of the style, all traces end blindly in the top of the inferior ovary. The lower portion of the corolla is tubular, of course, devoid of any vascular supply but in its upper part, it splits up longitudinally, both radially as well as tangentially to form about 10–15 setose lobes. Thus, this corolla is exactly similar to the setose pappus of this family which also consists of a lower short tubular portion bearing the setae at its top. The evolutionary history of pappus is matched by the ray-corolla of *Conyza japonica* (Singh 1994).

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JUGLONE INDEX – A POSSIBILITY FOR EXPRESSING ALLELOPATHIC POTENTIAL OF PLANT TAXA WITH VARIOUS LIFE STRATEGIES

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The classic example of literature on allelopathy is juglone, the effective substance of walnut (*Juglans regia* L.) tree. I found a quotient, the juglone index, suitable for expressing quantitatively whether an allelopathic plant extract or effective substance (allelochemical) has a stronger or a weaker effect than juglone. This index can be calculated only on the basis of the major characteristics of germination and the early stage of seedling-development, and it is suitable only for basic orientation, it expresses the strength of allelopathic potential, it is a ratio referring to the degree of inhibition activity.

It has been found that the *Sinapis alba* germination test is the most suitable for measuring the effect. The values for calculation are gained from shoot length (mm), root length (mm) and germination ability (%) measured on the 6th day after the beginning of germination (imbibition). The sum in the numerator is referring to the effect of 1 mM juglone, and the sum in the denominator is referring to the effect of a given allelochemical substance (1 mM, 1 or 5 g plant material extracted by 100 ml distilled water).

If the juglone index of a substance "x" is bigger than 1 ($Ij/x > 1$), the inhibition is stronger than that of juglone (the allelopathic potential is more expressed), if it is smaller than 1 ($Ij/x < 1$), the inhibition is weaker than that of juglone (the allelopathic potential is less expressed). If Ij/x is smaller than 0.5, we cannot practically speak about an allelopathic potential.

The juglone indexes obtained by the described simple calculation (exactly keeping the circumstances of biotesting) demonstrate the relative activity of the allelochemicals and extracts examined by us, and the gained values give a reliable orientation about the allelopathic potential.

The juglone indexes of plant extracts occurring in results are classified according to the behaviour types and naturalness values defined by Borhidi (1993).

Key words: allelopathy, germination test, juglone index, measuring of allelopathic effect

Introduction

Investigating the mutual effect of plants on each other is somewhat analogous with studying the effect of plants on microorganisms and consument animals. Man uses the bioactive substances of plants mainly as medicines. Today the so-called effective substances are in most cases

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known and classified at the molecular level (Hegnauer 1962–1996). However, in nature a much more complicated phenomenon takes place, because even in the case of allelopathy the idealised effect of a single molecule-type is very rare. In most cases, among changing abiotic and biotic ecological circumstances the complex effect of many kinds of substances and derivatives on the observed population can be detected even in the case of the most simple allelopathic phenomenon. Therefore it is very difficult to reach an unambiguous conclusion in each case. In the long run one can build only on proofs gained by different methods and many-sided approaches. Seeing the difficulties, the expensive investigations could be carried out only at a few research institutes in the world. Most research groups undertake exploratory basic survey studies which are essential from the point of view of further research into the natural vegetation and cultivated plants of a given country.

According to the articles of Narwal and Jain (1994), Willis (1994, 1995, 1996, 1997), Golovko et al. (1995), outstanding, school-founding scientists dealing with the study of allelopathy and the physiological and biochemical interactions among plants are the following scientists: Molisch, Pickering, Schreiner, Grodzinsky, Grümmer, Muller and Rice.

Today the two most important poles of research are the USA: Rice (1979, 1984, 1994), Putnam (1985, 1988), Waller et al. (1986, 1987) and India: Narwal and Tauro (1994). The books of Knapp (1954), Grümmer (1955) and Grodzinsky (1982) provide also today a valuable summary. Allelopathy has recently become part of ecological biochemistry (Harborne 1984, 1989, Schlee 1986).

The Hungarian results of allelopathy research have been summarised by Csontos (1997). In this paper he lists the scientists who published results or opinions concerning allelopathy (some of the authors do not even call the interaction allelopathy): Rapaics, Ubrizsy, Précsényi, Juhász-Nagy, Soó, Lukácsovics and Juhász-Nagy, Gáspár and Barthodeiszky, Csapody, Ujvárosi, Fekete, Terpó and P. Kotori, Hunyadi, Mikulás, Pozsgai, Béres, Szabó et al., Oborny, Csontos, Váradi et al., Pethő). According to his evaluation there have been initiatives for developing three trends: 1. The study of natural plant communities (forest and grassland), 2. Agricultural research (cultivated plants and weeds), 3. Search for phytochemical relations.

According to the Hungarian opinion concerning allelopathy, the phenomenon can be met with under laboratory circumstances, but in nature – especially in Hungarian conditions – it can rarely be observed (Borhidi 1969, Juhász-Nagy, Soó cit. in Csontos 1997).

Allelopathy in the wider sense is a well-known phenomenon: population-forming plant taxa with a specific chemical composition are in competitive chemical interaction with each other. Usually the development of two different taxa is observed, but in natural ecosystems the situation is more complicated, since in a given plant association more species live together. The majority of substances responsible for allelopathy is washed (leached) out easily in water from the tissues of the donor taxon and penetrates into the living tissues of the acceptor taxon, where through a short or long distance translocation they impose a certain kind of developmental physiological effect on the "target organism". In most cases this influence is of an inhibitory character. Rarely, the biologically active substances get into the medium (soil, air, water) via secretion.

The majority of allelochemicals dissolves well in water and they reach their target via a medium and exert their effect depending on their chemical character and concentration.

It is important to point out that the allelochemicals include mainly such groups of substances that are similar concerning certain chemical characters and physiological effects (e.g. most polyphenols are generally enzyme inhibitors because of their ability to denaturate proteins, cyanogenic substances, toxic alkaloids or terpenoids are frequently respiratory inhibitors influencing the mitochondrial electron transport). Not so frequently, specific effective substances (e.g. juglone) are held to be responsible.

From a practical point of view it is also important to know that decaying plant organs still contain a large amount of allelochemicals and so the substances washed out of dead plants or plant parts are potential inhibitors. At the same time they might serve as a carbon or nitrogen source for microorganisms as factors in regulating soil life in the natural ecosystems.

From an allelopathic point of view the following substance types are especially effective: phenoloids (polyphenols, phenolic acids), terpenoids (monoterpenes, sesquiterpenoid lactones, diterpenes, triterpene saponins), polyines, azotoids (alkaloids, chromoalkaloids, cyanogenic glucosides), glucosinolates. It has to be taken into consideration that the degree of effect always depends mainly on the concentration-relations of a given bioactive substance-group.

It is beyond argument that the life strategy of a given taxon is determined by the combination of many factors. The morphological and physiological characteristics determined by the genotype assert themselves in a complementary way during the competition. The allelochemical (phyto-

chemical) features belong to the physiological habit, if these features really contribute to the adaptive ability, reproduction, survival and propagation.

Borhidi (1993) set up a life strategy model for the vascular plants of Hungary, in which the plant taxa are categorised according to their natural habitats possible. The categories called social behaviour types express various states of naturalness or disturbances of the plant-habitat relationships. The given so-called naturalness values express this state and range from –3 to 10.

If the allelopathic strategy-types are grouped, it becomes clear that most taxa with an allelopathic inclination are alien to a region and flora, introduced cultivated plants. Most allelopathic phenomena were observed in agroecosystems. It is especially interesting that about one third of all native allelopathic plants is arborescent. Half of them (almost 50%) are specialists, natural competitors or generalists. Among them competitors are very important, because these arborescent species produce a large quantity of biomass, in a given stage of succession they are capable of the highest degree of competitiveness, control the potential scope of the accompanying species and in the long run they can stabilise the composition and operation of the association. Most species of the mentioned groups belong to the orders Fagales, Salicales, Pinales and Ericales. As mentioned before, these orders can be characterised by the great amount of polyphenols.

Beside the cultivated plants, disturbance-tolerant species and aggressive competitors form significant type-groups. Most taxa concerning all these groups belong to the orders Asterales, Fabales and Poales. The fact that these orders are rich in species is certainly in relation with the great diversity of their allelochemistry (allelochemicals).

Allelopathy can often be observed already during germination, but if the effect reaches the plant later, it can occur in any developmental stage. Most data and observations concern germination and seedling-development (Knapp 1954, Terpó and P. Kotori 1974, Williams and Hoagland 1982, Duke et al. 1983, Friedman and Waller 1985, Nandakumar and Rangaswamy 1985, Williamson and Richardson 1988, Haugland and Brandsaeter 1996). That is why so many germination bioassays have been worked out, for example: – for the germination of radish: coumarins, phenylpropanoids (Aliotta et al. 1993) and the allelochemicals of *Ruta graveolens* (Aliotta et al. 1994); – for the germination and seedling-development of lettuce: phenolic acids (Li et al. 1992, 1993); – germination of seeds containing allelochemicals together with seeds of lettuce or radish (Marcus and Burz 1994); – study of

the development and leaf growth of cucumber seedling under the influence of ferulic acid in relation to soil interaction (Blum et al. 1987).

The classic example of literature on allelopathy is juglone, the effective substance of walnut tree. I found the quotient, coined juglone index, suitable for expressing quantitatively whether an allelopathic plant extract or effective substance has a stronger or a weaker effect than juglone. This index can be calculated only on the basis of the major characteristics of germination and the early stage of seedling-development and it is suitable only for basic orientation: it expresses the strength of allelopathic potential, it is a ratio referring to the degree of inhibition activity.

Method

The *Sinapis alba* germination test has been found the most suitable for measuring the effect. The values for calculation are gained from shoot length (mm), root length (mm) and germination ability (%) measured on the 6th day after the beginning of germination (imbibition). The sum in the numerator is referring to the effect of 1 mM juglone, and the sum in the denominator is referring to the effect of a given allelochemical substance (1 mM, 1 or 5 g plant material extracted by 100 ml distilled water).

If the juglone index of a substance "x" is bigger than 1 ($Ij/x > 1$), the inhibition is stronger than that of juglone (the allelopathic potential is more expressed), if it is smaller than 1 ($Ij/x < 1$), the inhibition is weaker than that of juglone (the allelopathic potential is less expressed). If Ij/x is smaller than 0.5, we cannot practically speak about an allelopathic potential.

In the case of an "x" substance the juglone index is:

$$Ij/x = (Hj + Rj + Gj) / (Hx + Rx + Gx)$$

where Hj = shoot length on the effect of 1 mM juglone (mm), Rj = root length on the effect of 1 mM juglone (mm), Gj = germination ability on the effect of 1 mM juglone (%), Hx = shoot length on the effect of 1 mM (1% or 5%) substance "x" (or cold water extract), Rx = root length on the effect of 1 mM (1% or 5 %) substance "x" (or cold water extract), Gx = germination ability on the effect of 1 mM (1% or 5 %) substance "x" (or cold water extract).

The applied germination test: germination of *Sinapis alba* seeds (the undressed seed of the same cultivar) with 100% (or maximum) germination ability:

- determination of germination %: the average germination ability of 3 times 100 seeds (at the same temperature, possibly at 20 °C) on the 5th day following soaking in solution for 1 day (6th day counted from the beginning of imbibition),
- determination of shoot and root length: suitable mean values of seedlings measured on the 5th day following soaking in solution of 3 times 10 seeds for 1 day (6th day counted from the beginning of imbibition).

Substance "x": 1 mM known substance (allelochemical) or 1 or 5 g plant material extracted by 100 ml distilled water (1 hour soaking at 20 °C, shaken every ten minutes, finally filtered).

Germination: following imbibition (swelling) at 20 °C for 24 hours in Petri dishes with 10 cm diameter (by all means the same size), seeds placed on a double-layered filter paper, covered with one-layered filter paper, wetted with 5 ml solution – also used for swelling – and covered, in darkness, placed in a biological thermostat.

Keeping the above circumstances is very important, because we get values suitable for orientation only in this way. The juglone index for 1 mM indol-acetic acid is:

$$Ij/1\ mM\ IAA = 1.24$$

which means that auxin – as a well-known plant hormone – in 1 mM concentration is strongly growth and development inhibitor that is toxic. The index can be calculated on the basis of the following data:

H auxin (1 mM) = 15.6 mm H j = 21.0 mm
R auxin (1 mM) = 7.0 mm R j = 9.0 mm
G auxin (1 mM) = 77% G j = 94%
Ij/auxin (1 mM) = (21 + 9 + 94)/(15.6 + 7 + 77) = 124/99.6 = 1.24

1 µM indol-acetic acid is less toxic, its juglone index is smaller than 1, which means that its effect is weaker than that of 1 mM juglone. Calculated in a similar way:

H auxin (1 mM) = 30.9 mm
R auxin (1 mM) = 21.4 mm
G auxin (1 mM) = 81%
I j/auxin (1 mM) = (21 + 9 + 94)/(30.9+21.4+81) = 124/133.3 = 0.93

This example here is only to illustrate that a substance with a known physiological effect may also have an allelopathic quality, which means that its allelopathic potential can be quantitatively expressed, if compared with a known allelochemical. It is unquestionable that its effect depends on concentration.

The juglone indexes obtained by the described simple calculation (exactly keeping the circumstances of biotesting) demonstrate the relative activity of the allelochemicals and extracts examined by us and the gained values give a reliable orientation about the allelopathic potential.

Results and evaluation

The juglone indexes of plant extracts occurring in results are classified according to the social behaviour types and naturalness values defined by Borhidi (1993).

Abbreviations and values belonging to them (the whole set of values is detailed later): AC (–3) = alien competitors, aggressive invadors (–3); I (–1) = introduced crops running wild (–1); W (1) = anthropophilous elements of the native flora: native weed species (+1); DT (2) = disturbance tolerant plants of natural habitats (+2); G (4) = generalists (+4); C (5) = competitors (+5); S (6) = specialists (+6); Su (10) = unique (to be found only in the Hungarian flora) specialists (+10).

Allelopathic potential of aqueous extracts (1 and 5 g/100 ml) made of the green, dry shoot system (leaf, flower and stem parts) of herbaceous species characterised by the juglone index:

AC (–3)	*Echinochloa crus-galli* (L.) P. B.	1 g	0.65	5 g	0.73
	Solidago canadensis L.	1	0.66	5	0.90
	Stenactis annua (L.) Nees	1	0.61	5	8.77!
I (–l)	*Anethum graveolens* L.	1	0.58	5	1.31
	Avena sativa L.	1	0.59	5	0.71
	Cnicus benedictus L.	1	0.66	5	1.33
	Hyssopus officinalis L.	1	0.63	5	0.74
	Melissa officinalis L.	1	0.52	5	1.06
	Mentha aquatica L. var. *crispa* (L.) Benth.	1	0.58	5	1.14
	Mentha x piperita L.	1	0.58	5	1.11
	Petroselinum crispum (Mill.) A. W. Hill	1	0.65	5	1.36
	Rosmarinus officinalis L.	1	0.54	5	0.53
	Satureja hortensis L.	1	0.84	5	1.32
	Thymus vulgaris L.	1	0.60	5	1.11
	Triticum aestivum L.	1	0.79	5	1.06
W (1)	*Aristolochia clematitis* L.	1	0.71	5	1.08
	Artemisia absinthium L.	1	0.62	5	0.66
	Artemisia vulgaris L.	1	0.61	5	0.84
	Datura stramonium L.	1	0.78	5	5.00!
	Fumaria officinalis L.	1	0.50	5	0.74
	Galium aparine L.	1	0.64	5	1.22

	Melilotus officinalis (L.) Desr.	1	0.82	5	0.98
	Verbena officinalis L.	1	0.49	5	0.59
DT (2)	*Achillea millefolium* L.	1	0.56	5	0.85
	Agrimonia eupatoria L.	1	0.56	5	0.80
	Atropa bella-donna L.	1	0.62	5	1.27
	Equisetum arvense L.	1	0.60	5	0.65
	Euphorbia cyparissias L.	1	0.85	5	0.54
	Galega officinalis L.	1	0.52	5	0.72
	Geum urbanum L.	1	0.60	5	0.82
	Glechoma hederacea L.	1	0.76	5	0.88
	Humulus lupulus L.	1	0.72	5	10.70!
	Hypericum perforatum L.	1	0.69	5	1.13
	Origanum vulgare L.	1	1.03	5	1.04
	Plantago lanceolata L.	1	0.80	5	0.73
	Saponaria officinalis L.	1	0.67	5	0.81
	Trifolium pratense L.	1	0.66	5	1.04
	Trifolium repens L.	1	0.79	5	18.78!
G (4)	*Centaurium erythraea* Rafn.	1	0.61	5	1.92
	Hedera helix L.	1	0.46	5	1.61
	Helleborus odorus W. et K. ex Willd.	1	0.46	5	3.15!
	Viola tricolor L.	1	0.48	5	0.80
	Viscum album L.	1	0.60	5	0.89
S (6)	*Adonis vernalis* L.	1	0.71	5	1.05
Su (10)	*Paeonia banatica* Rochel	1	0.91	5	4.83!

