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JÁNOS DOHY (GÖLLNER)
1905—1990

On 6 March 1990, in the 85th year of his life János DOHY (Göllner) dr., emeritus professor died. With his death a rich life spent in valuable work came to an end.

The name of professor János DOHY is well-known in professional circles all over Hungary. He taught in Debrecen, Keszthely, Kolozsvár and Mosonmagyaróvár. I myself had the luck to attend his lectures on agricultural botany when a student in Mosonmagyaróvár, and to be a member of the study circle at his department. He imparted his agrobotanical knowledge with an extraordinary gift of teaching, and made his students like the profession. He was an ideal for me, who determined my career.

János DOHY was born in Kolozsvár on 19 October 1905. His father, János GÖLLNER, certificated engineer, was an agricultural academy teacher. The

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hungarization of professor Dohy's surname took place in 1944, when he adopted his paternal grandmother's family name.

He was educated partly in Kolozsvár, partly in Debrecen, and took his final examination at the Roman Catholic Piarist gymnasium, Debrecen. In 1926 he graduated from the Debrecen Agricultural Academy with excellent result. His state employment began in the same year as an unpaid, so-called unofficial assistant at the Crop Production Department of the Academy. On 1 January 1927 he gained a post at the Department for Professional Training, then on 1 September 1927 became assistant at the Department of Botany. In 1929 he was appointed professor's assistant, in 1937 assistant professor and in 1938 associate professor. During his service as professor's assistant he worked at the Department of Botany of the Magyaróvár Academy of Agriculture for more than 3 years (from 26 October 1929 to 31 December 1932). When working in Debrecen and Magyaróvár, respectively, he was given special permission to attend lectures at the philosophical faculty of the Debrecen University, where he prepared his doctor's dissertation "Study on the anthracnose of melon" at the department of professor Pál GREGUSS. He passed his examination for a doctor's degree with "summa cum laude" in botany as main subject as well as in chemistry and zoology as minor subjects. The examiner of the main subject was professor Rezső SOÓ, for whom János DOHY was the first doctorand.

János DOHY was appointed head of the Department of Botany and Plant Protection at the Kolozsvár Academy of Agriculture in 1940. It was there that he was promoted to full academy professor. When the Kolozsvár academy was developed into an Agricultural College, János DOHY was nominated for the direction of the Department of Plant Physiology and Phytopathology. In autumn 1944 -- to the order of national mobilization on 5 September 1944 -- he was assigned to a post at the Department of Plant Physiology and Phytopathology of the Keszthely Academy of Agriculture, where in 1945 he was appointed to an associated professorship. After the reorganizaton of the higher institutions of agricultural education he was given assignment to the Phytopathological Department of the Debrecen Section of the Agronomical Faculty of the University of Agricultural Sciences, as associate university professor. However, the provincial sections of the university were temporarily liquidated in 1949, and János DOHY was then placed on the unattached list and transferred to Kisvárda (NE-Hungary) as a research-worker at the Plant Breeding Station. In 1954 he was commissioned to head the

Botanical and Zoological Department of the reopened Agricultural Academy at Mosonmagyaróvár, where he acted at the same time as sub-rector.

The students of the Academy organized a demonstration on 25 October 1956 to express their support of the revolutionary movements of the students of the Budapest universities on 23 October. Next day they took part in the joint demonstration of the factory workers and students of schools in the city. At the barrack of the frontier guard the peaceful procession received volley-firing. Many of the demonstrators died -- with two students of the Academy among them --, and many were wounded. Professor DOHY was just as enthusiastic as his students. With wise advices and actions he protected the interests of the institution and the students. With his active participation the educational work was resumed. He was to deliver a lecture in botany when as sub-rector he was called in evidence to the law court of Győr. I myself heard him to give direction to his substitute with the remark: "the rest we shall talk about tomorrow". Next day he could not come, because he was condemned together with the other defendants on the basis of a made-up charge. His rising professional career had been broken. When granted amnesty his first visit was to the Department, but he was not reengaged.

In 1962 he was employed by the State Farm of Lábod, in 1963 was included in the staff of the Keszthely College of Agriculture, and worked as technical administrator at the potato breeding station of the College at Rinyatamás. In 1966 he was assigned to the Laboratory of the Plant Protection Research Institute, Keszthely, from where he retired as a scientific administrator in 1969. As a pensioner he worked for further 10 years as an external worker of the AGROINFORM.

In his higher educational service rich in success János DOHY taught crop science, plant physiology, phytopathology, microbiology, animal husbandry, animal pests of plants and genetics.

Beside his educational activity professor DOHY carried on researches and did other professional work. His main line was the fungal diseases of potato, first of all those caused by soil fungi, and the elaboration of the technique of control. On the basis of his results he qualified for a candidate's degree in agricultural sciences. His publishing activity was rich; he is author of six books, more than 30 scientific papers, nearly 100 articles and notes of practical knowledge and divulgation of science. He also played a role in scientific public life, was the member of the Society of Natural Sciences, the Hungarian Botanical Association, the Transylvanian

Hungarian Agricultural Society; and after the war he belonged to the membership of the Hungarian Microbiologists' Association, the Hungarian Committee of the International Congresses on Comparative Pathology, the Society for Public Science, the Hungarian–Soviet Cultural Association, the Microbiological Section of the National Society of Sylviculture, the Committee on Plant Protection of the Hungarian Academy of Sciences. He was in professional connection with phytopathologists of many countries.

Professor János DOHY not only took over his father's torch of professional activity but also passed it on to his children. Of his three children the elder son is today professor and head of the Department of Animal Husbandry at the Gödöllő University of Agricultural Sciences, the daughter is a horticultural engineer, and the younger son a land organizer.

On 6 March 1990 black flags were flown on the buildings of the Agricultural Academy of Magyaróvár. On the occasion of 15 March Mátyás Szűrös, provisory president to the Hungarian Republic, conferred honours in the Parliament's hall under the dome. Szécheny Prize was awarded to "Dr. János DOHY, candidate in agricultural sciences, emeritus professor, for his outstanding work done for decades in agricultural higher education and phytopathological research. (The prize was taken over by his son, Dr. János DOHY, professor, head of department.)"

In the name of all his students and colleagues living in Hungary and abroad we take leave of the excellent pedagogue, researcher, and man of distinction. His memory will be kept for the succeeding generations by the large number of his student and the history of agrobotany.

Gy. Czimber

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EL GENERO MOACROTON CROIZ. (EUPHORBIACEAE)

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(Recibido: 15. Diciembre, 1977)

Moacroton Croiz. is a serpentinicolous endemic genus of the Flora of Cuba with 7 species and several infraspecific taxa. The new treatment contains the description of two sections: sect. Moacroton and sect. Glaucifoliae Borhidi; a new species: M. gynopetalus n.sp. and a new taxonomic subdivision of the species M. lanceolatus Alain. Analytic key, distribution pattern and evolutionary remarks completes the study.

Introduccion

Las especies primeras del género Moacroton fueron descritas como taxa pertenecientes al género Croton (C. trigonocarpus Griseb., C. cristalensis Urb, C. ekmanii Urb.). CROIZAT (1942) reconoció que este grupo de especies cubanas posee un grupo de caracteres particulares, que permite la separación de ellas de los demas taxa del género Croton creando para ellas el genero Moacroton. Como especie típica del género fue designada la Moacroton leonis, una especie recientemente descrita por el mismo CROIZAT. En 1952 ALAIN H. LIOGIER añadió otras dos especies mas, y alfin BORHIDI & MUÑIZ completaron la lista de los taxa actualmente conocidos con la descripción de una especie y dos variedades nuevas para la cienca. Durante la revisión y reorganización del herbario de la Academia de Ciencias de Cuba (HAC) realizados por el autor y sus colaboradores, MAIRA FERNANDEZ y PEDRO HERRERA, apareció una especie no descrita de este genero, que presenta los caracteres mas primitivos entre todas las especies conocidas.

Moacroton gynopetalus Borhidi sp. nov.

Frutex monoicus, 2-3 m altus. Rami hornotini striati, dense lepidoti, veteriores teretes, striati, cortice brunnei. Folia alterna, 1-2 cm longe petiolata petiolis dense lepidotis, basi 3-4 mm crassis, apice glandulas binas gerentibus suffulta, ovata, ovato-elliptica vel lanceolata, apice breviter acuminata, obtusiuscula vel acuta et mucronata, basi attenuata

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et in petiolum protracta, 8-20 cm longa et 2,5-7 cm lata, nervo medio supra impresso, subtus crasse prominenti, lateralibus utroque latere 9-15 utrinque prominulis et ante marginem conjunctis, obsolete reticulatis, lamina supra glaucescens, opaca, sparse lepidota vel squamis delapsis impresso punctata, subtus chrysea, densius lepidota et minute prominenter punctata, utrinque glabra, margine obsolete glanduloso-denticulata, tenuiter recurva. Inflorescentia spicata vel racemosa, superne mascula, inferne feminea, axillaris vel terminalis, 4-15 cm longa, rachide crasso sparse lepidoto. Flores masculi in axillis bractearum triangularium fasciculati, pedicello gracili 2-4 mm longo, alabastro globoso, 0,6-0,8 mm in diametro; sepalis 4, 1 mm longis, lanceolatis, sparse lepidotis, petalis 4, obovatis, 0,7-0,8 mm longis, omnibus pellucide punctatis et apice barbatis; stamina 5, subsessilia, antherae ovatae. Flores feminei: perianthio duplo, sepala 5-6, lineari-lanceolata, elepidota, coriacea, petala 5-6, similia. Capsula leviter trilobata, 7-8 mm longa, coccis dorso carinatis, lepidibus sparsis ornata. Styli breves, basi connati, superne 2-partiti, stigmata horizontaliter dilatata, obovata.

HOLOTYPUS: ALAIN 7267 SV(HAC); Cuba; Prov. Oriente; Charasscos cerca de la Via Mulata, Quibijan, Baracoa. Leg.: ALAIN & LOPEZ FIGUEIRAS 3. enero 1960. Isotypus: HAC.

Specimina examinata: Ibidem, ALAIN 7283 HAC.

Obs.: Perianthio duplo floris feminei capsulaque non acuta inter omnes species huius generis insignis.

MOACROTON Croizat 1942 Journ. Arn. Arb. 23:220.

Arbustos o arboles pequeños de hojas alternas cartáceas o coriáceas, el envés cubierto de escamas radiadas. Flores masculinas con doble periantio, 3-5-meros, pétalos y sépalos subiguales; estambres 3-6, anteras sentadas o subsentadas, subhorizontales en la antesis; flores femeninas mayormente apétalas, los pétalos muy parecidos a los sépalos cuando presentes, sépalos 4-6; estilos cortos, 2-4-partidos; capsula obovada, mayormente 3-angulosa, nuececillas angulosas. Género endémico cubano con 7 especies.

Typus generis: Moacroton leonis Croizat

Sectio Moacroton: Foliis coriaceis supra lucidis in sicco nigris et plerumque elepidotis, subtus chryseis.

Typus sectionis: Moacroton leonis Croizat

Otras especies pertenecientes a esta sección: M. ekmanii (Urb.) Croiz., M. cristalensis (Urb.) Croiz., M. tetramerus Borhidi & Muñiz;

Sectio Glaucifoliae Borhidi sect. nova: Foliis cartaceis vel subcoriaceis, supra opacis et sparse lepidotis, in sicco glaucescentes.

Typus sectionis: Moacroton trigonocarpus (Griseb.) Croiz.

Otras especies pertenecientes a esta sección: M. lanceolatus Alain, M. gynopetalus Borhidi.

Clave analitica:

1 a Hojas de 8-20 cm, apiculadas y agudas en el apice, flores femeninas con sépalos no lepidotos mayormente con pétalos coriaceos 5-6, (Baracoa) ... .1. M. gynopetalus

b Hojas de 3-13 cm, redondeadas y obtusas en el apice, flores femeninas con sepalos densamente lepidotos, siempre apétalos 2

2 a Hojas sin abultamientos glandulares en le base, estambres 6 (Pinar del Rio: Cajalbana) .....2. M. trigonocarpus

b Hojas con abultamientos glandulares en la base 3

3 a Hojas cartáceas, azulosas y pálidas en el haz cuando secas, el envés completamente cubierto de escamas .....3. M. lanceolatus

aa Flores masculinas pediceladas, anteras triangulares (Oriente) ....3a. ssp. lanceolatus

aaa Hojas lanceoladas de 4-7 cm de largo (Moa) .....var. lanceolatus

aab Hojas oblongo-lanceoladas a lineales de 7-12 cm de largo (Sierra del Cristal .....var. longifolius

aac Hojas elipticas (Sierra de Nipe) .....var. ellipticus

aad Hojas lineales y lanceoladas en la misma rama (Holguin).var. varius

ab Flores masculinas sentadas o subsentadas, anteras obovadas (Matanzas) .....3b. ssp. revolutus

b Hojas coriáceas a muy coriáceas, nítidas en el haz, espaciadamente escamosas a estrellado-puberulas en el envés 4

4 a Hojas lineal-lanceoladas a oblongo-lineales de menos de 1 cm de ancho (Moa) .....4. M. leonis

b Hojas ovales, elípticas a elíptico-oblongas, mas anchas de 2 cm 5

5 a Inflorescencia y envés de las hojas estrellado-pubescentes (Sierra del Cristal) .....5. M. cristalensis

b Inflorescencia y envés de las hojas apretado-escamosas 6

6 a Hojas oblongas u ovales de 5-14 cm, flores masculinas 5-meras, estambres 6-(7), (Moa-Baracoa) .....6. M. ekmanii

b Hojas elípticas de 3-6 cm, flores femeninas 4-meras, estambres 5 (Moa) .....7. M. tetramerus

Conspectus specierum

1/ Moacroton gynopetalus Borhidi hoc loco
Typus: ALAIN 7267 (SV)HAC. Cuba: Oriente, Baracoa. Endémica

2/ Moacroton trigonocarpus (Wr. ex Griseb.) Croiz.
Basionym: Croton trigonocarpus Wr. ex Griseb. Pl. Wright I.: 173. Typus: CH. WRIGHT 1972. GOET. Cuba: Pinar del Rio: Mt. Cajalbana. Endémica

3/ Moacroton lanceolatus Alain Contrib. Ocas. Mus. Hist. Nat. Col. La Salle 11:4. 1952. Typus: LEÓN et al. 22635. (HAC); Cuba: Oriente, Matanzas. Endémica

A/ ssp. lanceolatus
Cuba: Oriente (Gu, Ho)

a/ var. lanceolatus (Sierra de Moa)

b/ var. longifolius Borhidi var. n.
A typo foliis oblongo-lanceolatis vel lineatis, 7-12 cm longis differt. Typus: BORHIDI 8462 HAC, isotypus: BP. Cuba, Oriente, Sierra del Cristal, Saca La Lengua, Leg.: BORHIDI, OVIEDO, VALES, 1976. 04. 11.

c/ var. ellipticus Borhidi & Muñiz Acta Bot. Acad. Sci. Hung. 17:10. 1971. Typus: BORHIDI & MUÑIZ s.n. 19. 07. 1970. Isotypus: BP 503302. (Sierra de Nipe)

d/ var. varius Borhidi Acta Bot. Acad. Sci. Hung. 22:306. 1976. Typus: BORHIDI 27793 HAC, Cuba: Oriente, Holguin, Cerro Galano, Leg.: BORHIDI, CAPOTE & OVIEDO 25. 09. 1975. Isotypus: HAC, BP.

B/ ssp. revolutus (Alain) Borhidi comb. n.
Basionym: Moacroton revolutus Alain Contrib. Ocas. Mus. Hist. Nat. Col. La Salle, 11:3. 1952. Typus: LEÓN 13136 (HAC); Cuba: Serpentinas de Matanzas. Endémica

4/ Moacroton leonis Croizat J. Arn. Arb. 23:220. 1942.
Holotypus: NY; LEÓN 20983, Cerro de Miraflores, Cuba: Moa. Endémica

5/ Moacroton ekmanii (Urb.) Croiz.
Basionym: Croton ekmanii Urb. Symb. Ant. 9:194. 1924.
Holotypus: B+, Lectotypus: EKMAN 4246, S; Loma de Cuaba, Baracoa; Cuba: Oriente: Sierras de Moa y Baracoa. Endémica

6/ Moacroton cristalensis (Urb.) Croiz.
Basionym: Croton cristalensis Urb. Symb. Ant. 9:197. 1924.
Holotypus: B+; Lectotypus: S; EKMAN 6793, Rio Lebisa, Cuba: Sierra del Cristal. Endémica

7/ Moacroton tetramerus Borhidi & Muniz Acta Bot. Acad. Sci. Hung. 17:10. 1971. Holotypus: UO 687. M. LOPEZ FIGUEIRAS Sierra de Iberia, Moa. Endémica

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Fig. 1. Holotype specimen of Moacroton gynopetalus Borhidi sp. n. (Alain 7267, HAC)

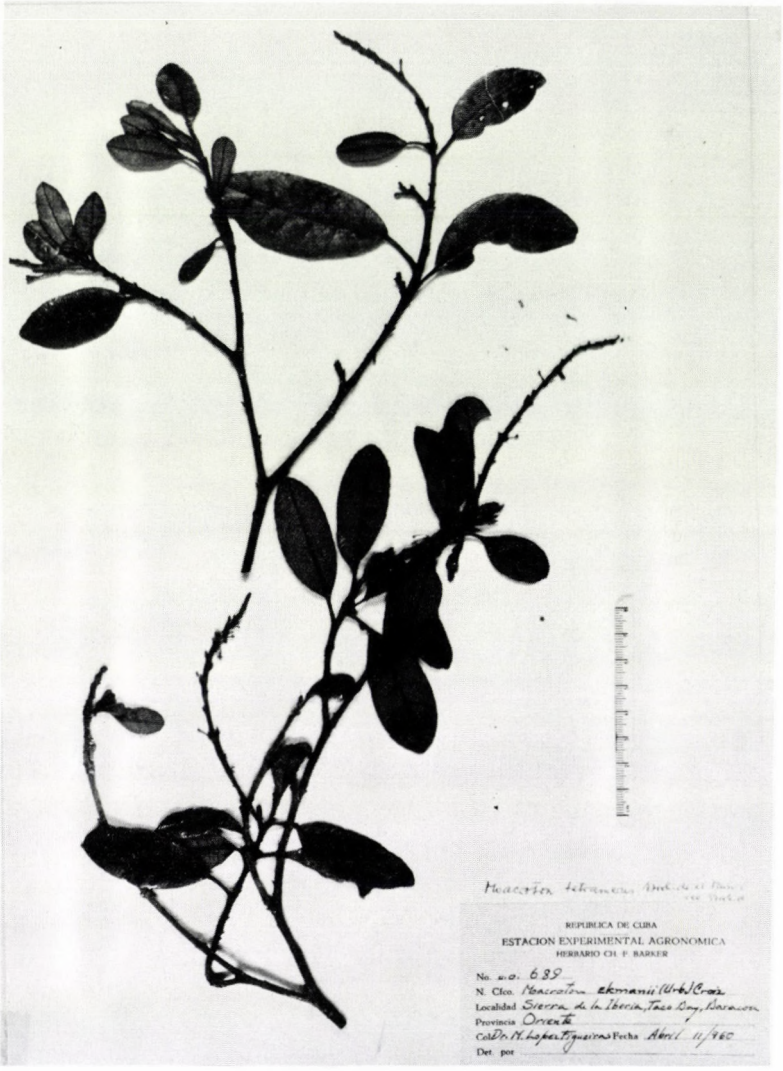

Fig. 2. Holotype specimen of Moacroton tetramerus Borhidi et Muñiz (UO 689 in HAC)

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TAXONOMIC REVISION OF GENUS LEUCOCROTON (EUPHORBIACEAE)

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(Received: 20 September 1978)

A new amplified description of the genus is described. It may be divided into 3 sections: Adeliocroton sect. nova, with two species, Lasiocrotonopsis sect. nova, with seven species and the section Leucocroton with 19 species. Seven new species and a new variety are described: L. anomalus sp. n., L. havanensis sp. n., L. incrustatus sp. n., L. longibracteatus sp. n., L. pachyphylloides sp. n., L. sameki sp. n., L. acunae sp. n., and L. virens var glaber var. n. A new analytic key is also added. The genus actually consists of 28 species, 27 of them are living in Cuba and 1 in Hispaniola.

Introduction

The author began to study this very interesting genus in 1970, when he collected with his collaborators an undescribed species in the Conservation Area of Cupeyal, South of the Moa range. It turned out, that the variability and specific richness of the genus is higher than it had been known. Its ecological importance is emphasized by the fact, that all the 28 species actually known are indicators: two of them are calciphilous plants, living exclusively on limestone, and the other 26 species are obligatory serpentinicolous plants. A preliminary study has shown, that 11 species are Ni-hyperaccumulators. All the species are endemic to Cuba or Hispaniola, many of them with a small, restricted area. The centre of diversification is apparently in the Nipe-Moa-Toa-Baracoa range, where 21 species are found. (Although the paper was ready in 1978, it has not been allowed to be published by the Commission of the Flora of Cuba Project.)

Leucocroton Griseb. in Plant. Wright. Mem. Acad. Amer. 8:160. 1860.

Dioeceous, rarely monoic shrubs or small trees up to 10 m height, with alternate triplinerved or penninerved leaves. Inflorescences in the axils of the terminal branches. Male racemes mostly many flowered, ended in a terminal group of sterile bracts, rarely subtending an uncomplete female

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flower. Male flowers with 3--5-parted calyx, calyx lobes valvate, stamens 5--28, mostly united at the base, sometimes free, connated to the mostly thin, membranous, rarely thick and fleshy disc; rudimental ovary mostly hairy, rarely glabrous. Pollen grains 3- or 6-colpate, oblate-sphaeroidal to prolate sphaeroidal with narrow and short operculum. Exine thick, sexine semitectate, finely reticulate. Muri very thin, simplibaculate. Female inflorescence generally a shorter raceme with many sterile lateral bracts and 1 (-2-3) terminal flower, sometimes with uncomplete male flowers at the axils of the sterile bracts. Female flowers with 5- or 6-lobulated calyx, disc annular, ovary 2--3-locular. Styles short, widened and entire or divided to ramified at the tip; ovules solitary. Capsel dividing into 2-valvate nuts; seeds without caruncula, cotyledons wide planate. 28 species, 27 in Cuba, 1 in Hispaniola, all endemics.

The genus may be divided into 3 sections:

A. Section: Adeliocroton Borhidi sect. nova

Foliis triplinervibus, floribus 5-meris, staminibus 8--10, filamentis liberis, disco carnoso.

Typus sectionis: Adelia microphylla A. Rich. = Leucocroton microphyllus (A. Rich.) Pax et Hoffm.

B. Section: Lasiocrotonopsis Borhidi sect. nova

Foliis triplinervibus, floribus (3-)-4-meris, staminibus 8--28, plerumque 12-16, filamentis basi connatis, disco membranaceo.

Typus sectionis: Leucocroton virens Griseb.

C. Section: Leucocroton

Foliis penninervibus, floribus 3--5-meris, staminibus 5--10, basi connatis, disco membranaceo.

Typus sectionis: Leucocroton wrightii Griseb.

Analytic artificial key for the genus:

1 a Leaves 3--5-nerved at the base 2
b Leaves penninerved (sect. Leucocroton) 10

2 a Leaves oblong, more than 2 times as long as broad, disc of the male flowers fleshy, stamens free (sect. Adeliocroton) 3
b Leaves ovate, cordate or suborbicular, less than 2 times as long as broad, disc of male flowers membranous, stamens fused at the base (sect. Lasiocrotonopsis) 4

3 a Ramified thorny shrub, leaves oblong-obovate to 3 cm long (Cuba)
1. L. microphyllus

b Unarmed shrubs leaves lineal oblong up to 7 cm long, densely yellow to ferruginous pubescent with large stellate hairs (Hispaniola) 2. L. leprosus

4 a Leaves membranous, deciduous developing mostly simultaneously with the inflorescence 5

b Leaves coriaceous or chartaceous, evergreen developing mostly independently of the inflorescence 7

5 a Leaves green, glabrous with stellate hairs on the nerves beneath 3. L. virens

aa Nerves hairy beneath var. virens

ab Nerves glabrous beneath var. glabra

b Leaves whitish and tomentose beneath with minute densely compressed stellate hairs 6

6 a Leaves with sparsely spread hairs above when young, inflorescence without basal bracts, male flowers pedicellate in groups of 4--5, pedicels 5-6 mm long 4. L. discolor

b Leaves glabrous above, inflorescence with lanceolate bracts at the base, yellow tomentose, flowers sessile in groups of 1--3 5. L. cordifolius

7 a Leaves hairy above 6. L. bracteosus

b Leaves glabrous above 8

8 a Inflorescence and bracts yellow to ferruginous tomentose, male flowers with 3 bracts, sepals 4, stamens 20--28 7. L. moncadae

b Inflorescence and bracts covered with rufous to brown scales, male flowers with 1 bract, bracts fleshy or absent, stamens 9--20 9

9 a Leaves oblong-ovate or oblong-obovate, rounded to truncate at the base; male sepals 4(5), stamens 16--20 8. L. subpeltatus

aa petiole subpeltate a/ var. subpeltatus

ab leaf emarginate at the base b/ var. epeltatus

ac leaf glabrous on both surfaces c/ var. nudifolius

b Leaves ovate, suborbicular or obovate, truncate to cordate at the base; male sepals 3, stamens 9--10 9. L. incrustatus

10 a Leaves ovate, elliptic or obovate to lanceolate, mostly broader than 2 cm 11

b Leaves lineal-elliptic to lineal, narrower than 2 cm 20

11 a Leaves lanceolate to oblong, covered with adpressed scales beneath . 12

b Leaves with other type of indument 13

12 a Leaves, bracts and inflorescence white-scaled, stamens 8--11, filaments hairy 10. L. wrightii

b Leaves and inflorescence yellow-scaled, bracts shiny, glabrous, stamens 7--8, filaments glabrous 11. L. longibracteatus

13 a Leaves oblong-lanceolate, covered with adpressed yellow stellate hairs beneath 12. L. flavicans

b Leaves covered with fine white, yellow or ferruginous aracnoid indument beneath 14

14 a Monoic plants, leaves elliptic, 3--5 cm broad, moderately coriaceous, flattened margin 13. L. brittonii

b Dioceous plants, leaves rigidly coriaceous, margin revolute 15

15 a Leaves obovate 16

b Leaves elliptic to lanceolate, seldom oblanceolate, when obovate then densely reticulate beneath 17

16 a Leaves very thick coriaceous, mostly white tomentose beneath, glabrous above, male flowers with 3 sepals and pedicels 2--6 mm long 14. L. pachyphyllus

b Leaves less coriaceous, yellow tomentose beneath, stellate hairy above when young, male flowers with 4 sepals, pedicels 6--10 mm long 15. L. obovatus

17 a Male flowers in pedinculate heads, white aracnoid-tomentose 16. L. ekmanii

b Male flowers in elongated racemes 18

18 a Leaves oblanceolate to elliptic, 3--6 cm long, male flowers with 3 sepals, glabrous inside 17. L. sameki

b Leaves oblong-elliptic, longer than 6 cm, male flowers with 4 sepals, villous on both surfaces 19

19 a Leaves with prominently reticulate venation above 18. L. comosus

b Leaves without conspicuous venation above 19. L. pachyphylloides

20 a Leaves covered with scales beneath 21

b Leaves tomentose to villous with stellate hairs beneath 24

21 a Leaves and inflorescence with yellow to brown scales 20. L. stenophyllus

b Leaves covered with white scales beneath 22

22 a Leaves lineal, less than 1 cm broad 21. L. pallidus

b Leaves lineal-elliptic to lineal-lanceolate, broader than 1 cm 23

23 a Male flowers sessile, sepals 4 stamens 6, filaments glabrous 22. L. anomalus

b Male flowers with pedicels 2--3 mm long, sepals 3, stamens 8, filaments hairy at the base 23. L. saxicola

24 a Leaves filiform up to 5 mm width, strongly revolute at the margin 24. L. linearifolius

b Leaves broader 25

25 a Leaves 1--2 cm broad, white to yellow tomentose beneath 26

b Leaves up to 1 cm broad, hairy beneath, strongly revolute at the margin 27

26 a Leaves and inflorescence densely aracnoid-tomentose, leaves very coriaceous, revolute at the margin, male flowers in pedinculate heads 25. L. moaensis

b Leaves and inflorescence finely stellate tomentose, leaves chartaceous, flattened at the margin or slightly recurved, male flowers in elongated racemes 26. L. havanensis

27 a Male flowers and inflorescence covered with yellow stellate hairs, flowers sessile or subsessile 27. L. revolutus

b Male flowers and inflorescence white hairy to glabrescent, flowers pedicellate, pedicels 2--3 mm long 28. L. acunae

Leucocroton anomalus sp. n.

Arbor parva usque ad 3—4 m alta. Rami hornotini profunde striati, flavo-nitidi, psarse lepidoti, veteriores cinerei, longitrorse striati. Folia 5—10 mm longe petiolata, petiolis pallide viridibus, nitidis, sparsissime albo-lepidotis, supra profunde canaliculatis suffulta, ea plantae femineae lanceolata vel oblongo-lanceolata, basi cuneata, ea plantae masculinae elliptica vel oblongo elliptica, utrinque obtusa et mucronata, rariter rotundata vel truncata, 5—10 cm longa et 1—2.5 cm lata, nervo medio supra impresso, subtus crassiuscule prominenti, lateralibus numerosis utrinque prominulis et dense reticulatis, lamina supra viridis, glabra, subtus ea plantae masculinae lepidibus multiradiatis albis, plantae femineae lepidibus eradiatis albis, margine membranaceis densissime obtecta, margine revoluta, chartacea vel subcoriacea.

Inflorescentiae masculinae ad apicem ramorum ex axillis hypsophyllorum abeuntes, numerosae. Hypsophylla anguste lanceolata vel sublinearia, flavo-lepidota. Pedunculi 0.5—1 cm longi et 1 mm lati, dense flavo-lepidoti. Bracteae 1—2 mm longae, crassae, concaviusculae, lanceolatae, flores masculini 3 in quaqua bractea sessiles vel subsessiles, flavo-lepidoti. Alabastra ovata, sepala 4, triangularia, intus albo-pilosa, stamina 6, in centrum disci inserta, filamenta glabra, basi breviter connata, discus tenuis, annularis, glaber, margine liber, obscure crenulatus, rudimentum ovarii bene evolutum, 2-lobatum, albo-villosum. Apice inflorescentiae masculinae flos femininus unus sterilis terminalis praesens. Inflorescentiae femininae ex axillis hypsophyllorum numerosae sub anthesi 1—2 cm longe pedunculatae, apicem versus bracteosae, apice uniflorae. Bracteae lanceolatae 3—4 mm longae, flavo-lepidotae; flos femininus: sepala 5, lanceolata, 4 mm longa, utrinque flavolepidota, discus annularis obscure 5-gonus. Ovarium 3-loculare, leviter depressum, 2 mm altum et 2.5 mm in diametro, flavo-lepidotum. Styli 3, e basi breviter multiramosi; baccae tricoccae.

Holotypus: BORHIDI 27804 SV-HAC!; Prov. Oriente, in fruticetis semper-virentibus serpentinosis montis Cerro Galano prope opp. Holguin. Leg.: A. BORHIDI, R. CAPOTE et RAMONA OVIEDO, 25. 9. 1975.

Obs.: L. saxicolae Britt. affinis, qui a specie nostra floribus masculinis 2--4 mm longe pedicellatis, sepalis 3, staminibus 8, atque inflorescentia homogama abunde differt. L. anomalus floribus femineis terminalibus sterilibus in inflorencentia masculina disposita ab omne specie huius generis differt.

Leucocroton incrustatus sp. n.

Frutex. Rami veteriores teretes, lenticellis oblongis breviter longitrorse striati et cicatricibus orbicularibus foliorum delapsorum obsiti, flavido grisei; hornotini lepidibus flavis vel ferrugineis minutis, margine multiradiatis incrustati. Stipulae nullae. Folia petiolis 1.2—5 cm longis, longitrorse striatis, supra planis vel leviter sulcatis, usque ad 2—2.2 mm crassis, flavo-lepidotis suffulta, ovalia, suborbicularia vel obovata, basi truncata vel breviter cordata, apice rotundata, 3—10 cm longa et 2.5—8 cm lata, basi 5-nervia; nervo medio supra tenuiter impresso, lateralibus utrinque latere 4—7 supra prominulis et venis obsoletis conjunctis, subtus crassiuscule prominentibus, venis transversis subhorizontalibus et dense reticulato-anastomosantibus, lamina supra glabra et nitida, in sicco viridis, subtus pilis stellatis cinereis vel lutescentibus tomentosa, margine tenuiter revoluta, coriacea.

Inflorescentia masculina tantum visa; ex axillis foliorum singulatim prodiens et apice ramorum ex axillis hypsophyllorum bracteiformium fasciculatae. Hypsophylla lanceolata, crassa, acuta, lepidibus rufis incrustata, 5—8 mm longa, pedunculi 8—15 mm longi, profunde striati, lepidoto-incrustati. Bracteae 1—1.5 mm longae, late triangulares, crasse carnosae, incrustatae. Flores sessiles solitarii in axillis bractearum. Alabastra globosa 2—3 mm longa, in rhachidem racemi impressa, densissime brevissimeque rufo-puberula, calycis lobi 3, valvares, intus glabri, stamina 9—10, filamenta glabra, basi coalita, discus 3-lobatus, glaber. Cetera non visa.

Holotypus: ACUÑA 12489 SV-HAC; Cuba, Prov. Oriente, Moa, Playa de Vaca. Leg.: J. ACUÑA, 11. 4. 1945.

Obs.: Habitu L. cordifolio (Britt et Wils.) Alain similis, sed indumento foliorum atque structura florum, disco sepalisque intus glabris ab omnibus speciebus aliis huius generis clare differt.

Leucocroton pachyphylloides sp. n.

(L. pachyphyllus León et Alain p.p. non Urb.)

Frutex vel arbor parva, 4—5 m alta. Rami hornotini squamulis multiradiatis albidis vel pallide ferrugineis et pilis longe stipitatis multiramosis albis interjectis dense villosis, veteriores teretes, cinerei, glabri. Folia 6—17 mm longe petiolata, petiolis supra leviter sulcatis, muricatis, pube ramorum indutis suffulta, oblongo-oblanceolata vel oblongo-elliptica, basi cuneata vel obtusiuscula, antice rotundata vel attenuata et in mucronem pungentem 1—1.5 mm longum excurrentia, 4—10 cm longa et 1—2.7 cm lata, nervo medio supra impresso, subtus crasse prominenti, lateralibus utroque latere 10—16 sub angulo 70—85^{0} pinnatim abeuntibus et irregulariter directis, supra inconspicuis vel obsoletis, subtus bene prominentibus et dense

anastomosanti-reticulatis, lamina supra in sicco pallide viridis, glabrescens, nitida, punctis crassiuscule prominulis irregulariter dispositis rugulosa, subtus pilis e basi multiramosis dense tomentosa, albicans, flavescens vel pallide ferruginosa, margine recurva, crasse et rigide coriacea.

Inflorescentiae masculinae tantum visae, ex axillis hypsophyllorum singulatim abeuntes, 1.5—5 cm longae. Pedunculus 1—2 cm longus, albo- vel flavescenti villosus. Bracteae linearilanceolatae, acuminatae crassae, arcuatae, concavae, usque ad 3 mm longae, in axillis 3—6-florae. Flores masculini 0.5—1.5 mm longe pedicellati. Alabastra globosa; sepala 4, lanceolata, crassa, utrinque albo-villosa. Stamina 5—6, filamenta filiformia, basi connata et in centrum disci inserta, albo-villosa, superne glabra. Discus 6-lobatus, antherae quadratae, 0.2—0.3 mm longae connectivum longum. Ovarii rudimentum bene evolutum, trilobatum, albo-villosum.

Holotypus: LEÓN 22614 LS-HAC; Cuba; Prov. Oriente, Sierra de Moa, charrascales del Coco. Leg.: LEÓN, CLEMENTE, ALAIN et CRISÓGNE, 3. 8. 1945.

Specimina examinata: ACUÑA 12491 SV-HAC; Cuba, Prov. Oriente, Moa, Rio Yagrumaje. Leg.: ACUÑA, 17. 4. 1945. — ACUÑA 12941/a Moa: Cayo Guam Leg.: ACUÑA, 17—18. 4. 1945.

Obs.: L. pachyphylli Urb. affinis, qui foliis 2—6 mm longe petiolatis, obovatis, 2.5—7 cm longis et 1.5—3.5 cm latis, nervis lateralibus utroque latere 8—12 sub angulo 60—70° abeuntibus, perellelis rectis, laminis supra foveolatis, sepalis masculinis 3, staminibus 6—7, ovarii rudimento nullo omnino differt.

Leucocroton longibracteatus sp. n.

Frutex vel arbor parva. Rami hornotini longitrorse striati, flavescentes, nitidi, glabri, veteriores cinerei. Folia 1—2 cm longe petiolata, petiolis profunde canaliculatis, nitidis, flavis glabribusque suffulta, lanceolata, apice acuta vel obtusiuscula, basi longe cuneata et in petiolum protracta, 7—17 cm longa et 2—5 cm lata, nervo medio supra per totam longitudinem impresso, lateralibus utroque latere 9—11, arcuatis, supra tenuiter impressis, subtus bene prominentibus, ante marginem conjunctis et reticulato-anastomosantibus, supra glabra, nitida, viridia, subtus lepidibus flavis margine membranaceis non multiradiato-pilosis densissime obtecta, rigide coriacea, margine tenuiter recurva.

Flores masculi tantum visi. Inflorescentiae terminales numerosi, racemosi, 4—5 cm longi, ex axillis hypsophyllorum bracteiformium prodeuntes. Hypsophylla lanceolata, 8—12 mm longa, flava, nitida, glabra. Bracteae patentes, lanceolatae, apice subulato-acutae, 4—7 mm longae, glabrae floribus pedicellatis longiores. Pedicelli 2—4 mm longi, albo-lepidoti. Alabastra globosa. Sepala 4, lepidota, intus glabra. Stamina 7—8, filamenta uniseriata complanata, basi 0.6—07 mm longe connata, 2 mm longa, glabra, in centrum disci inserta. Discus complanatus, leviter 4-lobatus, margine breviter ciliatus. Rudimentum ovarii apice columnae filamentorum minutum, 2—3-lobatum, basi minutissime pilosum.

Holotypus: SMITH 620 HAC. Cuba; Prov. Oriente, Region de Moa, Bahia de Taco. Leg.: EARL SMITH, 9. May, 1952.

Obs.: Leucocrotoni wrightii Griseb. habitu simillimi, qui a specie nostra foliis subtus lepidibus multiradiatis, albo-tometosis, hypsophyllis et bracteis albo-lepidotis, bracteis pedicellis brevioribus, sepalis utrinque squamosis, staminibus 9—10, filamentis pilosis, disci villoso omnino differt.

Leucocroton sameki sp. n.

Frutex dioicus. Rami veteriores nigrescentes, puberuli, postremo cinerei, glabri, hornotini angulati, apice lepidibus irregulariter multiradiatis brunneis densissime induti. Folia 3—7 mm longe petiolata petiolis subtus convexis, supra tenuiter sulcatis, muricato-granulatis pube ramorum obtectis suffulta, oblongo-elliptica vel oblongo-oblanceolata, rariter obovata 3—6 cm longa et 1—2 cm lata, basi obtusa, antice attenuata nervo medio supra impresso, subtus carinato-prominenti, lateralibus utroque latere 5—8, sub angulo 80—90° abeuntibus, dimidio apicem versus arcuatis et irregulariter ramificatis et dense anastomosanti-reticulatis, supra conspicue prominulis, subtus bene prominentibus, supra in sicco viridia, lucida epunctata, subtus pilis stellatis adpressis aureo-brunneis dense obtecta, mox nigrescentia et ad nervos glabrescentia, inter nervos cinereo-tomentosa, margine incrassata, recurva, crasse et rigide coriacea.

Inflorescentiae masculae tantum vise, quae ex axillis hypsophyllorum singulatim prodeuntes 2—3 cm longae. Pedunculi angulati, 1—2 cm longi, squamis multiradiatis ferrugineis vel aureo-brunneis, postremo cinerascentibus obtecti. Bracteae lineari-lanceolatae, concavae, arcuatae, 2.5—3 mm longae, 3—5-florae. Flores masculi: sepala 3, suborbiculares, extus dense ferrugineo vel brunneo stellato-tomentosa, intus glabra. Stamina 6, filamenta filiformia, basi villosa et in discum inserta, superne glabra. Antherae 0.5—0.6 mm longae, connectivum breve. Discus parce evolutus, non lobulatus, albo-villosus. Ovarii rudimentum subnullum.

Holotypus: 27062 HAC. Cuba; Prov. Oriente, charrascales serpentinosos, Yamanigüey, entre Moa y Baracoa. Leg.: Grupo SAMEK.

Obs.: Leucocrotoni pachyphylli Urb. affinis, qui foliis obovatis, nervis lateralibus supra inconspicuis, viridibus et irregulariter punctatis, nervis lateralibus rectis, sub angulo 60—70° abeuntibus, indumento inflorescentiae albo- vel flavo-villoso, sepalis masculis intus puberulis certe specifice differt.

Leucocroton acunae sp. n.

Frutex dioicus, 1—2 m altus. Rami hornotini angulati, longitrorse sulcati, flavi lucide glabri. Folia petiolis 3—5 mm longe petiolata, oblongo-elliptica vel lineari-elliptica, 3—9 cm longa et 5—15 (-20) mm lata, basi longe cuneata et in petiolum contracta, apice angustata et obtusa, plerumque breviter mucronata, nervo medio supra impresso, subtus crassiuscule prominenti, lateralibus utroque latere numerosis subrectangulari abeuntibus, utrinque breviter prominulis, supra densissime reticulatis, lamina supra nitida, viridis, glabra, subtus pilis multiradiatis minutis flavisque densissime et valde adpressis tomentosa, margine valde revoluta, rigide coriacea.

Inflorescentiae (masculinae tantum visae) ex axillis hypsophyllorum prodeuntes, hypsophylli anguste lanceolati vel lineares, usque ad 1 cm longi, glabri, pedunculis 3—4 cm longi, glabri, bracteae lanceolatae 2—3 mm longae, squamosae et stellato-pilosae, pedicelli in quaqua bractea 2—3, usque ad 3 mm longi, flore caduco persistentes. Alabastra globosa stellato-pilosa et squamosa. Sepala 4—5, intus dense et longe albovillosa, discus evolutus, crassus, villosus. Stamina 10, filamenta 1-seriata, basi pilosa et in columellam 1—1.5 mm longam connata, antherae subquadratae. Ovarii rudimentum bene evolutum, villosum.

Holotypus: UO 2334 HAC. Cuba; Prov. Oriente, Canadas de Quibiján, Baracoa. Leg.: LÓPEZ FIGUEIRAS, 28—29. 7. 1960.

Specimina examinata: ALAIN 7579, charrascos de la Ermita al Este del Yunque de Baracoa. Leg.: ACUÑA, ALAIN y RAMOS, 15. 01. 0960. ALAIN 7272, Arroyo del charrascal junto a la carretera de Quibiján (Via Mulata), Baracoa. Leg.: ALAIN y LÓPEZ FIGUEIRAS, 3. 01. 1960. — ALAIN 7611, Pinares al Norte del Yunque de Baracoa. Alt. 250 m. Leg.: ACUÑA y ALAIN, 13. 01. 1960.

Obs.: Habitu Leucocrotoni stenophylli Urb. simillimus, qui a specie nostra foliis subtus lepidotis, inflorescentia flavo-lepidota, sepalis 3—4, staminibus 7—8, solummodo basi connatis certe specifice differt. Leucocroton pallidus Britt. ramis puberulis, foliis subtus albo-lepidotis statim discernendus.

Leucocroton virens Griseb. var. glaber var. n.

A typo differt: foliis utrinque perfecte glabris, chartaceis, inflorescentiis masculis in statu nascendi foliorum elongatis, laxis, usque ad 3 cm longis.

Holotypus: ALAIN 7759 HAC. Cuba; Prov. Oriente, Sierra de Nipe. Charrascal de la Loma de la Bandera. Alt. aprox. 300 m. Leg.: ACUÑA et ALAIN, 19. 4. 1960.

Leucocroton havanensis sp. n.

(Leucocroton flavicans auct. cub. non Muell. Arg., L. angustifolius auct. cub. non Pax et Hoffm., nec. L. angutifolius Britt.)

Frutex vel arbor parva usque ad 5 m alta. Rami hornotini profunde striati, albo-tomentosi et sparse flavideque lepidoti, veteriores cinerei et longitudinaliter fissurati. Folia 5—10 mm longe petiolata, petiolis flavescentibus nitidis, sparsissime lepidotis, lamina oblongo-elliptica, utrinque obtusa et rotundata, apice ipso obtusa et mucronata, basi sensim angustata et obtusa, 5—13 cm longa et 1—2.5 cm lata, nervo medio supra impresso, subtus crassiuscule prominenti, lateralibus numerosis, utrinque prominulis et dense reticulatis, lamina supra viridis, glabra, subtus pilis stellaribus albis atque simplicibus interjectis longioribus dense obtecta, et ad nervos flavo-lepidota, margine plana, chartacea. Inflorescentiae masculinae ad apicem ramorum ex axillis hypsophyllorum abeuntes, numerosae. Hypsophylla anguste lanceolata vel sublinearia, albo stellato-tomentosa, pedunculi 1—2 cm longi, albo-tomentosi et sparse flavo-lepidoti. Bracteae 1—3 mm longae, lanceolatae, flores masculini 2—3, sessiles in axillis bractearum. Alabastra ovata, albo-tomentosa et flavo lepidota, sepala 3 (4), ovata, intus longe albo-pilosa, stamina 7—9, filamenta 2 mm longa, glabra ad marginem disci inserta, discus albo-villosus, ovarii rudimentum 2—3-lobulatum, villosum. Inflorescentiae femineae ex axillis hypsophyllorum numerosae, sub anthesi 2—4 cm longe pedunculatae, bracteis sterilibus suffultae, apice uniflorae. Bracteae lanceolatae, 3—4 mm longae, flos femineus sepalis 5 lanceolatis, 4—5 mm longis, utrinque albo-tomentosis. Ovarium 3-loculare, 2—3 mm altum, albo-tomentosum, styli 3, apice trifurcati, baccae tricoccae.

Holotypus: EKMAN 16425/a, S; Cuba centralis; Prov. Havana, in serpentinosis collis Loma de Coca, Campo Florido. Leg.: E. L. EKMAN, 27. 05. 1923. Paratypus: EKMAN 16425/b, S; Loma de Coca, 27. 05. 1923.

Specimina examinata: EKMAN 956, Loma de Coca, EKMAN 13243 Loma de Coca, Rio Quesada; BORHIDI, MUÑIZ et VAZQUEZ Loma de Coca, 10. 10. 1969; EKMAN 10913, San Miguel de Habana; Prov. Matanzas: EKMAN 18587, 18588, Ceiba Mocha, Canasi; LEÓN 12968, Espinal de Canasi; BORHIDI 12. 04. 1984, Piedra Sola, Corral Nuevo.

Obs.: Species intermediaria inter L. flavicans Muell. Arg. et L. revolutus Wr. in Sauv. A specie nostra L. flavicans Muell. Arg. foliis lanceolatis, latioribus et utrinque acuminatis acutisque differt. L. revolutus Wr. in Sauv. foliis minoribus valde revolutis et coriaceis atque floribus masculis 5--6-staminatis, filamentis basi puberulis et connatis clare distinguitur.

Conspectus specierum

1. Leucocroton microphyllus (A. Rich.) Pax & Hoffm. Pflanzenreich IV. 147. VII. 1914. — Basionym: Adelia microphylla A. Rich. in Sagra: Hist. Fis. Nat. Pol. Cuba 11: 209. 1850. — Syn.: Bernardia microphylla Muell. Arg. in Linnaea 34: 172. 1865. et in DC. Prodr. 15/2: 917. 1866. — Holotype: SAGRA 58. Habana, (P).
2. Leucocroton leprosus (Willd.) Pax & Hoffm. Pflanzenreich IV. 147. VII. 1914. — Basionym: Croton leprosus Willd. Spec. Pl. 4: 553. 1805. — Syn.: Bernardia leprosa Muell. Arg. in Linnaea 34: 172. 1865. et in DC. Prodr. 15/2: 917. 1866. — Holotype: POITEAU s.n. Haiti, (G).
3. Leucocroton virens Griseb. Diagn. in Nachr. Ges. Wiss. Goett. 1865: 175. — Holotype: CH. WRIGHT 1978.
   — var. virens
   — var. glaber Borhidi hoc loco. — Holotype: ALAIN 7759 HAC
4. Leucocroton discolor Urb. Symb. Ant. 9: 203. 1924. — Holotype: B +; Lectotype: EKMAN 5963, Cuba: Rio Piedra, Sierra de Nipe, (S).
5. Leucocroton cordifolius (Britt. & Wils.) Alain in Contr. Ocas. Mus. Hist. Nat. Col. La Salle 11: 5. 1952. — Basionym: Lasiocroton cordifolius Britt. et Wils. Mem. Torr. Bot. Club 16: 76. 1920. — Holotype: SHAFER 1724. Cuba: Sierra de Nipe, Paso Estancia, (NY).
6. Leucocroton bracteosus Urb. Symb. Ant. 9: 204. 1924. — Holotype: SHAFER 1736. Cuba: Paso Estancia, (NY).
7. Leucocroton moncadae Borhidi in Borhidi et Muñiz Acta Bot. Hung. 21: 222. 1975. — Holotype: BORHIDI et MONCADA 27688/A HAC Cuba: Prov. Habana, Loma de Coca.
8. Leucocroton subpeltatus (Urb.) Alain Contr. Ocas. Mus. Hist. Nat. Col. La Salle 11: 5. 1952. — Basionym: Lasiocroton subpeltatus Urb. Symb. Ant. 9: 205. 1924. — Holotype: B +; Lectotype: EKMAN 4809, Cuba: Sierra de Nipe, Rio Piedra, (S):
   — var. subpeltatus
   — var. epeltatus (Urb.) Alain loc. cit. 11: 5. 1952.
   Basionym: URBAN sub Lasiocroton, in Feddes Rep. 28: 222. 1930. — Holotype: B +; Lectotype: EKMAN 15309, Cuba: Sierra de Nipe, Rio Barigua (S).
9. Leucocroton incrustatus Borhidi hoc loco. Holotype: ACUÑA 12489 HAC; Cuba: Playa de Vaca, Moa.
10. Leucocroton wrightii Griseb. Abh. Ges. Wiss. Goett. 9: 21. 1860. et Pl. Wr. Mem. Amer. Acad. 8: 160. 1860. — Holotype: CH. WRIGHT 1994. GOET Cuba: Monte Verde.
11. Leucocroton longibracteatus Borhidi hoc loco. Holotype: SMITH 620 HAC; Cuba: Taco Bay, Moa.
12. Leucocroton flavicans Muell. Arg. in DC. Prodr. 15/2: 757. 1866. — Syn.: L. flavicans var. latifolius Muell. Arg. in DC. Prodr. l.c., L. wrightii León et Alain in Flora de Cuba 3: 91—92. — Holotype: CH. WRIGHT 1424 Cuba, (GH).
13. Leucocroton brittonii Alain Contr. Ocas. Mus. Hist. Nat. Col. La Salle 11: 5. 1952. — Holotype: SHAFER 4139. Cuba: Moa, (NY).

14. Leucocroton pachyphyllus Urb. Symb. Ant. 9: 202. 1924. — Holotype: B +; Lectotype: EKMAN 3816. Cuba: Minas de Iberia, Moa, (S).
15. Leucocroton obovatus Urb. Symb. Ant. 9: 200. 1924. — Holotype: B +; Lectotype: EKMAN 6786. Cuba: Sierra del Cristal: Rio Lebisa, (S).
16. Leucocroton ekmanii Urb. Symb. Ant. 9: 199. 1924. — Holotype: B+; Lectotype: EKMAN 4284. Cuba: Lomas de Cuaba, Baracoa, (S).
17. Leucocroton sameki Borhidi hoc loco. — Holotype: SAMEK 27062 HAC; Cuba: Yamanigüey, Moa.
18. Leucocroton comosus Urb. Symb. Ant. 9: 201. 1924. Syn.: L. dictyophyllus Urb. loc. cit. 202. — Holotype: B +; Lectotype: EKMAN 4769. Cuba: Sierra de Nipe, Rio Canapu, (S).
19. Leucocroton pachyphylloides Borhidi hoc loco. Syn.: L. pachyphyllus León et Alain in Flora de Cuba 3: 91. 1953. non Urb. — Holotype: LEÓN 22614 HAC. Cuba: Cayo Coco, Moa.
20. Leucocroton stenophyllus Urb. Ber. Deutsch. Bot. Ges. 36: 505. 1919. — Syn.: L. angustifolius Britt. Bul. Torr. Bot. Club. 44: 14. 1917. non Pax et Hoffm. 1914. — Holotype: SHAFER 3636. Cuba: Sierra de Nipe, Rio Guayabo, (NY).
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28. Leucocroton acunae Borhidi hoc loco. — Holotype: UO 2334 HAC; Cuba: Quibijan, Baracoa.

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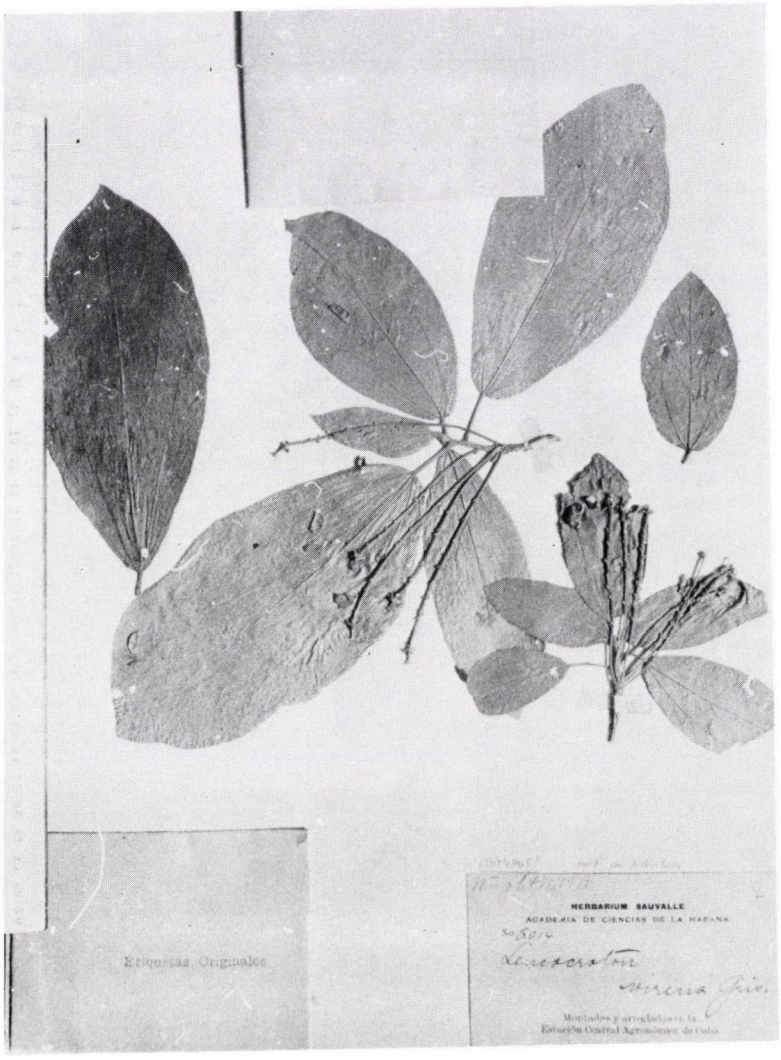

Fig. 1. Leucocroton virens Griseb. isotype specimen in HAC; (Ch. Wright 1978)

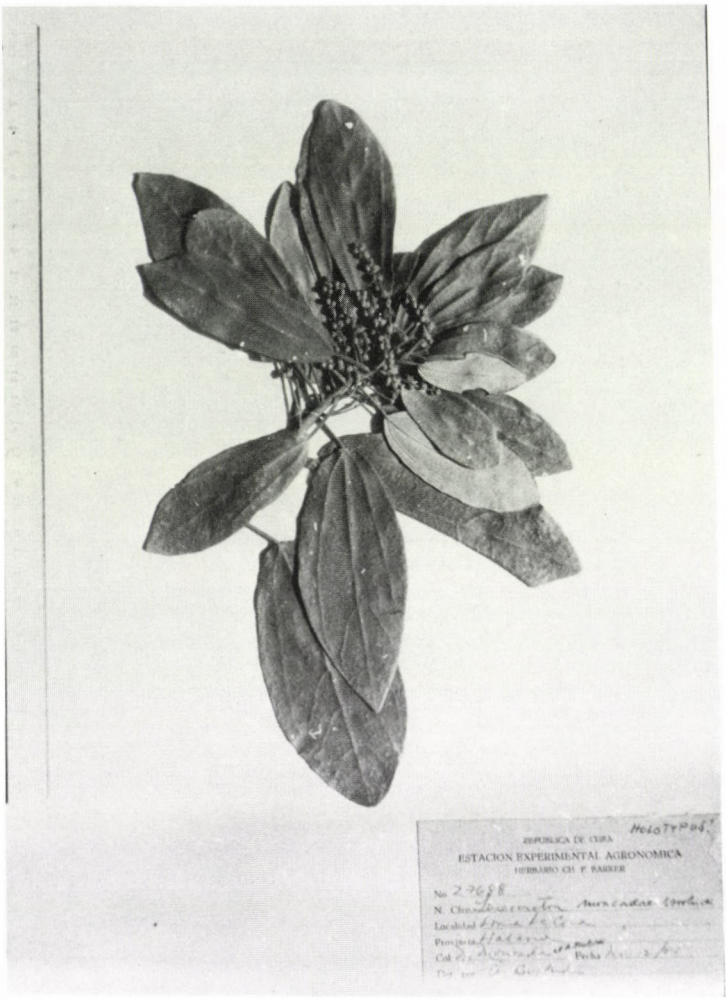

Fig. 2. Leucocroton moncadae Borhidi, holotype specimen, Moncada & Borhidi 27688/A in HAC

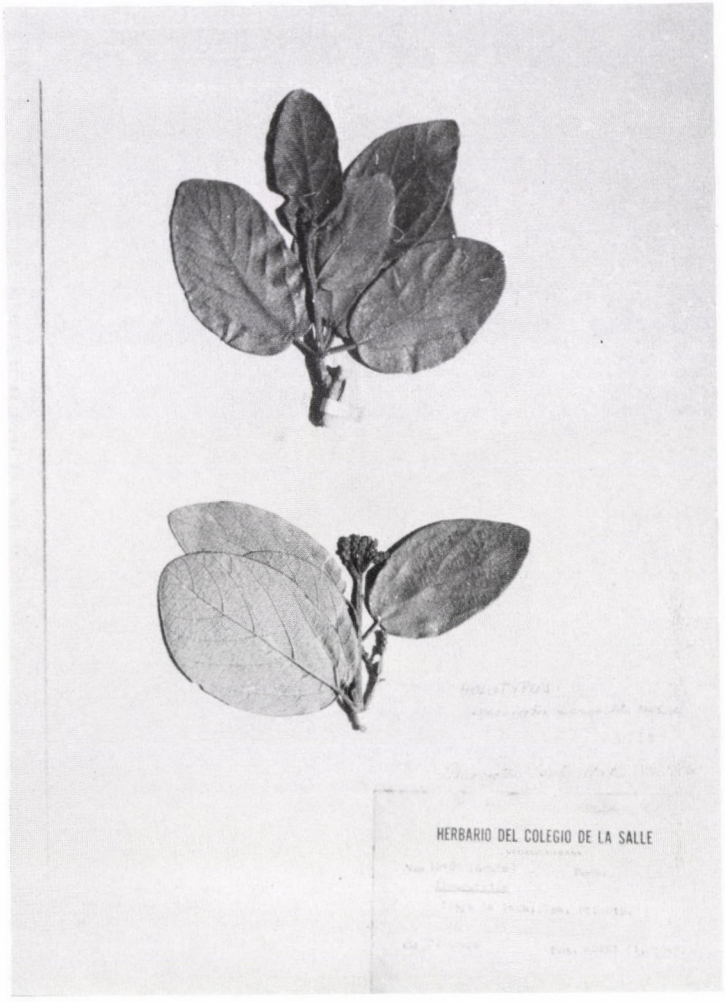

Fig. 3. Leucocroton incrustatus Borhidi, holotype specimen, Acuña 12489, HAC

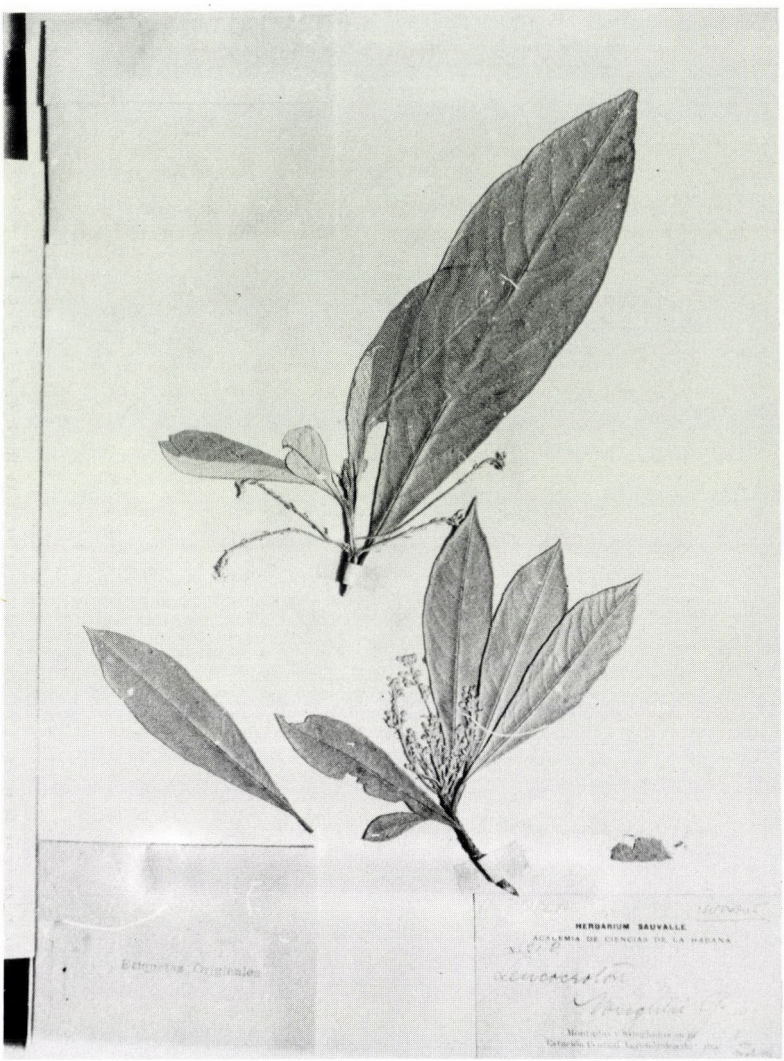

Fig. 4. Leucocroton wrightii Griseb., isotype specimen in HAC; Ch. Wright 561

Fig. 5. Leucocroton longibracteatus Borhidi, holotype specimen, Smith 620 in HAC

Fig. 6. Leucocroton flavicans Muell. Arg. isotype specimen in HAC, Ch. Wright 1994