On the basis of the list it can be stated that a stronger allelopathic potential can be observed when using more concentrated extracts.

From the DT- and W-type species two taxa can be characterised by the juglone index much higher than 1: *Trifolium repens* and *Humulus lupulus*, but the following species also represent high values: *Datura stramonium*, *Atropa bella-donna*, *Hypericum perforatum*, *Galium aparine*, *Aristolochia clematitis* and *Trifolium pratense*. Slightly weaker than juglone were *Melilotus officinalis*, *Achillea millefolium*, *Glechoma hederacea*, *Agrimonia eupatoria*, *Euphorbia cyparissias*, *Geum urbanum*, *Saponaria officinalis*, *Plantago lanceolata*, *Fumaria officinalis* and *Galega officinalis*.

From the AC- and I-type species the highest (higher than 1) Ij-value was found in the case of 5 g/100 ml extracts of *Stenactis annua*, *Petroselinum crispum*, *Cnicus benedictus*, *Satureja hortensis*, *Anethum graveolens*, *Mentha aquatica* var. *crispa*, *Thymus vulgaris*, *Mentha* × *piperita*, *Triticum aestivum*, *Melissa officinalis*. The value was slightly lower than 1 at *Solidago canadensis*, *Echinochloa crus-galli*, *Hyssopus officinalis*, *Avena sativa*.

It is worth emphasising that from the generalist and specialist species *Paeonia banatica* and *Helleborus odorus* had an especially strong activity concerning allelopathic potential. Furthermore *Adonis vernalis*, *Centaurium*

erythraea and *Hedera helix* showed strong activity (the latter only in the form of a more concentrated extract).

Allelopathic potential – characterised by juglone index – of aqueous extracts (1 and 5 g/100 ml) made of dry leaf of arborescent plant species and of the shoot system of cryptophyta (G) and hemicryptophyta (H) from the Mecsek Mts:

I (–1)	*Juglans regia* L. (green)	1 g	0.64	5 g	0.91
	Juglans regia L. (litter)	1	0.68	5	1.20
	Elaeagnus angustifolia L.	1	0.60	5	0.75
DT (2)	*Rubus caesius* L.	1	0.56	5	0.93
C (5) – MM	*Betula pendula* Roth	1	0.81	5	1.00
	Carpinus betulus L.	1	0.72	5	0.69
	Fagus sylvatica L.	1	0.70	5	0.86
	Quercus cerris L.	1	0.66	5	0.85
	Quercus petraea (Matt.) Liebl.	1	0.60	5	1.00
	Quercus robur L.	1	0.66	5	0.76
C (5) – G	*Aegopodium podagraria* L.	1	0.63	5	0.88
	Allium ursinum L.	1	0.56	5	1.00
	Corydalis cava (L.) Schw. et K.	1	0.86	5	5.87!
	Galium odoratum (L.) Scop.	1	0.61	5	0.98
	Melica uniflora Retz.	1	0.63	5	0.70
	Oxalis acetosella L.	1	0.56	5	1.44
C (5) – H	*Carex pilosa* Scop.	1	0.56	5	0.61
	Festuca drymeia M. et K.	1	0.65	5	1.15

The allelopathic potential of deciduous trees belonging mostly to species with natural competitor strategy and character species of natural *Fagetum* communities studied by us mostly approaches that of juglone – in the case of more concentrated extracts. Among tree species especially *Betula pendula* and *Quercus petraea* are active. From the G- and H-character species *Corydalis cava* can be characterised by a strong allelopathic potential (even in a diluted form). Furthermore *Oxalis acetosella, Festuca drymeia, Allium ursinum, Aegopodium podagraria* and *Galium odoratum* have a high allelopathic potential in the order of weakening effect.

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EFFECT OF MICROFUNGI ON STABILITY OF ALLELOCHEMICALS

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According to our phytochemical investigations the stability of some known allelochemicals was different during a short time (18 days) after in vitro incubation of *Aspergillus niger* and *Penicillium expansum*.

A specific allyl-cysteine sulfoxide, alliin was sensitive, its quantity decreased quickly. Atropine (1000 ppm atropine sulphate) comparatively is a stable allelochemical. Trans-cinnamic acid (1000 ppm) and thymol (500 ppm) greatly inhibited the mycelium growth of microfungi during the incubation. In case of a greater concentration t-cinnamic acid was stable but in 100 ppm decreased especially at the end of incubation. Rutin slightly stimulated the growth of both microfungi. The quantity of rutin decreased to zero gradually until the end of incubation.

By the help of simple microbiological in vitro test and thin-layer chromatographic-densitometric method it was found that well-known allelochemicals have different stability. It seems the role of microfungi in allelopathy is very expressive not only in vitro but in the natural or also agroecosystems.

Key words: allelopathy, alliin, *Aspergillus niger*, atropine, cinnamic acid, decreasing effect, in vitro test, rutin, *Penicillium expansum*

Introduction

The manifestation of allelopathy can significantly be modified by ecological factors (temperature, light, soil moisture, pH, nutrient supply, degree of oxygen supply, biological activity of soil, salinity, etc.). At the same time these factors influence the getting out of the allelochemicals (volatility, washing out, secretion, decomposition, chemical transformation). Stress also modifies allelopathic sensitivity, reaction significantly.

The influence of the chemical and biological characteristics of soil on chemical transformation of phenolic acids and on changes in connection with ion uptake was studied in detail (Hoagland and Williams 1985, Balke 1985, Waller et al. 1987, Blum and Shafer 1988, Shafer and Blum 1991,

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Wardle and Nilsson 1997). From an ecological point of view terpenoids are of particular importance. Among them, in detoxification of monoterpenes, *Aspergillus niger* plays an important role (Moleyar and Narasimham 1987), but microfungi also take part in microbiological degradation of glucosinolates with a herbicid character (Smits et al. 1993, Brown and Morra 1995). The bacterial transformation and change in effect of juglone, well-known for its allelopathic effect, was studied by several researchers (Schmidt 1988, 1990, Neave and Dawson 1989, Williamson and Weidenhamer 1990), but there are new attainments also about microbiological transformation of glycyrrhizin belonging to tetraterpenes (Yamada et al. 1994).

It is known that reproduction of saprotrophic microfungi is possible in a quite broad pH-interval. For this reason they play a significant role in cellulose decomposition, since their cellulase activity insures it in a more or less satisfying degree. In general, however, it is little known, how microorganisms living in soil inactivate substances of a natural origin, having a broad scale of chemical composition. Obviously they may decompose, transform and utilise them (as a C- and N-source). Our factual knowledge of this kind is very insufficient, there are data rather about microbiological transformation of xenobiotics. A few allelopathic examples have already been mentioned in the summary of literature. Hence saprotrophic microfungi surely play an important role not only in cellulose decomposition, but also in neutralising allelopathically active substances.

Due to their frequency and ecological importance, two well-known microfungi, *Aspergillus niger* and *Penicillium expansum*, were chosen for our model experiments (Szabó et al. 1996, Szabó 1997).

Material and methods

Incubation

All the substances used in the in vitro mycological studies are water-soluble and can be sterilised by heat. Alliin (= (+)-S-allil-L-cysteine-sulfoxide) can be identified from the hot water extract of *Allium ursinum*. So a hot water extract was used. Atropine (DL-hyoscyamine in the form of atropine sulphate) was applied in a concentration of 1000 ppm, thymol in 50 and 500 ppm (its solubility limits do not permit testing higher concentration in this way), the other studied phenoloids (caffeic acid, trans-

cinnamic acid, rutin) in 100 and 1000 ppm, dissolved in the medium of microfungus cultures.

The medium of the stationer liquid cultures contained 2 g sucrose and 1 g peptone in 100 ml. Substances usually applied for setting the pH were not given to the substances dissolved in distilled water, in order to avoid the disturbing effect of metal ions. The starting pH value was 5.6–5.7. The inoculum was a 5-mm disc cut out of the 2-day culture of fungi (*Aspergillus niger, Penicillium expansum*) on PDA medium. At this stage spores are not formed yet and mycelium however weaves through the whole surface, insuring in this way a homogeneous inoculum of nearly the same quantity.

Inoculation was carried out under sterile circumstances, under a laminar box, and filtration was also made under sterile circumstances during sample taking.

Incubation was carried out in a biological thermostat, in 10-ml medium in test-tubes of the same size, at 25 °C, in 4 parallels in the case of each variant.

At each parallel the freshly filtrated mycelium was dried in an exsiccator set for 40 °C and measured precisely with analitical scales following the basic rules of gravimetry. Effect studies were executed at point of times following each other, that is why the suitable untreated control parallels were set separately. The obtained results were compared with these controls.

Thin layer chromatography and densitometry

In the studies with in vitro microfungus-cultures, quantitative changes of the allelochemicals remaining in the medium were traced by densitometry (Camag TLC Scanner II) following thin layer chromatographic separation.

TLC-characteristics (plate: Kieselgel by Merck, 20 × 20 cm):

- alliin – mobile phase: etanol – n-propanol – glacial acetic acid – water (2:1:1:1); alliin test Rf = 0.6; detection: solution of ninhydrin reagent with butanol, dissolved in acetic acid (activating at 110 °C for 5 minutes), measured at 490 nm under tungsten lamp,
- atropine – mobile phase: 0.2 M sodium acetate – methanol – chloroform (1:6:3); detection: Dragendorff's reagent with tartaric acid, measured at 530 nm under tungsten lamp,
- t-cinnamic acid – mobile phase: hexane – ethyl acetate – formic acid (30:10:1); detection: at 250 nm under deuterium lamp,

– rutin – mobile phase: ethyl acetate – formic acid – acetic acid – water (100:11:11:27); detection: at 340 nm under deuterium lamp.

Results

Both fungi were grown under sterile circumstances as described in methods. Their growth and mycelium increase quickly began, in the short-term serial studies a sample was used each time for phytochemical analysis. On these occasions the allelochemicals dissolved in medium was detected by thin layer chromatography and densitometry. Mycelia were measured, as well. Going into details is not necessary here, but it is worth mentioning that in each case the fresh weight of mycelium was almost the same already on the 12th day as on the 18th day, at the end of the experiment. In the table values measured on the 18th day are given (Table 1).

Table 1

Increase of mycelium weight (expressed in % of control)

Name of allelochemical	ppm	*Aspergillus niger*	*Penicillium expansum*
Alliin	160	106.8	101
Atropine	1000	100.5	100.6
T-cinnamic acid	1000	0	0
	100	100.7	73.8
Caffeic acid	1000	98.8	84.5
	100	99	87.6
Rutin	1000	109.6	93.5
	100	106.1	94.6
Thymol	500	0	0
	50	91.8	63.6

Mycelium weight of control containing no effective substance – mean of 10 parallels – on the 18th day: *Aspergillus niger* = 0.3875 g = 100%, *Penicillium expansum* = 0.3432 g = 100%. Note: the given quantity of alliin is the result of a measurement, it corresponds with the original concentration, there were only a few µg-s of the test available. Data (molecule weight in parentheses): – alliin = (+)-S-allil-L-cysteine-sulfoxide (177) = 160 ppm = 0.90 mM; – atropine = atropine sulphate.H_2O (695) = 1000 ppm = 1.44 mM; t-cinnamic acid (148) = 100 ppm = 0.68 mM – 1000 ppm = 6.75 mM; – caffeic acid (180) = 100 ppm = 0.55 mM – 1000 ppm = 5.55 mM; – rutin = quercetin-3-O-rutinoside (665) = 100 ppm = 0.15 mM – 1000 ppm = 1.5 mM; – thymol (150) = 50 ppm = 0.33 mM – 500 ppm = 3.33 mM

It can be seen that 1000 ppm t-cinnamic acid and 500 ppm thymol completely inhibited mycelium growth from the beginning which means that they are fungicide for the applied species at a relatively high concentration. The same substances in a tenfold dilution did not inhibit mycelium in-

crease or only to a small extent. From the two fungi *Penicillium expansum* proved to be more sensitive. This sensitivity can also be seen in the case of the hardly inhibiting caffeic acid and rutin. Alliin and atropine slightly increased mycelium weight, rutin, however, increased only the mycelium weight of *Aspergillus niger* significantly (rutin as a glycoside can be utilised due to its sugar component, as an easily accessible carbon source for the fungus).

Hence the majority of the applied allelochemicals did not inhibit reproduction of fungi in this study.

The quantity of effective substances remaining in the medium was detected by densitometry following separation by thin layer chromatography, according to the previous description of the method.

Densitograms shown in figures prove the observations summarised below.

In vitro stability of alliin (Fig. 1)

We have seen it already at the beginning of incubation that the amount of alliin gained from the leaf of *Allium ursinum* was smaller, if incubation was made together with the leaf (suspended in 2% mass ratio, approximating 1 g in the sample volume of the solution). If alliin was extracted in a hot water extract (being relatively stable on heat effect), its amount in the applied volume of the solution was around 3 g. It has to be mentioned that when extracting with water, obviously other nutritive organic and inorganic substances dissolve, as well. These must have contributed to the increase in the reproductive vigour of the applied fungi. However, alliin in the leaf of *Allium ursinum* often reaches 0.5% (for dry matter), which is not a small quantity! It means that an allelopathic effect of *Allium ursinum* is possible in the case of such concentration! (It is known that alliin is a direct precursor of allicin and other derivatives, which are rather lipophilous substances and the direct elicitors of allelopathic and "fitoncide" effect. The higher the quantity of alliin, the higher is the probability of formation of such substances.)

Hence at the start in the cultures uniformly inoculated with *Aspergillus niger* inoculum the presence of alliin is obvious.

On the 3rd day of incubation there was no significant change in the quantity of alliin. On the 6th day, however, with the increasing growth of *Aspergillus niger*, there remained significantly less not decomposed alliin in the medium (0.1–0.2 g in 5 l = 2–4 mg%) than on the 3rd day.