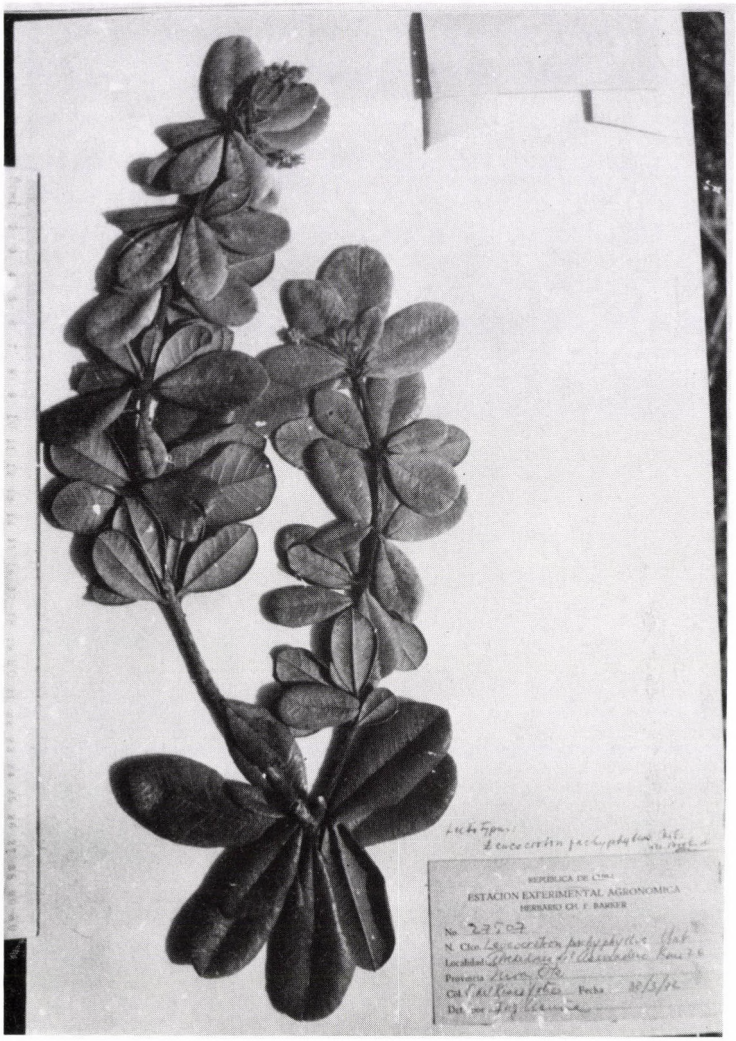

Fig. 7. Leucocroton pachyphyllus Urb. authentic specimen from HAC, Del-Risco et al. 27507

Fig. 8. Leucocroton pachyphylloides Borhidi, holotype specimen, León et al. 22614, HAC

Fig. 9. Leucocroton anomalus Borhidi, holotype specimen, Borhidi et al. 27804 HAC

Fig. 10. Leucocroton stenophyllus Urb. Isotype specimen of L. angustifolius Britt. non Pax & Hoffm. Shafer 3626 in HAC

Fig. 11. Leucocroton linearifolius Britt. authentic specimen, Acuña 12486, in HAC

Fig. 12. Leucocroton acunae Borhidi, holotype specimen, UO 2334, M. Lopez Figueiras, in HAC

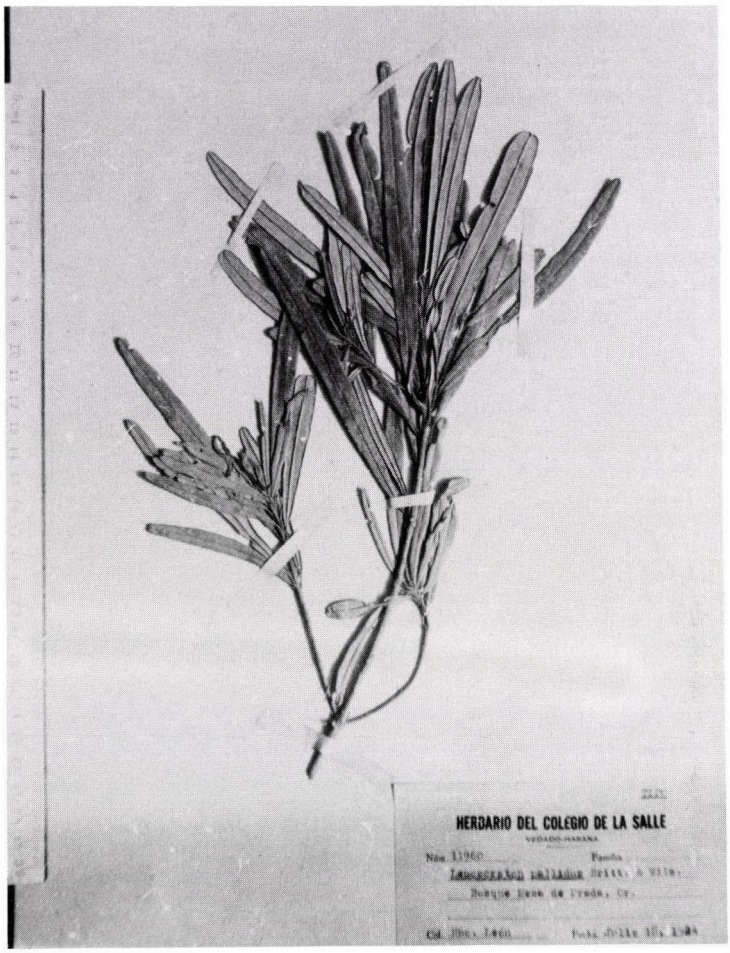

Fig. 13. Leucocroton pallidus Britt & Wils. isotype specimen, León 11960 in HAC

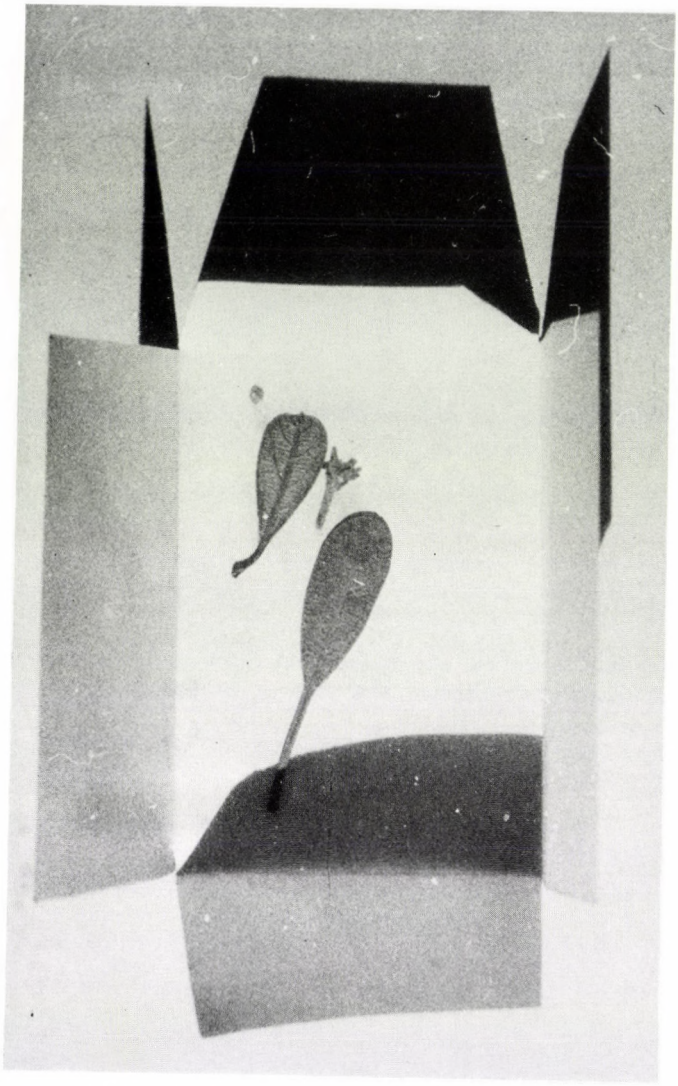

Fig. 14. Leucocroton brittonii Alain, isotype specimen, Shafer 4139 in HAC

Fig. 15. Leucocroton moaensis Borhidi & Muñiz, isotype specimen

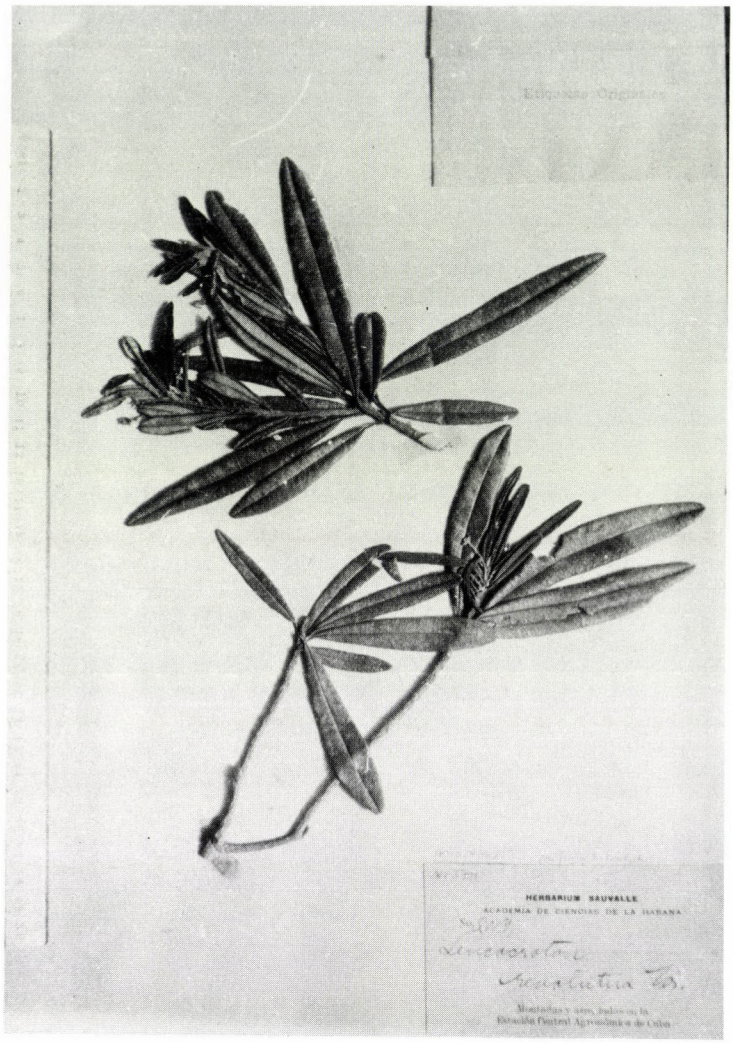

Fig. 16. Leucocroton revolutus Wr. in Sauv. isotype specimen, Ch. Wright 3701 in HAC. (L. angustifolius Muell. Arg.)

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NEW SPONTANEOUS TAXA OF THE GENUS CLERODENDRUM BROWN EX L. (VERBENACEAE) IN CUBA

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The new revision of the Cuban Clerodendrum taxa confirms the existence of 8 indigenous species and adds the description of 7 new infraspecific taxa. These are 1 subspecies: C. grandiflorum (Hook.) Schauer ssp. cajalbanense; 4 new forms within C. aculeatum (L.) Schlecht: var. aculeatum: — f. acutifolium, — f. mucronatum, — f. lanceolatum, and — f. rotundatum; 1 new form within C. aculeatum (L.) Schlecht var. gracile Griseb. ex Mold.: — f. orientale. The author suggests a new combination: C. cubense Schauer var. brachypus (Urb.) Kereszty. He includes C. calcicola Britt. under the earlier described C. tuberculatum A. Rich. being the two taxa conspecific.

Introduction

The oldest known species of the genus is the C. aculeatum (L.) Schlecht. (1831) a widely spread New World species mentioned under different names based on specimens collected at the end of the 17th century. It is described at first in Linné's works as Volkameria aculeata found in Jamaica and Barbados (cf. 1748, 1764 and 1780). Further three species from Cuba collected by forest-rangers got to the De Candolle's Herbarium, and J. C. SCHAUER as monographer of the Verbenaceae described from them C. cubense, C. sagraei and C. grandiflorum in De Candolle: Prodromus 1847. These descriptions were taken over in shortened form into the work of RAMON DE LA SAGRA, and two further new species were added by A. RICHARD: C. tuberculatum and C. lindenianum (1850). In the Prodromus SCHAUER referred to the great similarity between C. sagraei and C. grandiflorum, though he described them as different species. Since W. HOOKER had earlier described a similar taxon under the name of Aegiphila grandiflora (1846), the correct name for the two species became C. grandiflorum (Hook.) Schauer.

In the 2nd half of the 19th century C. Wright's collections enriched the herbaria with further specimens. His material was supervised by A. H. R. GRISEBACH in Göttingen. After the decades of the Cuban wars of independence

Akadémiai Kiadó, Budapest

N. L. BRITTON and A. SHAFER made important collections starting from 1909. Based on these collections BRITTON described C. calcicola of W-Cuba and subsequently C. anafense with P. WILSON from the Anafe hills as new taxa (BRITTON 1920). Synchronously with these activities E. L. EKMAN started exploring the flora of Cuba and Hispaniola. His materials were studied and described by I. URBAN in the Botanical Institute of Berlin-Dahlem, who described 2 further new species: S. brachypus and C. nipense (URBAN 1924). After the 30ies priests of the College of La Salle continued the exploration of the Cuban flora. Based on their efforts Bro. LEÓN and Bro. ALAIN got to publish the first whole Flora of Cuba in 5 volumes (1946--1962). In the 4th volume they gave a treatment of 9 indigenous and several cultivated species, taking into consideration the notes of H. N. MOLDENKE, the well-known Verbenaceae expert, including C. denticulatum Mold. and other infraspecific taxa described by him 1940.

Starting from the 40ies the Herbarium of the Experimental Station of Agronomy in Santiago de Las Vegas (SV) became an important collection, due to the enthusiastic work of Ing. J. ACUÑA, the excellent Cuban botanist and agronomist. After the establishment of the Botanical Institute of the Cuban Academy of Sciences (1971) this herbarium (SV), the collection of Brother LEÓN and ALAIN (LS) and the Sauvalle-Herbarium (Ch. WRIGHT's collection) has been united into a larger collection, the Herbarium of the Academy of Sciences (HAC). Simultaneously with the re-organization of the herbarium carried out by A. BORHIDI, Maira FERNANDEZ, P. HERRERA and Ramona OVIEDO, a preliminary taxonomic revision was also implemented. As result of this work many new species (including Verbenaceae) were added to the knowledge of the Cuban flora (BORHIDI 1976, BORHIDI & KERESZTY 1979). From 1976 a new international Flora Project was started by the Cuban Academy of Sciences and the University of Havana with the participation of German, Russian, and Hungarian experts in order to prepare the new Flora of Cuba. This work belongs also to the framework of the New Flora of Cuba project, suggested also by the bilateral agreement between the Hungarian and Cuban Academies of Sciences.

Herbarium of the Cuban Academy of Sciences (HAC) and the Herbarium of the National Botanic Garden (HAJB) under the auspices of the University Havana, as well as on materials of the following herbaria: REGNELL's in Stockholm (S), HAUSSKNECHT's in Jena (J), TURCZANINOV's in Kiew (KW) and Herbarium of the Hungarian Natural History Museum (BP). Approximately 250 specimens of these Herbaria have been studied. The species C. aculeatum,

C. cubense and C. grandiflorum turned to be of highly great variety which made necessary a new taxonomic treatment, while C. calcicola and C. tuberculatum proved to be conspecific.

Clerodendrum aculeatum (L.) Schlecht.

The leaves of the specimens collected in Cuba and the Caribbean Islands show a great variation. Although the high variation of the leaf-form and leaf-size, even within the same individual, is well known, the dominancy of one leaf-form nevertheless can be revealed (cf. Moldenke 1985). However, some leaf-forms appear to be overwhelming on West Indian Islands certain, their reliable geographical separation needs more studies. Since these taxa proved to be consistent in other characters, they are considered and described as taxonomic forms. Within the species a smaller sized and hairy leaved variety was named by GRISEBACH and described later by MOLDENKE (1940) from RUGEL's collection in Matanzas as C. aculeatum (L.) Schlecht. var. gracile. It is much more frequent in Oriente than around its classic locality. Some specimens from the recent collections in S-Oriente differ from the type by having smaller leaves and a different structure of leaf epidermis. Therefore I consider them belonging to a new form of the var. gracile.

Description of the plant

Deciduous, mostly vinelike, usually much-branched spinescent shrubs. Branches slender, straggling or long-arching with opposite 3-8 mm long stout, yellowish spines beneath the articulation of the petiole. Leaves opposite or three in a whorle on reduced lateral branches. Blades different in size and shape, from narrowed lanceolate to nearly rounded, 1-8 cm long and 0.6-3 cm wide, obtuse to mucronate at the apex, truncate to cuneate at the base, hairy or glabrous above, puberulous below. Veins obsolete, densely glandulous on both surfaces. Stomata anomocytic, characteristically fasciculated. Inflorescences flowered axillary cymes with peduncles up to 2.5 cm long. Pedicels slender, puberulent, 6-14 mm long. Flowers less than 3 cm long, densely glandulous. Calyx about 3 mm long, campanulate; lobes reflexed, triangular, acute, one-third as long as the tube. Corolla salverform, white, about 1.8 cm long, densely glandulous, the tube thin, ribbed, the lobes 6 mm, reflexed. Stamens long-exserted; filaments purple, unequal, 2.4-4 cm long. Fruit 5-7 mm long, 4-grooved, splitting into 2 parts at maturity (CORREL 1982).

Widespread in dry wastes, coastal thickets everywhere from Mexico throughout the West-Indies to northern South America (ADAMS 1972 and BRITTON 1918).

Key to the varieties and forms of C. aculeatum

1.5-3 m tall vinelike shrub with oval and terete lenticells densely emerged on the puberule branches. The leaf-edges unrolled, blades shiny scarcely puberulent with thin long cylindrical hairs on both surfaces. Areoles with parallel micro-wrinkled, radially settled filaments above, straight and cushioned below. Calyx lobes crenate. By reason of the dominant leaf-shape 4 diverse forms can be separated. Distribution: common in dry coastal thickets of Cuba

-- var. aculeatum

Leaf ovate, elliptic, acute at the apex, cuneate at the base, densely glandulous on both surfaces. Glands with cross-shaped impression. The upper leaf surface shiny, dark-green and glabrous, reticulation obsolete. The lower leaf surface flat, lighter and scarcely hairy. Distribution: the most common form in the dry coastal thickets of Cuba

-- -- f. acutifolium

Leaf ovate, ovate-elliptic, mucronate at the apex, cuneate at the base, sparsely glandulous, reticulation conspicuous on the both surfaces. Simple thin hairs occasionally only beneath. Areoles small, straight, cushionform forming a dense network.
Distribution: common in Santo Domingo

-- -- f. mucronatum

Leaf lanceolate, narrow lanceolate, acute at the apex, attenuate at the base. Weakly reticulated and scarcely glandulous on both surfaces. Bundles around the stomata are inconspicuous.
Distribution: frequent in Santo Domingo, rare in Cuba

-- -- f. lanceolatum

Leaf obovate, subacute to rounded at the apex, cuneate at the base. Glands and hairs very rare, stomata with 2-3 smooth bundles.
Distribution: common in Puerto Rico, rare in Cuba

-- -- f. rotundatum

Smaller shrub up to 1.5 m tall. Branch yellowish puberulent or setose with sparsely dispersed ovate lenticelles. Leaf narrow lanceolate, dull, acute or obtuse at the apex, cuneate at the base, edges rolled. Both surface densely glandulous and covered with simple, sometimes articulated hairs and with hairs of thickened base beneath. Areoles "gravelshaped", dark-green and of equal hairs above, dull with unequal hairs and projecting "bundlecytic" stomata beneath. Calyx lobes acute. Inflorescence similar to the type. Distribution: current of all the West Indies in coastal thicket

-- var. gracile

Leaves without articulated hairs, only simple, small, stout, scattered hairs above with characteristic radiate epidermis-cells. Areoles regular, cushion-shaped and densely glandulous. Densely villous with various shaped, stout to long, flat and articulated hairs beneath, stomata small and unconspicuous (Fig. 6).
Distribution: on the south-central Oriente in Cuba

-- -- f. orientale

C. aculeatum (L.) Schlecht. var. aculeatum f. acutifolium Kereszty, forma nova

A typo distinguitur foliis ovatis vel ellipticis, apice acutis, basi cuneatis, dense glandulosis et utrinque vix reticulatis; supra nitidis atrovirentisque subtus opacis viridisque, glandulis cruciformiter impressis obstitis. Frequentissima forma in areae.

Holotypus: Jone PAULSEN 283, Ltl. Princess, St. Croix (S) (Fig. 1).

Specimens examined: CUBA sine loc.: WRIGHT 2174 (S); PAULSEN 283 (S) — PINAR DEL RIO: Lago de Piedras: ACUÑA 15631, 18280 (HAC); ALAIN 1942, 6052 (HAC); Peninsula Guanahacabibes: BISSE et al. 2039, 2060 (J); Las Vegas: BISSE et al. 4390 (J) — HABANA: EKMAN 298 (S); GALLO 2118 (HAC) — ISLA DE PINOS: BORHIDI et al. 518805 (BP) — Prov. GRANMA Cabo Cruz: EKMAN 7794 (S); Sierra Maestra: BISSE et al. 13397 (J) — SANTIAGO DE CUBA: BAKER 24579 (HAC); BISSE et al. 3119, 7819, 8141 (J); CLEMENTE 86 (HAC); EKMAN 8899 (S) — GUANTANAMO: BISSE et al. 8304 (J); Maisi: DIETRICH 48078 (HAJB); Baracoa: BISSE et al. 30912 (HAC); BORHIDI et al. 11214, 31564 (HAC); LEÓN 9516, 12050, 12051 (HAC); HOLGUIN: Manati: ACUÑA 17182 (HAC).

C. aculeatum (L.) Schlecht. var aculeatum forma mucronatum Kereszty, forma nova

A typo distinguitur foliis ovatis vel ovatoellipticis, apice mucronatis, basi cuneatis, sparse glandulosis utrinque manifeste reticulatis, areolis densis laevibusque pulvinatis, subtus pilis simplicibus sparsisque brevissimis, rarissime dispositis. Proprius in Santo Domingo.

Holotypus: EKMAN 15345, Hispaniola: Peninsula Samaná (S) (Fig. 2).

Specimens examined: VILLA CLARA: Rio Zaza: LEÓN 1388 (HAC); Manacal: ACUÑA 11383 (HAC).

Fig. 1. C. aculeatum (L.) Schlecht. var. aculeatum f. acutifolium, Kereszty — PAULSEN 283. Ltl. Princess. (S)

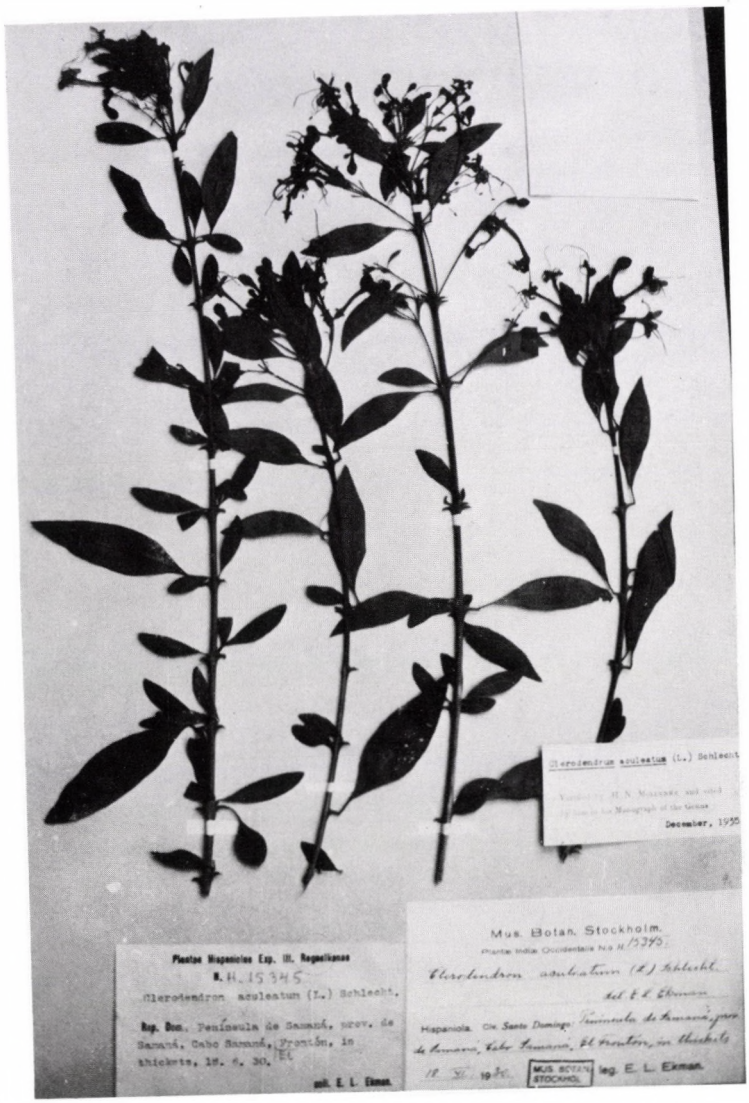

Fig. 2. C. aculeatum (L.) Schlecht. var. aculeatum f. mucronatum, Kereszty — EKMAN 15345. Hispaniola. (S)

C. aculeatum (L.) Schlecht. var. aculeatum f. lanceolatum Kereszty, forma nova

A typo distinguitur foliis lanceolatis vel anguste lanceolatis, apice acutis, basi attenuatis, reticulatione inconspicua et structura stomatarum vix fasciculata. Proprius in Santo Domingo, Puerto Rico.

Holotypus: SWARTZ (sine num.), St. Bartholomew (S).

Specimens examined: ORIENTE: Loma del Gato: CLEMENTE 4289 (HAC); SANCTI SPIRITUS: Banao: BISSE et al. 28957, 30911 (HAC).

C. aculeatum (L.) Schlecht. var. aculeatum f. rotundatum Kereszty, forma nova

A typo distinguitur foliis obovatis, apice subacutis vel rotundatis, basi cuneatis, minime glandulosis et capillatis, stomatis fasciculis 2-3 laevibus praeditis. Proprius in Puerto Rico, rariter in Cuba.

Holotypus: Sintenis 579. Puerto Rico, Salinas de Cabo Rojo (S) (Fig. 3).

Specimens examined: PUERTO RICO: Ponce: HELLER et al. 6122 (S); CUBA: Habana: GALLO 1090 (HAC); Sra. de Anafe: BISSE et al. 28077 (J); EKMAN 13024 (S).

C. aculeatum (L.) Schlecht. var. gracile Griseb. et Moldenke f. orientale Kereszty, forma nova

A typo differt laminis foliorum supra pilis articulatis absentibus et tantum setosis simplicibusque dispositis subtus pilis robustis villosis suffultis stomata occultis. Rariter in Prov. Santiago de Cuba.

Holotypus: BORHIDI et MUÑIZ 518915. Cuba: Santiago de Cuba, Aguadores (BP) (Fig. 4).

Specimens examined:
C. aculeatum var. gracile (Fig. 5)
CUBA (sine loc.): WRIGHT 3174 (HAC); HABANA: ACUÑA 24423 (HAC); MOLDENKE 19859 (HAC); MATANZAS: Peninsula Zapata: BISSE et al. 33834 (HAC); Roig 7368, 7919 (HAC); CAMAGUEY: Loma del Gato: CLEMENTE 4289 (HAC); LEÓN 4089 (HAC); SANTIAGO DE CUBA: Laguna de Baconao: EKMAN 8253 (S); GUANTANAMO: Cerro del Fraile: LEÓN 22787 (HAC); Imias: BISSE et al. 30908, 30909, 33178 (HAC); Jauco: LEÓN 11751 (HAC); Macambo: ACUÑA 27304 (HAC); Maisi: ACUÑA 17349 (HAC); Monte Christo: ACUÑA 17345 (HAC).

C. aculeatum var. gracile f. orientale

SANTIAGO DE CUBA: CRYSOGONE 2684 (HAC); LÓPEZ 263 (HAC); Sardinero: CRYSOGONE 6083 (HAC).

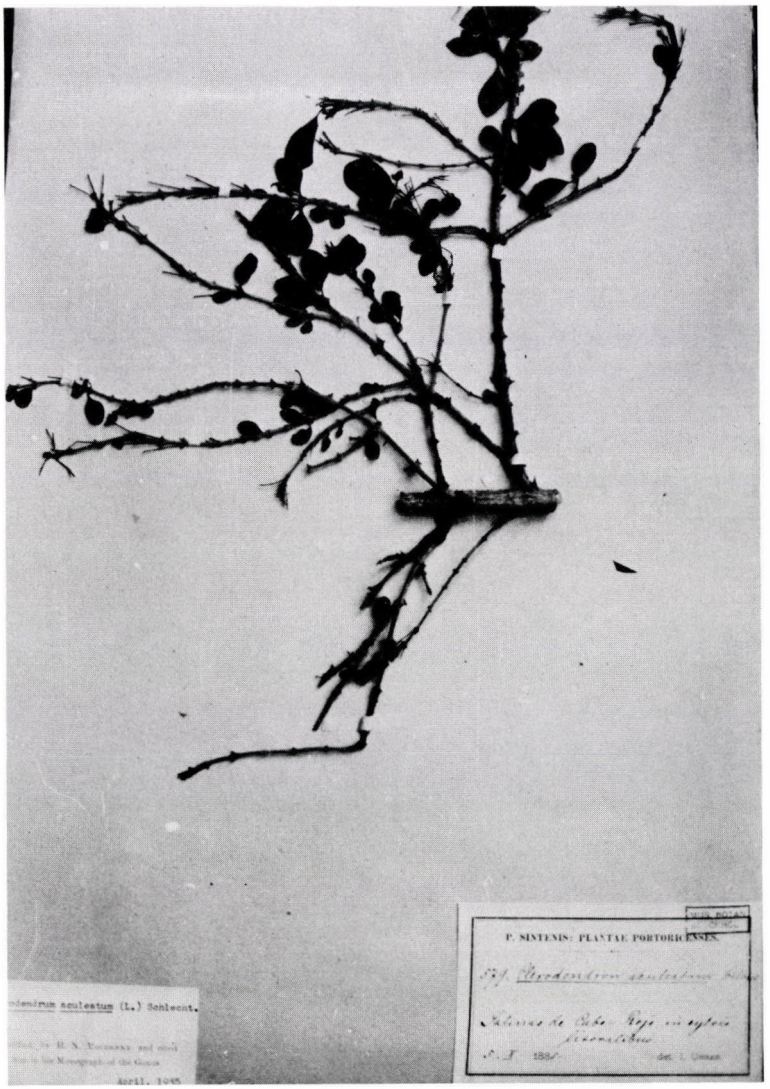

Fig. 3. C. aculeatum (L.) Schlecht. var. aculeatum f. rotundatum Kereszty — SINTENIS 579. Puerto Rico. (S)

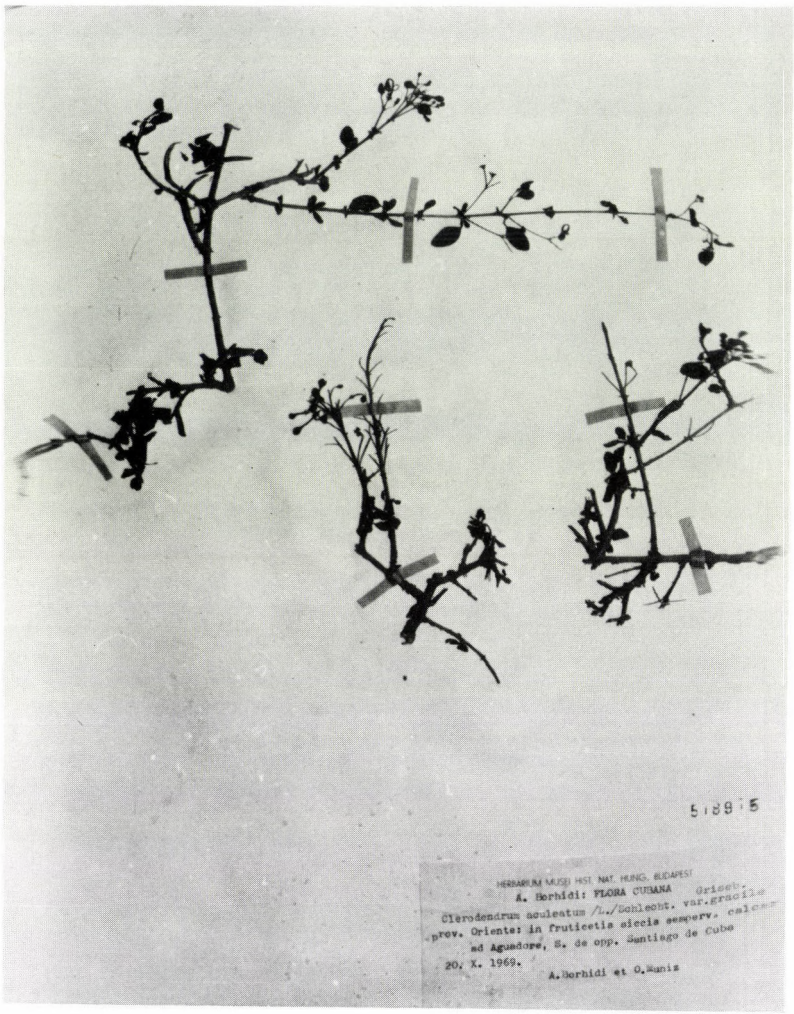

Fig. 4. C. aculeatum (L.) Schlecht. var. gracile Griseb. et Moldenke f. orientale Kereszty — BORHIDI et MUÑIZ 518915. Santiago de Cuba (BP)

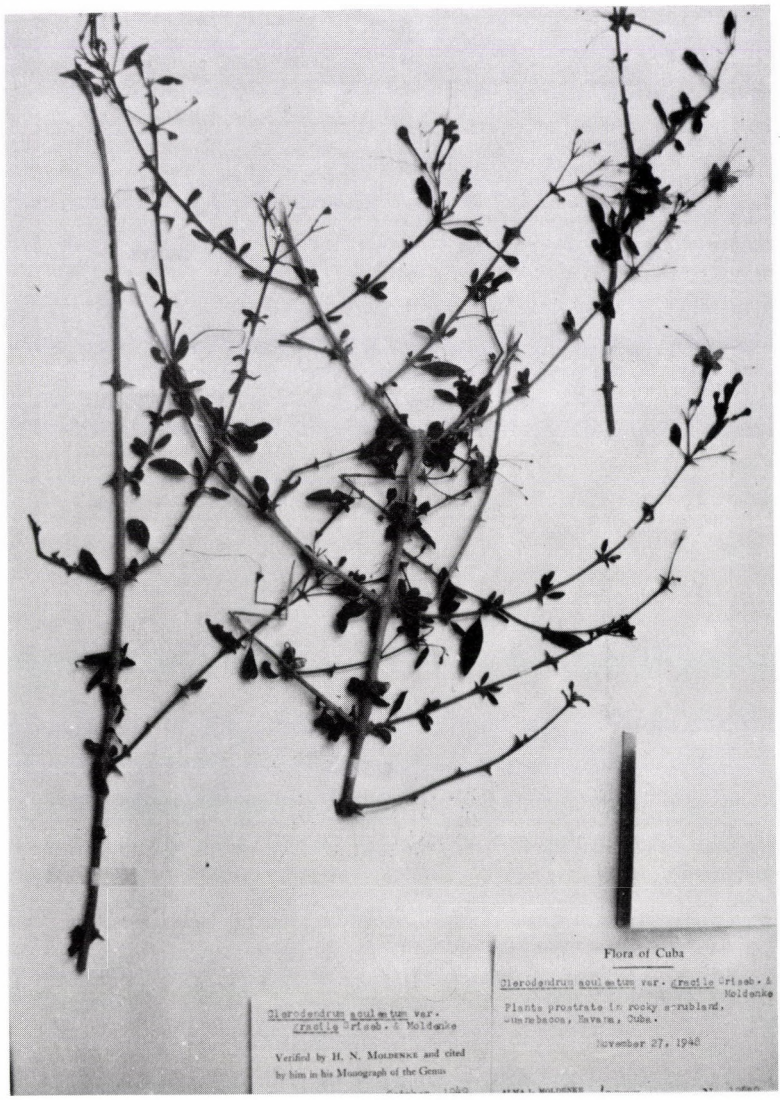

Fig. 5. C. aculeatum (L.) Schlecht. var. gracile Griseb. ex Moldenke — MOLDENKE 19850. Habana. (HAC)

Clerodendrum grandiflorum (Hook.) Schauer

Comparing my specimen collected in 1985 in Cajalbana with the collections of LEÓN, ALAIN and ACUÑA, it turned out that the plants living on the serpentine rock of the Mt. Cajalbana, were different from living on the limestome of the Sierra del Rosario. The morphological differences of this taxon combine with its geographical distribution. Therefore it is reasonable to consider this endemic plant as a subspecies.

Trees and shrubs with cylindric greyish-white, bark and lentil-shaped emergent lenticells on the longitudinal ribs. Leaves shiny, entire, opposite, coriaceous with short petiole, glandulated and glabrous with cushion-shaped areolas above; dull, hardly reticulated and glandulous beneath with stomata secreting in the pits. Inflorescence axillary cyme with a pubescent and ribbed peduncle up to 12 cm long. Pedicels glandulous with 2 awn-like bracts. Calyx 3-5 mm long, campanulate, setose. Corolla 3-5 cm long, yellow, pubescent outside, tube gradually widening upwards, the margine of the limbs undulate. Stamen long outstanding. Fruit 1 cm long, ovate, glabrous with 2-4 seeds.

Occurs in West and Central Cuba, in tropical broadleaved forest, pine woodlands and tropical deciduous limestone forest on conic karsts.

Key to the subspecies of C. grandiflorum

— Shrubs or small trees of 2-4 m height. Stem sparsely pubescent, provided with ovate and terete glabrous lenticells. Leaves dark green, more than 6 cm long, obovate to ovate, mostly acute or subacute at the apex, cuneate at the base. Glabrous, scarcely reticulated and sparsely glandulous above. Inflorescence many flowered. Calyx with 5 acute lobes puberulous and glandulous. Corolla glandulous.

— ssp. grandiflorum

— 2-3 m high shrub, branches densely setose with pubescent ovate lenticells. Leaves 6-8 cm long, shape variable, mostly elongated elliptic, acute or obtuse at the apex, obtuse or subcordate at the base. Strongly reticulated and densely glandulous above, with simple and articulate hairs beneath. Pedicels pubescent but without glands. Calyx cut or undulated at the margin. Corolla thin without glands. Endemic on the Mount Cajalbana on old serpentine ferritic soils.

— ssp. cajalbanense

C. grandiflorum (Hook.) Schauer ssp. cajalbanense Kereszty, subspecies nova

A typo differt habitu fruticoso, ramis pubescentibus, lenticellis ovatis pubescentibus protectis, foliis variabilibus, 6-8 cm longis, plerumque ellipticis, apice acutis vel obtusis, basi truncatis vel subcordatis, supra valde reticulatis et glandulosis subtus pilis simplicibus et articulatis; pedicellis puberulis, eglandulosis, calice truncato vel margine undulato, corolla eglandulosa. Endemicus in monte Cajalbana solo serpentinoso, ferritico.

Holotypus: ACUÑA 16416, Cuba, Pinar del Rio, Mt. Cajalbana (HAC) (Fig. 6).

Specimens examined:
C. grandiflorum ssp. grandiflorum (Fig. 7).
CUBA, sine loc.: SAGRA, sine num. (Isotype, KW); WRIGHT 3176 (HAC); PINAR DEL RIO: ALAIN 495, 2008, 4274; MISSE et al. 15591 (HAJB); EKMAN 18158 (S); LEÓN 910 (HAC); SAUVALLE 1779, 1780 (HAC); Bahia Honda: BISSE et al. 29154 (HAJB); EKMAN 10405, 10542, 12644 (S); LEÓN 20974 (HAC); Cayajabos: LEÓN 6024, 12566 (HAC); Las Hojas: BISSE et al. 36274 (HAJB); Loma Pelada: LEÓN 12540 (HAC); Mogote de Soroa: LEÓN 12901 (HAC); Pan de Guajaibón: ACUÑA 10626, 10799 (HAC); BISSE et al. 31319 (HAC); EKMAN 29079 (S); Playa Morrillo: EKMAN 17386 (S); Rangel: ACUÑA 18320 (HAC); ALAIN 21, 110, 20091 (HAC); LEÓN 488, 12640 (HAC); Sra. de Cañada: BISSE et al. 41965 (HAJB); Sra. de la Guasasa: EKMAN 16620 (S); Sra. del Rosario: EKMAN 16391 (S); IMCHANITZKAJA 33574 (HAC); Sta. Catalina: HERMANN 3247 (HAC); Sta. Cruz: LEÓN 7417 (HAC); Vinales: ALAIN 6875 (HAC); LEÓN 17605 (HAC); HABANA: YERO 553 (HAC); ISLA DE PINOS: EKMAN 12503 (S).
C. grandiflorum ssp. cajalbanense
PINAR DEL RIO: Cajalbana: ACUÑA 16417 (HAC); ALAIN 1166, 1203, 4499, 24421 (HAC); BISSE et al. 14869 (HAJB); LEÓN 465 (HAC); YERO 575 (HAC).

Clerodendrum cubense Schauer

This species is very rare in the thickets on the old slatey sandstone of the Sierra de los Organos. When studied the EKMAN's collection URBAN recognized that the typical form was substituted by a morphologically different one in the limestone-thickets on the plateaus of the same mountains. He described this form as a separated species under the name: C. brachypus. According to our studies this taxon must be considered only as a taxonomic form of the type.

2 m high shrub with verrucated, setose branches, table-shaped bark. Young branches flattened, densely ribbed with terete lenticells. Leaves glabrous or occasionally pilose, obovate to ovate elliptic, obtuse or rounded at the apex, sometimes slightly acuminate, truncate or subcordate at the base. Edge conspicuously rolled with small distant teeth. Strongly reticulated, veins pilose, areolas shiny without glands above. The veins emergent glabrous or with 1-2 hairs, the surface dull, areolas cushioned, densely glandulous beneath. Stomata protruding on the ribbes without radiate bundles. Inflorescence is an axillary cyme shorter than the leaves,

Fig. 6. C. grandiflorum (Hook.) Schauer ssp. cajalbanense Kereszty — ACUÑA 16416. Pinar del Rio, Cajalbana. (HAC)

Fig. 7. C. grandiflorum (Hook.) Schauer ssp. grandiflorum — SAUVALLE 1780. Cuba. (HAC)

peduncle short, pubescent with 2 bracts at the base. Pedicels short with 2 small bracts in the middle. Calyx campanulate, upward widening, glabrous or puberule. Corolla white, glabrous, stamens long projecting. Rare in W-Cuba and Isla de Pinos.

Key to the Varieties of C. cubense

— Leaves 6-18 cm long, obovate-elliptic, obtuse, rounded or apiculate at the apex, obtuse or subcordate at the base. Peduncle as long as the flower. Cyme many-flowered. Calyx truncate above, corolla 4-5 cm long, tube 2.5 cm, limb 1 cm long. Rare on acidic rocks and soil

— var. cubense

— Leaves 4-7 cm long, narrow obovate, obtuse or subtruncate at the base. Peduncle less than 3 mm long. Cyme few flowered. Calyx cylindric, lobes 1/3 of the calyx-tube. Corolla not longer than 3 cm. On limestone of the W-Cuban mountains and lowlands and in Isla de Pinos very rare.

— var. brachypus

C. cubense Schauer var. cubense (Fig. 8).

DC. Prodromus XI: 658 (1847)

Isotype: 3175 Cuba, Habana WRIGHT (HAC)

Specimens examined: CUBA (sine loc.): SAGRA, sine num. Isotype (KW); SAUVALLE 1779 (HAC); PINAR DEL RIO: San Julian: EKMAN 18728 (S); Srra de los Organos, Ancón: ALAIN 6875 (HAC).

C. cubense Schauer var. brachypus (Urb.) Kereszty combinatio nova

Basionym: C. brachypus Urban — Rep. Nov. Spec. Gen. 1924. XX: 347

Type: EKMAN 16673. Pinar del Rio, Ensenada de Vega Cuchilla (S) (Fig. 9).

Specimens examined: PINAR DEL RIO: Las Martinas: ACUÑA et ROIG 10873 (HAC); LEÓN et ROIG 455 (HAC); Cayo Mono: CREMATA 7514 (HAC); Srra. del Rosario: IMCHANITZKAJA 33603, 33604 (HAC); ISLA DE PINOS: Rio Yucaro: ALAIN et VICTORIN 28 (HAC); Sta Fe: ACUÑA 17679 (HAC).

Specimens from Provincia Oriente determinated as C. cubense proved to be C. lindenianum: CLEMENTE 4961. Holguin, Moa (HAC); LEÓN et VICTORIN 19814. Holguin, Srra. de Nipe (HAC).

Clerodendrum calcicola Britton

The great similarity between the C. calcicola collected in W-Cuba mostly from the limestone on the peninsula Guanahacabibes and the C. tuberculatum living on limestone in the surroundings of the Zapata Peninsula and

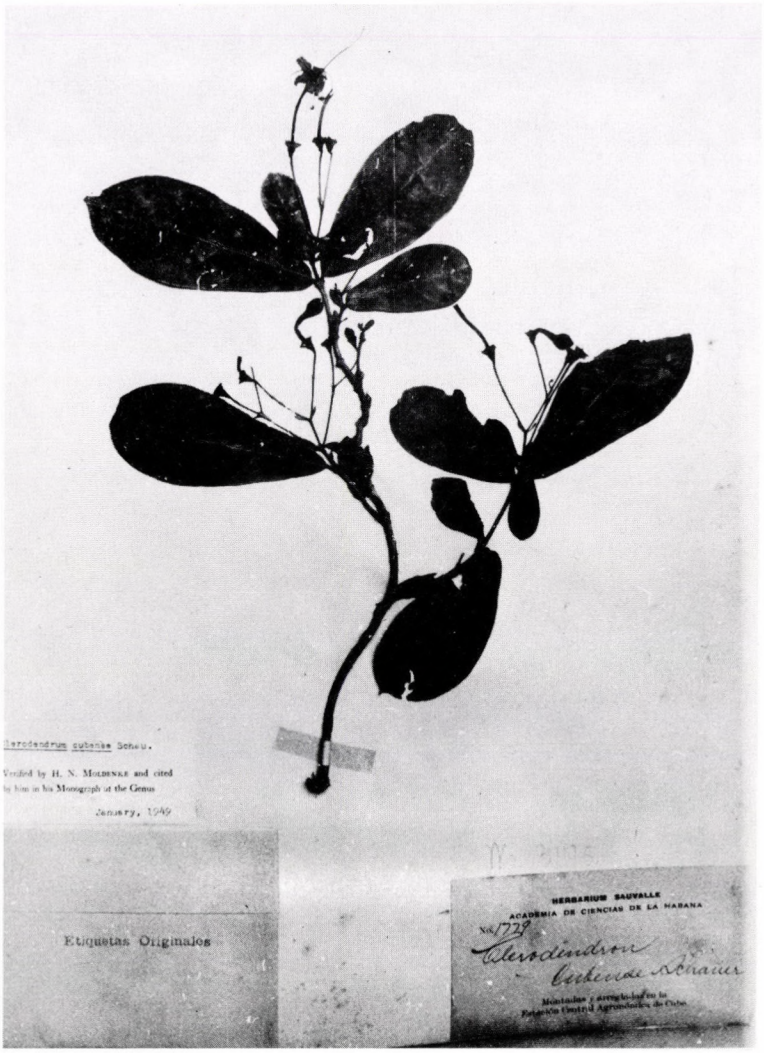

Fig. 8. C. cubense Schauer var. cubense — CH. WRIGHT 3175. Cuba. (HAC)

Fig. 9. C. cubense Schauer var. brachypus (Urb.) Kereszty — EKMAN 16673. Pinar del Rio. (S)

Fig. 10. C. calcicola Britton — YERO 26518. Pinar del Rio. (HAC)

Fig. 11. C. tuberculatum A. Rich. — LEÓN 13654. Habana. (HAC)

the southern coast of Central-Cuba is well known. C. tuberculatum was described by A. RICHARD (1850) based on the specimens collected on the western slopes bordering the Cienfuegos Bay. BRITTON was not able to study the type specimen in Paris because transatlantic mailing problems by the world-war. He had had to base his decision on the short description of A. RICHARD which turned to be unsufficient. BRITTON was convinced that the specimens received from the Guanahacabibes peninsula represented a new species and described it under the name C. calcicola. Recent studies including those on the leaf anatomy suggest to consider the two taxa being conspecific, and therefore I unite them under the earlier name of A. RICHARD as C. tuberculatum A. RICH.

Specimens examined:
C. calcicola: PINAR DEL RIO. Guanahacabibes peninsula: ACUÑA 19933 (HAC), 30907 (HAJB); ALAIN 6964 (HAC); YERO 26518 (HAC); BISSE et al. 34199 (HAJB); DIAZ 31885 (HAC); EKMAN 18799 (S); El Morillo: ALAIN 19349 (HAC); La Iguana: BISSE et al. 33283 (HAJB); MATANZAS: EKMAN 17208 (S); OVIEDO 33694 (HAC). (Fig. 10).
C. tuberculatum: HABANA: LEÓN 294, 13654 (HAC); VILLA CLARA: Srra. del Escambray: DANERT 243 (HAC); ALAIN 985 (HAC). (Fig. 11).

Acknowledgements

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XYLOTOMICAL EXAMINATION OF LIGNIFYING SHOOTS AND ROOTS FOR AGE DETERMINATION OF GRASSLANDS — FUMANA PROCUMBENS AND EUPHORBIA SEGUIERIANA+

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(Received: 13 November 1986)

In the course of cenological investigations of vegetation dynamics the demand for getting information on the age of a given stand of grasslands is frequently raised. Fortunately, species with lignifying shoots may be of help in such cases. Author studied the extensive perennial Festucetum vaginatae and its two lignifying species (Fumana procumbens and Euphorbia seguieriana) on the sandy areas of the Great Hungarian Plain with anatomical methods to this end. In the first place Fumana procumbens can be used for determining the age of grasslands.

In investigations of time dynamics which recently have become more and more widespread the age of the grassland examined is of importance. While the questions is familiar when it concerns a forest, it is confusing when it is about a grassland. Yet, the lignifying perennials and dwarf shrubs which can be studied with xylotomical methods may be of help here, too, and may give a clue to the age of the cenosis through the age determination of the individual plants.

Material and Method

The perennial Festucetum vaginatae danubiale on the plains of the Carpathian basin is an extensive plant association, for edaphic reasons with semi-arid character. Its species are characterized by numerous contrasting life-forms; many of the species have lignifying shoots and roots. Two species of this kind chosen for the purpose of examination are: Fumana procumbens and Euphorbia seguieriana (site of collection: sandy area of Fülöpháza, so-called "young" and "old" grassland, 1980). The two species — perhaps just because of the lower rate of their ontogenesis — do not appear in the first stages of vegetation development. In the later stages of succession on the driftsand areas of lowlands (e.g. on the sandy area of Fülöpháza in the Kiskunság National Park) they are cenologically separated according to their slightly different demands. In Festuca vaginata grasslands developing on lighter sands Fumana, while with a closed vegetation Euphorbia is the dominant species. Although the individual plants are very different in age — as is obvious from visual observations —, the age distribution is characteristic of the population, while the age of the oldest plants may give almost the only information about the age of the association.

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From the root-necks, stems and shoots 1 cm samples were taken for the purpose of softening. The softened material was inbedded in paraffine, then sections were prepared from them. The sections were stained with a 3% alcohol solutions of toluidine-blue micro-colouring agent (SÁRKÁNY and SZALAI 1964). Of the sections suitably magnified photoes were taken.

Wood anatomy

Fumana procumbens (Dun) Gr. et Godr.

A sub-shrub, 10-12 cm in height, with a woody stem easy to examine.

Woody stem of Fumana procumbens

In the cross section the wavy borders of annual rings are clearly visible. Immediately at the borders of annual rings -- in the spring timber -- relatively large vessels generally arranged in one or two continuous rows can be found (Fig. 1). The wood can therefore be said more or less ring-porous. In the autumn timber the vessels are smaller, generally set in singles, sometimes in twos. The mass of the annual rings consists of thinner-walled fibre tracheids in the spring timber, and of thicker-walled ones in the autumn timber. The medullary rays are one- or two-layered, rather thick. The longitudinal parenchyma is metatracheal (cf. GREGUSS 1945 and METCALFE and CHALK 1950).

Comparative study of the "young" and "old" grass originating from the samples of Fumana procumbens.

As seen in Figs 1 and 2 the two samples are of the same age, 8 years old. The fact that the one from the "old" grass is thicker is due to the broader annual rings rather than to the older age. The microscope photoes are magnified at the same rate, so the difference in thickness between the shoots is shown well in the figures.

Euphorbia seguieriana NECKER

A perennial species with only the roots surviving for years, the shoots, though lignify, wither every year.

Root head of Euphorbia seguieriana above ground level

In cross section the lignified part of the root head can be regarded as diffuse-porous because of the hardly visible, slurred borders of annual rings. In the sample examined the borders of annual rings are indicated by

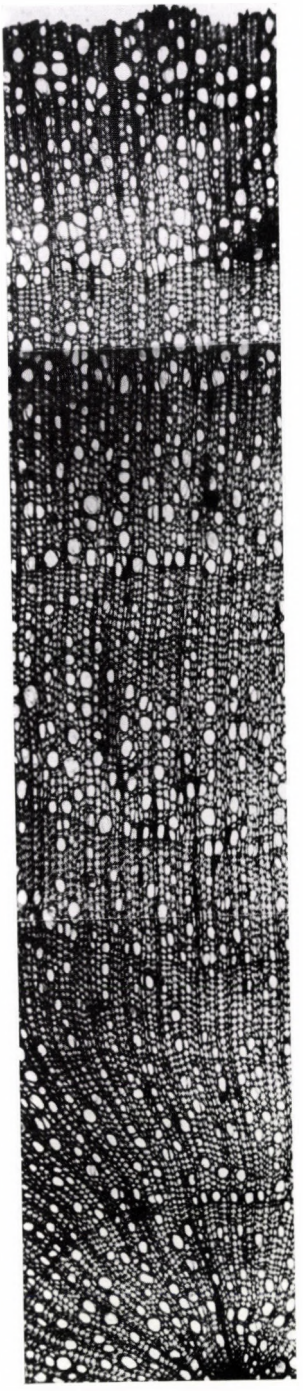

Fig. 1. Cross section of Fumana procumbens stem, 120 x. Collected at Fülöpháza, 1980, "young" grassland

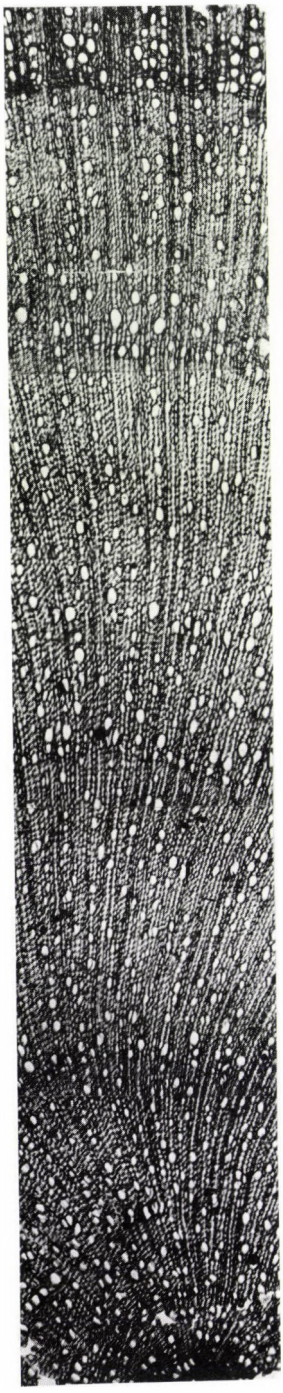

Fig. 2. Cross section of Fumana procumbens stem, 120 x. Collected at Fülöpháza, 1980, "old" grassland

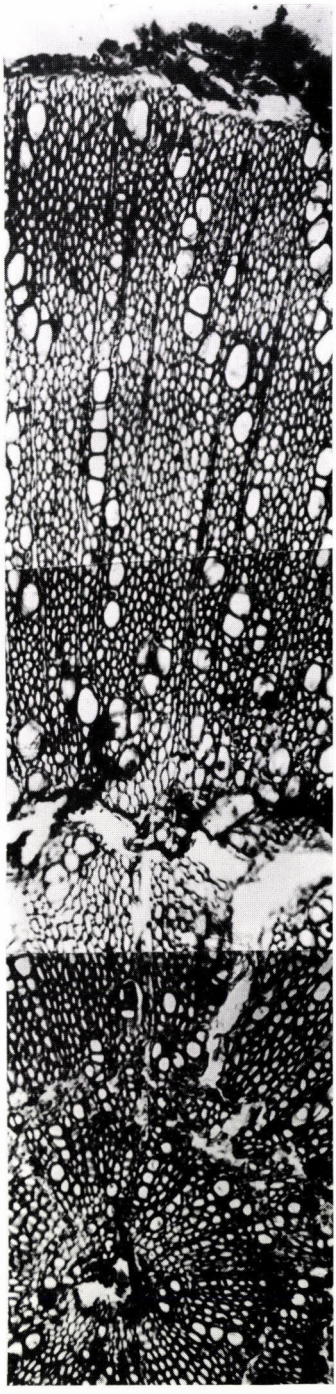

Fig. 3. Cross section of Euphorbia seguieriana root head, 120 x. Collected at Fülöpháza, 1980

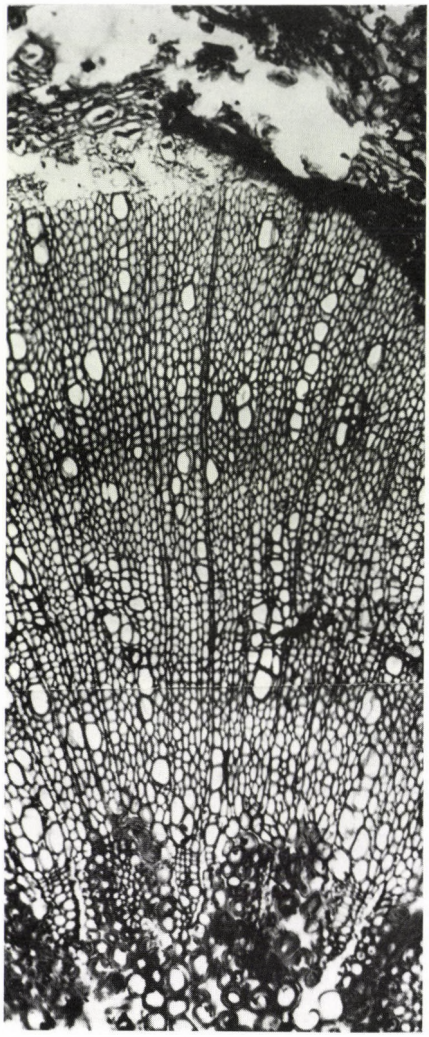

Fig. 4. Cross section of Euphorbia seguieriana shoot of 1979, 120 x.
Collected at Fülöpháza, 1980

smaller or larger lysigenic tissue deficiencies (secretion-holding cavities) (Fig. 3). The vessels in the annual rings are set in singles, twos or in radial groups of 4-6. The vessels grow in size with age. The medullary rays are one- or two-cell wide. The basic substance is formed of thin-walled fibres and fibre tracheids. Metatracheal longitudinal parenchyma. The longitudinal parenchyma cells are difficult to distinguish from the medullary ray cells, since both are highly elongated and relatively narrow.

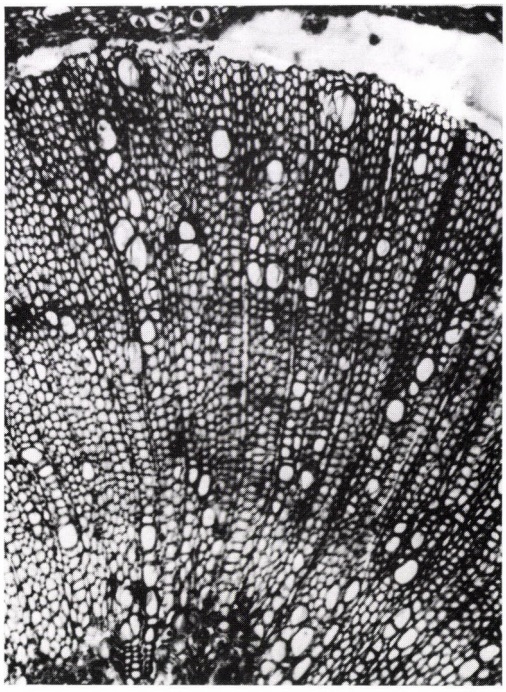

Fig. 5. Cross section of Euphorbia seguieriana shoot of 1980, 120 x.
Collected at Fülöpháza, 1980

Shoots of Euphorbia seguieriana

The shoots of E. seguieriana lignify, but according to the xylotomical examination are not suitable for age determination. This fact is shown in Figs 4 and 5. The shoots from 1979 and 1980 are of the same tissue structure. The difference in thickness between the shoots can be seen well in the figures, because the microscope photoes were magnified at the same rate.