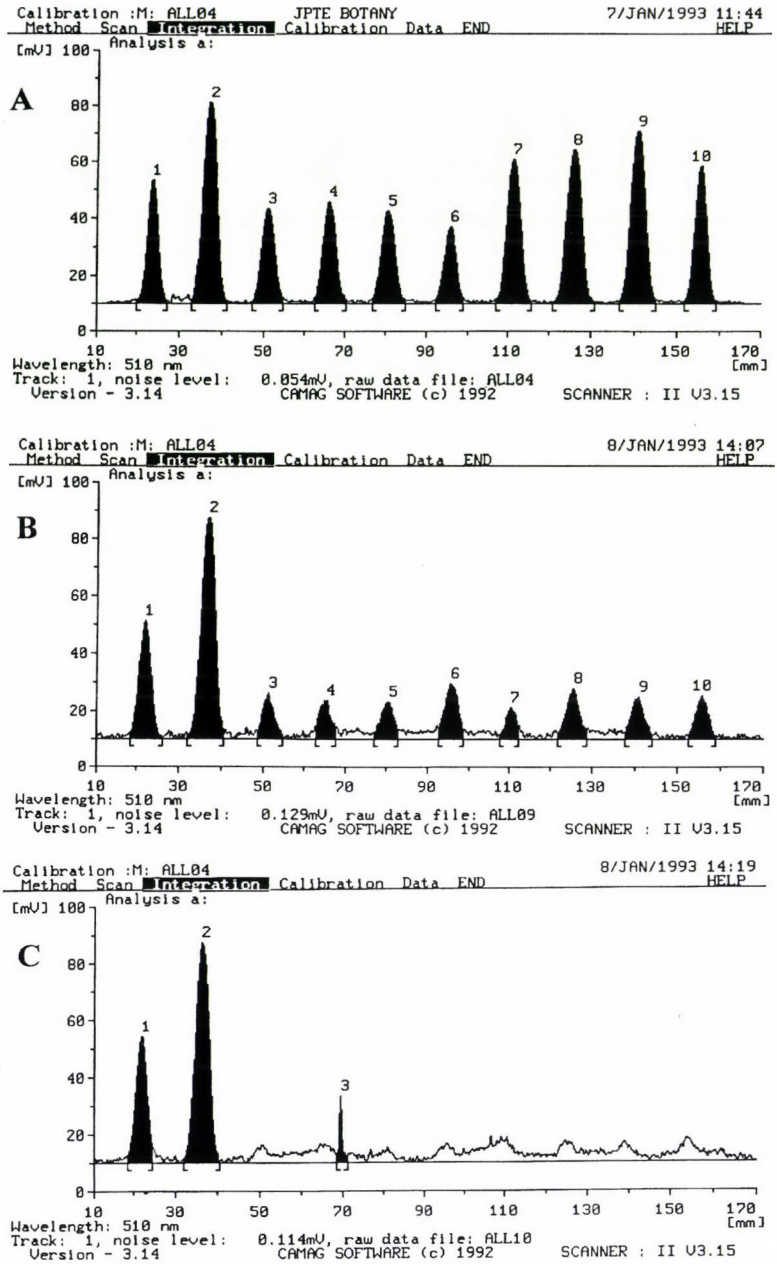


Fig. 1. In vitro stability of alliin (= (+)-S-allyl-L-cysteine sulfoxide. Microfungus: *Aspergillus niger*. – 1: 1 µg alliin, 2: 2 µg alliin, 3–6: alliin (in 2 µl) from herb of *Allium ursinum*, 7–10: alliin (in 2 µl) from hot water extract, A: at the beginning of incubation, B: in 6 days of incubation, C: in 12 days of incubation

On the 12th day alliin practically could not be detected which means it was decomposed, presumably the fungus utilised it as a C-, N- and S-source.

Incubation of *Penicillium expansum* gave completely similar results. There was however an important difference from *Aspergillus niger*: alliin was completely decomposed already on the 6th day. It means that in its presence the stability of alliin, the precursor responsible for allelopathy is even smaller.

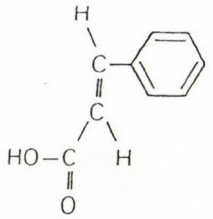
In vitro stability of t-cinnamic acid (Fig. 2)

It was necessary to ascertain the stability relations of the toxic 1000 ppm t-cinnamic acid, which inhibits the mycelium increase of both studied fungus species, without fungus-inoculation, as well. For this reason t-cinnamic acid was identified from the fungus-free medium on the 3rd and 6th day. The same quantity of t-cinnamic acid was found in the aliquot volume of medium samples applied besides tests in chromatography, which proves that t-cinnamic acid in itself remains stable throughout the experimental procedure and it undergoes no change in the medium. (On the chromatogram we found no spots referring to decomposition, either.) The figure also makes clear that on the effect of *Aspergillus niger* the quantity of this substance was the same on the 3rd day, which is in accordance with the fact that it has an inhibiting effect in a concentration of 1000 ppm.

The situation is similar in the case of *Aspergillus niger* on the 6th day and in the case of *Penicillium expansum* on the 3rd day. It means that the latter fungus species does not decompose t-cinnamic acid either. The measurements carried out on the 18th day are in accordance with all these.

The situation is different when applying t-cinnamic acid in a concentration of 100 ppm. Also here the stability studies without fungus inoculation were carried out on the 3rd and 6th day. Its quantity did not change. However, due to the effect of *Aspergillus niger* there remained only traces of it in the medium on the 3rd day.

There was no t-cinnamic acid to be found in the medium on the 6th day due to the effect of *Aspergillus niger* and on the 3rd day due to the effect of *Penicillium expansum*. The same is true for the 18th day.



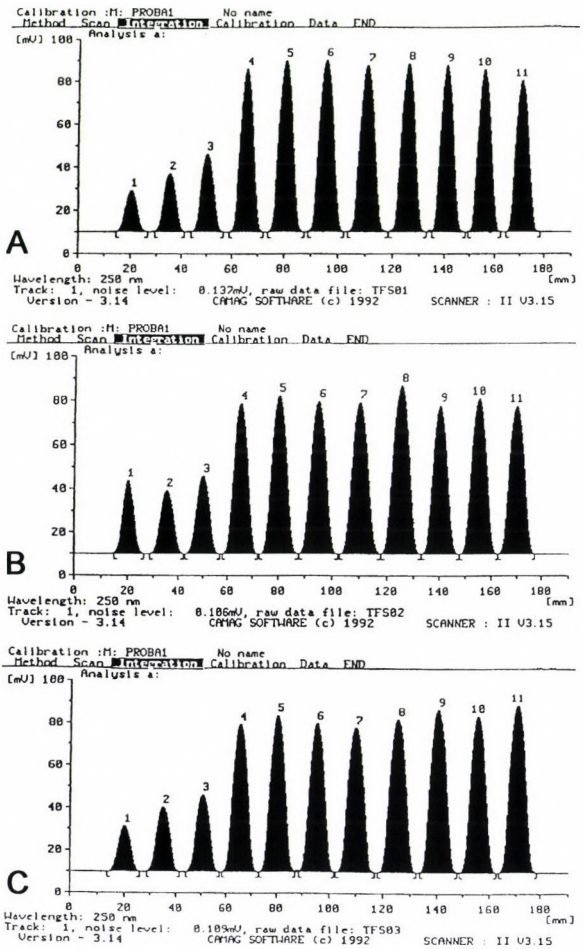


Fig. 2a. In vitro stability of trans-cinnamic acid 1000 ppm. – A: 1: 3 µg, 2: 5 µg, 3: 7 µg, 4–5: without fungi at the beginning of incubation, 6–7: without fungi in 3 days of incubation, 8–9: without fungi in 6 days of incubation, 10–11: *Aspergillus* in 3 days of incubation; B: 1: 3 µg, 2: 5 µg, 3: 7 µg, 4–7: *Aspergillus* in 6 days of incubation, 8–11: *Penicillium* in 3 days of incubation; C: 1: 3 µg, 2: 5 µg, 3: 7 µg, 4–7: *Aspergillus* in 18 days of incubation, 8–11: *Penicillium* in 18 days of incubation

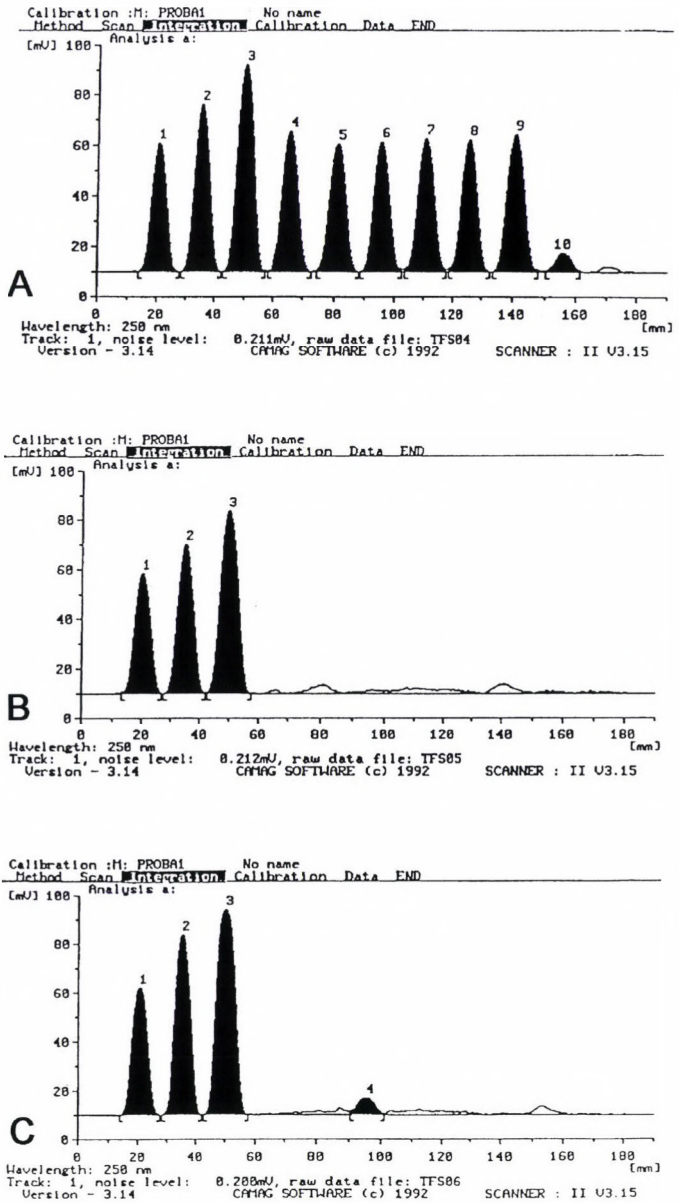


Fig. 2b. In vitro stability of trans-cinnamic acid 100 ppm. – A: 1: 5 µg, 2: 7 µg, 3: 10 µg, 4–5: without fungi at the beginning of incubation, 6–7: without fungi in 3 days of incubation, 8–9: without fungi in 6 days of incubation, 10: *Aspergillus* in 3 days of incubation; B: 1: 5 µg, 2: 7 µg, 3: 10 µg, 60–120 mm: *Aspergillus* in 6 days of incubation, 120–180 mm: *Penicillium* in 3 days of incubation; C: 1: 5 µg, 2: 7 µg, 3: 10 µg, 60–120 mm: *Aspergillus* in 18 days of incubation, 120–180 mm: *Penicillium* in 18 days of incubation

In vitro stability of rutin (Fig. 3)

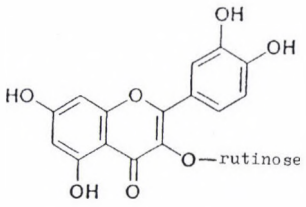
If only 100 ppm rutin was present in the applied medium, i.e. incubation took place without inoculation, another spot besides rutin could be detected at a suitable wavelength (340 nm). This chemically not identified substance (it may be a flavonoid or a phenolic acid), characteristic of the substrate, was present until the end of incubation as an "accompanying" substance. For this reason the stability of rutin in the medium was studied in more detail. If the medium did not contain rutin, in the course of growing the fungi the presence of this "contaminating" substance could be detected on the 12th day, too (and also a flat peak can be observed on the densitogram at about 30 mm distance, corresponding to an unknown substance, which is however present only in traces). Without fungus growing, there was no change in the amount of rutin in the rutin-containing medium on the 3rd day, and besides, the previously mentioned accompanying substance was present, as well (sample 3 is the base of comparison, which corresponds to the medium containing no fungi or rutin).

On the 12th and 18th day of incubation without fungi, rutin (and the "accompanying" substance) were present without any change, as proved also by the tests (3, 5 and 7 g rutin) present on the chromatogram and the densitogram.

In possession of previous knowledge the effect of fungi on quantitative changes of rutin was studied by us. Compared to the applied tests it can be seen that on the 3rd day of incubation *Aspergillus niger* and *Penicillium expansum* did not decrease the amount of rutin. On the 6th day, however, rutin remained stable only in the case of the *Penicillium expansum* culture (it seems likely that this substance is not utilised so intensively as alliin and t-cinnamic acid), and a small decrease could be experienced in the presence of *Aspergillus niger*.

The in vitro stability of rutin was in accordance with the above described situation on the 12th day, too. Due to the effect of *Aspergillus niger*, rutin was practically decomposed (in all likelihood it was utilised by the fungus). Its amount decreased also on the effect of *Penicillium expansum*.

At the end of the experimental period, i.e. on the 18th day of incubation, rutin remained only in traces even in the case of *Penicillium expansum* (curves 8–10), in the presence of *Aspergillus niger* it was completely decomposed. It is interesting that the decrease in the amount of nutritives was followed by the significant decrease in the amount of the "accompanying substance" as well, although its traces can still be detected on the 18th day on the densitogram in the case of *Aspergillus niger* (curves 4–7). *Aspergillus*



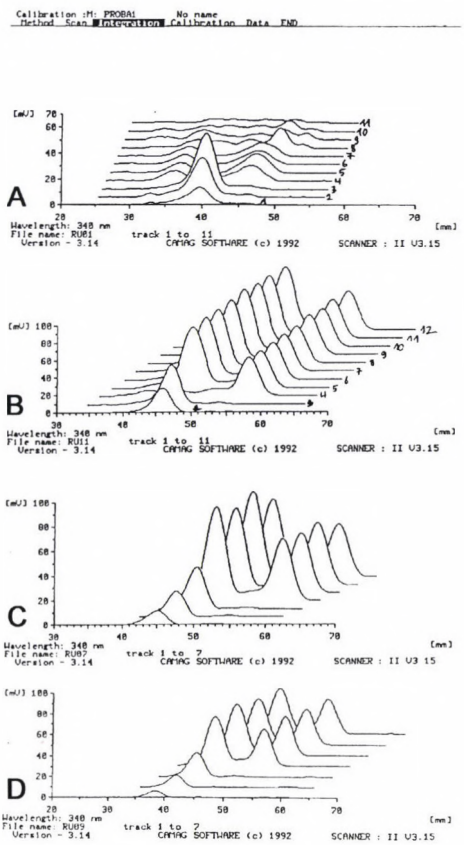


Fig. 3. In vitro stability of rutin (= quercetin 3-O-rutinoside) 100 ppm. – A: 1: 3 µg, 2: 5 µg, 3: 7 µg, 4–7: *Aspergillus* without rutin, 8–11: *Penicillium* without rutin; B: 2: 5 µg, 3: 7 µg, 4: medium without rutin and fungi, 5–8: only rutin at the beginning of incubation, 9–11: only rutin in 3 days of incubation; C: only rutin in 12 days of incubation; D: only rutin in 18 days of incubation; E: 1: 7 µg, 2: 10 µg, 3: without rutin and fungi, 4–7: *Aspergillus* in 3 days of incubation, 8–11: *Penicillium* in 3 days of incubation; F: 1: 7 µg, 2: 10 µg, 3: without rutin and fungi, 4–7: A*spergillus* in 6 days of incubation, 8–11: *Penicillium* in 6 days of incubation; G: 1: 5 µg, 2: 7 µg, 3: 10 µg, 4–7: *Aspergillus* in 12 days of incubation, 8–11: *Penicillium* in 12 days of incubation; H: 1: 5 µg, 2: 7 µg, 3: 10 µg, 4–7: *Aspergillus* in 18 days of incubation, 8–11: *Penicillium* in 18 days of incubation