Conclusions

On the basis of the xylotomical examinations it can be established that the Fumana procumbens can be properly used for the age determination of so-called "young" and "old" grasses. In the case of Euphorbia seguieriana only the root head can be used for age determination.

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XYLOTOMICAL EXAMINATIONS OF SOME VENEZUELAN SPECIES OF TREE BELONGING TO THE CAESALPINIACEAE, FABACEAE AND MIMOSACEAE FAMILIES — CAESALPINIACEAE II

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(Received: 30 October 1987)

Authors make known the exterior morphological and ecological characteristics, habitat, and main anatomical features of the xylem of four Venezuelan Caesalpiniaceae species, namely Cassia siamea Lam., Cassia emarginata L., Cercidium praecox (R. et P.) Harms., Parkinsonia aculeata L.

Material and Method

Blocks made from the wood of the four species were softened in a mixture of water and glycerin, in Brinzer's autoclave, at 1.5-2.0 atm., then transversal, tangential and radial sections were prepared from theme. The sections were stained with an alcoholic solution of Toluidin-blue. The maceration of tissues was made with the Schulze-method (SÁRKÁNY and SZALAI 1964).

Length of fibres and vessel-elements, tangential and radial diameters of vessels, width and height of medullary rays and other characteristics were determined from 50-100 measurements. Enlarged microphotographs were made of each section.

External morphology and distribution are based on descriptions by Prof. L. J. C. Cumana.

External morphology

Cassia siamea Lam.

Tree 6-12 m high, young branches somewhat puberulent. Leaves alternate, pinnately compound, the pinnae 1-10 pairs, 5-9 cm long, 1.5-2.0 cm wide, oblong, emarginated or apiculate apically, shiny below. Inflorescence a terminal corymb-like panicle, 15-50 cm long, with numerous yellow flowers. Sepals 5, imbricated, 4-5 mm long, 3-4 mm wide, two of them smaller, suborbicular, puberulent. Petals 5, imbricated, 1-2 cm long, 1.0-1.2 cm wide, unguiculate, glabrous, unequal, suborbicular. Stamens 10, 3-12 mm long, 3 of

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them sterile, subcurved, anthers dehiscent by pores. Gynoecium 1.0-1.5 cm long, curved, glabrescent. Legume linear-undulate, subligneous, puberulent, swollen margin, acute, 15-35 cm long, 1.0-1.5 cm wide.

Exotic species known as "Acacia del Siam"; having demand by its fast growing, easily adaptable character up to altitudes higher than 1500 masl.

Cassia emarginata L.

Shrub or small tree 3-5 m high, foliage caducous, unarmed, pubescent toward young branches. Leaves alternate, pinnately compound, petiole and rachis puberulent, the pinnae 3 pairs, 3.0-6.5 cm long, 3-4 cm wide, elliptic or obovate-elliptic, rounded or emarginated apically, pubescent. Inflorescence corymb-like racemes with 4-7 yellow flowers, pedicels 1 cm long, puberulent. Sepals 5, imbricated, 3-5 mm long, unequal, puberulent. Petals 5, imbricated, 8.0-9.5 mm long, shape and size unequal. Stamens 10, 7 of them bigger, fertile, 4-6 mm long, 3 smaller, modified as staminods, 2-3 mm long, anthers with scattered hairs, dehiscent by pores. Gynoecium 1 cm long, curved. Legume subligneous, linear, swollen margins, 10-25 cm long, 1 cm wide.

Autochthonous species known as "Brusca"; frequent in warm regions of Venezuelan North, in xerophytic and trophophyll forests. Flowering generally occur after leaves fall.

Cercidium praecox (R. et P.) Harms.

Tree 2-6 m high, foliage caducous, wide treetop, armed, cortex green. Leaves alternate, bipinnately compound, petiole and rachis puberulent, the pinnae 1-3 pairs, the leaflets 6-7 pairs, 3-7 mm long, 1-2 mm wide, oblong, obtuse or subacute apically, pilose marginally. Raceme 1-2 cm long with 2-6 yellow flowers, pedicels 4 mm long, puberulent. Sepals 5, valvated, 5-8 mm long, acute puberulent. Petals 5, imbricated, 1 cm long, oblong or suborbicular, unguiculate, adaxial petal conspicuously ornamented. Stamens 10, 1 cm long, filaments pilose at the base, anthers longitudinally dehiscent. Gynoecium 8-9 mm long, erect, glabrous. Legume chartaceo-membranous, oblong or oblanceolate, 3-5 cm long, 1 cm wide.

Autochthonous species known as "Cuica", "Yabo", "Palo Verde"; typic of xerophytic regions of the Venezuelan North, very common in coastal forests where it stands out by the green-yellowish colour of the stem and by the showy flowering that occurs after leaves fall.

Parkinsonia aculeata L.

Shrub or small tree 3-5 m high, armed, cortex green, branches laxly arranged and somewhat pending. Leaves alternate, subsessile bipinnately compound, the pinnae 2 pairs, the leaflets numerous, 3-4 mm long, 1.0-1.5 mm wide, linear-oblong, glabrous, arranged on a laminar rachis, main rachis rudimentary and spinose. Raceme axilar or terminal with 6-12 yellow flowers. Sepals 5, imbricate 6-7 mm long, 2.5-3.0 mm wide, oblong. Petals 5, imbricate undulate, 1.0-1.2 cm long, 0.25-0.30 cm wide. Stamens 10, 7-8 mm long, filaments conspicuously pilose toward the base, anthers longitudinally dehiscent. Gynoecium 7-9 mm long, erect, ovary pubescent. Legume subcylindric, constricted between seeds, acute, 6-13 cm long, 8-9 mm wide.

Autochthonous species known as "Espinillo", abundant in low hot soils, close to swamps of flooded places, also in zones close to xerophytic forests, and adventive on uncultivated lands.

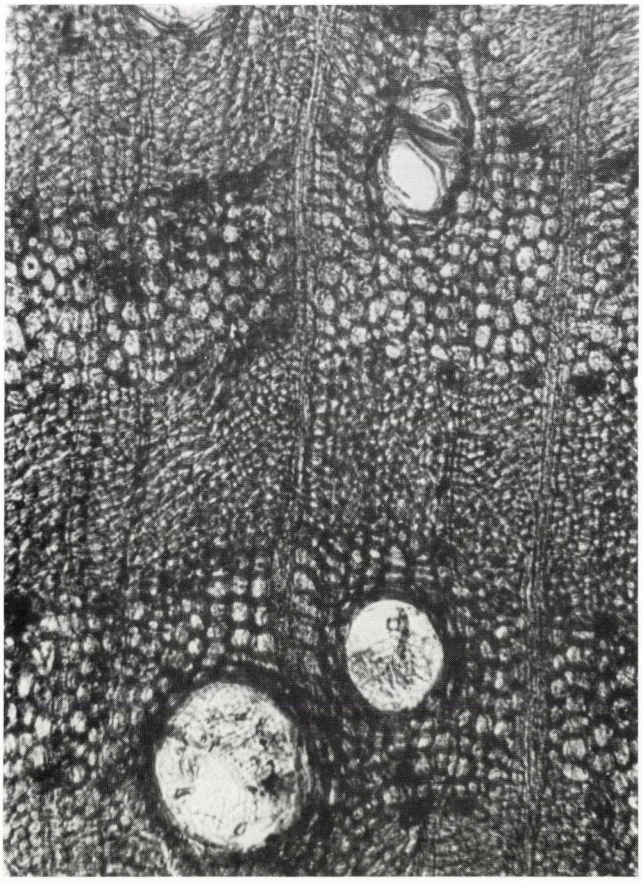

Fig. 1. Cassia siamea Lam. Cross-section 120 x. Vessels, medullary rays, fibres. Wide aliform-confluent longitudinal parenchyma

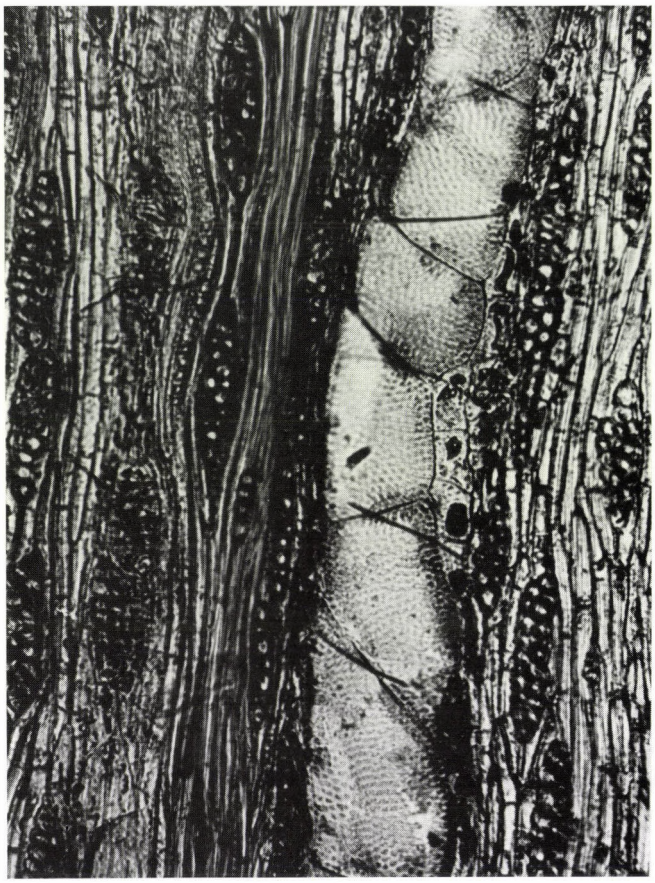

Fig. 2. Cassia siamea Lam. Tangential longitudinal section 120 x. One-, two- and three-cell wide medullary rays, vessel and fibres. In the wall of the vessel medium-size, slightly elongate bordered pits of alternate position

Wood anatomy

Cassia siamea Lam.

Wood porous. The bulk of wood is given by fibres of polygonal shape and relatively thick wall. Aliform-confluent longitudinal parenchyma in considerable amounts (METCALFE--CHALK 1950). The medullary rays are one-, two- and three-cell wide (Fig. 1).

The tracheae are oval or roundish, in groups of 2-4 tangentially flattened. They are 6-10.9-16/mm^2 in number. The tangential diameter is 51.1-102.3-144.1 µm, the radial diameter 41.8-113.1-153.4 µm. The vessel

Fig. 3. Cassia siamea Lam. Radial longitudinal section 120 x. Homogeneous medullary rays, longitudinal parenchyma and fibres. Septal crystal-holder fibre (sf) and septal crystal-holder longitudinal parenchyma (sp)

members are 80.5-197-8-322-0 μm long, with medium large, somewhat elongate bordered pits of alternate position in the walls. In the tracheae mastic material is rarely found. The perforate plate is simple.

Medullary rays 1-2-3-cell wide with homogeneous, or sometimes heterogeneous structure; height 57.5-142.8-241.5 μm; width: 14.5-20.8-34.5 μm (Figs 2, 3).

The fibres are arranged in radial rows; the diameter is 9.3-14.1-18.6 μm, the wall thickness 2.3-5.0-9.3 μm. The total length of fibres is 497.0-729.8-994.0 μm. The tips of fibres are smooth. Septal crystal-holding fibres are not unfrequent (marked sf in Fig. 3).

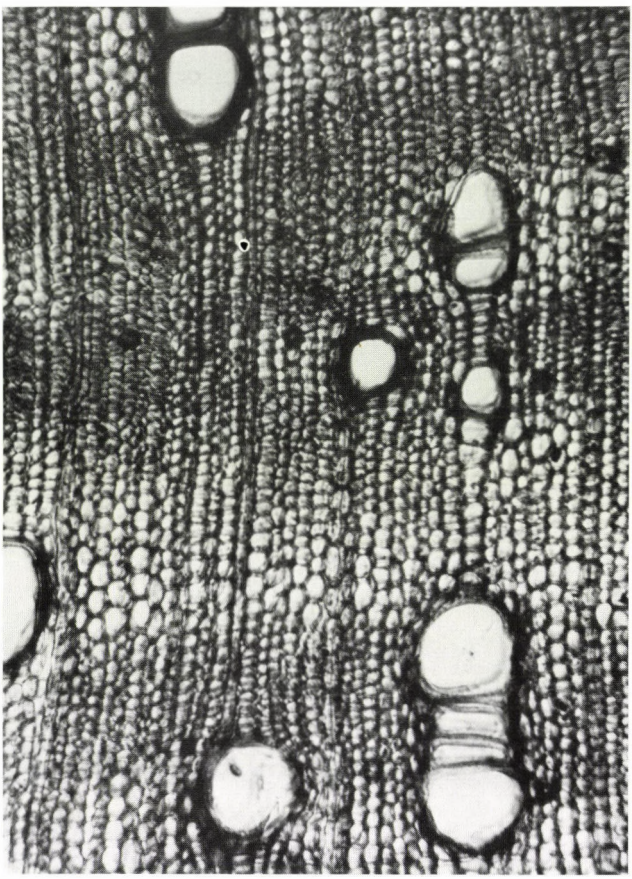

Fig. 4. Cassia emarginata L. Cross-section 120 x.
Vessels, medullary rays, fibres. Aliform-confluent longitudinal parenchyma

The tangential diameter of cells in the longitudinal parenchyma is 6.9-15.7-32.5 μm, their height is 23.2-64.2-93.0 μm. Septal crystal holders are not unfrequent (marked sp in Fig. 3).

Cassia emarginata L.

Porous wood mostly composed of fibres of polygonal shape and medium thick wall. Aliform confluent longitudinal parenchyma is present in considerable amounts. The medullary rays are 1-2-, seldom 3-cell wide (Fig. 4).

The tracheae are roundish or oval, in groups of 2-4 tangetially flattened. They are 11-17.6-27 per mm^2 in number. The tangential diameter is 41.8-87.4-120.9 μm, the radial diameter 74.4-114.0-148.8 μm. The members of

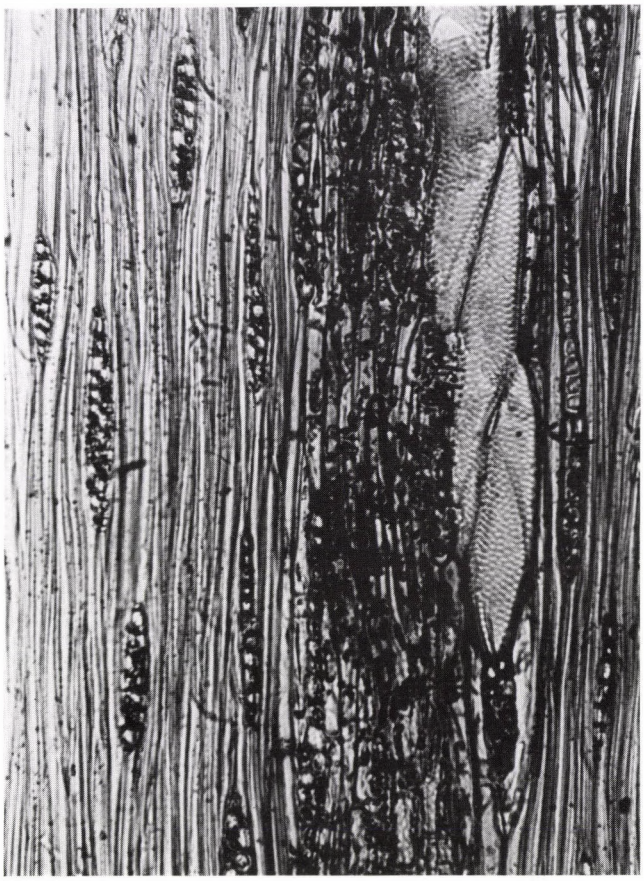

Fig. 5. Cassia emarginata L. Tangential longitudinal section 120 x.
One- and two-cell wide medullary rays, longitudinal parenchyma, vessel and fibres. In the wall of the vessel small, elongate bordered pits of alternate position

vessels are 172.5-260.6-345.0 μm long, with medium-size, slightly elongate bordered pits of alternate position in the walls. Mastic material of dark colour is rarely found in the tracheae. The perforate plate is simple.

The medullary rays are 1-2-, rarely 3-cell wide with homogeneous and heterogeneous structure. They are 57.5-202.7-563.5 μm high and 11.5-20.1-34.5 μm wide. The medullary ray cells often contain dark mastic material (Figs 6, 7).

The fibres are arranged in radial rows. The diameter is 9.3-14.5-19.5 μm, the wall thickness 2.3-3.8-4.6 μm. The total length of fibres is 426.0-578.6-781.0 μm. They end generally in smooth, sometimes in serrate tips.

Fig. 6. Cassia emarginata L. Radial longitudinal section 120 x.
Homogeneous medullary rays, vessel, longitudinal parenchyma and fibres. In the longitudinal parenchyma- and medullary ray cells dark mastic material. Septal crystal-holder longitudinal parenchyma (sp)

The tangential diameter of the longitudinal parenchyma cells is 9.3-15.9-23.3 μm, their height 41.8-79.2-120.9 μm. Septal crystal holders are not unfrequent (marked sp in Fig. 6).

Cercidium praecox (R. et F.) Harms.

Porous wood the bulk of which is made up by polygonal, thin-walled fibres with medium lumen. Contact-vasicentric and aliform-confluent longitudinal parenchyma. The medullary rays are 1-2- and 5-cell wide (Fig. 7).

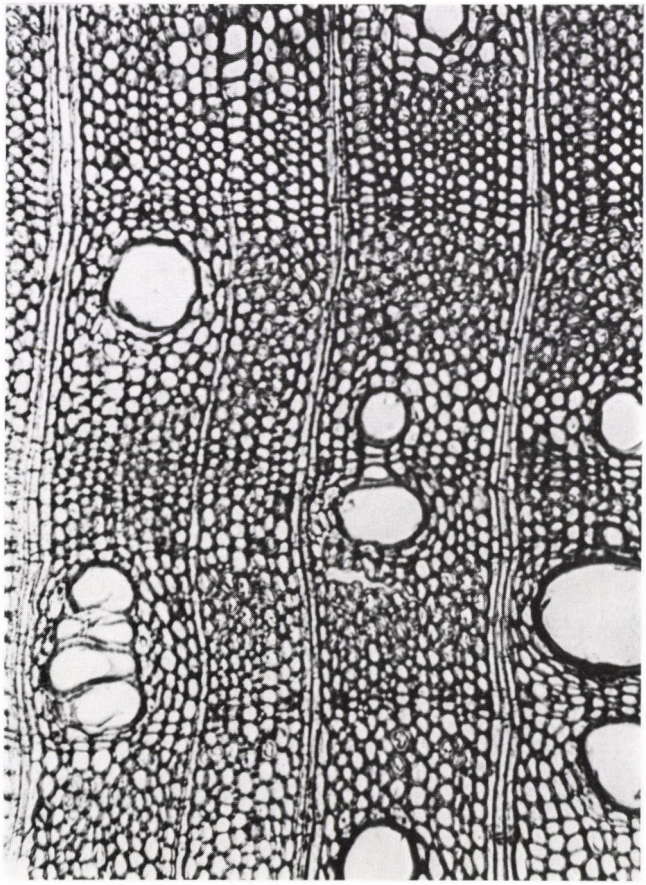

Fig. 7. Cercidium praecox (R. et P.) Harms. Cross-section 120 x.
Vessels, medullary rays, fibres. Contact-vasicentric and very narrow confluent longitudinal parenchyma

Tracheae round or oval, in groups of 2-4 tangentially flattened. They are 16-21.4-31/mm^2 in number. The tangential diameter is 41.8-82.6-111.6 μm, the radial diameter 41.8-86.3-134.8 μm. The members of vessels are 92.0-172.5-230.0 μm long, with medium-size, elongate bordered pits of alternate position in the walls. The perforate plate is simple.

The medullary rays are 1-2- and 5-cell wide, of homogeneous structure; 57.5-308.5-609.5 μm high and 11.5-36.0-57.5 μm wide. Dark mastic material in the cells of medullary rays is not unfrequent (Figs 8, 9).

Fig. 8. Cercidium praecox (R. et P.) Harms. Tangential longitudinal section 120 x. One- and several-cell wide medullary rays, longitudinal parenchyma, vessel and fibres. In the wall of the vessel small, elongate bordered pits of alternate position

Fibres arranged in radial rows. Their diameter is 13.9-18.9-23.2 μm, their wall thickness 1.8-2.9-4.6 μm. The total length of fibres is 355.0-563.7-781.0 μm. The tips of the fibres are smooth, pointed, though sometimes serrate at one end.

The tangential diameter of the longitudinal parenchyma cells is 6.9-12.3-18.6 μm, their height 32.5-55.8-102.3 μm. Septal crystal-holding longitudinal parenchyma is not unfrequent (marked sp in Fig. 9).

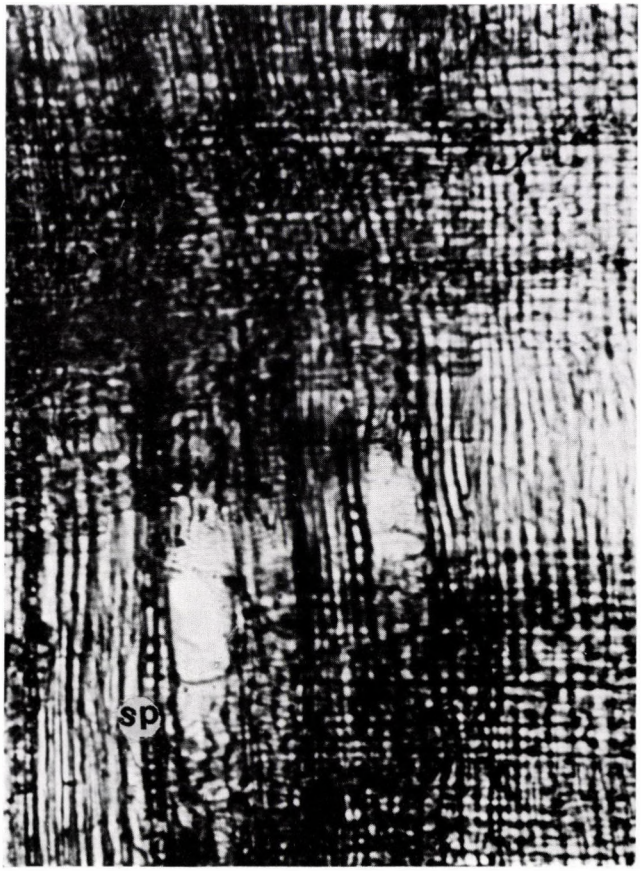

Fig. 9. Cercidium praecox (R. et P.) Harms. Radial longitudinal section 120 x. Homogeneous medullary rays, vessels, longitudinal parenchyma and fibres. Crystal-holder longitudinal parenchyma (sp)

Parkinsonia aculeata L.

Porous wood the bulk of which is made up by thin-walled polygonal fibres with large lumen. The medullary rays are 1-2-cell wide. The longitudinal parenchyma is paratracheal, contact-vasicentric, scanty (Fig. 10).

The tracheae are round or oval, in groups of 2-4 and 6-8 tangentially flattened; they are 17-25.5-50/mm^2 in number. The tangential diamater is 32.5-60.6-83.7 μm, the radial diameter 51.5-86.3-106.9 μm. The members of vessels are 80.5-190.9-241.5 μm long. There are highly elongated bordered pits of alternate position in the walls of the vessels. Simple perforate

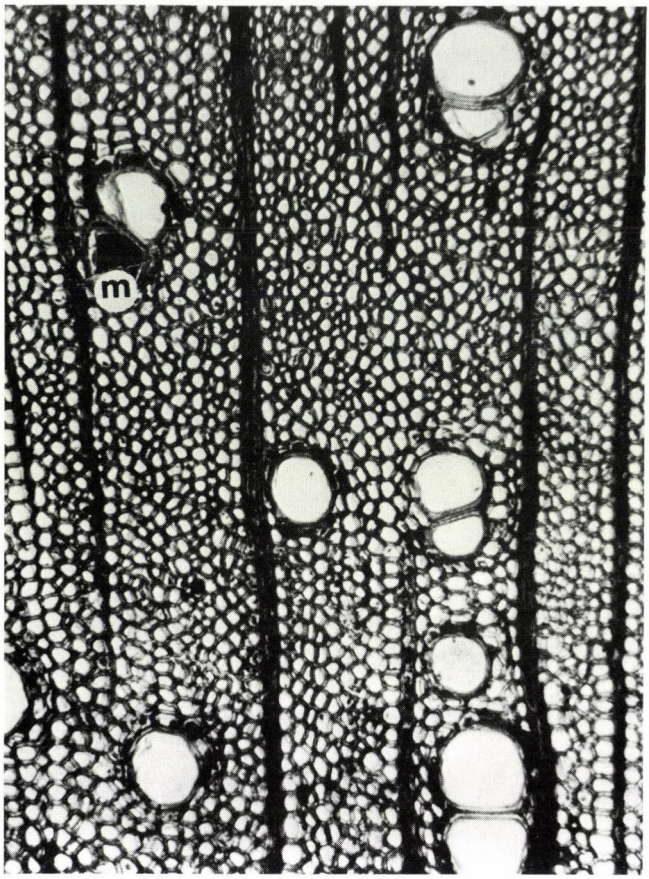

Fig. 10. Parkinsonia aculeata L. Cross-section 120 x.
Vessels, medullary rays, fibres. Contact-vasicentric longitudinal parenchyma. In one of the vessels dark mastic material (m)

plates. In the vessels dark mastic material is rarely found (marked m in Fig. 10).

The medullary rays are 1-2-cell wide, of homogeneous structure. They are 23.0-187.4-310.5 μm high and 11.5-14.9-23.0 μm wide. In the cells of the medullary rays dark mastic material may occur (Figs 11, 12).

The fibres are irregularly arranged. Their diameter is 13.9-20.1-27.9 μm, their wall thickness 1.8-3.0-4.6 μm. The total length of fibres is 284.0-585.7-852.0 μm. The tip of the fibre is smooth, pointed.

Fig. 11. Parkinsonia aculeata L. Tangential longitudinal section 120 x.
One- and two-cell wide medullary rays, longitudinal parenchyma, vessel and fibres. In the wall of the vessel highly elongated bordered pits of alternate position. In the medullary ray cells dark mastic material

The tangential diameter of cells in the longitudinal parenchyma is 9.3-13.9-23.5 μm, their height 27.9-48.3-83.7 μm. Dark mastic material may occur in the cells.

Detailed anatomical characteristics of the wood in the above four tree species, and their measurements are contained in Tables 1 and 2.

Fig. 12. Parkinsonia aculeata L. Radial longitudinal section 120 x.
Homogeneous medullary rays, vessels, longitudinal parenchyma and fibres. In the medullary ray cells dark mastic material

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Table 1

Anatomical features of the species examined

Wood elements	Features	Cassia siamea	Cassia emarginata
Trachea	arrangement	diffused, solitary or in radial groups of 2-4 members	diffused, solitary or in radial groups of 2-4 members
	shape	roundish or oval shaped, in groups tangentially flattened	roundish or oval shaped, in groups tangentially flattened
	tangential diam.	51.1-102.3-144.1 μm	41.8-87.4-120.9 μm
	radial diameter	41.8-113.1-153.4 μm	74.4-114.0-148.8 μm
	length of vessels	80.5-197.8-322.0 μm	172.5-260.6-148.8 μm
	number per mm^2	6.0-10.9-16.0	11.0-17.6-27.0
	wall thickness	2.3-4.9-9.3 μm	2.3-4.9-9.3 μm
	intervascular pitting	elongated bordered	elongated bordered
	perforate plate	simple	simple
	content	rarely mastic material	rarely mastic material
Medullary rays	width	narrow	narrow
	number of cells	1-2-3	1-2, rarely 3
	classification	homogeneous, rarely heterogeneous	homogeneous, rarely heterogeneous
	height	57.5-142.8-241.5 μm	57.5-202.7-563.5 μm
	width	14.5-20.8-34.5 μm	11.5-20.1-34.5 μm
	content	—	mastic material
Fibers	arrangement	radial rows	radial rows
	shape	polygonal	polygonal
	full thickness	9.3-14.1-18.6 μm	9.3-14.5-19.5 μm
	wall thickness	2.3-5.0-9.3 μm	2.3-3.8-4.6 μm
	full length	497.0-729.8-994.0 μm	426.0-578.6-781.0 μm
	type of pitting	small, bordered	small, bordered
Longitudinal parenchyma	arrangement	aliform-confluent	aliform-confluent
	diameter	6.9-15.7-32.5 μm	9.3-15.9-23.2 μm
	height	23.2-64.2-93.0 μm	41.8-79.2-120.9 μm
	number of cells	4-6	2-4
	content	calcium-oxalate crys.	mastic material
	others	septal crystal-holder long. parenchyma	septal crystal-holder long. parenchyma

Table 2

Anatomical features of the species examined

Wood elements	Features	Cercidium praecox	Parkinsonia aculeata
Trachea	arrangement	diffused, solitary or in radial groups of 2-4, rarely 8 members	diffused, solitary or in radial groups of 2-3, rarely 6-8 members
	shape	roundish or oval shaped, in groups tangentially flattened	roundish or oval shaped, in groups tangentially flattened
	tangential diam.	41.8-82.6-111.6 µm	32.5-60.6-83.7 µm
	radial diameter	41.8-86.3-134.8 µm	51.1-86.3-106.9 µm
	length of vessels	92.0-172.5-230.8 µm	80.5-190.9-241.5 µm
	number per mm^2	16.0-21.4-31.0	17.0-25.5-50.0
	wall thickness	4.65 µm	2.3-3.4-4.6 µm
	intervascular pitting	elongated bordered	elongated bordered
	perforate plate	simple	simple
	content	—	rarely mastic material
Medullary rays	width	wide	narrow
	number of cells	1-5	1-2
	classification	homogeneous	homogeneous
	height	57.5-308.5-609.5 µm	23.0-187.4-310.5 µm
	width	11.5-36.0-57.5 µm	11.5-14.9-23.0 µm
	content	mastic material	rarely mastic material
Fibers	arrangement	radial rows	radial rows
	shape	polygonal	polygonal
	full diameter	13.9-18.9-23.2 µm	13.9-20.1-27.9 µm
	wall thickness	1.8-2.9-4.6 µm	1.8-3.0-4.6 µm
	full length	355.0-563.7-781.0 µm	284.0-585.7-852.0 µm
	type of pitting	small, bordered	small, bordered
Longitudinal parenchyma	arrangement	contact-vasicentric and confluent narrow	contact-vasicentric scanty
	diameter	6.9-12.3-18.6 µm	9.3-13.9-23.5 µm
	height	32.5-55.8-102.3 µm	27.9-48.3-83.7 µm
	number of cells	2-4-6	2-4-8
	content	calcium-oxalate	mastic material
	others	septal crystal-holder long. parenchyma	—

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XYLOTOMICAL EXAMINATIONS OF SOME VENEZUELAN SPECIES OF TREE BELONGING TO THE FAMILIES CAESALPINIACEAE, FABACEAE AND MIMOSACEAE — CAESALPINIACEAE III. — FABACEAE

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Authors make known the exterior morphological and ecological characteristics, habitat and major anatomical features of the xylem in three Venezuelan species: Delonix regia (Bojer.) Raf. /Caesalpiniaceae/, Peltophorum inerme (Roxb.) Naves /Caesalpiniaceae/, Gliricidia sepium (Jacq.) Stand. (Fabaceae).

Material and Method

Blocks made from the wood of the three species were softened in a mixture of water and glycerin, in Brinzer's autoclave, at 1.5-2.0 atm., then transversal, tangential and radial sections were prepared. The sections were stained with the alcoholic solution of Toluidin-blue. The maceration of tissues was made with the Schulze method (SÁRKÁNY and SZALAI 1964).

Length of fibres and vessel elements, tangential and radial diameters of vessels, width and height of medullary rays and other characteristics were calculated from 50-100 measurements. Enlarged microphotographs were made of each section.

External morphology and distribution are based on descriptions by Prof. L. J. C. CUMANA.

External morphology

Delonix regia (Bojer.) Raf.

Tree 6-12 m high, wide treetop, slightly fragrant, foliage caducous, unarmed, stem short and very thick. Leaves alternate, bipinnately compound, glabrous, 30-50 cm long, pinnae 10-25 pairs, leaflets 18-40 pairs, 6-10 mm long, 2-3 mm wide, oblong, subacute apically, asymmetric basally. Inflorescence axillary or terminal corymb-like racemes with numerous showy scarlet flowers, pedicels 4-8 cm long, the inferior longer. Sepals 5, valvated, green outside and red inside, 2.0-3.5 cm long, 9-10 mm wide. Petals 5, imbricated, 5-7 cm long, 4-5 cm wide, unguiculate, petal adaxial,

very conspicuous. Stamens 10.3-5 cm long, filaments red, pubescents at the base, anthers longitudinally dehiscent. Gynoecium 4-6 cm long, erect, glabrous or inconspicuously pilose. Legume ligneous, pending, linearly compressed, 30-60 cm long, 4-7 cm wide. Exotic species known as 'Flamboyant', 'Josefina', 'Acacia'; widely cultivated in tropical regions as ornamental; easily adapted to different soils; very resistant to drought. Widely distributed in Venezuela.

Peltophorum inerme (Roxb.) Naves

Tree 5-12 m high, unarmed, foliage dense and brilliant, conspicuosly ferruginous-tomentose toward young branches. Leaves alternate, bipinnately compound, pinnae 9-18 pairs, leaflets 10-20 pairs, 0.9-1.8 cm long, 5-7 mm wide, oblong, asymmetric basally, emarginated or obtuse apically, brilliant below. Inflorescence a terminal penicle with numerous flowers of a very intense yellow colour. Sepals 5, imbricated, 0.9-1.0 cm long, 5-7 mm wide, ovate-elliptic, pale green below and shiny green above, unequal, ferruginous-puberulent with membranous margins. Petals 5, imbricated, 2.0-2.5 cm long, 1.0-1.5 cm wide, membranous, yellow translucent, obovate, undulate or crenate, conspicuously ferruginous-tomentose toward the base, unequal. Stamens 10, 1.5-2.0 cm long; filaments ferruginous-tomentose at the base, anthers longitudinally dehiscent. Gynoecium 1.5-2.0 cm long, ovary compressed, puberulent, style curved, stigma conspicuous, expanded, peltate. Fruit coriaceous, compressed, margins laminated, conspicuously alate, 6-9 cm long, 2.0-2.3 cm wide.

Exotic species known as 'Acacia', 'San Francisco'; mainly distributed toward hot regions of the country where it is cultivated as ornamentals.

Gliricidia sepium (Jacq.) Stand.

Shrub or tree, 4-10 m high, unarmed, foliage caducous, slightly fragant, generally wide treetop. Leaves alternate, imparipinnately compound, glabrous, shiny green in the adaxial- and pale green in the abaxial surface, leaflets 7-17, 3-8 cm long, 2-2.5 cm wide, oblong-lanceolate or elliptic, acuminate or acute apically, obtuse basally, margin revolute. Inflorescence a loose, pendulous raceme with numerous pinkish flowers, generally before leaves appear. Sepals 5, valvated, fused in a tube, 8-10 mm. Petals 5, imbricated, papilionaceous, standard 28-29 mm long, 15-16 wide, wings 23-24 mm long, 5-6 mm wide, keels 22-23 mm long.

Wood anatomy

Delonix regia (Bojer) Raf.

Wood diffuse porous. The bulk of the wood is composed of thin-walled fibres of polygonal shape. The amount of longitudinal parenchyma is not considerable. The arrangement of the longitudinal parenchyma is contact vasicentric and very scanty aliform confluent (two-cell wide with diamond-shaped calcium oxalate crystals in the cells). Medullary rays one- or two-cell wide (Fig. 1).

Tracheae round or oval, solitary, often duplicate. In groups (of 3-4) seldom to be found they are tangentially flattened. They are 10-13.2-17/mm^2

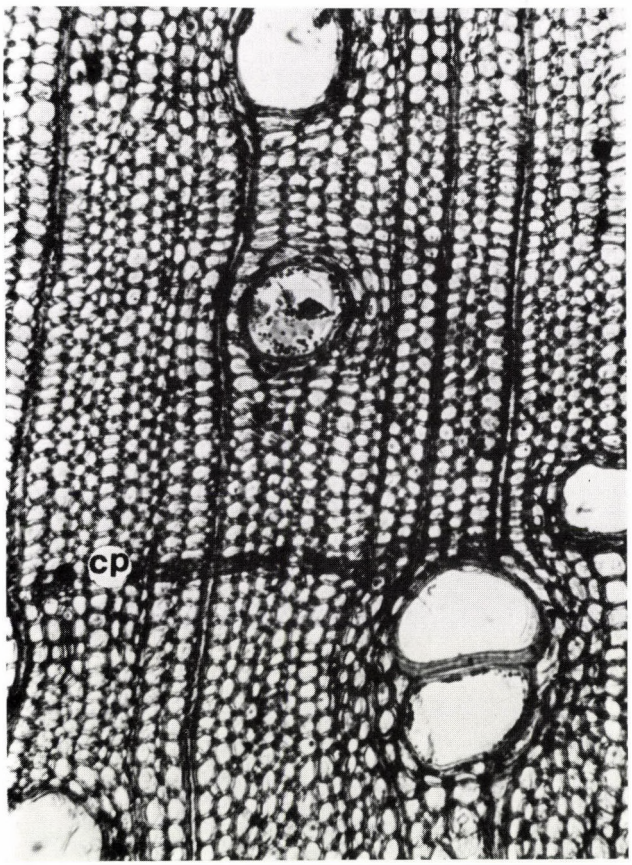

Fig. 1. Delonix regia (Bojer) Raf. Cross section 120 x.
Vessels, medullary rays, fibres. Contact-vasicentric and scanty aliform-confluent longitudinal parenchyma with crystals (crystal-holder longitudinal parenchyma = cp)

Fig. 2. Delonix regia (Bojer) Raf. Tangential longitudinal section 120 x.
One- or two-cell wide medullary rays, vessel, longitudinal parenchyma and fibres. In the walls of vessels medium size, elongated, alternately set bordered pits. Longitudinal parenchyma with crystals (crystal-holder longitudinal parenchyma = cp)

in number. Their tangential diameter is 5.15-78.21-102.30 μm, their radial diameter 46.50-90.86-134.85 μm. Vessel members 161.0-273.7-368.0 μm long with medium size elongate bordered pits of alternate position in the walls. On rare occasions mastic material is found in the tracheae. The perforation plate is simple.

Medullary rays 1-2-cell wide, of homogeneous structure; 57.5-184.92-345.0 μm high, 11.50-14.72-23.0 μm wide (Figs 2, 3).

Fibres arranged in radial rows; their diameter is 9.3-18.87-27.9 μm, their wall thickness 2.32-3.18-4.65 μm, their total length 497.0-708.5-923.0 μm. The fibres end in smooth tips.

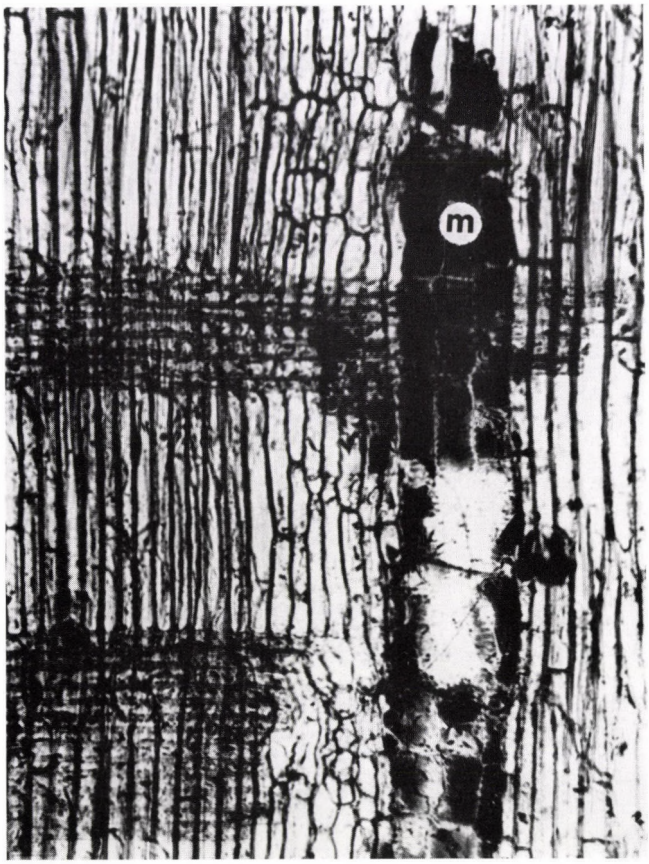

Fig. 3. Delonix regia (Bojer) Raf. Radial longitudinal section 120 x. Homogeneous medullary rays, vessel, longitudinal parenchyma and fibres. In the longitudinal parenchyma cells and in the vessels dark mastic material (mastic material = m)

The tangential diameter of the longitudinal parenchyma cells is 9.3-16.83-27.9 μm, they are 37.3-74.80-101.80 μm in height. Crystal-holder longitudinal parenchyma is not unfrequent.

Peltophorum inerme (Roxb.) Naves.

Wood diffuse-porous. Half of the volume of wood is made up by polygonal shaped fibres with thinner walls, the other half is composed of aliform-confluent longitudinal parenchyma. The medullary rays are one-, two- and three-cell wide, respectively (Fig. 4).

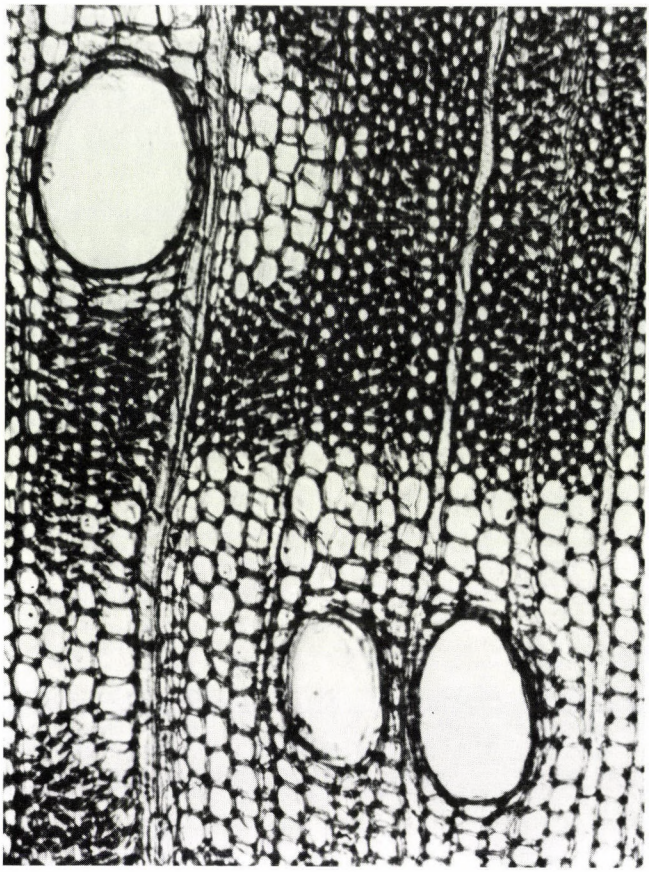

Fig. 4. Peltophorum inerme (Roxb.) Naves. Cross section 120 x.
Vessels, medullary rays and fibres. Wide aliform-confluent longitudinal parenchyma

The oval tracheae are set one by one or in twos. Groups of three are rare; in this case the tracheae are flattened in tangential direction. They are 5-8.73-19/mm^2 in number. The tangential diameter is 74.4-114.39-190.65 μm, the radial one 88.35-148.42-223.20 μm. The vessel members are 138.0-192.28-253.0 μm long with alternately or sometimes irregularly set medium size elongated bordered pits in the walls. The perforation plate is simple.

The medullary rays are one-, two- and three-cell wide, with heterogeneous structure, 69.0-236.44-575.0 μm high and 11.5-31.97-57.5 μm wide (Figs 5, 6).

The fibres are arranged in radial rows. The diameter of fibre is 18.6-29.06-46.5 μm, the thickness of wall 1.16-2.83-4.65 μm. Septate fibres

Fig. 5. Peltophorum inerme (Roxb.) Naves. Tangential longitudinal section 120 x. One-, two- and three-cell wide medullary rays, vessel, longitudinal parenchyma and fibres

are not infrequent. The fibres end in smooth tips. The total length of the fibres is 355.0-653.9-852.0 μm (METCALFE and CHALK 1950).

The tangential diameter of the longitudinal parenchyma cells is 9.3-20.46-32.55 μm, the height of cell is 46.5-84.99-113.9 μm. Septate crystal-holder longitudinal parenchyma is met with.

Gliricidia sepium (Jacq.) Stand.

Wood diffuse-porous. The bulk of wood is made by polygonal shaped fibres with thinner wall and medium lumen. Contact-vasicentric longitudinal parenchyma; medullary rays one- or two-cell wide (Fig. 7).

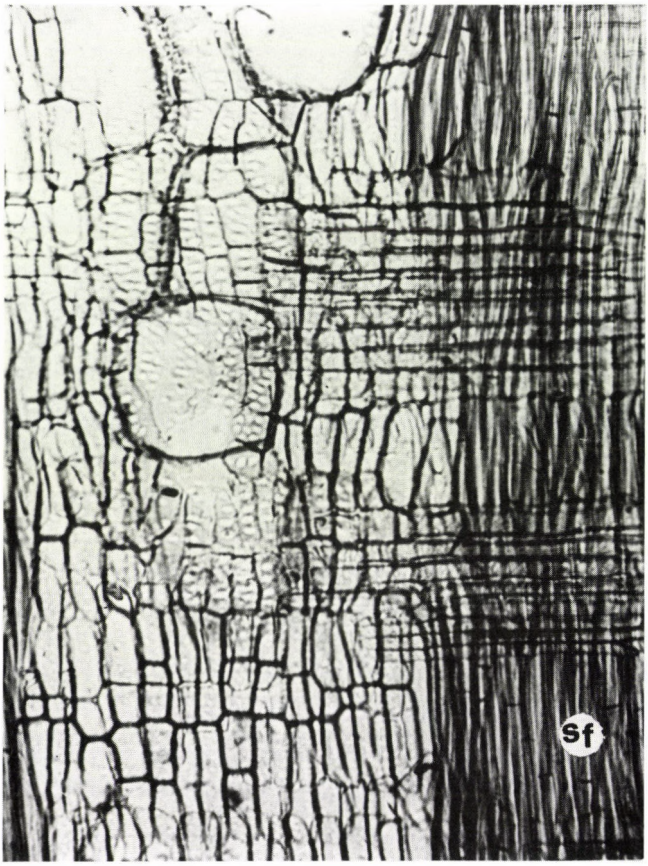

Fig. 6. Peltophorum inerme (Roxb.) Naves. Radial longitudinal section 120 x. Heterogeneous medullary rays, vessels, longitudinal parenchyma, fibres and septal fibres (septal fibre = sf)

The tracheae are oval or round, in the groups (of 2-4) tangentially flattened. They are 9-21.73-41/mm^2 in number. The tangential diameter is 51.15-86.02-125.55 μm, the radial diameter 55.8-99.6-139.5 μm. The vessel members are 207.0-319.24-483.0 μm long, with alternately set medium size bordered pits in the walls. The perforation plate is simple.

The medullary rays are one- or two-cell wide, of heterogeneous structure, 57.5-246.1-425.5 μm long and 11.5-17.71-23.0 μm wide (Figs 8, 9).

The fibres are arranged in radial rows. The diameter of fibre is 9.3-14.06-23.25 μm, the thickness of wall 1.16-2.06-3.48 μm. The total length of fibre is 426.0-778.8-1065.0 μm. The fibres generally end in

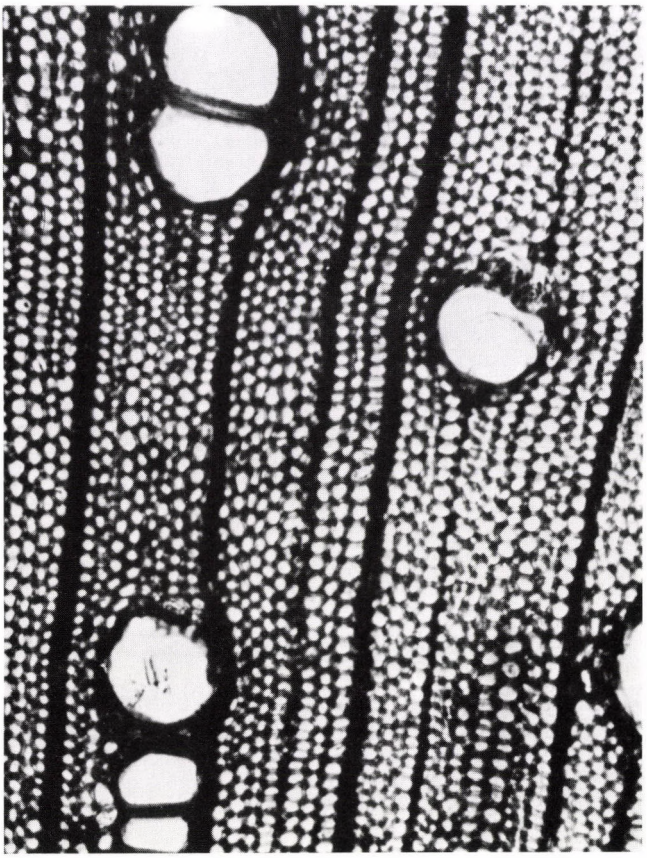

Fig. 7. Gliricidia sepium (Jacq.) Stand. Cross section 120 x.
Vessels, medullary rays, fibres. Contact-vasicentric longitudinal parenchyma

smooth tips though sometimes branch off at one end. Septate fibres often occur.

The tangential diameter of the longitudinal parenchyma cells is 9.3-14.06-18.6 μm; the height of cell is 51.15-92.43-116.0 μm. Septate crystal-holder longitudinal parenchyma is met with, though on rare occasions.

Detailed anatomical characteristics and their measurements for the wood of the three tree species are contained in Tables 1 and 2.

Fig. 8. Gliricidia sepium (Jacq.) Stand. Tangential longitudinal section 120 x. One- and two-cell wide medullary rays, longitudinal parenchyma, vessels and fibres. In the walls of vessels bordered pits of alternate position. In the cells of medullary rays dark mastic material. Septal fibre (septal fibre = sf)

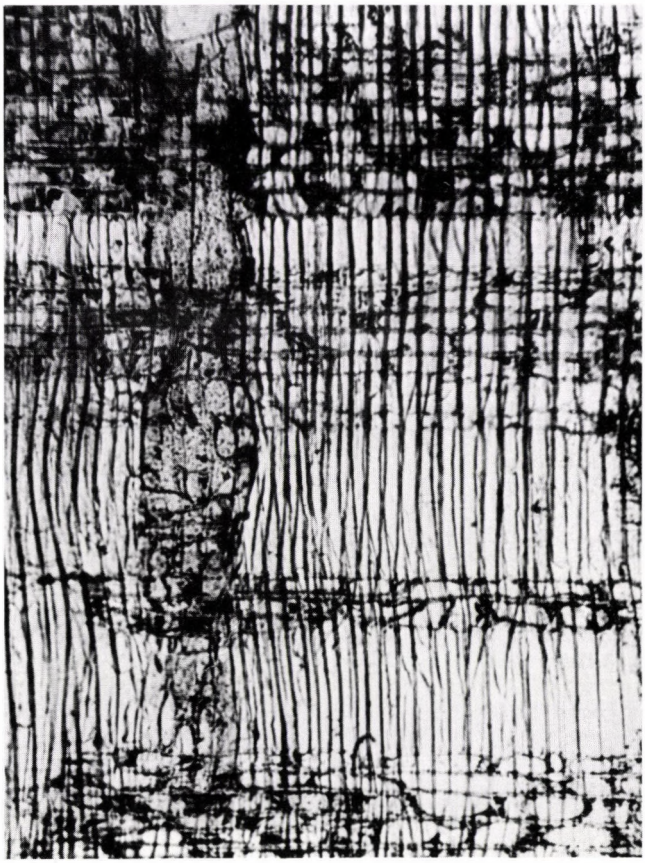

Fig. 9. Gliricidia sepium (Jacq.) Stand. Radial longitudinal section 120 x. Heterogeneous medullary rays, vessel, longitudinal parenchyma and fibres. In the cells of medullary rays dark mastic material

Table 1

Anatomical features of the species examined

Wood elements	Features	Delonix regia	Peltophorum inerme
Trachea	arrangement	diffused, solitary and duplicate, rarely in radial groups of 3-4 members	diffused, solitary and duplicate, rarely in radial groups of 3 members
	shape	roundish or oval shaped in groups in tangential direction flattened	oval shaped in groups in tangential direction flattened
	tangential diam.	51.15-78.21-102.30 μm	74.4-114.39-190.65 μm
	radial diameter	46.50-90.86-134.85 μm	88.35-148.42-223.20 μm
	length of vessels	161.0-273.7-368.0 μm	138.0-192.28-253.0 μm
	number per mm^2	10.0-13.2-17.0	5.0-8.73-19.0
	wall thickness	4.65-7.32-9.30 μm	4.65-7.81-9.3 μm
	intervascular pitting	elongated bordered	elongated bordered
	perforate plate	simple	simple
	content	rarely mastic material	—
Medullary rays	width	narrow	narrow
	number of cells	1-2	1-2-3
	classification	homogeneous	heterogeneous
	height	57.5-184.92-345.0 μm	69.0-236.44-575.0 μm
	width	11.5-14.72-23.0 μm	11.5-31.97-57.5 μm
	content	—	—
Fibres	arrangement	radial rows	radial rows
	shape	polygonal	polygonal
	full thickness	9.3-18.87-27.9 μm	18.6-29.06-46.5 μm
	wall thickness	2.32-3.18-4.65 μm	1.16-2.83-4.65 μm
	full length	497.0-708.5-923.0 μm	355.0-653.9-852.0 μm
	type of pitting	small, bordered	small, bordered
	other	—	septate fibre
Longitudinal parenchyma	arrangement	contact-vasicentric	aliform-confluent
	diameter	9.3-16.83-27.9 μm	9.3-20.46-32.55 μm
	height	37.2-74.8-101.8 μm	46.5-84.99-113.9 μm
	number of cells	3-5	2-3
	content	rarely mastic material	calcium-oxalate crystal
	other	septal crystal-holder long. parenchyma	—

Table 2

Anatomical features of the species examined

Wood elements	Features	Gliricidia sepium
Trachea	arrangement	diffused, solitary or in radial groups of 2-4 members
	shape	oval or roundish shaped in groups in tangential direction flattened
	tangential diameter	51.15-86.02-125.55 μm
	radial diameter	55.8-99.6-139.5 μm
	length of vessels	207.0-319.24-483.0 μm
	number per mm^2	9.0-21.73-41.0
	wall thickness	4.65-6.90-9.30 μm
	intervascular pitting	bordered
	perforate plate	simple
	content	—
Medullary rays	width	narrow
	number of cells	1-2
	classification	heterogeneous
	height	57.5-246.1-425.5 μm
	width	11.5-17.71-23.0 μm
	content	mastic material
Fibres	arrangement	radial rows
	shape	polygonal
	full thickness	9.3-17.34-23.25 μm
	wall thickness	1.16-2.06-3.48 μm
	full length	426.0-778.8-1065.0 μm
	other	septate fibre
Longitudinal parenchyma	arrangement	contact-vasicentricus
	diameter	9.3-14.06-18.6 μm
	height	51.15-92.43-116.0 μm
	number of cells	2-3
	content	calcium-oxalate crystal
	other	—

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COMPARISON OF THE POLLEN OF VARIOUS ANGIOSPERMOUS TAXA AND THE SPORAE OF FERNS FOR PROLINE CONCENTRATION AND QUALITY

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Extracts of pollen from 23 angiospermous and 10 gymnospermous species were analysed for concentrations of free proline and total amino acid content. Microstaining with the "normal" and the new "total" isatin reagent was also carried out. The normal stain which indicates the quality is only positive when the proline concentration of the extracts is above 1.0 per cent. When the total reagent is used, the staining is isatin positive even when the proline concentration of the pollen grains is between 0.2 and 1.0 per cent, but below 0.2 per cent not a single pollen grain will be stained (isatin negative groups). There are species not only among the angiosperms but among the gymnosperms too, in which the proline concentration of the pollen is above 1.0 per cent. We measured the proline concentration and reaction with total isatin stain of sporae from 17 fern species. The sporae of these fern species contain either very little or no proline. The total staining of sporae is negative too. According to our examinations there is no transition between the very high (isatin positive) and very low (isatin negative) proline concentration of the pollen grains. Our new total stain is suitable to classify the pollen grains on the basis of their proline contents. The normal reagent may indicate the quality of the pollen grains whereby it can be used to select not only those varieties which produce good quality i.e. fertile pollen but also the ones that produce male sterile pollen. In isatin positive species the dominance of proline compared to the total amino acids ranges between 52 and 65 per cent. From the total amino acid concentration without proline it turns out that proline accumulates not at the expense of other amino acids, but appears as a plus.

Introduction

It has been pointed out for numerous plant species that the concentration of free proline in their mature pollen grains is extremely high, and the amount of proline may express the degree of their quality (YAMADA and KONO 1976; AHOKAS 1978; ALARKON et al. 1978; HESLOP-HARRISON 1979; KURSAKOV and RYZHKOV 1980; DASHEK and MILLS 1981; ZHANG et al. 1982; ZHANG and CROES 1983).

However, from the above works it does not become evident what the term high or extremely high means in respect of the proline concentration of

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pollen. Nor can it be found out from them whether the high proline content of pollen grains applies to all plant species, or there are species whose mature pollen grains contain little proline. A further question: what concentration of proline is meant by the term "little"? It is not known either, whether there is any transition between the high and low proline concentrations of the various species.

To answer all these questions by analysing pollen extracts from all plant species for proline concentration is a very difficult, almost unfeasible task.

As a first step we developed a simple and quick staining method by which the pollen grains take considerably different colours on the very basis of their proline concentrations (PÁLFI and PÁLFI 1982; PÁLFI and KÖVES 1984; PÁLFI and MIHALIK 1985; GULYÁS and PÁLFI 1986a,b; PÁLFI et al. 1987a,b).

In the staining- or other methods used so far to determine the quality of the pollen grains fresh, live pollen grains had to be used in each case. Our technique, on the other hand, gives appraisable results for the proline content of pollen grains even when the grains are fixed and dried at 90 ^{o}C within 5 days after the date of collection; staining can then be carried out any time.

On the basis of the results of examinations on 167 species from 36 families of Angiospermae (PÁLFI and KÖVES 1984; PÁLFI and GULYÁS 1985; PÁLFI and MIHALIK 1985; GULYÁS and PÁLFI 1986a,b; PÁLFI et al. 1987a, b), from the point of view of the proline concentration of pollen grains the species can be divided in two large groups: 1) "Isatin positive" are those species in which the proline concentration of mature and vital pollen grains exceeds 1.0 per cent of the dry matter; 2) the proline content of pollen grains in the "non-isatin positive" species was found to remain much below 1.0 per cent (generally does not even exceed 0.2 per cent).

In the experiment here described we studied the proline concentration and staining with normal and total isatin reagent of pollen grains from angiospermous families and species not so far examined by us.

Analyses were also performed with pollen grains of lower species, i.e. with those of 10 gymnospermous species. Furthermore, sporae of ferns were also examined for proline concentration and for responsiveness to staining on the basis of proline content. Namely, GEMMRICH (1975) pointed out a very high proline concentration in fern sporae. According to his results 55 per cent of the total amino acid content of the sporae was made up by proline

alone. In the course of our work we continued to study the non-isatin positive species not so far examined by us. We also tried to find out whether there was any transition between the isatin positive and isatin negative type of pollen in the individual species. With 18 species we looked into the question whether the high proline accumulation of the isatin positive pollen grains occurred at the expense of other amino acids and of the total amino acid content, respectively.

Material and Method

The pollen grains were collected in the Botanical Garden of our University. The names of the families and species are given in the figures and tables. Two kinds of isatin reagent giving intensive staining with proline were prepared: 1) The "normal" isatin reagent is a mixture of 18 ml acetone, 2 ml isopropanol and 0.6 ml concentrated acetic acid, in which 0.2 g isatin is dissolved. This reagent stains the "mature" pollen grains and indicates their quality in those species which belong to the isatin positive type. 2) The "total" isatin reagent is composed of 20 ml methanol, 0.8 ml concentrated acetic acid, and 0.25 g isatin dissolved in the mixture of the two. Total staining is only suitable to decide whether the species examined is of isatin positive type or not. If the pollen of the species is of isatin positive type, the pollen grains (and even the immature but already developed microspores) stain almost uniformly (98%) black. On the other hand, if the species is not isatin positive, not a single pollen grain will stain black.

The details of the staining technique were described in earlier papers (PÁLFI and KÖVES 1974; Pálfi and MIHALIK 1985; GULYÁS and PÁLFI 1986a, b). Staining was evaluated in the visual field of the microscope at 100-300 x magnifying. For the qualification colour data of 5 visual fields (about 100 pollen grains 5-times) were averaged. Qualification of normal staining: pollen grains stained black and dark blue represent the excellent and good quality pollen. Medium blue and bluish-green pollen grains are indicative of medium quality, while those left unstained, that is the yellow, light brown and other colours suggest poor quality. The proline concentrations of the amino acid extracts of pollen were determined by the method of PALEG and ASPINALL (1981) while the amount of total amino acid was measured after ROSEN (1957).

Results and Discussion

With insect- and self-pollinating plant species it is often difficult to tell how many of the pollen grains contained in the anthers are mature. If proline type but immature pollen grains are stained with the normal (quality) isatin reagent, often a quite negative staining may be obtained. Namely, the proline content of the pollen grains is still low -- 0.2 to 1.0 per cent. Staining the same immature but developed pollen grains with total reagent gives in each case positive i.e. black colour if the pollen of the species is of isatin positive type. If the pollen is not of isatin type, none of the pollen grains will stain black.