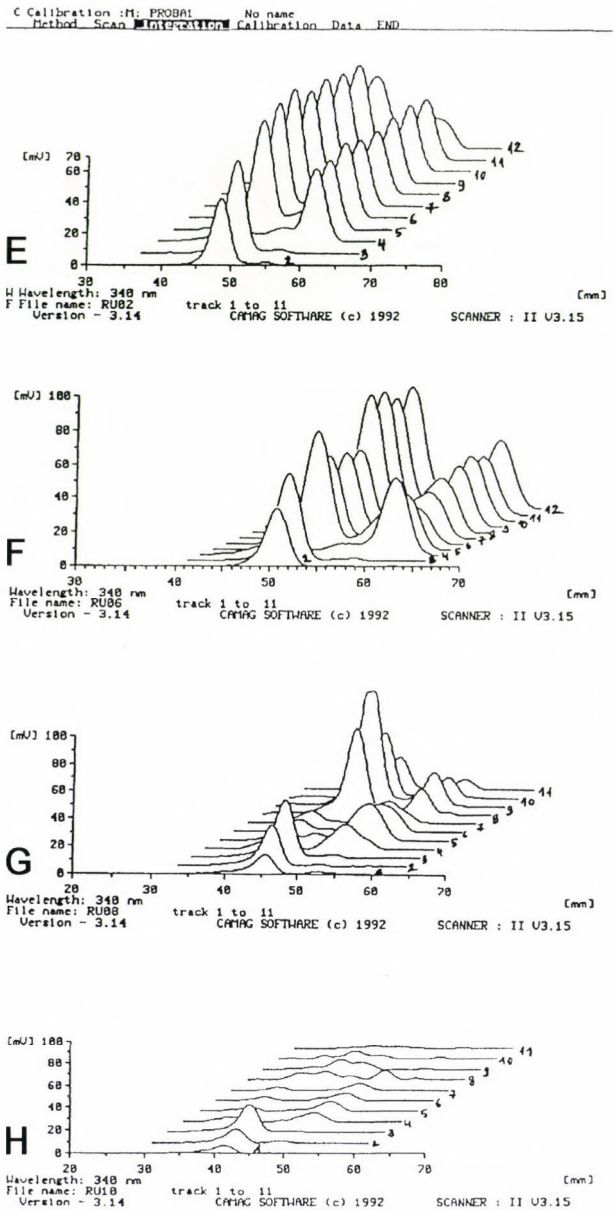


Fig. 3. In vitro stability of rutin (continued). E: 1: 7 µg, 2: 10 µg, 3: without rutin and fungi, 4–7: *Aspergillus* in 3 days of incubation, 8–11: *Penicillium* in 3 days of incubation; F: 1: 7 µg, 2: 10 µg, 3: without rutin and fungi, 4–7: *Aspergillus* in 6 days of incubation, 8–11: *Penicillium* in 6 days of incubation; G: 1: 5 µg, 2: 7 µg, 3: 10 µg, 4–7: *Aspergillus* in 12 days of incubation, 8–11: *Penicillium* in 12 days of incubation; H: 1: 5 µg, 2: 7 µg, 3: 10 µg, 4–7: *Aspergillus* in 18 days of incubation, 8–11: *Penicillium* in 18 days of incubation

niger reached the maximal value of mycelium growth at the same time. It can also be seen in the table that at this time the mycelium growth of *Penicillium expansum* did in fact not reach 100%, so it is not surprising that there remained some rutin in the medium.

If there was rutin abundantly (1000 ppm) in the medium, the above described characteristics cannot be observed, rutin can further be detected, since it is present in an excess amount, but there is not so much from it as to inhibit the growth of myceliums. It means that rutin is well tolerated by the fungus species used in the experiment.

In vitro stability of atropine (Fig. 4)

Because of its weaker allelopathic effect, atropine was applied only in higher (presumably more effective) concentration. (As a sulphate salt it is a S-source, as well!)

Without inoculation, the presence of atropine could be detected in the medium at the beginning of the incubation. On this day neither the inoculated *Aspergillus niger*, nor *Penicillium expansum* changed. A small decrease occurred particularly due to the effect of *Aspergillus niger* on the 6th day. This decrease could be observed later also in the case of *Penicillium expansum*, but atropine was not decomposed on the effect of either fungus on the 18th day. It means that during this period, when mycelium growth reached its maximum, a part of atropine remained in the medium, which renders it probable that part of atropine (atropine sulphate) may be utilised as a N- and C-source (and S-source), but it may also happen that it undergoes a smaller degree of decomposition.

Discussion

Saprotrophic microfungi play a very important role in biological cellulose decomposition mainly because of their frequency and pH-tolerance. From the allelochemical substances (alliin, atropine, t-cinnamic acid, caffeic acid, rutin, thymol) applied in a lower and higher concentration in the course of in vitro (laboratory) growing, only 500 ppm thymol and 1000 ppm t-cinnamic acid inhibit completely the mycelium growth of *Aspergillus niger* and *Penicillium expansum*. The other substances do not significantly inhibit the growth of fungi either in a smaller or a higher concentration.

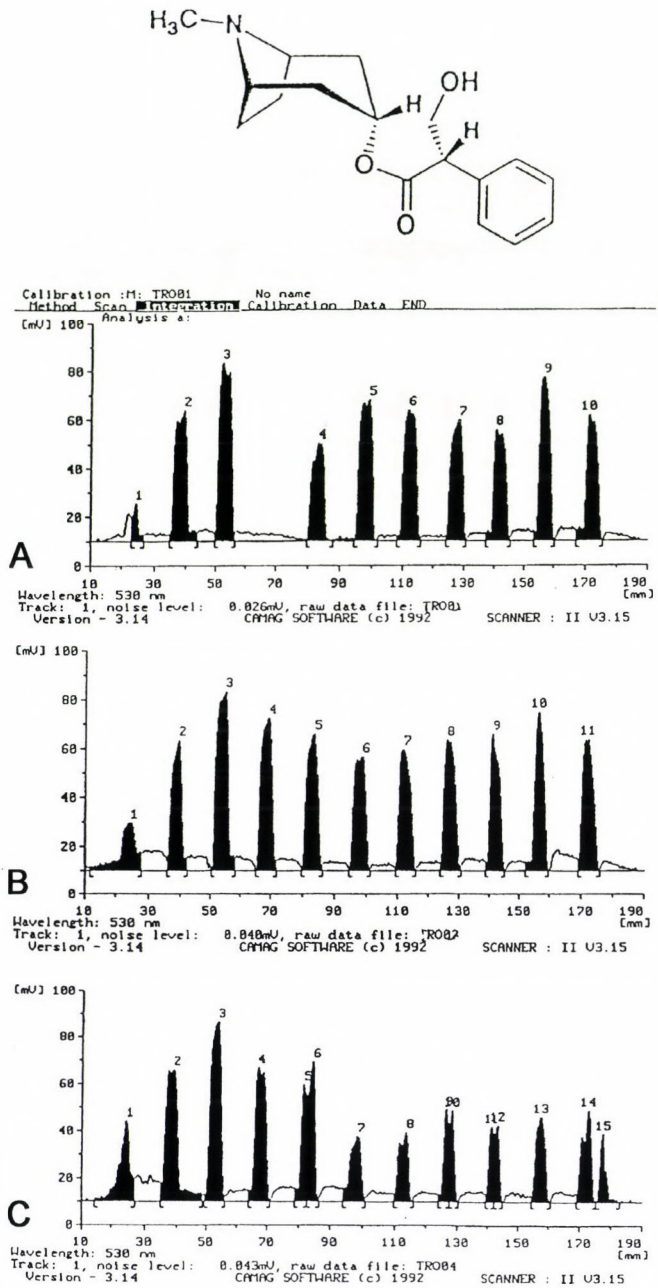


Fig. 4. In vitro stability of atropine (= DL-hyoscyamine) sulphate 1000 ppm. – A: 1: 2 µg, 2: 4 µg, 3: 8 µg, 4–7: without fungi, 8–10: *Aspergillus* at the beginning of incubation; B: 1: 2 µg, 2: 4 µg, 3: 8 µg, 4–11: *Penicillium* at the beginning of incubation; C: 1: 2 µg, 2: 4 µg, 3: 8 µg, 4–10: *Aspergillus* in 18 days of incubation, 11–15: *Penicillium* in 18 days of incubation

Phytochemical measurements (thin layer chromatography and densitometry) can prove that during the 3–18-day-long incubation period – from 5 samples (taken on the 3rd, 6th, 12th and 18th day), analysing 4 parallels in each case – among the character substances alliin, as the special amino acid of *Allium ursinum*, decomposes quickly in the culture of both fungi. Already on the 6th day the amount of alliin decreases to 10–20% of the original concentration. The decomposing activity of *Penicillium expansum* is particularly powerful, because due to its effect no alliin can be detected on the 6th day already. In the case of *Aspergillus niger*, the same can be experienced later, on the 12th day.

T-cinnamic acid in higher concentration is stable (fungicide), its amount changes very little during incubation. However, when applying it in 100 ppm concentration, its amount significantly decreases at the end of incubation. Its weak fungistatic effect can be observed also at this concentration.

Rutin slightly increases the growth of fungi in both concentrations. 100 ppm rutin gradually decomposes, especially in the case of *Aspergillus niger*. It is likely that microfungi cleave particularly the sugar component of rutin and utilise it as a carbon source.

On the basis of our phytochemical studies, atropine, having a medium-level allelopathic effect, in a concentration of 1000 ppm decomposes only partly in the in vitro cultures.

Hence, plant metabolites with an allelopathic effect (e.g. special amino acids, phenoloids, alkaloids) may undergo significant changes under in vitro circumstances. In these changes saprotrophic microfungi, frequently occurring in nature, play an important role.

Acknowledgements

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DETERMINANTS OF STRUCTURE IN A NIGERIAN MANGROVE FOREST

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The aim of this study was to examine the variation in mangrove vegetation structure, in relation to measured environmental variables. To achieve this, a relatively undisturbed mangrove swamp was analysed. Vegetation variables included species frequency, density, coverage, tree height and basal area while environmental measurements were soil texture, bulk density, root content of soils, salinity, organic matter, carbonate and exchangeable cations of soils. Multiple regression analysis revealed the importance of salinity and soil texture as the determinants of mangrove structure. Nutrient cations of soils and field moisture were relatively less important. Canonical correlation analysis revealed that the vegetation structure was significantly related to the measured variables.

Key words: determinants, mangrove, Nigeria, structure

Introduction

Having studied vegetation and soils of mangrove swamps in western Australia, and West Malaysia, Clarke and Hannon (1967), and Dietmont and van Wijngaarden (1974) concluded that soils did not offer satisfactory explanation for the observed vegetation structure. This conclusion was obvious, considering the difficulty of obtaining sufficient vegetation and soil data in tidal mangroves. The mangrove swamp soils, being regularly flooded is often extremely soft, with high water table. The dense entanglement of *Rhizophora* props present difficulty of movement while sampling time is limited by the tidal cycles. Consequently, data, particularly in the vegetation subsystem, are usually not obtained in forms that could permit statistical correlations (Naidoo 1980). The soil data are often derived from laboratory analysis which may not give a true indication of field conditions (Ukpong 1992). Since mangroves are often discretely zoned along sea coast littorals, most studies have adopted the simplified approach of emphasising vegetation structure in relation to isolated soil variables obtained within the zonation e.g. soil salinity (Cintron et al. 1978, Ukpong 1991).

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In the present study a small, relatively undisturbed, densely vegetated mangrove forest was sampled for vegetation, soil and other environmental attributes. The aim was to examine the variation in vegetation structure in relation to the measured environmental variables.

Study site

The occurrence of mangroves (*Rhizophora mangle, R. racemosa, R. harrisonii, Avicennia africana*), commonly codominant species with *Nypa fruticans, Raphia hookeri, Raphia vinifera* and *Phoenix reclinata* in the brackish/saline estuarine forests of Nigeria reach their northernmost limits in the Creek Town Creek/Calabar River Swamp (Fig. 1). Located between latitudes 4°55′N and 5°00′N, about 20 km from the Atlantic Ocean coast, the climate is humid tropical with mean annual rainfall of 4021 mm. Although rainfall occurs throughout the year, there are peaks from May to August (1880

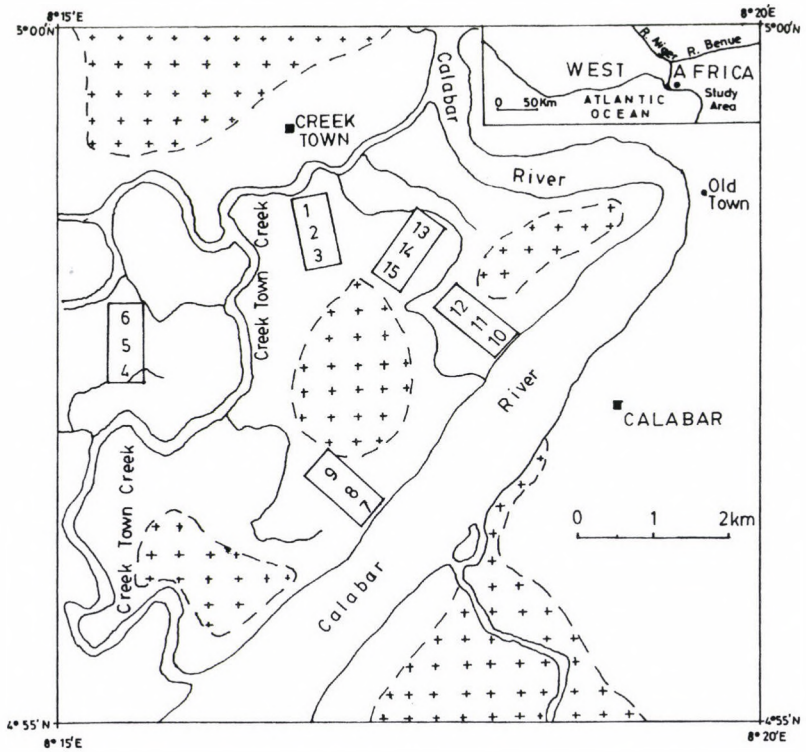

Fig. 1. Map of the study area showing approximate location of sample stands (1–15)

mm): lowest values (240 mm) occur from December to February. Temperatures are uniformly high with a maximum of 30 °C and minimum of 23 °C (FRN 1998). The swamps experience regular diurnal tides with mean amplitude of 0.75 m at Creek Town.

Methodology

Vegetation sampling

To obtain vegetation data, the Creek Town Creek/Calabar River mangrove swamp was sampled in the relatively dry months between November and January. The criteria for stand selection were (i) mangroves consisted members of the canopy layer and (ii) vegetation showed evidence of least disturbance within at least 0.5 hectare in size. The vegetation was stratified into A stratum (> 3 m tall), B stratum (1–3 m tall) and C stratum (< 1 m tall).

The mangroves were measured in fifteen 200 m^2 quadrats randomly located within the swamp. Quadrat location was determined by accessibility through tributary channels. Tree frequency, density, tree height, diameter at breast height and crown cover were obtained in 25 m^2 subquadrats established within the larger quadrats. Coverage for the C strata and seedling density were obtained in a further 1 m^2 subdivision.