On Plate I microphotoes show the result of "normal" (quality) staining of pollen from 10 species for which a previous "total" staining had undoubtedly pointed out the isatin positive type of pollen.

Plate I shows the result of staining pollen grains with quality isatin reagent for dicotyledonous species of angiosperms in Figs 1 to 6 and for monocotyledonous species of them in Figs 7 to 10. It can be established that many of the pollen grains in the pictures are stained black, that is, they generally are mature and of good quality (in the pictures pollen grains stained dark blue also appear to be black). Poor quality pollen grains show various greyish shades in the photoes. Anyway, it must be noted that colours well differentiated by quality can be seen under the microscope. The photo, on the other hand, shows the dark grey and dark brown pollen grains uniformly black in the case of intensive development.

The proline concentrations of amino acid extracts of pollen and the results of isatin staining are given in tables below.

Table 1 contains 13 dicotyledonous and 10 monocotyledonous species form 14 families, with isatin positive type pollen. It can be seen that the proline concentrations of the pollen extracts range between 1.18 and 2.25 per cent, and the percentage values (from 28 to 91%) of the positive isatin reaction change in direct ratio to the proline concentrations. Further, the table makes it clear that the proline concentrations of extracts from mature pollen grains of isatin positive type species are really above 1.0. The pollen grains of the 23 plant species included in Table 1 were stained with the normal (quality) isatin reagent, and the colours were evaluated by microscope examination.

According to TUPY (1963), STANLEY and LINSKENS (1974), BRITIKOV (1975), AHOKAS (1978), KURSAKOV and RYZHKOV (1980), ZHANG et al. (1982) and ZHANG and CROES (1983) from the proline content of the pollen grains conclusions on the quality can also be drawn. Namely, the authors mentioned found the pollen grains with high proline concentrations to be more resistant to drought and to low air humidity. PALEG and ASPINALL (1981), THEBUD and SANTARIUS (1981), ZHANG et al. (1982), CLOUTIER and SIMONOVITCH (1983) and VAN SWAAIJ et al. (1985) point out that the high proline content of the pollen grains provides protection from cold and frost. ANBAZHAGAN et al. (1988) poisoned the atmosphere of rice plants of 3 varieties with SO_2, NH_3 and NO_2 gas for 4 days over 2 hours a day; then measured the free amino acid concentrations in several shoots of each variety, and gathered in the yield of the remaining plants. He found the highest proline concen-

Table 1

Proline concentrations of amino acid extracts of pollen from di- and monocotyledonous species, in terms of dry matter percentage, and percentage of isatin positive reactions given by the pollen trains with "normal (quality) stain". Isatin positive reaction is given by those pollen grains which with the normal reagent stain black on the basis of their high proline contents

Families	Species	Proline concentration of the extracts	Positive reaction with isatin
		(per cent) %	
Paeoniaceae	Paeonia officinalis	1.56	55
Rosaceae	Rosa canina	2.08	89
Fabaceae	Medicago sativa	1.64	67
	Pisum sativum	1.53	54
	Phaseolus vulgaris	1.46	47
Elaeagnaceae	Elaeagnus angustifolia	1.51	53
Hippocastanaceae	Aesculus hippocastanum	1.96	85
Umbelliferae	Anthriscus caucalis	1.42	47
Caprifoliaceae	Sambucus nigra	1.37	44
Labiatae	Glechoma hederacea	1.71	68
Violaceae	Viola arvensis	1.18	28
Fagaceae	Fagus silvatica	1.24	32
Salicaceae	Populus robusta	1.29	35
Liliaceae	Lilium longiflorum	1.36	43
	Lilium regale	1.41	46
	Colchicum arenarium	1.79	80
	Allium sativum	1.62	65
Iridaceae	Iris pseudacorus	1.37	43
Gramineae	Sorgum vulgare	2.25	91
	Sorgum halepense	2.04	87
	Glyceria maxima	1.57	58
	Puccinellia distans	1.32	29
	Alopecurus pratensis	1.34	42

There is a close positive correlation between the two variables.

The value of the correlation coefficient: r = 0.97528, is significant at 0.1% level. The regression equation: y = 668449x - 486849.

tration in the variety most resistant to air pollution. According to the author, the proline is such a stress-amino acid which gives protection not only against water deficiency and dry air, or against high salt content of soil and effect of cold, but also in the case of air pollution. In accordance with the above, in Table 1 the positive isatin reaction, that is the percentage values of pollen grains stained dark blue and black express the proportion of excellent and good quality pollen grains. This fact is supported by the results of LINSKENS (1974), MASCARENHAS (1975), DASHEK and MILLS (1981) too.

Table 2

Proline concentrations of amino acid extracts of pollen from di- and monocotyledonous species, in terms of dry matter percentage, and "isatin negative" reactions of pollen grains with total stain. In the case of isatin negative reaction none of the pollen grains stain black even when the total stain is used

Families	Species	Proline concentration of the extracts (per cent) %	Positive reaction with isatin
Convolvulaceae	Convolvulus arvensis	0.05	—
Boraginaceae	Cynoglossum officinale	0.07	—
Hypericaceae	Hypericum perforatum	0.05	—
Compositae	Cirsium arvense	0.07	—
	Bellis perennis	0.09	—
Polygonaceae	Rumex crispus	0.05	—
	Rumex pulcher	0.07	—
	Rheum palmatum	0.06	—
Gramineae	Dactylis glomerata	0.06	—
	Agropyron repens	0.07	—
	Bromus inermis	0.05	—
	Poa pratensis	0.06	—
	Holcus lanatus	0.08	—
	Festuca pratensis	0.06	—
	Festuca arundinacea	0.05	—
	Festuca vaginata	0.06	—

Average deviation being below ± 5 per cent; n = 3.

In the course of further investigations we studied species with pollen belonging to the "non-isatin positive type" (Table 2).

As seen from Table 2 the proline concentrations of amino acid extracts from the pollen of di- and monocotyledonous species did not even reach 0.2 per cent, therefore we obtained negative results not only with the normal but also with the total isatin staining, that is, not a single pollen grain stained black.

We then studied the isatin staining of sporae from fern species and of pollengrains from some gymnospermous species. Several pictures of total isatin staining are shown in Plate II.

Figures 1 to 6 on Plate II show that the sporae of the fern species examined do not stain at all with the total isatin reagent, that is, their proline contents either are very low, or this amino acid is not even present in them.

Figures 7 and 8 on Plate II represent pollen grains from higher plants, from 2 gymnospermous species. As seen in the pictures, the pollen

Table 3

Proline concentration of amino acid extracts of sporae from fern species and pollen from gymnospermous species, in terms of dry matter percentage; and isatin negative and isatin positive reactions, respectively, given by the sporae and pollen grains with "total stain"

Families	Species	Proline concentration of the extracts (per cent) %	Positive reaction with isatin %
	Spores		
Ophioglossaceae	Botrychium lunaria	—	—
Polypodiaceae	Polypodium crassifolium	Trace	—
	Polypodium brasiliense	—	—
	Platycerium alcicorne	Trace	—
	Platycerium aureum	Trace	—
	Pyrrosia angustata	—	—
Pteridaceae	Pteris longifolia	0.05	—
Demstaedtiaceae	Pteridium aquilinum	Trace	—
Aspidiaceae	Dryopteris filix-mas	—	—
	Dryopteris assimilis	—	—
	Phegopteris connectilis	Trace	—
Aspleniaceae	Asplenium nidus	—	—
	Asplenium trichomanes	—	—
	Asplenium viride	—	—
	Cystopteris fragilis	0.06	—
Athyriaceae	Athyrium filix-femina	Trace	—
Nephrolepidaceae	Nephrolepis exaltata	0.07	—
	Pollens		
Taxaceae	Taxus baccata	Trace	—
Abietaceae	Picea abies	1.16	32
	Pinus silvestris	1.27	38
	Pinus nigra	1.43	46
	Pinus strobus	1.38	41
	Pinus mugo	1.25	35
	Pinus griffithii	1.41	44
Cupressaceae	Juniperus communis	Trace	—
	Juniperus virginiana	Trace	—
	Juniperus chinensis	Trace	—

There is a positive correlation between the two variants of pollen.
The value of the correlation coefficient: r = 0.98073 is significant at 0.1% level. The regression equation: y = 492949x - 255717

grains contain considerable amounts of proline, as they have stained uniformly black with the total reagent. Thus, the pollen of the 2 gymnospermous species is of isatin positive type.

We analysed the amino acid extracts of sporae from 18 fern species and of pollen from 10 gymnospermous species for proline concentration, and

Table 4

Proline- and total amino acid concentrations (the latter with- and without proline) of amino acid extracts of pollen from gymnospermous and angiospermous species, and percentage ratio of proline to total amino acids. The pollen is isatin positive if its proline concentration exceeds 1.00 per cent of the dry matter content

Species	Proline	Total amino acid with proline	Proline in per cent of the total amino acid	Total amino acid without proline
Gymnosperm — isatin positive and negative				
Taxus baccata	Trace	1.18	—	1.18
Picea abies	1.38	2.64	52.27	1.26
Pinus nigra	1.37	2.62	52.29	1.25
Pinus strobus	1.38	2.65	52.07	1.27
Pinus mugo	1.53	2.87	53.31	1.34
Juniperus communis	0.06	1.47	4.08	1.41
Juniperus virginiana	0.05	1.43	3.50	1.38
Juniperus chinensis	0.05	1.31	3.81	1.26
Angiosperm, dicotyledon, isatin positive				
Rosa canina	2.16	3.48	62.07	1.32
Tilia cordata	1.49	2.74	54.38	1.25
Petunia hybrida	1.34	2.54	52.75	1.20
Papaver rhoeas	1.52	2.84	53.52	1.32
Dahlia variabilis	1.40	2.66	52.63	1.26
Corylus avellana	1.55	2.92	53.08	1.37
Juglans regia	1.37	2.59	52.89	1.22
Salix babylonica	1.63	2.94	55.44	1.31
Monocotyledon — Isatin positive				
Colchicum autumnale	2.18	3.51	62.11	1.33
Allium cepa	1.96	3.38	57.99	1.42
Iris germanica	1.37	2.62	52.29	1.25
Secale cereale	1.30	2.48	52.42	1.18
Zea mays	2.38	3.62	65.74	1.24
Sorgum halepense	2.19	3.56	61.52	1.37
Dicotyledon — Isatin negative				
Brassica napus	0.08	1.53	5.23	1.45
Begonia semperflorens	0.06	1.44	4.17	1.38
Cucurbita pepo	0.06	1.49	4.03	1.43
Cucurbita maxima	0.05	1.46	3.42	1.41
Helianthus annuus	0.07	1.34	5.22	1.27
Monocotyledon — Isatin negative				
Tulipa germanica	0.05	1.21	4.13	1.16
Narcissus pseudo-narcissus	0.08	1.38	5.79	1.30
Dactylis glomerata	0.05	1.40	3.57	1.35
Agropyron repens	0.07	1.31	5.34	1.24
Bromus inermis	0.06	1.53	3.92	1.47
Festuca pratensis	0.08	1.46	5.47	1.38

The values for the gymnospermous species are highly varied. The deviation from the monocotyledonous and dicotyledonous species of Angiospermae is significant at 0.5% only. There is no significant difference in the isatin positive and isatin negative groups between monocotyledons and dicotyledons. Between the isatin positive and isatin negative groups, on the other hand, there is significant difference at 0.1% level.

carried out the total staining of sporae and pollen grains. The results are shown in Table 3.

Accordingly, the amino acid extracts of sporae from the fern species examined contain very little or mere traces of proline, if this amino acid is present at all. This result is confirmed by the evaluation of the total isatin staining which was completely negative.

Further, Table 3 shows that the proline concentration of pollen from 6 of the 10 gymnospermous species examined exceeded 1.0 per cent. The pollen grains of these 6 species gave positive reaction to staining with the normal (quality) isatin (32-46%), in proportion to their proline contents.

The 5 Pinus species included in Table 3 gave uniform positive and the 3 Juniperus species negative isatin staining (in the case of either normal or total staining). This confirms the conclusion of our earlier works (PÁLFI and GULYÁS 1985; PÁLFI and MIHALIK 1985; GULYÁS and PÁLFI 1986a,b; PÁLFI et al. 1987a,b) that the pollen grains of species belonging to the same genus are all either of isatin positive or of isatin negative type. This fact can be considered as a chemotaxonomic character.

The ratio of proline to the total amino acid content is shown in Table 4.

Table 4 makes it obvious that the pollen of species can be really divided in two large groups on the basis of proline concentration: 1 = isatin positive, and 2 = isatin negative group.

A further evidence of Table 4 is that between these two groups of proline concentration in the pollen of the species examined no transition (from 0.08 to 1.18%) could be pointed out, as it turns out from Tables 1, 2, and 3 and from our earlier papers (PÁLFI and MIHALIK 1985; PÁLFI and GULYÁS 1985; GULYÁS and PÁLFI 1986a,b; PÁLFI et al. 1987a, b) too.

The essence of Table 4 is that the large amount of free proline is synthetized not at the expense of other amino acids, but appears as a plus. This is supported by the fact that the total amount of amino acids without proline is almost the same for the species of the isatin positive, and isatin negative group. Consequently, the total amino acid concentration (including proline) of the isatin positive group is substantially higher than that of the isatin negative group (2.48—3.62% compared to 1.18—1.53%). The proline content of the isatin positive pollen makes up by itself more than half of the total amino acid content (52.07—65.74%). The corresponding value for the isatin negative pollen ranges between 3.42 and 5.79%.

Our present method, similarly to other staining- and tube-germinating techniques used in studying the fertility of pollen, is a sort of indicator for pollen quality. However, it can be used for quality determination only with the pollen of isatin positive species. Of course, most of the farm crops can be placed in the isatin positive type (e.g. all cereals, food- and feed plants belonging to the family Fabaceae, food plants of Solanaceae, forest trees, etc.).

Attempts have recently been made to select, or produce by chemical treatment male sterile varieties for plant breeding purposes. TUPY (1963), STANLEY and LINSKENS (1974), AHOKAS (1978), ALARKON et al. (1978) found the pollen of male sterile plants to contain very small amounts of or no proline. It follows that our isatin reagents can be used to identify male sterile varieties, as supported by the works of RAI and STOSKOFF (1974).

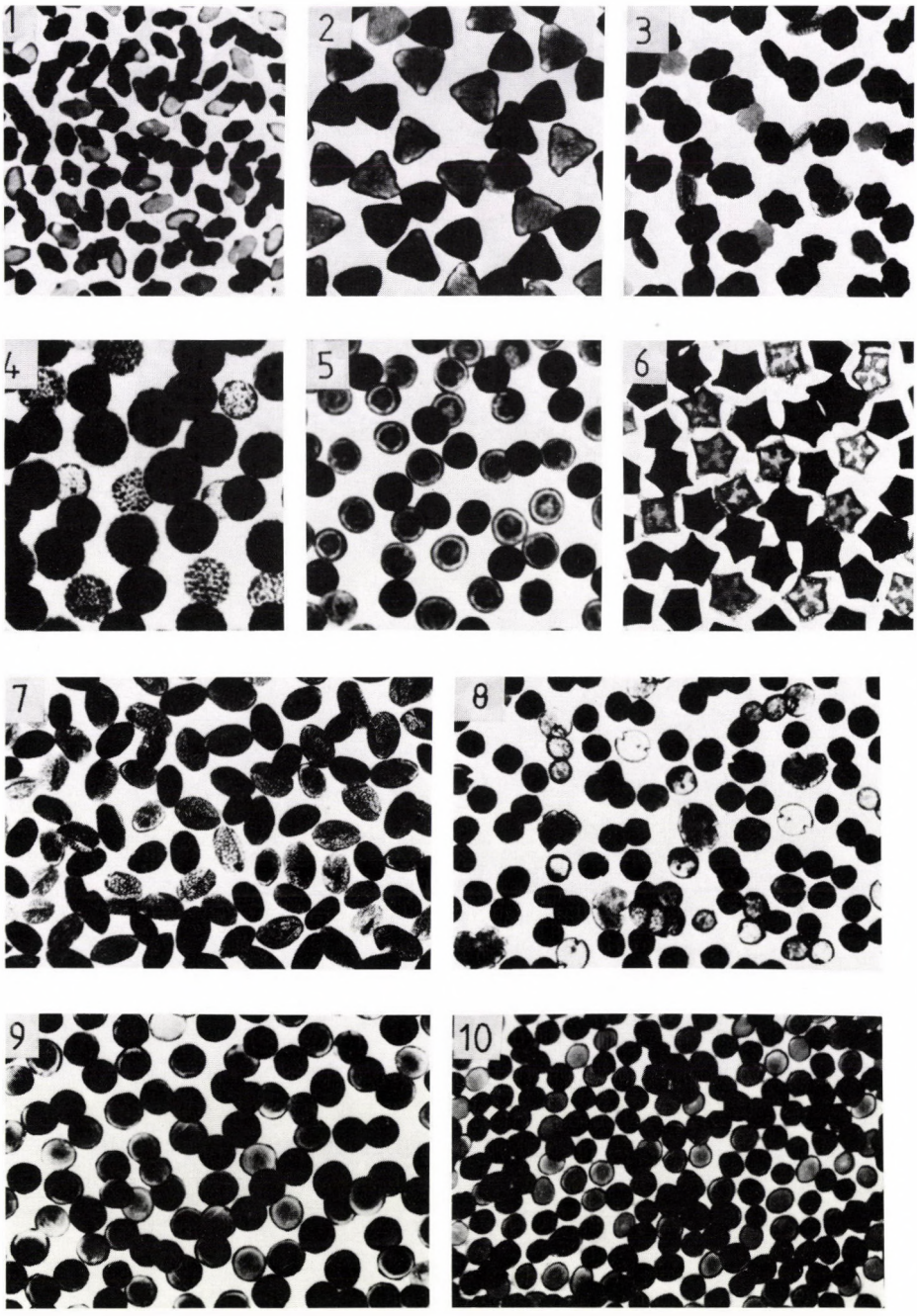

Plate I

"Isatin positive" staining of 6 dicotyledonous and 4 monocotyledonous species in the case of using the "normal reagent". The good and excellent quality pollen grains stained black, the poor ones show shades of grey.

Fig. 1. Anthriscus caucalis Fig. 2. Persica vulgaris Fig. 3. Glechoma hederacea Fig. 4. Abutilon theophrasti Fig. 5. Juglans regia Fig. 6. Viola arvensis Fig. 7. Lilium longiflorum Fig. 8. Iris germanica Fig. 9. Sorghum halepense Fig. 10. Triticum aestivum

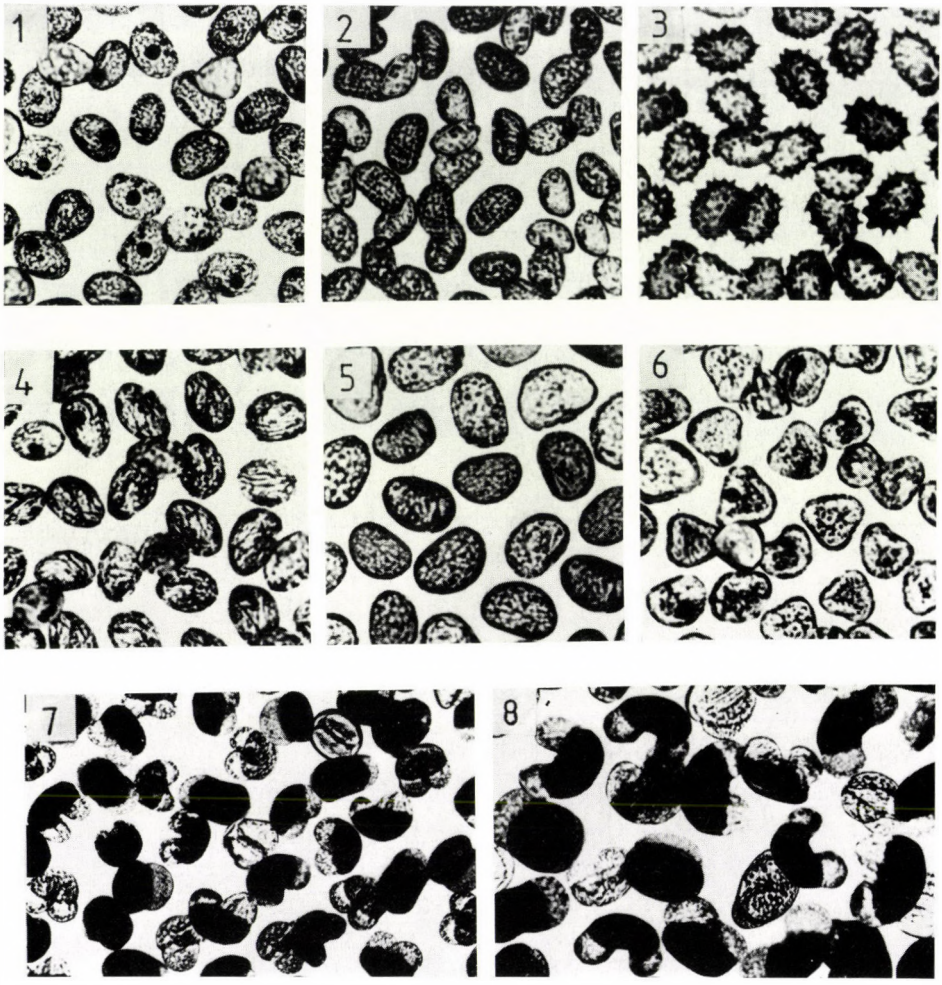

Plate II

Sporae of the 6 fern species gave negative staining with the "total isatin reagent" (Figs 1 to 6), none of the sporae stained black. The pollen grains of the two gymnospermous species showed positive (black) staining with the total reagent. The pollen of these species is "isatin positive".

Sporae: Fig. 1. Polypodium crassifolium Fig. 2. P. brasiliense Fig. 3. Cystopteris fragilis Fig. 4. Pyrrosia angustata Fig. 5. Nephrolepis exaltata Fig. 6. Botrychium lunaria

Pollen: Fig. 7. Pinus silvestris Fig. 8. Picea abies

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CUNNINGHAMIA R.BR. IN THE POLLEN SPECTRA OF CENTRAL EUROPE

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Cunninghamia R.Br. represents an exotic conifer with its present area of distribution in the southeastern part of Asia, and in the middle and southern China and in Taiwan. It was one of the common accessory elements of the European mixed forest during the Tertiary. Its pollen grains are distinct in morphology enough to be differentiated from the other inaperturate pollen types of Taxodiaceae. The morphology of modern and fossil pollen and their taxonomy are discussed, and the new species Cunninghamiaepollenites janinae sp. nov. is described.

Introduction

During our palynological investigation of sediments from the Tertiary basins in Bohemia and Poland we have found several pollen grains that differ from the bulk of inaperturate pollen and are not fully assignable to any artificial taxa of pollen known up to now. In detailed study of their morphology in the light microscope (LM) we have recognized the near relationship with the modern pollen of a Chinese conifer Cunninghamia. This relation stimulated the comparative study of modern pollen of Cunninghamia lanceolata (Lamb.) Hook. The modern pollen material was derived from the Limprecht Herbarium located at sporotheca of the W. Szafer Institute of Botany, Polish Academy of Sciences in Kraków and from the cultivated specimens of the Botanical Garden, Charles University, Prague, Czechoslovakia. On the basis of these studies we have concluded that it was possible to identify Cunninghamia in the Tertiary pollen spectra as a botanical taxon on the level of the genus. Therefore we review the main morphological features of modern pollen grains, their size and morphological variability, contributing to the identification of fossil pollen. The taxonomy and nomenclature of fossil pollen is given.

Modern Cunninghamia is one of the very interesting elements of the conifer and mixed mesophytic forests in southeastern China and Taiwan.

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Its contemporary area demonstrates a relict character in comparison with Tertiary distribution. It was also spread in Europe during Tertiary, especially in Neogene. The rests of twigs, cones and needles are known. The first pollen grains compared and assigned to Cunninghamia were announced from the Green River Formation, U.S.A. by WODEHOUSE (1933), in Europe they were described from the sediments bearing macroscopic plant remains of Cunninghamia at the locality Stare Gliwice in Upper Silesia, South Poland (OSZAST in SZAFER 1958; OSZAST 1960).

This work was carried out on the basis of an official cooperation prgramme between the Institute of Geology and Geotechnics, Dpt. of Paleontology, Czechoslovak Academy of Sciences, Prague and the W. Szafer Institute of Botany, Dpt. of Palaeobotany, Polish Academy of Sciences, Kraków.

Cunninghamia in the modern forest

The genus Cunninghamia is represented only by two species -- C. lanceolata (Lamb.) Hook. and C. konyshii Hayata. The last one is an endemic species whose area of distribution is limited to the island of Taiwan, where it is a rare element of the mountain forest. C. lanceolata (Fig. 1) has much larger area of distribution: central and southwestern parts of China and rarely in north Vietnam. It is an element of mixed mesophytic forest of the warm temperate zone (Figs 2, 3). Cunninghamia occurs there as an admixture tree associated with Picea morris, Tsuga chinensis, Taxus wallichiana, Pinus sp.div., Pseudotsuga wilsoniana a.o., mainly in the highlands and in the mountains, at the altitude between 800-1300 m (DALLIMORE-JACKSON 1934). In the valleys, especially in the Yang-Tse Valley it forms a dense coniferous forest together with Pinus species. In the lower mountain valleys, ut to 1000 m it is associated with Pinus massoniana, Liquidambar formosana, Cephalotaxus fortunei, Taxus chinensis, Pseudolarix amabilis, Cryptomeria japonica, Ginkgo biloba, Liriodendron chinense, Magnolia officinalis, Machillus ichgangensis, Nyssa sinensis, Pistacia chinense, Sassafras tsunen, Tilia japonica, T. oblongifolia, Ulmus arbifolia, U. davidiana, Zelkova sinica (WANG-CHI-WU 1961).

The finds of Cunninghamia remains in Central Europe (Fig. 4)

The macrofossils of the genus Cunninghamia were described by SZAFER (1958) from the Tertiary sediments at the locality Stare Gliwice in Upper Silesia as a new species C. europaea Szafer together with cones, twigs,

Fig. 1. Morphology of Cunninghamia lanceolata (Lamb.) Hook.
(ex KRÜSSMANN 1972, after BEISSNER)

needles, cuticles and pollen grains compared with Cunninghamia lanceolata (Lamb.) Hook., (OSZAST in SZAFER 1958). The accompanying assemblage of spores and pollen spectra of Stare Gliwice, inclusive Cunninghamia pollen, were described in more detail by OSZAST (1960). Both authors (SZAFER and OSZAST 1958) demonstrated besides the fossil remains of the genus also the modern species of Cunninghamia, C. lanceolata (Lamb.) Hook. The macroscopic plant remains of Cunninghamia were there found for the first time together with pollen grains in the same sedimentary horizon. Nevertheless, pollen grains of Cunninghamia were mainly overlooked in the European spectra or they were fused together with other inaperturate types. Outside of Europe pollen grains of Cunninghamia were reported from Japan and lower Tertiary of U.S.A., wherefrom they were originally described (WODEHOUSE 1933). They are occurring from the Tertiary up to the Recent time in the southern part of Asia.

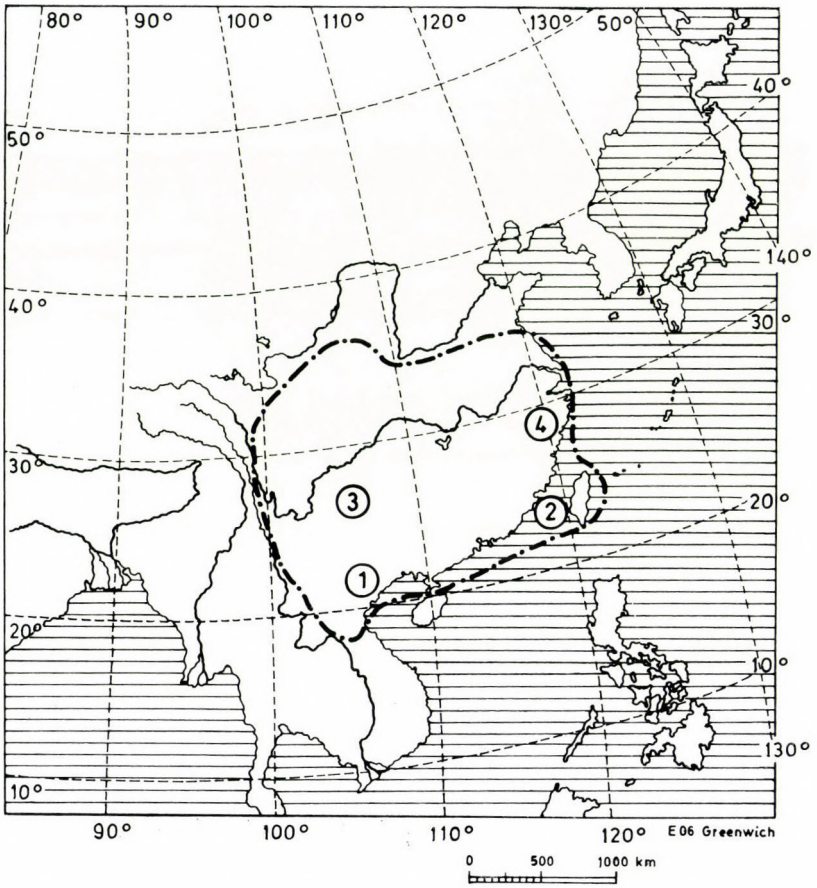

Fig. 2. Recent distribution of the modern genus Cunninghamia R.Br. (after TRIFONOVA 1978)

The macroscopic plant remains of Cunninghamia from Central Europe have been known since the last century (ETTINGSHAUSEN 1872). Further finds, beside that of SZAFER (1958, 1961) have been reported e.g. from the Badenian of Wieliczka (ZABŁOCKI — aff., oral communication of M. ŚRODONIOWA), from the Egerian locality Krumvír in Moravia (KNOBLOCH 1975), Egerian near Linz in Austria (KOVAR 1982), West Germany (KLIPPER 1968), East Germany (SCHNEIDER 1974), Hungary (ANDREÁNSZKY 1959), Bulgaria (PALAMAREV, PETKOVA, USUNOVA 1978), from the Pannonian of Roumania (GIVULESCU 1968) a.o. (Fig. 5) and Neogene of U.S.S.R. (SVESHNIKOVA and BUDANCEV 1959).