Environmental sampling and laboratory methods

Using a semi-cylindrical corer, three soil samples to a depth of 60 cm were obtained from each quadrat and bulked for laboratory analysis: particle size distribution (% sand, silt, clay) was obtained by the hydrometer method (Bouyoucos 1962); bulk density in steel cores of volume 550 cm^3, and field moisture, from weight of oven-dry samples; organic matter by the Walkley–Black method (Walkley and Black 1934); chloride content by $AgNO_3$ titration method (USDA 1969). Exchangeable cations were extracted in 1 N ammonium acetate at pH 7 from which the concentrations of potassium, magnesium and calcium were determined by atomic absorption and sodium by flame photometry (Isaac and Korber 1971); cation exchange capacity was obtained as the summation of the exchangeable cations and exchange acidity. Exchangeable acidity was extracted with barium acetate and titration with NaOH (Jackson 1962). Carbonate was determined by the Bromo Thymol Blue indicator titration method. Root content

was determined by passing known weight of soil through a 2 mm sieve, weighing the roots retained in the sieve and expressing the weight as percentage of soil (Ukpong 1995a). Total salinity was measured at the water table in the swamp using a portable refractometer calibrated against a Bisset Berman salinometer. At the same sites pH measurements were obtained using a portable pH meter with glass electrode.

Synthesis of data

A hypothesis was formulated that the structural development of mangrove vegetation was influenced by variation in environmental properties. To test the hypothesis, multiple regression analysis was used to investigate statistically the primary relationship between each structural property of the vegetation and a series of measured environmental variables. Multicollinearity among the variables was eliminated using Hauser's (1974) criteria. The multiple linear equation was of the form:

$$Y = a + b_1x_1 + b_2x_2 + \ldots + b_nx_n + SE,$$

where Y = dependent (vegetation) variable; a = Y intercept, b = partial regression coefficient, X = independent variable (environment), and SE = standard error of estimate.

To enable the more important environmental variables that accounted for mangrove structure to be discerned, the environmental variables selected by the regression analysis were linked with the vegetation data using canonical correlation analysis (COR). This analysis was aimed at exposing the joint structures of the two data sets (ter Braak and Prentice 1988). The environmental variables, being positively skewed in their distributions were transformed to log10 in order to meet the requirements of normality for parametric statistics (Gregory 1974).

Results

Vegetation analysis

The mangrove swamp is dominated by *Nypa fruticans* (Table 1). *Nypa fruticans* appears to thrive best, initially on soft tidal mud which is converted to firmer soil as the rootmat becomes complex. *Rhizophora mangle* is associated with *Nypa* in the inner swamp while *R. racemosa*, with extensive prop roots are adapted to channel margins. *Avicennia africana* could be

found in pure stands along channel margins although it dominates in the inner somewhat less inundated swamps. *Rhizophora racemosa* apparently tolerates salt more than the other species and is regarded as the pioneer colonizers of mudflats (Keay 1953). Generally tree height decreased from the channel inlands, correlating with decreasing crown cover and basal area. Three species (*Avicennia africana, Rhizophora mangle, R. racemosa*) clearly show dominance in terms of basal area (Table 1).

Table 1

Mensuration data of vegetation in fifteen 200 m^2 quadrats

Species	Frequency (%)	Density (stems/ha)	Mean coverage (%)	Mean tree height (m)	Mean basal area (m^2/ha)
A Stratum					
Nypa fruticans	60.0	153	29.0	4.8	–
Rhizophora mangle	66.7	100	16.3	4.9	4.3
Rhizophora vinifera	44.7	87	18.7	5.8	–
Rhizophora racemosa	44.7	87	16.7	7.7	2.8
Avicennia africana	60.0	80	7.3	5.2	3.5
B Stratum					
Rhizophora mangle	66.7	237	26.3	–	0.8
Nypa fruticans	66.7	213	20.3	–	–
Rhizophora racemosa	53.3	307	13.3	–	0.8
Avicennia africana	53.3	146	15.0	–	1.2
Conocarpus erectus	40.0	113	14.7	–	0.3
Phoenix reclinata	33.0	47	8.2	–	0.3
C Stratum					
Mangrove saplings	93.3	393	0.3	–	
Hypa fruticans	80.0	300	0.8	–	
Raphia spp.	53.3	200	0.6	–	
Conocarpus erectus	46.7	120	0.2	–	
Phoenix reclinata	46.7	120	0.2	–	
Vassia cuspidata	33.3	46	0.2	–	
Acrostichum aureum	26.7	144	0.5	–	

In the B stratum there was the expected increase in stem density, usually associated with understorey vegetation. *R. mangle* was dominant although *R. racemosa* had the highest density on account of fringing the creeks in almost pure stand. Inland, *Phoenix reclinata*, *Conocarpus erectus* and *Avicennia africana* were associated in mixed stands. The importance of mangrove and *Nypa* saplings in the C stratum was obvious: these species respond to dynamic tide transport due to buoyancy of their propagules. The occurrence of ferns, grasses and sedges (*Acrostichum aureum*, *Vossia cuspidata*, etc.) contrasts with the open ground layer often reported for littoral swamps (Giglioli and Thornton 1965).

Environmental characteristics

Thirteen environmental variables were measured and their variability assessed using the coefficient of variation (Table 2). The mangrove substrate was loamy in texture with low variability of the particle size fractions, indicating uniform sedimentation from similar sources. Bulk density

Table 2

Summary of environmental data in fifteen 200 m^2 quadrates

Variable	Mean	Standard deviation	Coefficient of variation
Sand (%)	35.60	4.80	13.4
Silt (%)	43.20	9.20	21.2
Clay (%)	21.20	3.50	16.5
Bulk density (g cm^{-3})	0.75	0.16	21.3
Field moisture (%)	125.80	19.60	15.6
Organic matter (%)	6.22	0.90	14.5
Root content (%)	8.45	3.70	43.8
Salinity (soil water %)	2.91	1.60	54.9
Chloride content (soil %)	2.05	0.65	31.7
Carbonate (g/100 g)	7.20	2.50	34.7
Exchangeable acidity (me/100 g)	3.2	1.10	34.3
pH (field moist)	6.1	0.14	2.3
Cation exchange capacity (CEC) (me/100 g)	42.8	3.64	8.5

increased with sand content of soils while field moisture decreased with an increase in sand content. pH values indicated increasing alkalinity in *Avicennia* sp. stands than in *Rhizophora* spp. stands. The average field moist pH value stood at 6.1.

Laboratory determined chloride content of soils was lower than field soil water salinity. However, variability in salinity could be accounted for by freshwater inputs from upland streams, distance from ocean tides (Ukpong 1991) and subsurface seepage of freshwater across the mangrove high forest ecotone (Semeniuk 1983). The high values of organic matter showed a correlation with the fibrous root content of soils. Both values decreased with soil depth and were reflected in the peaty nature of the mangrove soils.

The soils had a large sink for cations as indicated in the high cation exchange capacity values.

Effect of environmental factors on vegetation structure

An attempt was made using multiple regression analysis to discern the environmental factors that account for variation in mangrove vegetation structure. The multiple regression utilised stepwise elimination procedures to develop predictive models for vegetation response to the environment. As a search procedure, the stepwise technique identifies those independent (environmental) variables having the strongest relationship with the dependent (vegetation) variables. The results are shown in Table 3. All the regressions presented have a multiple coefficient of determination (R^2) of 0.55 and higher. Since (R^2) is equal to the percentage of variation which the multiple regression is accountable for, i.e. the level of explanation, it follows that all the regression equations account for 55% or above of the

Table 3

Regression models for vegetation structure determinants in the mangrove swamps

Tree density	6.02 + 0.38 SAL – 0.34 BD + 2.51 OM ± 23.4% (R^2 = 58.8%)
Tree height	–12.44 + 0.78 SAN – 0.46 SAL + 0.37 BD ± 20.5% (R^2 = 66.3%)
Basal area	0.51 + 0.18 SAL – 0.11 CEC + 0.31 SAN + 0.07 OM ± 20.4% (R^2 = 84.3%)
Cover	90.20 – 0.62 FM – 0.24 SAL – 0.15 SI ± 26.4% (R^2 = 76.5%)
Seedling density	53.41 + 1.77 SI + 0.84 SAN – 0.21 SAL ± 12.6% (R^2 = 84.2%)

Where SAL = salinity, BD = bulk density, OM = organic matter, SAN = sand, CEC = cation exchange capacity, FM = Field moisture, SI = silt

Table 4

Percentage contribution of the independent (environmental) variables to the total variance of the regression equations

Vegetation properties	% Contribution of independent variables							Total variance %
	SAL	SAN	BD	OM	CEC	FM	SI	
Tree density	41.2***		11.4*	6.2*	–	–	–	58.8
Tree height	20.5**	32.7**	1.7**	–	–	–	–	66.3
Basal area	54.3***	10.3*	–	5.5**	15.2**	–	–	84.3
Cover	18.2**	–	–	–	–	48.3*	10.0*	76.5
Seedl. density	10.0*	14.0**	–	–	–	–	60.2***	84.2

Where SAL = salinity, SAN = sand, BD = Bulk density, OM = organic matter, CEC = cation exchange capacity, FM = field moisture, SL = silt. *** = Significant at the 0.1% level; ** = Significant at the 1% level; * = Significant at the 5% level

variance. Table 4 shows the percentage contribution of each independent variable to the total variance of the regression equations.

The most frequently occurring environmental parameter in the equations is salinity. Vegetation response to salinity is well marked particularly with respect to tree density and basal area where salinity accounts for 41.2% and 54.3% of the total variances for both equations as shown in Table 4. Substrate texture as represented by silt is important for the rooting and establishment of mangrove propagules. Silt contributes 60.2% to the total variance of 84.2% extracted for the seedling density equation. Besides salinity, sand texture was next in importance, retained in three equations but being particularly marked for the tree height equation where it accounted for 32.7% of the total variance of 66.3%. Field moisture was an important determinant of plant cover, contributing 48.3% to the total variance of 76.5%. Bulk density, organic matter and exchangeable cations were relatively less significant determinants of the mangrove structural characteristics (Table 4). Other environmental properties measured but not reflected in the regression equations were eliminated from the analysis on the basis of multicollinearity (Hauser 1974).

As a further step towards determining the effects of the measured environment on the vegetation, canonical correlation analysis (COR) was performed directly on the data. The matrix of environment and vegetation data was now described in terms of vectors with eigenvalues greater than 0.42 (which together explained 82.4% of the variance in the initial environment/vegetation data matrix). Primarily, this eigenvalue technique pre-

Table 5

Canonical factor structure of environment-vegetation relations (only weights > 0.03 on two correlations are reported)

Environmental weights		Vegetation weights	
r_1			
Sand	–435	Basal area (A)	+582
Salinity	–572	Seedling density	+433
Carbonate	–398	Tree density	+387
pH	–427	Coverage (B)	+425
Organic matter	+388	–	–350
r_2			
Root content	+422	Coverage (A)	+523
Field moisture	+625	Seedling density	+423
Bulk density	+342	Coverage (C)	+308
Silt	+511	Tree height	+411
CEC	–336	Density (C)	+394

(A) = A stratum; (B) = B stratum; (C) = C stratum

sented the canonical weights in terms of vectors. However, since the relationship of vectors to the initial data is known, the canonical weights have been expressed in terms of the environmental and vegetation properties as shown in Table 5. The first correlation (r_1 = 88.5%) and second correlation (r_2 = 76.2%) are significant with p less than 0.01. This implies that the environment and vegetation are related in two significant ways. Table 5 reports the canonical weights (+0.3) on the two significant correlations.

On the first correlation, environmental properties with large weights are salinity, sand and pH. The vegetation properties with large weights are basal area, seedling density and coverage. The positively weighted vegetation properties reflect high performance in relation to negatively weighted and diminishing importance of the environmental properties. The positive weights on the vegetation properties also indicate adaptation or tolerance such that vegetation increases are not affected by hostile environmental conditions dominated by salinity, coarse substrates and increasing acidity.

On the second correlation, both environmental properties and vegetation properties are positively weighted showing that vegetation increases occur as the environmental properties are enhanced, particularly field

moisture, silt content of substrates and root content. Most responsive to these environmental properties are seedling density, tree height and density of C stratum species. These are highly significant joint structures which explain structural variations in the mangrove vegetation.

Conclusion

Multiple regression analysis and canonical correlation analysis have been used to investigate possible environmental determinants of vegetation structure in mangrove forests. A previous applications of multiple regression was in the prediction of species distribution in relation to soil nutrients (Ukpong 1995b). Likewise, canonical correlation (COR) was applied to soil-species studies in estuarine mangroves (Ukpong 1994). However, inferences in these studies were not related to vegetation biomass characteristics. The present study has shown that soil water salinity, substrate textural characteristics, field moisture and bulk density of soils are important determinants of variations in tree basal area, tree coverage, tree height, seedling density and other structural characteristics of the mangrove forest. Primarily, the study is a possible input into mangrove ecosystem management and conservation procedure.

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MANGROVE VEGETATION AND SOILS ALONG AN ACCRETING PART OF THE COAST OF WEST AFRICA AND THEIR IMPLICATION TO CONSERVATION

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Mangrove vegetation and soil along an accreting estuary in West Africa were studied with a view to understanding the community structure and soil characteristics of the ecosystem. To obtain representative data the vegetation and soil were sampled on the basis of habitat differentiation. Species dominance varied between the habitats. Overlapping boundaries between habitats resulted in mixed interface vegetation. Although *Rhizophora racemosa* appeared to be the pioneer colonizer of mudflats, the mature vegetation was structurally more complex; species occurrences consisted of mixed stands of tree species and aberrants of shrubs and ferns. *Nypa fruticans*, an introduced species was ecologically more important than other species. Soil analysis indicated hypersalinity although the surface water system generally varies from brackish/fresh to saline. The soils are silty, organic and peaty with a high cation sink in terms of exchangeable magnesium. The distinguishing characteristics of the habitats were mainly in the substrate texture. The findings could be used to initiate baseline studies aimed at the conservation of estuarine mangrove forests.

Key words: conservation, mangrove, soil, W Africa

Introduction

Mangroves are halophytic in nature, occupying brackish and saline shorelines in the tropics and subtropics. But in West Africa the coastline does not favour extensive mangrove growth due to the presence of fault lines and rising sea level (Ibe and Antia 1983), and subsidence outstripping the supply of sediments with the result that the coastline displays an erosional trend (Hoyt 1967). However, along the deltas and estuaries, shallow water depths lead to reduced wave energy resulting in inlets predominantly depositional. In this area, sedimentation has tended to keep pace with tidal inundation resulting in the formation of mudflats which support mangrove vegetation. Due to the predominance of inlets, Keay (1953) maintained that the most mature mangroves in the West African mangrove subregion occur along the Nigerian–Cameroun coast where the Cross River estuary is found (Fig. 1).

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The growth of mangrove is associated with substrate transformation; upon colonization of mudflats, the soil becomes firm through entrapment of sediments and addition of organic detritus as the mangrove develop. This study aims at analysing the mangrove vegetation and soil with a view to understanding the community structure, soil characteristics of accretive mangrove swamps and their implications to the conservation of coastal/ estuarine vegetation in general.

Study area

The Cross River estuary in West Africa (Fig. 1) typifies a tropical ever-wet mangrove swamp with an annual rainfall of 3886 mm, relative

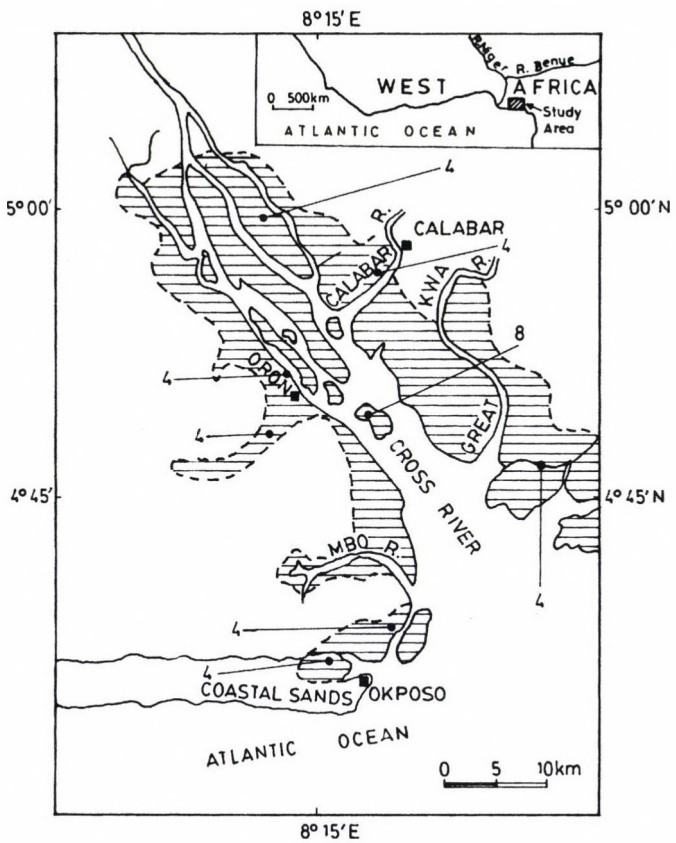

Fig. 1. Map of the study area showing location of transects and number of quadrats on each transect

humidity averaging 80% and mean annual temperature of 27 °C. Tidal amplitude in the estuary averages 2.01 m at spring tides and 1.07 m at neap tides (Nigerian Navy 1986).

Methods

Aerial photographs (scale 1 : 5,000) of the study area were used in the investigation of vegetation distribution patterns. Environmental transects were located on physiographic mangrove habitats identified and modified after Lugo and Snedaker (1974) as: (i) Tidal inlets/hinterland dune habitats – consisting of ebbflood rills and adjacent "old" levees where intense sedimentation had caused channels to abandon their former courses; (ii) Braided channel habitats, which are vegetated longitudinal intertidal bars found within the river channel, and (iii) Wooded levee habitats – consisting of accreting natural channel levees that merge inshoreward into the low freshwater swamps. The transects were at least 100 m long across the habitats and were oriented at right-angles from the water channels inland. On each transect 10 m × 10 m quadrats were established at regular 20 m intervals. A total of thirty-six quadrats were sampled (see Fig. 1). Habitat classification eliminated a bias for sampling only the more accessible forest types.

The mangrove vegetation was categorised into overstorey (> 3 m tall), understorey (1–3 m tall) and groundlayer (< 1 m tall). Vegetation measurements included crown cover of each species, obtained by the crown-diameter method (Mueller-Dombois and Ellenberg 1974); species density, frequency and tree basal area excluding *Rhizophora* props); and tree height using Hagar altimeter. The understorey and groundlayer species coverage were estimated visually in 25 m^2 and 1 m^2 subplots, respectively. The relative measures of frequency, density, and coverage were summed to obtain the ecological importance values of each species in the mangrove swamps (Stephenson 1986). In the dry season (December/January) during low tides, soil samples were obtained from each quadrat using a corer. Each observation consisted of three bulked samples obtained at 20 cm intervals to a depth of 60 cm, which appeared to be the mean rooting depth for the mangrove species. Soil properties analysed for were: percentage of sand, silt and clay (Bouyoucos 1962); percentage of field moisture from oven-dry weight of field moist samples; bulk density, in cores of volume 550 cm^3; percentage of organic carbon by the Walkley–Black method, and pH in 1:2

soil to water suspension using glass electrode (Jackson 1962). Exchangeable cations (K, Mg, Ca, Na) were extracted in 1 N ammonium acetate at pH 7 and their concentration determined by atomic absorption and flame photometry; cation exchange capacity (CEC) was obtained by the summation method; exchangeable acidity was determined by NaOH titration and aluminium by EDTA titration (Jackson 1962). Soluble sulphate was by turbidimetric determination (Tabatabai 1974). Chloride content was determined by $AgNO_3$ titration while total salinity was calculated as total water soluble salts, mainly chlorides + sulphates (USDA 1969). Carbonate content was measured using the Bromo Thymol Blue indicator titration method. Available phosphorus levels were obtained by the Bray method while total nitrogen was measured by the Macro-Kjeldahl method (Jackson 1962).

Results

Vegetation analysis

The results of vegetation analysis of the overstorey are shown in Table 1. The most important species in this layer is *Nypa fruticans* with a density of 151 stems/hectare and ecological importance value (I.V.) 81.5. *Avicennia africana*, *Rhizophora mangle* and *Rhizophora racemosa* had closely similar frequencies of occurrence. The relatively rare species included *Rhizophora*

Table 1

Summary of vegetation analysis of the overstorey in 36 quadrats (100 m²)

Species	Frequency (%)	Density (stems/ha)	Mean coverage (%)	Mean tree height (m)	Mean basal area (cm²)	Importance value (I.V.)
Nypa fruticans	70.0	151	28.1	2.5 (54)	–	81.5
Avicennia africana	51.4	105	14.6	6.1 (39)	258.1 (31)*	52.3
Rhizophora mangle	54.8	81	13.5	6.1 (29)	216.7 (21)	47.8
Rhizophora racemosa	46.2	79	14.2	5.2 (18)	305.2 (22)	45.2
Raphia vinifera	27.2	51	10.3	3.3 (11)	–	29.5
Pandanus candelabrum	9.6	31	6.4	4.0 (5)	159.3 (8)	15.8
Phoenix reclinata	13.4	14	1.7	5.0 (3)	94.8 (3)	8.9
Conocarpus erectus	9.0	9	1.0	8.5 (4)	189.7 (2)	5.8

* Numerals in parentheses indicate actual number of trees measured

Table 2

Summary of vegetation analysis of the B and C strata in 36 quadrats (100 m^2)

Species	Frequency (%)	Density (stems/ha)	Coverage (%)	Basal area (cm^2)	Importance value (I.V.)
Understorey					
Nypa fruticans	82.0	201	24.5	–	86.9
Avicennia africana	61.8	167	16.8	45.6	65.8
Rhizophora mangle	58.4	96	16.2	38.5	53.0
Rhizophora racemosa	52.8	92	12.6	37.3	46.1
Phoenix reclinata	38.2	42	6.4	40.9	26.8
Groundlayer					
Acrostichum aureum	46.2	–	1.5	–	59.6
Nypa frusticans	51.4	350	0.8	–	42.9
Mangrove saplings	70.0	480	0.4	–	39.7
Raphia saplings	54.8	160	0.6	–	38.8

harrisonii, *Laguncularia racemosa* and *Triumfetta rhomboideae* with frequencies of occurrence < 5%. Although the vegetation was generally shrubby, the forest contained tall trees, e.g. *Rhizophora harrisonii* (8.5±2.3 m), *R. racemosa* (6.9±5.9 m), *R. mangle* (6.6±2.8 m) and *Avicennia africana* (6.1±2.7 m).

In the understorey (Table 2), there was the expected increase in stem density usually associated with lower stratum vegetation. However, the sequence of ecological importance for the major species was similar to the overstorey structure.

Groundlayer analysis revealed the overwhelming importance of *Acrostichum aureum*, *Nypa*, mangrove and *Raphia* saplings, which contracts with the generally open groundlayer reported in the literature for littoral mangrove swamps (Tomlinson 1986).

Soil analysis

The summary of soil analysis is reported in Table 3. Bulk density was low (72.8±0.05 g cm^{-3}), and the soils were dominantly silty. While total salinity and chloride values indicate hypersalinity, high field moisture values reflect waterlogging of the soils. The mangrove soils have a high sink for cations. Total nitrogen values are low, but organic carbon (5.7±2.8%)

Table 3

Physical and chemical properties of soil in the mangrove swamp (values are means±SE)

Soil properties	Values	Soil properties	Values
Bulk density (g cm^{-3})	72.8±0.05	Magnesium (meq/100 g)	18.4±1.5
Field moisture (%)	119.2±10.4	Potassium (meq/100 g)	0.23±0.1
Sand (%)	25.8±2.8	Sodium (meq/100 g)	1.7±0.2
Silt (%)	56.5±4.5	Total nitrogen (%)	0.8±0.3
Clay (%)	17.7±3.9	Phosphorus (µg ml1)	7.2±1.3
Clay (%)	5.3±0.1	Organic carbon (%)	5.7±2.8
pH total salinity (%)	4.1±0.2	Aluminium (meq/100 g)	0.21±0.1
Chloride (%)	2.9±0.2	Sulphate (meq/100 g)	0.06±0.1
CEC (meq/100 g)	34.4±2.5	Exchange acidity (meq/100 g)	0.87±0.2
Calcium (meq/100 g)	13.1±1.7	Carbonate (meq/100 g)	6.7±1.8

and available phosphorus values (7.2±1.3 µg ml^{-1}) are high. The high carbonate contents are indicative of saline soils with a high molluscan population.

Discussions

Since *Nypa fruticans* is an introduced species (Mercer and Hamilton 1984), its importance indicates the extent of human interference in the ecology of mangroves in West Africa. Generally in West Africa *Rhizophora racemosa* is important along shorelines, while *Avicennia africana* and *Rhizophora mangle* tend to favour elevated and somewhat more inland portions of the swamp. Occurrence of *Raphis vinifera*, basically an upland freshwater species in the upper estuary, reflects a salinity gradient between the wet and relatively dry periods of the climate during which the swamps vary from nearly fresh to saline. Tall trees occur particularly on channel levees where *Rhizophora racemosa* stands > 25 m tall were observed. *R. mangle* were tallest in the inner swamp but shrubby on the channel margins, while *Avicennia africana* showed no observable height gradient across the swamps. The height structure of *Nypa fruticans* and *Raphis vinifera* were similar. *Rhizophora racemosa* (excluding props) had the highest mean basal area, although *Avicennia africana*, usually with straight trunks had the widest range. The spatial distribution of understorey species apart from fre-

quency and density increases was similar to the overstorey. In the groundlayer, *Acrostichum aureum* was frequent on topographic mounds and sandy deposits (Ukpong 1995a). The distribution of mangrove saplings did not reflect a relationship with mature mangrove stands since saplings were most numerous within *Nypa fruticans* stands. Entrapment of propagules within the dense *Nypa* growth, in contrast to the generally open groundlayer in mature mangrove stands appeared to account for this distribution.

Silt was the dominant particle size fraction in the West African mangrove soils, consequent to a slow rate of sedimentation of fine particles out of suspension in the swamp. The low bulk density and high field moisture values indicate daily inundation of the soils by tides as well as the relatively low sand content of the sample profiles (Ukpong 1995b, Anderson 1995). The soils (air-dry) are acidic; the low pH values indicate acidity conditions usually associated with empoldered mangrove soils (Boto and Wellington 1984). The relatively large organic carbon contents are indicative of the peaty nature of waterlogged soils and preponderance of fibrous roots and pneumatophore. The mangrove soils have a high capacity to adsorb cations, particularly magnesium, probably due to the abundance of magnesium in tidal waters. Exchangeable potassium and sodium are comparatively small. As the soils are appreciably acidic (pH 5.3±0.1), phosphorus is probably fixed by aluminium. Aluminium and soluble sulphate probably were the main contributors to hydrogen ion concentrations in the soil; exchangeable acidity value stood at 0.87±0.2 meq/100 g. The high carbonate value reflects precipitation of carbonate mud in soil with fresh saline water wedge (Clarke and Hannon 1967). The soils are hypersaline on account of soil salinity in mangroves being usually much higher than that of the surface water (Ukpong 1991).

According to the Soil map of Africa (D'Hoore 1963) estuarine mangroves occur on soils described as "Juvenile soils on marine alluvium", classified in the order "Weakly developed soils". In the elevated landward segments of the swamp, the soils classify as "Halomorphis soils" because the uppermost horizons are not flooded during part of the year (in the dry season) and the profiles become differentiated. According to Soil Survey Staff (1990), the soils classify as Aquents since they are wet at depths < 50 cm. Soils close to the channel margins fall into the great soil groups of Hydraquents. Depending on their texture, degree of flooding and topographic position some of the soils may be classified as Epiaquents and Psammaquents.

Table 4

Mean values of species coverage (%) and stem density (stems/hectare) (across all strata) of the mangrove vegetation in three physiographic habitats

Species	Tidal inlets/hinterland dunes (12)		Braided channel (12)		Wooded levee (12)	
	Cover (%)	Density (stems/ha)	Cover (%)	Density (stems/ha)	Cover (%)	Density (stems/ha)
Nypa fruticans	6.6	82	24.8*	152	6.2*	94
Avicennia africana	8.4*	79	6.1	58	12.7*	110
Rhizophora mangle	10.2*	68	2.8	41	–	–
Rhizophora racemosa	14.5*	60	6.4	98	4.6	54
Raphia vinifera	3.2	42	–	–	4.4	46
Phoenix reclinata	2.4	32	1.8	24	3.6	38
Pandanus candelabrum	2.8	26	1.9	17	1.2	11
Triumfetta rhomboideae	1.5	16	–	–	0.4	8
Conocarpus erectus	0.2	6	0.2	8	0.3	10
Acrostichum aureum	1.4	–	0.6		0.8	–

+ Numerals in parentheses indicate number of quadrats/observations in each habitat
* Indicate the dominant species in each habitat

Aerial photograph analyses revealed that the mangroves occur in defined physiographic habitats although due to gradual sediment accretion, the habitat boundaries overlap. The habitats are geomorphic landforms arising from the fluvial processes of channel lengthening, abandonment and downwarping of sediments (Thom 1967). There appear to be a correlation between species structure and fluvial habitat types (Neave et al. 1995), and soil types (Ukpong 1995a). Table 4 shows the vegetation characteristics computed for each physiographic habitat, while Table 5 shows the corresponding soil properties.

The most luxuriant growth of *Rhizophora* spp. is found on the tidal inlets/hinterland dune habitat (Table 4). Mixed stands of *Rhizophora* spp. and *Avicennia africana* are fronted by dense growth of *Nypa fruticans, Raphia vinifera* and *Rhizophora harrisonii* at the foot of the "old" hinterland dunes. With increasing distance from diurnal flooding, the mangroves become shrubby, occurring with *Acrostichum aureum* as groundlayer vegetation. In the landward portions of this habitat, true mangroves (with pneumatophore/viviparous fruits) are completely replaced by *Raphia* spp. The most

distinguishing characteristics of the tidal inlets/hinterland dune soils (Table 5) are the relatively high bulk density values (78.5±0.08 g cm^{-3}), high sand and low clay contents. Clearly, bulk density increases as sand content increases. The lowest CEC values occur in this habitat (30.6±3.5 meq/100 g) which reflects the low calcium and magnesium concentrations when compared with the other habitats. The tidal inlets have gradients > 0.5 in 500 m. At mean low water neap tides (1.04 m) the hinterland dunes are flooded while the inlets remain flooded by mean low water spring tides (0.45 m).

Table 5

Mean values of soil physical and chemical properties in three physiographic mangrove habitats (values are means±SE)

Soil properties	Tidal inlets/hinterland dunes (12)	Braided channel	Wooded levee (12)
Bulk density (g cm^{-3})	78.5±0.08	66.2±0.12	70.8±0.06
Field moisture (%)	120.8±11.3	124.6±2.2	115.2±9.4
Sand (%)	41.5±8.2	58.4±5.8	32.8±2.6
Silt (%)	33.7±3.4	18.0±4.9	40.2±4.8
Clay (%)	14.8±4.6	5.3±0.1	27.0±3.2
pH	5.4±0.1	4.3±0.2	5.6±0.1
Total salinity (%)	4.0±0.3	3.1±0.2	4.3±0.2
Chloride (%)	2.9±0.4	3.1±0.2	3.0±0.1
CEC (meq/100 g)	30.6±3.5	36.8±4.8	37.2±1.2
Calcium (meq/100 g)	11.8±1.4	14.2±1.5	15.8±1.8
Magnesium (meq/100 g)	15.6±2.1	198±1.8	18.4±1.2
Potassium (meq/100 g)	0.24±0.1	0.33±0.2	0.26±0.1
Sodium (meq/100 g)	2.0±0.4	1.9±0.3	1.9±0.5
Total nitrogen (%)	0.8±0.2	0.7±0.1	0.8±0.4
Phosphorus (μg ml^{-1})	7.5±2.3	8.1±1.5	6.6±1.3
Organic carbon	5.4±1.5	6.8±2.9	5.6±2.4
Aluminium (meq/100 g)	0.22±0.1	0.22±0.3	0.20±0.1
Sulphate (meq/100 g)	0.08±0.01	0.7±0.01	0.07±0.01
Exchange acidity (meq/100 g)	1.0±0.4	0.6±0.1	1.2±0.2
Carbonate (meq/100 g)	5.1±2.8	7.5±2.5	8.2±1.6

( ) Parentheses indicate number of quadrats/observation in each habitat

The braided channel habitat is dominated by *Nypa fruticans* (Table 4), in association with *Avicennia africana* and *Rhizophora racemosa*. The species may occur in pure stands along those segments of the channels favourable for their establishment and growth; e.g. on the raised braided levees, *Avicennia africana* is the dominant species, while *Nypa fruticans* and *Rhizophora racemosa* are adapted to the constantly submerged margins on account of their extensive fibrous and prop roots. Species distribution shows close relationship with occurrence of distributary channels; e.g. *Nypa* and mangrove propagules are seasonally dispersed along the minor channels, resulting in greater competition among species in the inner swamp. The distinguishing soil characteristics of this habitat are the relatively low bulk density values (Table 5), low sand content (23.6±2.2%) and high silt value (58.4±5.8%). The high organic carbon value (6.8±2.9%) correlate with high phosphorus value (8.1±1.5 $\mu g\ ml^{-1}$) and dense vegetation cover (24.8%). The braided depositional bars have gradients < 0.05 in 500 m. Silting and permanent ponds characterise these "mangrove islands" which are flooded by all diurnal tides.

The wooded levee habitat is dominated by *Avicennia africana* in association with *Nypa fruticans*, *Rhizophora racemosa* and *Raphia vinifera*. Although *Avicennia africana* is dominant, *Nypa fruticans* and *Rhizophora racemosa* with extensive props occupy the submerged channel margins in mixed stands, while *Avicennia* sp. adapts to elevated segments. The distinguishing characteristics of the wooded levee soils are the relatively high clay (27.0±3.25%) and carbonate (8.2±1.6 mg/100 g) contents. Other soil attributes are similar to the braided channel on account of their topographic similarities. The highest levees have gradients > 0.05 in 500 m and are flooded by tides > mean low water neap (1.04 m). The low tidal mudflats are flooded by all diurnal tides.

Conclusions

Mangrove vegetation along accreting shores shows a high level of structural complexity, compared to the shrubby vegetation usually associated with erosional coastlines (Semeniuk 1980, Ukpong 1992). Forests on accreting shores correspond with the classification of Davy (1938) and Pool et al. (1977) as "tropical mangrove woodlands". These forests are found below high-tide mark in the estuaries. The tropical mangrove woodland habitat is usually estuarine mud. Comparatively, forests on eroding shores

classify as "tropical littoral woodlands" which is synonymous with strand vegetation in which few species occur in pure stands along sandy beaches (Walsh 1974).

In the eroding coastal mangroves of Australia, Clarke and Hannon (1967) reported predominantly sandy (> 75%) soils. This contrasts with the result of this study where the mean sand proportion in the accreting swamp is 25.8±2.8%. In Australia CEC was extremely low, ranging from 0.38 to 0.84 meq/100 g, while in this study the mean CEC value is 34.4±2.5 meq/100 g. In South America, Moorman and Pons (1974) reported clayey texture in soils, which contracts with the dominantly loamy texture of the West African swamp. These differences are also accounted for by geogenetic factors, e.g. soils of adjacent areas or hydraulic characteristics of channels and tidal influence. Salinity is mainly of importance because it excludes competition from non-mangroves thereby enabling mangroves to be perpetuated along the shorelines.

Unless physiography habitats are differentiated in accretive swamps, the relationships between the soil and species distribution and structure may be obscured. The exchangeable cations could be important in habitat differentiation but they are also influence by location of stands relative to marine influences (Ukpong 1995c). The sedimentation pattern and consequently soil texture probably determines which species dominate on the different habitats. Hence, baseline studies for the purpose of initiating conservation should emphasise identification of habitats/forest types and the associated physical environmental variables which evolved the peculiar microtopographic/topographic variations. Conservation models should therefore take into account the dynamic mangrove habitats which are continually modified by sediment accretion.

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SPATIAL DISTRIBUTION OF SOIL NUTRIENTS AND EPHEMERAL PLANTS UNDERNEATH AND OUTSIDE THE CANOPY OF *ARTEMISIA MONOSPERMA* (ASTERACEAE) SHRUBS IN THE EGYPTIAN DESERTS

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Soil nutrients, plants density and biomass of annual plants underneath and outside *Artemisia monosperma* shrubs were measured at the end of the growing season in two different habitats in the Egyptian deserts. Levels of soil nitrogen, phosphorus and organic matter were significantly higher underneath than outside the shrubs, but they depended on the climatic conditions at the habitats.

In regards to the plant density differed not much between underneath and outside the shrubs in both habitats. But in many cases plant density was lower underneath than outside the canopy. Difference in water supply as well as allelopathic effects of leachates from the shrubs may be the causes of the differences in plant density.

Key words: desert, distribution, Egypt, ephemerals

Introduction

The shedding of plant parts is an essential source of the minerals which are necessary for plant life. Release of mineral elements during decomposition of plant litter alters the chemical properties of deserts and increases the fertility of soils under shrub canopies (Charley 1972, Charley and West 1975, Romney et al. 1977). But at the same time the volatile oil which is washed from some shrubs can negatively affect the establishment of ephemeral seedlings and the plant growth underneath these shrubs (Gutiérrez et al. 1993).

The supply of available nitrogen and phosphorus for the plants depends on the turnover of organic matter and results immediately from the mineralisation of organic compounds of these elements. Principally, the supply with these nutrients is positively correlated to the amount and turnover rates of above- and belowground organic matter.

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Both the amounts and turnover rated of soil organic matter are very low in desert areas. This is due to the aridity of these regions. As a consequence, production of biomass and decomposition of organic substances (and thus formation of soil organic matter) occurs at very low rates compared to ecosystems in humid regions (Noy-Meir 1985). Desert ecosystems frequently loose organic matter and nutrients by wind drift of dead biomass.

Arish Valley and the desert around Cairo–Suez desert road are very different in their climatic character. Accordingly, plant distribution and species composition differ also considerably between both habitats. This investigation is mainly focused on factors affecting seed germination, seedling establishment and plant growth underneath and outside *A. monosperma* shrub which dominate in both habitats. Additionally, plant nutrients are analysed in the soil underneath and outside the shrub canopy.

Material and methods

The study areas

Two different habitats have been compared: one is Arish Valley (northeast of Cairo), the other is situated 70 km east of Cairo (around the Cairo–Suez desert road). Where as the annual rainfall does not exceed 25 mm/year in Suez, it is usually nearly 100 mm/year in Arish Valley (Fig. 1).

The rainy season begins in Egypt in October, and ends in March. The plants under study begin to shed their leaves shortly after the end of the rainy season.

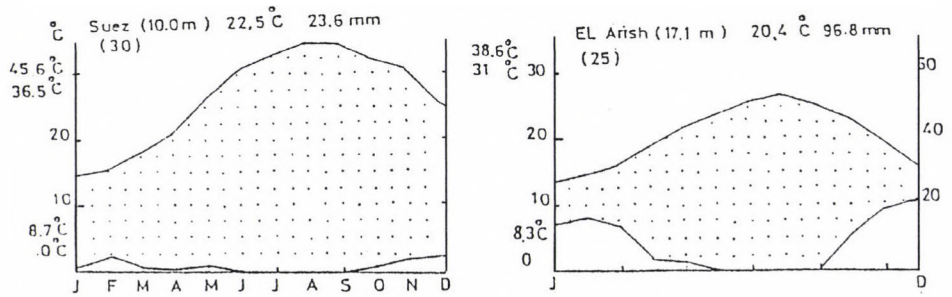

Fig. 1. Climatic diagrams of the two stations in areas inhabitad by *Artemisia monosperma*

Soil sampling

Two samples from the soil surface (weight about 1 kg) were taken under the canopy of each of five randomly chosen *A. monosperma* shrubs. A similar number of soil samples was taken exactly in the middle between the *Artemisia* shrubs. The samples were transported immediately to the laboratory for chemical analysis. The pH was determined after dilution of soil subsample with distilled water (1:5, w/v). Organic matter was calculated from organic carbon estimation by oxidation with dichromate in presence of H_2SO_4, N-content was analysed with a nitrogen-carbon analyser (Model Na 1500). Phosphorus was colourimetrically determined according to Gericke and Kurmies (1952).

Plants sampling

The different plant species were collected in spring 1995 (at the end of the growing season), from the same places as the soil collection. The plant material was immediately transported to the laboratory where it was oven-dried at 65 °C for 48 hrs. The dried material was ground in a Wiley mill to pass a 0.1 mm mesh sieve and was kept in closed bottles until the chemical analysis.

Results

Chemical and physical characteristics of the soils

Concentrations of nitrogen (N), as well as of phosphorus (P), and of organic matter (OM) were significantly higher in soils of the Arish region (Table 2). At the same time there was 3 times more nitrogen, twice more organic matter and 1.5 times more phosphorus underneath than outside the canopies. However, in the Suez region soil samples did not indicate significant differences. Also pH values indicate no great differences in both habitats. Soils underneath as well as outside the shrubs are sandy soils, but there was a slight difference in both habitats, the soils underneath were less gravely than those outside the canopies.

Species composition, density and biomass

A total of 9 species grow outside the *Artemisia* canopy and 8 species underneath in the Suez region, and in the Arish region 7 species grow out-

side as well as underneath the canopy. The number of species in Arish region per unit area significantly greater than of Suez where was nearly 1.5 greater than that of Suez region. The number of species per unit area was outside greater than underneath the *Artemisia* canopies. Some species were greatly affected: some species either disappeared in both habitats (*Silene* sp., Caryophyllaceae), or grew weakly (*Trigonella stellata*, Leguminoseae). As a role, the biomass of the different species was significantly greater underneath the canopy of *Artemisia monosperma* than outside, with the exception of *Trigonella* whose biomass in the Suez region was outside twice greater than underneath, and in the Arish region was three times greater outside than underneath. *Silene* sp. was totally disappeared underneath the canopies in both habitats.

Discussion

It is well-known that *A. monosperma* is well adapted to arid conditions with low winter rainfall and high evaporation. But nevertheless, water supply seems to be the main controlling factor that effects the distribution of *A. monosperma* in both habitats.

Soil texture has an obvious effect on seedling emergence. Coarse textured soil has been found optimal for seedling emergence. This applies to both habitats. But silt and clay percentage underneath the canopies was much higher than outside the canopies (Table 1).

Table 1

Texture of soil (%) taken underneath and outside the canopy of *Artemisia monosperma* growing around the Cairo–Suez desert road (70 km from Cairo) and in Arish Valley (mean±SE)

Location	Gravel		Sand		Silt		Clay	
	Suez	Arish	Suez	Arish	Suez	Arish	Suez	Arish
Outside	6.8 ±0.21	6.9 ±0.24	79.2 ±4.0	83.1 ±3.1	9.1 ±0.41	8.3 ±0.38	4.9 ±0.25	2.7 ±0.1
Underneath	7.9 ±0.30	8.3 ±0.39	80.4 ±3.8	82.2 ±3.9	8.5 ±0.36	7.6 ±0.33	3.2 ±0.13	1.9 ±0.017

Went (1942) observed that while some herbs were strongly associated with certain shrubs, other were not. This association appeared to be related to the organic matter accumulation under shrubs, which provided a nutri-

Table 2

pH, organic matter, nitrogen and phosphorus contents of soil samples taken underneath and outside of *Artemisia monosperma* canopies near the Cairo–Suez desert road and in Arish Valley in spring 1995 (mean ± SD)

Location	pH		Organic matter (%)		N (%)		P (%)	
	Suez	Arish	Suez	Arish	Suez	Arish	Suez	Arish
Outside	7.7 ±0.30	6.3 ±0.28	3.1 ±0.19	4.6 ±0.21	0.03 ±0.001	0.04 ±0.002	0.02 ±0.001	0.03 ±0.001
Underneath	7.8 ±0.36	7.3 ±0.31	5.5 ±0.21	6.8 ±0.33	0.04 ±0.002	0.08 ±0.005	0.02 ±0.002	0.04 ±0.002

ent rich microhabitat for annuals. Parker et al. (1982) found higher density and greater biomass of many annuals grown with high levels of nitrogen under a *Lorrea* sp. canopy.

In contrast to these observations our results show a higher plant density outside than underneath the canopies of *A. monosperma*. This species contains many volatile substances that retard germination of many seeds. Fahmy et al. (1960) isolated four crystalline compounds from powdered leaves and flowering tops of *A. monosperma*, Maksudov et al. (1962) determined essential oils, organic acids, tannins and sugars in blooms of *Artemisia* sp., Saleh et al. (1985) identified and isolated a number of flavone compounds from *A. monosperma*. Hammouda et al. (1978) isolated an acetophenone derivative coumarin from *A. monosperma*. Some of the above compounds can retard the seed germination of some plant species. This would be the cause of lower plant underneath than outside the canopies, especially in the Arish region, where the leaching can be stronger (Fig. 1; the rainfall 4 times higher than in the Suez region).

Underneath the canopies in spite of soil richness in organic compounds (Table 2). The growth was lesser and the biomass was greater in compared to that outside the canopy (Table 3).

The leguminous species *Trigonella stellata* as well as *Silene* sp. (Caryophyllaceae) were possibly affected by the leachates. The growth of *Trigonella* was greatly retarded and *Silene* showed no growth at all underneath *Artemisia* canopies. Therefore, species richness was lower under shrub canopies.

Higher nutrient availability under shrubs was demonstrated by the soil analysis apparently increased the growth of some annual species. But it would also contribute to the reduction in species richness. This inverse

Table 3

Density (plant/m²) and biomass (g. dry weight/m²) of different species underneath and outside *Artemisia monosperma* shrubs growing in different habitats (mean ± SE)

Species	Suez				Arish			
	Density		Biomass		Density		Biomass	
	o	u	o	u	o	u	o	u
Plantago ovata	2.1 ±0.1	1.3 ±0.09	0.11 ±0.01	0.18 ±0.01	3.92 ±0.2	2.2 ±0.1	0.23 ±0.01	0.11 ±0.01
Filago desertorum	2.35 ±0.15	1.14 ±0.08	0.12 ±0.01	0.19 ±0.01	2.35 ±0.2	1.95 ±0.1	0.18 ±0.01	0.11 ±0.01
Senecio desfontainei	1.77 ±0.08	1.17 ±0.08	0.22 ±0.01	0.18 ±0.01				
Trigonella stellata	1.05 ±0.08	0.93 ±0.03	0.18 ±0.02	0.08 ±0.001	2.18 ±0.14	2.27 ±0.15	0.26 ±0.015	0.09 ±0.001
Bassia muricata	0.83 ±0.07	0.50 ±0.02	0.33 ±0.02	0.32 ±0.02				
Cotula cinerea	1.07 ±0.08	0.88 ±0.04	0.09 ±0.001	0.09 ±0.001				
Matthiola livida	0.64 ±0.04	0.52 ±0.04	0.09 ±0.001	0.09 ±0.001				
Centaurea pallescens	0.61 ±0.03	0.58 ±0.03	0.09 ±0.001	0.09 ±0.001				
Silene sp.	0.31 ±0.01		0.18 ±0.02		1.19 ±0.08		0.38 ±0.02	
Ononis serrata					1.07 ±0.08	0.95 ±0.05	0.18 ±0.01	0.06
Erodium laciniatum					2.81 ±0.2	2.11 ±0.1	0.31 ±0.02	0.26
Schisus barbatus					2.1 ±0.1	1.75 ±0.1	0.06 ±0.003	0.05 ±0.003

o = outside; u = underneath

relationship between production and species richness, known as the paradox of enrichment (Rosenzweig 1987), may result because the dominant species are often to utilise an increased nutrient availability to increase their biomass or density at the expense of other species.

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BOOK REVIEWS

Editor: K. T. KISS

ACEDO, C. and LLAMAS, F. (1999): The genus *Bromus* L. (Poaceae) in the Iberian Peninsula. – Phanerogamarum Monographiae 22. J. Cramer, Berlin and Stuttgart, 293 pp.

In Chapter I the authors of the taxonomic monograph give a summary of the historical evolution of the concept of the genus *Bromus*. The genus and the first 11 species were described by Linnaeus, so he is considered to be the "godfather" of the genus as well, although Plinius had already used the same Latin form of the name (which has Greek origin and means 'food'). Only two years after the Species Plantarum had been issued, Linnaeus was again, who described the next three new species and just from the territory of Hispania. These has been followed by numerous ones during the centuries, and nowadays about 150 species belongs to the genus. In this summary all of the authors are mentioned in chronological order who studied the genus, described new species, set up new genus for some taxa or listed others back to the genus *Bromus*. The authors completed a similar review about the "vicissitudes" of the species found in the Iberian Peninsula. The row of the newly described taxa is closed by two species separated by Acedo and Llamas recently.

For the taxonomic revision the authors have examined about 4,000 specimens, most of them borrowed from the most important herbaria (about 40) of the Iberian Peninsula and a minor part from areas outside the study area. Field observations were also made on almost all the taxa. As much morphological data as possible were gathered from each specimen examined. The anatomical studies were carried out on the leaves and culms (transverse section) of live material collected by the authors. The epidermis, silica and cork cells, stomata were observed by means of SEM, and chromosome studies were carried out on root apices. All these are completed by palinological studies and phenological observations (Chapter II, Material and Methods).

In Chapter III the results obtained are commented, discussed and compared with the authors' who carried out similar studies describing in detail the taxonomic character of the genus and producing with them a key for identification of the Iberian species. In some cases the authors criticise the previous results demonstrating that certain features are inadequate to use for identification or can be used restricted only to some groups of taxa.

It follows by the description of the 23 *Bromus* species (belonging to 4 subgenera) occurring in the Iberian Peninsula. (14 of the taxa occur in Hungary as well.) The correct name, the original full description, the indication of the type locality, the data of the type, the list of the previously published illustrations are given for each taxon. Based on the studied material, a full morphological and anatomical description are given, followed by the indication of the similarities and differences with the more related taxa, the variability within the species, the ecological and phytosociological behaviour, the distribution (with map), the list of species examined and finally the list of bibliographical reports.

In this excellent taxonomic monograph only the illustrations leave a lot to be desired. The drawings about the spikelets, glumes, lemma, palea and caryopsis could have been more detailed. But what is missing most are the drawings about the entire plants (there is only one about a new taxon). The bibliography occupies 23 pages (Chapter VI).

At last, let me cite the thoughts worth keeping in mind of the famous anatomist, Metcalfe, from the first page of the monograph chosen by the authors as a motto:

"...As we read the open book of nature we should remember that, as taxonomist, it is our duty to discover and interpret the natural systematic order that actually exists and the course that evolution has already taken and is still following. Our task is to reveal and not to invent."

We can recommend the monograph to everyone who deals with taxonomy of Poaceae and we also suggest to have a look at it when compiling the revised edition of the manual of the Hungarian Flora (considering that 14 species are included in the book of the 20 *Bromus* taxa occurring in Hungary).

GY. SZOLLÁT

HALL, R. D. (ed.) (1999): Plant cell culture protocols. – Methods in molecular biology 111. Humana Press, Totowa, New Jersey, 421 pp. (Humana Press Inc. 999 Riverview Drive, Suite 208, Totowa, New Jersey 07512. ISBN 0–89603–549–2.)

Besides research institutes dealing with plant biotechnology (Institute of Plant Biology of Hungarian Academy of Sciences in Szeged, Institute of Agricultural Biotechnology of Hungarian Academy of Sciences in Gödöllő, Institute of Viticulture in Kecskemét) more and more Hungarian universities take part in teaching the basis of this field of science. That is why the excellent book recently published means a great help since the reader may get protocols to each branch of plant cell and tissue culture.

We grew richer with an indispensable work, we can be grateful to the Publisher, the Editor and all the contributing authors!

In this book, Robert Hall (CPRO-DLO, Wageningen, The Netherlands) and a panel of experts present a comprehensive collection of the most frequently used and widely applicable techniques for plant cell and tissue culture. Readily reproducible and extensively annotated, the methods cover culture initiation, maintenance, manipulation, application, and long-term storage, with emphasis on techniques for genetic modification and micropropagation. Many of these protocols are currently used in major projects designed to produce improved varieties of important crop plants. In addition, a number of specialised protocols have been included to illustrate the diversity of the techniques available and their widespread applicability.

Plant Cell Culture Protocols is aimed at scientists involved in all aspects of plant biotechnological research, as well as those working in other areas of agriculture and horticulture who are interested in expanding their technical repertoire to include in vitro methodology.

Contents: I. An introduction to plant cell culture: pointers to success. II. Cell culture and plant regeneration. Callus initiation, maintenance, and shoot induction in rice. Callus initiation, maintenance, and shoot induction in potato: monitoring of spontaneous genetic variability in vitro and in vivo. Somatic embryogenesis in barley suspension cultures. Somatic embryogenesis in *Picea* suspension cultures. Direct, cyclic somatic embryogenesis of *Cassava* for mass production purposes. Immature inflorescence culture of cereals: a highly responsive system of regeneration and transformation. Cryopreservation of rice tissue cultures. Non-cryogenic, long-term germplasm storage. III. Plant propagation in vitro. *Micropropagation of strawberry via axillary shoot proliferation. Meristem-tip culture for propagation and virus elimination. Clonal propagation of orchids. In vitro propagation of succulent plants. Micropropagation of flower bulbs: Lily and Narcissus.* Clonal propagation of woody

species. Spore-derived axenic cultures of ferns as a method of propagation. IV. Applications for plant protoplasts. Protoplast isolation, culture, and plant regeneration from *Passiflora*. Isolation, culture and plant regeneration of suspension-derived protoplasts of *Lolium*. Protoplast fusion for symmetric somatic hybrid production in Brassicaceae. Production of cybrids in rapeseed. Microprotoplast-mediated chromosome transfer for the direct production of monosomic addition lines. Guard cell protoplasts: isolation, culture, and regeneration of plants. In vitro fertilization with isolated single gametes. V. Protocols for genomic manipulation. Protocols for anther and microspore culture of barley. Microspore embryogenesis and in vitro pollen maturation in tobacco. Embryo rescue following wide crosses. Mutagenesis and the selection of resistant mutants. The generation of plastid mutants in vitro. VI. Protocols for the introduction of specific genes. *Agrobacterium*-mediated transformation of *Petunia* leaf disks. Transformation of rice via PEG-mediated DNA uptake into protoplasts. Transformation of wheat via particle bombardment. Plant transformation via protoplast electroporation. Transformation of maize via tissue electroporation. Transformation of maize using silicon carbide whiskers. VII. Suspension culture initiation and the accumulation of metabolites. *Directing anthraquinone accumulation via manipulation of Morinda* suspension cultures. Alkaloid accumulation in *Catharanthus roseus* suspension cultures. Betalains: their accumulation and release in vitro.

L. GY. SZABÓ

HODGSON, D., VYVERMAN, W. and TYLER, P. (1997): Diatoms of meromictic lakes adjacent to the Gordon River, and of the Gordon River estuary in southwest Tasmania. – In: LANGE-BERTALOT, H. and KOCIOLEK, J. P. (eds): Bibliotheca Diatomologica 35. J. Cramer, Berlin and Stuttgart, 173 pp.

Planktonic, benthic, and epiphytic samples were collected and their diatom assemblages investigated by the authors from different locations in the lower Gordon River estuary and some adjacent lakes. Two of these lakes are meromictic, one vacillates between meromixis and holomixis, and the last one is holomictic (though might have also been meromictic in the past). As the authors think, these lakes and another one of their sampling sites from the estuary, a back-water, represent different developmental stages of meromictic lakes.

The authors used Detrended Correspondance Analysis to ordinate their data; the book presents diagrams showing the grouping of the sample sites and of the found diatom-taxa. In the introduction these results are discussed shortly, and in the taxonomic part a species list is presented, that contains 272 taxa. Taxonomic (e.g. valve dimensions, striae density) and ecological (particularly about salinity tolerance) data are given about them, and also their distribution in the collected samples can be read here. Of the most taxa there is also a light- or electron-micrograph at the end of the book.

Besides these floristical data the book also presents the results of the diatom investigations of a 17 m long core extracted from one of the two meromictic lakes. Comparing the found assemblages to those found in their recent samples, they could reconstruct the development of the place from perhaps a channel to a lake and also the occurrence of meromixis.

All in all, the book should be of interest not only for those that investigate Tasmania's diatomflora, but also for algologists interested in taxonomy and/or palaeolimnology and anyone else, as the investigated system, the estuary and the adjacent lakes, is really interesting and rare.

B. BESZTERI

LANGE-BERTALOT, H. and GENKAL, S. I. (1999): Diatoms from Siberia I. Islands in the Arctic Ocean (Yugorski-Shar Strait). – In: LANGE-BERTALOT, H. (ed.): Iconographia Diatomologica. Annotated Diatom Micrographs 6. Phytogeography–Diversity–Taxonomy. A. R. G. Gantner Verlag K. G., 292 pp.

When turning over the pages of this conscientiously edited and nicely made up book which is usual from the author, we realize how little is known about the Pennales species existing outside Europe. During examination of the Siberian district what possesses an enormous diatom-diversity, 42 new species and one new genus (*Neidiopsis*) have been described. Besides that many presumably new and previously known taxa were found, altogether 490. These are illustrated by 941 EM and LM photographs of high quality. The descriptions of new species and replaces of known ones are instructive even if we cannot agree with the author in some points of view and we cannot exactly find out what was the basis for the identification of known taxa. As recently there is not any widely accepted and even up-to-date diatom identification book at the disposable of taxonomists, it would have been practically very useful to include such references.

For taxonomists who practice a classical branch of this science, the flora of the vastly investigated European freshwaters offer comparatively little novelties, because the uncleared taxonomic questions in this field cannot be solved only with classical taxonomic methods.

Opposite to this, Siberian waters give the opportunity to find specialities because of the particular and variable environmental conditions such as cold climate, periodically salty or fresh waters, brackwaters. Halophilic and halophobic species often live together in the same waterbody.

This publication like the previous books by Lange-Bertalot about diatoms of exotic districts makes flashed the huge Pennales-diversity, serves as a support for the later research and finally enlarges the geobotanical knowledge relating to the climatically different zones of the Earth.

It cannot be neglected that this work widens the range of vision of algologists of every kind and at the same time it is very pleasant and enjoyable to read and to watch.

K. SZABÓ

LENZENWEGER, R. (1999): Desmidiaceenflora von Österreich. Teil 3. – In: KIES, L. and SCHNETTER, R. (eds): Bibliotheca Phycologica 104. J. Cramer, Berlin and Stuttgart, 218 pp.

The third volume of this book deals with the important genus of *Cosmarium* and the small one of *Cosmocladium*. First, the author presents in detail the morphology and terminology of cell shape and cell wall ornamentation types of different *Cosmarium* species. Seven species-groups are differentiated: first based on global view of cell shape, then combining the cell shape and cell wall structure. After that, keys of these groups differentiating

the species, are found. Many drawings complete the keys, to distinguish and determine the species. Among the species, many varieties are included in the keys, too.

The main part of the taxonomic chapter contains the description of species. There is the name and synonym(s) of the taxon, the important literature concerning on it, the morphological characteristics, and its occurrence with a few words about the ecology. More than 2,000 drawings are present on 66 Tables to help the determination of 193 species, and several varieties of *Cosmarium* genus. The complete references of the whole book is found in this volume.

Many *Cosmarium* species are important in biological water qualification and several of them are enumerated in red lists. This book deals with Austrian Desmidiaceae, but it is useful all over the world. Therefore, I warmly recommend this excellent manual to all algologists and applied hydrobiologists working with phytoplankton and periphyton. This book is useful for university education, too.

K. T. KISS

METZELTIN, D. and LANGE-BERTALOT, H. (1998): Tropical Diatoms of South America I. – In: LANGE-BERTALOT, H. (ed.): Iconographia Diatomologica. Annotated Diatom Micrographs 5. Diversity–Taxonomy–Geobotany, Koeltz Scientific Books, Königstein, 695 pp.

Research of the diatom flora in the tropical region lags behind that of the European waters, which have been observed in details for a long time. While the recent taxonomic research of diatoms in Europe means above all the new combinations of well-known taxa, a high amount of tropical species is unknown for the science. That is why this work fills a long-felt gap and can be regarded as a huge initiative.

As it is mentioned several times by the author himself, this book does not aspire to the completeness, moreover, cosmopolitan species in South Africa, which are already known from the Holarktis and the Centrales are intentionally neglected. New, endemic and pantropical species are much more emphasised.

A total of 202 new taxa (among them one new genus, Sieminskia) are documented here, these are accompanied with 2515 LM and EM photos of a high standard. While describing the taxa, a great number of taxonomic problems are brought up and many proposals for their solution are also recommended. The author stands against the "all-splitting" taxonomic way, which is inclined to ignore the evolutionary relationship and parallel with this, the rationality, too.

As we get to know when reading this book, previous specialist books about tropical diatoms identify these species with similar ones from the Holarktis, although genetical differences might be hidden behind similar external appearances.

Among others, this work tries to revise these faults and to point to the unravelled, luxuriant great variety of the tropics.

This beautifully presented book is a valuable treasure of the library even of those diatomists and algologists, who do not specialise on tropical algae.

K. SZABÓ and É. ÁCS

ZALOCAR DE DOMITROVIC, Y. and N. I. MAIDANA, N. I. (1997): Taxonomic and ecological studies of the Paraná River diatom flora (Argentina). – In: LANGE-BERTALOT, H. and KOCIOLEK, J. P. (eds): Bibliotheca Diatomologica 34. J. Cramer, Berlin and Stuttgart, 122 pp.

This book presents the results of investigations on the planktonic diatom flora of the so-called High and Lower sections of the Paraná River, made between 1976 and 1984. Unlike most other investigations on this river before, the authors identified the taxa on species (or variety) level.

The first part of the book is the list of the 145 found species with some words about their dimensions, geographical distribution and ecological preferences, supplemented with the authors' special observations in some cases. In the next part a drawing of each taxon can be found, indicating also the sizes.

The third part contains the ecological results: the mean values of the temperature, Secchi depth, conductivity, pH, Centrales-, Pennales- and total phytoplankton density at the different sampling stations; the course of hydrometric level, Secchi depth, Centrales-, Pennales density and Bacillariophyceae density relative to total phytoplankton; the found correlation and another species list indicating the occurrences at the 12 sampling sites.

In my opinion the book might become a great help for hydrobiologists, algologists working on rivers, provides useful information on the collected diatoms and also on the main yearly trends of floods and low water periods, other ecological data and their effect on phytoplankton.

B. BESZTERI

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