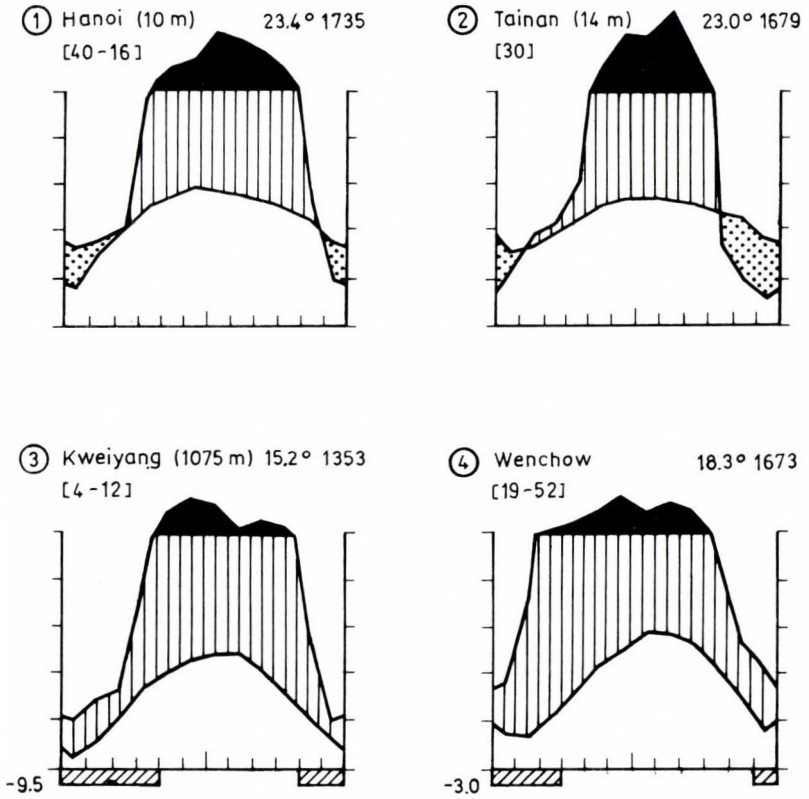

Fig. 3. Climatic diagrams of some localities within the area of Recent distribution of Cunninghamia R.Br.

Remarks on systematic of pollen grains

The taxonomic suggestions of the fossil pollen were based on morphological studies of Cunninghamia lanceolata pollen from the recent collections and on published data (ERDTMAN 1943, 1957, 1965). The fossil material is documented by microphotographs and detailed descriptions. The Cunninghamia pollen are well distinguished from other inaperturate types of the group Taxodiaceae-Cupressaceae, when they are preserved with the perine present. Some complications are connected with the nomenclature of fossil pollen grains. WODEHOUSE (1933) described, for the first time, fossil pollen of this genus from the Eocene sediments of the U.S.A. and named it Cunninghamia concidipites sp.nov. According to the nomenclature rules this name is invalid for the fossil taxon. NAGY (1969) described this type of pollen

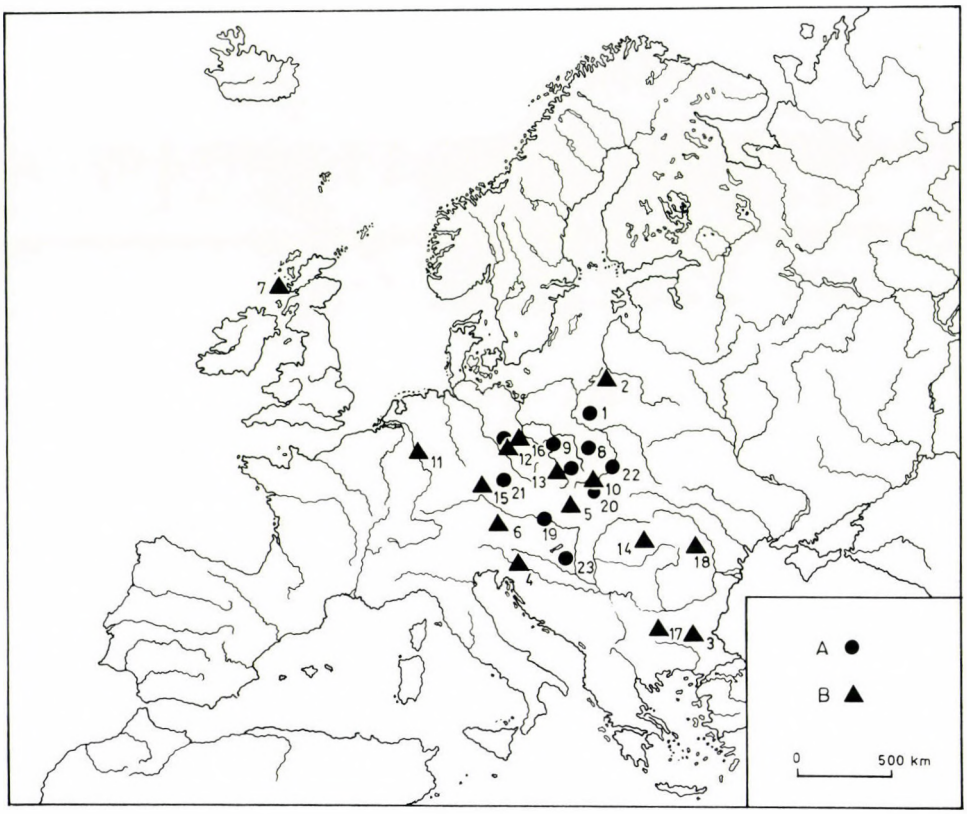

Fig. 4. Tertiary fossil finds of Cunninghamia in Europe (after KOVAR 1982 — supplemented). A = pollen; B = macroscopic remains: 1/ Rypin, NW Poland (STUCHLIK 1964); 2/ Peninsula Kaliningrad, USSR (SVESHNIKOVA et al. 1959, SVESHNIKOVA 1963); 3/ Bulgaria (PALAMAREV et al. 1966); 4/ Zavina, Yugoslavia (ETTINGHAUSEN 1872); 5/ Krumvíř, ČSSR (KNOBLOCH 1969, 1975); 6/ Ebelsberg, Pucking, Austria (KOVAR 1982); 7/ Mull Island, Scotland (JOHNSON 1936); 8/ Bełchatów, Central Poland (STUCHLIK et al. in press); 9/ Oczkowice, Western Poland (ZIEMBIŃSKA-TWORZYDŁO 1974); 10/ Wieliczka, South Poland (ŁAŃCUCKA-ŚRODONIOWA 1984); 11/ Rheineland, West Germany (KILPPER 1968); 12/ Lower Lusatia, GDR (LITTKE 1964); 13/ Stare Gliwice, Upper Silesia, Poland (SZAFER 1958, 1961, OSZAST 1960); 14/ Cluj and Timpa, Romania (GIVULESCU 1973); 15/ Oberpfalz BRD (GREGOR 1978); 16/ Bitterfeld and Lower Lusatia, GDR (KRUTZSCH 1971, SCHNEIDER 1974, 1979); 17/ NW Bulgaria (PALAMAREV et al. 1978); 18/ Valea Neagru and Deleureni, Romania (GIVULESCU 1962, 1968, 1975); 19/ Zillingsdorf, Austria (KLAUS 1951); 20/ Czarny Dunajec, Western Carpathians, Poland (OSZAST and STUCHLIK 1977); 21/ Cheb Basin, Northern Bohemia, ČSSR (KONZALOVA and STUCHLIK 1983); 22/ Piaseczno near Tarnobrzeg, East Poland (OSZAST 1967); 23/ Mecsek Mts, Hungary (NAGY 1969, 1985)

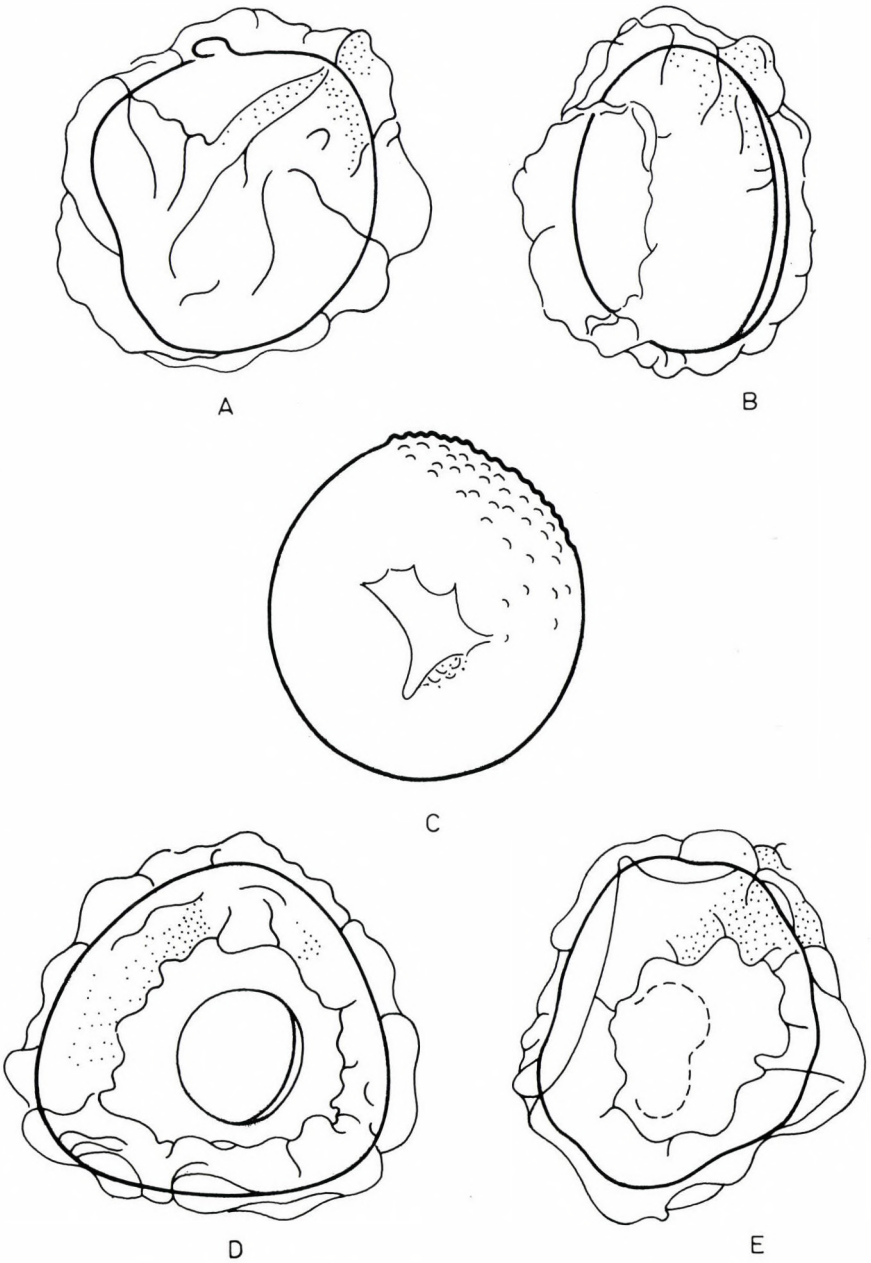

Fig. 5. Sketches of pollen grains of modern Cunninghamia lanceolata (Lamb.) Hook

as Cunninghamiaepollenites gen.nov. with the type species C. lignitus sp.nov. Unfortunately, her description is based on one specimen only. KRUTZSCH (1971) gathered all these types of pollen grains under one form-genus Inaperturopollenites Th. et Pf. and described a new formspecies I. radiatus sp.nov. without any suggestion of botanical affinity for the species. NAGY (1985) did not accept the validity of KRUTZSCH's taxon I. radiatus and considered it to be a synonym to the species Cunninghamiaepollenites lignitus Nagy (1969). Beside the holotype of this species she noticed one more specimen in the Hungarian Neogene. After our comparison of all described and illustrated pollen grains of I. radiatus W.Kr. with the Recent Cunninghamia lanceolata pollen we came to the conclusion that a part of KRUTZSCH's specimens, e.g. I. radiatus, may belong to the genus Cunninghamia.

In pollen spectra from the Bohemian and Polish Neogene we have encountered specimens, with some comparable to C. lignitus, but most of these specimens are closely comparable with the Cunninghamia lanceolata type, the fossil morphotype that we described as a new formspecies.

For Cunninghamiaepollenites lignitus as well as for the synonymous Inaperturopollenites radiatus the radially arranged folds are typical and considered as one of the diagnostic feature. Nevertheless, the modern pollen of Cunninghamia mostly lack this feature. It is extremely rare in the modern material (among 200 pollen with perine it was observed only once). On the other side foramina are present in both cases, in the fossil as well as in the recent material. Therefore we consider it to be useful to retain the lignitum/radiatus species with the radially arranged folds as one morphotype of Cunninghamia pollen and the morphotype with the perine forming irregular folds and enveloping the exine as a new morphotype species of Cunninghamiaepollenites. When the perine is lacking (not visible), it is very difficult to recognize Cunninghamia type at all in the fossil material.

Description of **Cunninghamia lanceolata** (Lamb.) Hook. pollen grains

For the description the following material has been used: Cunninghamia lanceolata (Lamb.) Hook., sporotheca Kraków Cunninghamia lanceolata (Lamb.) Hook., Botanical garden of the Botanical Faculty of the Charles University of Prague — cultivated specimen, pollen collected 3 June 1986 by the authors.

Pollen grains spheroidal, diameter 42 µm (36 µm — 46 µm), inaperturate or with rounded foramen, diameter about 10 µm. Exine about 2 µm thick distinctly divided into two layers. Ectexine as thick as endexine, granulate to scabrate, mostly covered by perine. The perine divided from the

exine, loosely arranged, forming a velum-like or fringe-like envelope with many small and larger folds on the proximal face. The leptoma area is without perine. On the perineless leptoma area a small (2 μm long) slightly curved papilla may be present (Pl. I, Figs 1, 2, Pl. II, Figs 4--6, Pl. IV, Figs 1--3), see also Fig. 5.

SEM-description (after KVAVADZE 1988, emended and simplified). Pollen grains concave--convex, on the distal face with a pore up to 2 μm in diameter. Leptoma large, covering nearly the whole distal face of the grain. Ectexine granulated, perine in the form of orbicules distributed on the whole surface of the grain. In the leptoma area orbicules more densely spaced. Orbicules 0.1-0.2 μm in diameter, covered by small spinules on its surface. Spinules very thin with broad basis as long as the basis are broad. On 1 μm^2 of the orbicules surface more than 25 spinules often fused together. TEM-description (after KVAVADZE 1988, emended and simplified). Ectexine granulated on the proximal side thicker. Maximal thickness 1.1-1.3 μm. Granules of irregular shape, polyangular, forming a 0.5-0.7 μm thick tectum supported by single columellae which are fused with the foot layer (Pl. VI, Fig. 2). On the distal face the leptoma area with very small granules. Endexine lamellated, 0.3-0.4 μm thick contains 6-7 parallel lamellae more or less of the same thickness. On the surface of pollen grain perine developed as rounded orbicules with electron transmitting center and electron absorbing margin. Orbicules covered by many small spinules.

The only species at our disposal was Cunninghamia lanceolata (Lamb.) Hook. The fresh material of Cunninghamia konishii Hayata was not available, therefore we rely on the description and the illustrations given by TSENG-CHIENG HUANG (1972) in the pollen flora of Taiwan. Both species were taken into consideration as the comparative material for our fossil specimens.

(The main observations were carried out in the light microscope Amplival, Carl Zeiss, Jena and completed, as far as possible, by the SEM scanning electron micrographs and by TEM of ultra thin sections.)

Description of fossil pollen grains

For the complete study of fossil pollen grains related to Cunninghamia the diagnosis given by NAGY (1969, 1985) and KRUTZSCH (1971) should be considered. In the paper of NAGY (1985) the synonymy and the occurrence of the taxon C. lignitum is given, but the complete description is missing. Therefore we present the following description that is the compilation of NAGY's (1969) and KRUTZSCH's (1971) diagnoses including our remarks.

Cunninghamiaepollenites lignitus NAGY 1969

1969 NAGY, MÁFI Évk. 52, 2, p. 153--154, Pl. XXXIV, Fig. 6.

1971 KRUTZSCH, Inaperturopollenites radiatus sp. nov., Atlas VII, p. 199, Table 12, Pl. LXIII, Figs 1--19.

1985 NAGY, Geol. Hung., Ser. Palaeont., 47, p. 146, Pl. LXXX, Fig. 15.

Pollen grains spheroidal, diameter 25-40 µm, foramen (porus after NAGY) rounded 5-7 µm in diameter. The wall of the grains of one layer, sculpture irregularly very fine punctate up to chagrenate (after KRUTZSCH), radial folds spreading from the equator to the foramen. NAGY mentioned the ectexinous very delicate finely intrapunctate veil surrounding the body of the grain. Nevertheless this feature is not visible on the Hungarian type material. Occurrence: Hungary: Miocene of the Mecsek Mts., Hidas village, the Middle Badenian coal bearing sequences and the Carpathians, Germany (G.D.R.): Lower and Middle Miocene, single finds in the Upper Oligocene (Chattian), for the localities see KRUTZSCH (1971).

Cunninghamiaepollenites janinae sp. nov.

Pl. V, Figs 1--12.

Holotype: Pl. 5, Figs 10--12.

Locus typicus: Bełchatów (Central Poland), profile IXc, slide 31b, crosstable: 93.6/17.8.

Stratum typicum: Carpathians.

1958 OSZAST in SZAFER, p. 7, Pl. II, Figs 16, 17.

1960 OSZAST, Monogr.Botan. **IX**, 1, p. 12--13, Pl. III, Fig. 15, cf. 17--19, Cunninghamia sp.

1964 STUCHLIK, Cunninghamia sp., Acta Palaeobot. **V**, 2, p. 29, Pl. X, Fig. 13.

1983 KONZALOVÁ-STUCHLIK, Cunninghamia-Cunninghamiaepollenites sp., p. 366, Table 1, Pl. I, Figs 3, 5.

Derivatio nominis: In honor of Dr. habil. JANINA OSZAST, the first palynologist who found and identified pollen grains in the European Neogene.

Diagnosis: Pollen grains sphaeroidal, diameter 25-40 µm, inaperturate with a rather large-sized leptoma on the distal face. The rim of leptoma is often envolved to the innert part of the grain, giving the impression of the pore. Exine 2 µm thick, ectexine faintly granulate, as thick as nexine, the outer part of the grain covered by loosely arranged perine with charac-

teristic ridges or folds on the surface. In several cases a small low papilla visible 2 up to 2.5 µm.

Remarks: Cunninghamiaepollenites janinae sp. nov. differs from C. lignitus Nagy by the absence of radial folds, the perine exhibits irregular folds, forming very often a velum- or fringe-like envelope, not overlapping the leptoma area, making a fringe-like rim. The small-sized papilla is often observable in the leptoma area on the distal face. The term perine is used in the morphology of the taxon in consistence with the opinion of UENO (letter communication 1987, and KVAVADZE 1988).

According to the specimens found in our material two forms may be distinguished. Forma minor 25-30 µm (Pl. VI, Figs 1—4, 10—12), forma maior 35-40 µm (Pl. VI, Figs 5—9).

As far as we know, pollen grains of C. janinae have been found in the Oligocene to the Upper Miocene in northern Poland, in the Middle Miocene of Central Poland (unpublished) and in the Lower Miocene of Bohemia. Some of the specimens described from Germany (G.D.R.) as Inaperturopollenites concedipites (Wodehouse) W. Kr. (1971, Pl. LXV, Figs 23—28 and 31—33) and also a part described as Sequoiapollenites gracilis W. Kr. (1971, Pl. LXIX, Figs 30—34) may belong to Cunninghamiaepollenites janinae.

Acknowledgement

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EXPLANATION OF PLATES

Plate I

Cunninghamia lanceolata (Lamb.) Hook.

Figs 1, 2. Specimen at two foccus levels. Faintly granulate surface with secondary folds and small papilla visible. 1000 x

Figs 3, 4. Another specimen at two foccus levels. Leptoma and the fringe-like rim of perine visible. 1000 x

Figs 5, 6. Two specimens without perine. The rest of it may be preserved on the surface. 1000 x

Plate II

Cunninghamia lanceolata (Lamb.) Hook.

Figs 1—3. Specimen in oblique distal view. The perine with many secondary folds. 1000 x

Figs 4—6. Specimen in lateral view with loose folded perine and papilla. 1000 x

Plate III

Cunninghamia lanceolata (Lamb.) Hook.

Figs 1—4. Specimen with foramen in distal view. 1000 x

Figs 5, 6. Another specimen in oblique distal view. 1000 x

Plate IV

Cunninghamia lanceolata (Lamb.) Hook.

Figs 1—3. Specimen in lateral view with small papilla. 1000 x

Figs 4—6. Specimen in proximal view with many secondary folds. 1000 x

Plate V

Cunninghamia lanceolata (Lamb.) Hook.

Fig. 1. SEM — distal face 2400 x

Fig. 2. TEM — fragment of sporoderm 13500 x

Figs 3, 4. SEM-fragment of distal face with leptoma, fringe-like rim and orbicules; 3. 7800 x; 4. 7200 x

Plate VI

Cunninghamia lanceolata (Lamb.) Hook. TEM — fragments of sporoderm

Fig. 1. — 30000 x

Figs 2, 3. — 24000 x, L: lamellated endexine; F: foot layer; C: columellae; T: tectum; O: orbicules

Plate VII

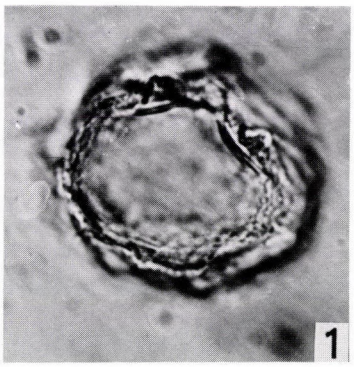
Cunninghamiaepollenites janinae sp. nov. 1000 x

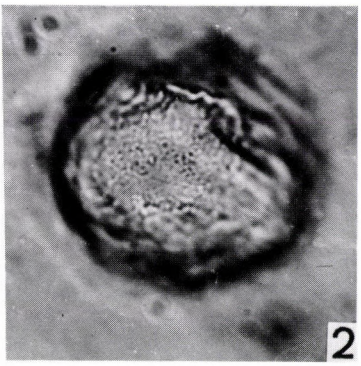
Figs 1—4. forma minor; 1, 2 Rypin II (STUCHLIK 1964 Pl. X, Fig. 13) 3, Rypin II, depth 58.40—58.60 m; 4, Rypin II depth 60.60—61.00 m

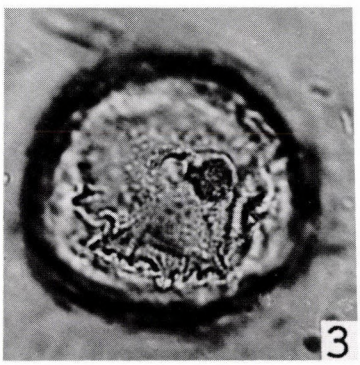
Figs 5—9. forma maior, Cheb Basin (Northern Bohemia) — profil V_3, 5, 6 — depth 70.00 m; 7—9, depth 75.00 m

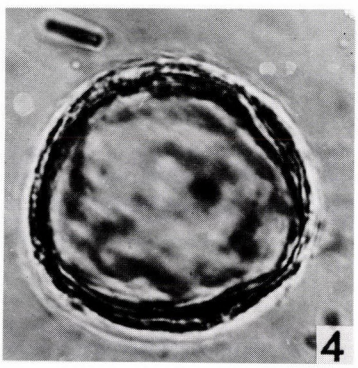
Figs 10—12. Holotype — Bełchatów (Central Poland), Miocene profil IXc, slide 31b — cross-table 93.6/17.8, film 65/158

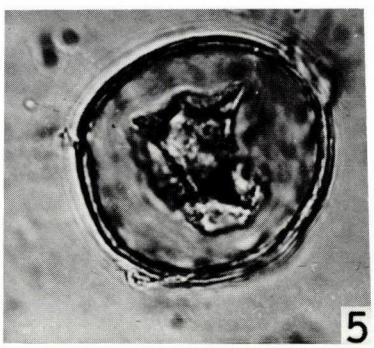
Plate I

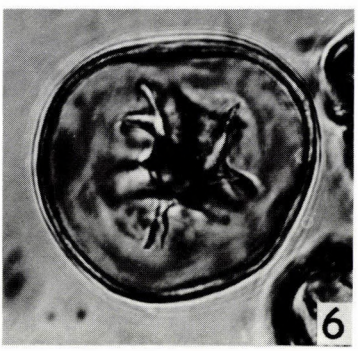












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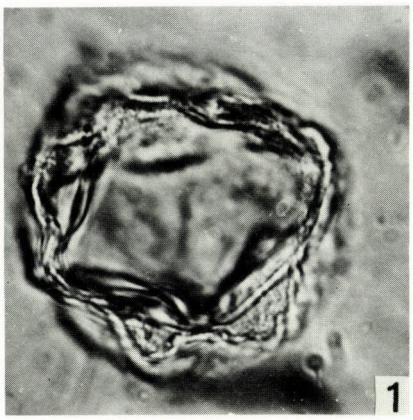

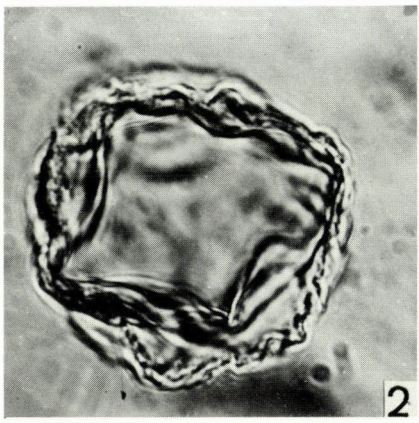

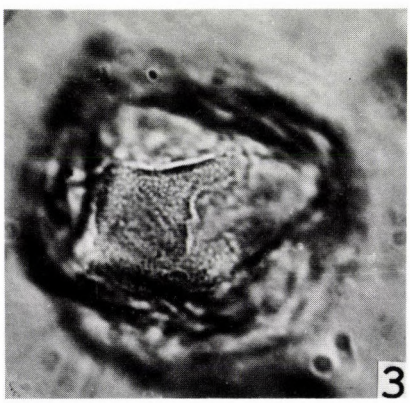

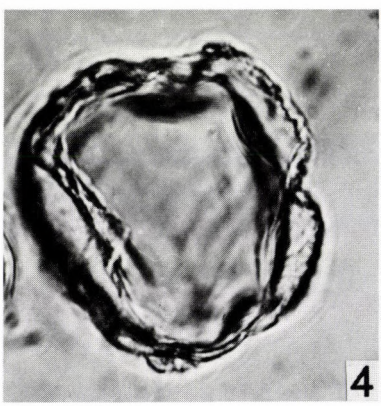

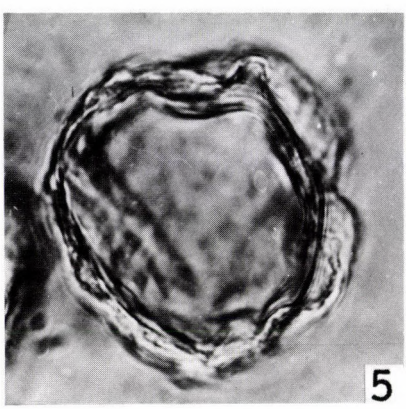

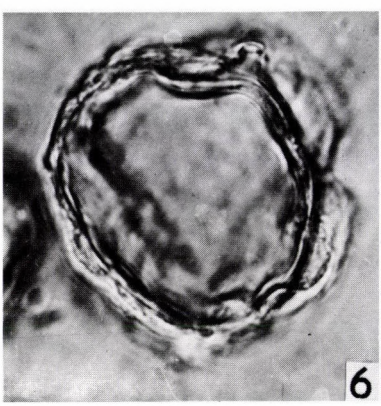

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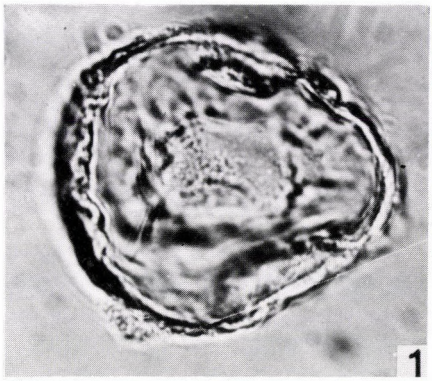

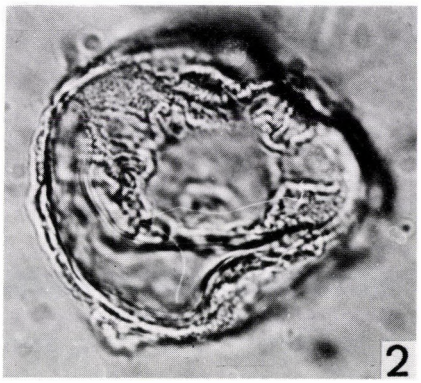

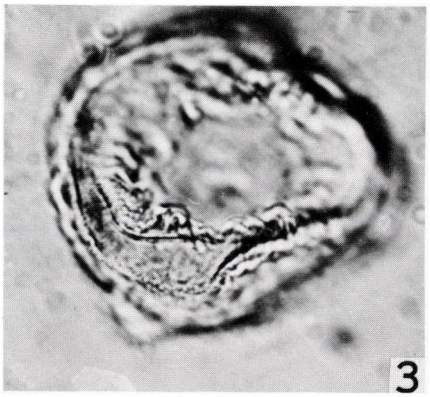

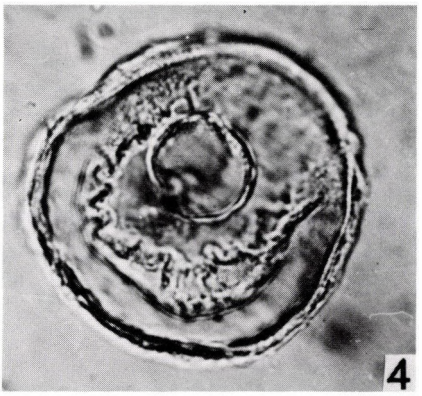

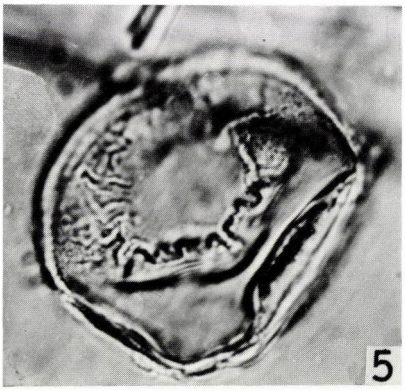

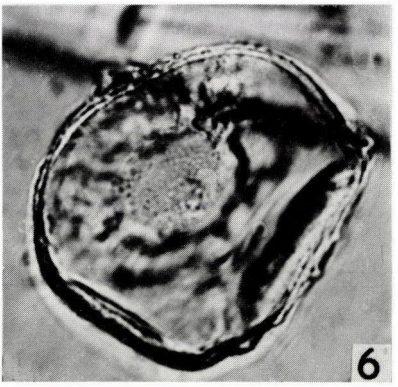

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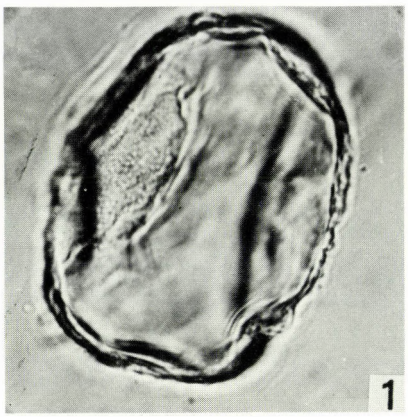

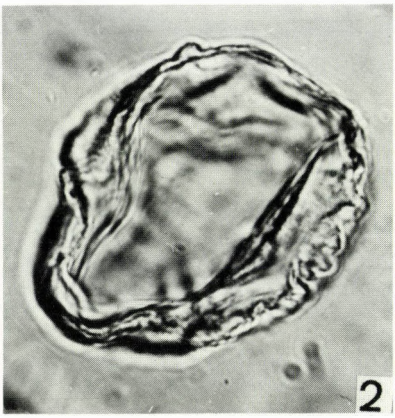

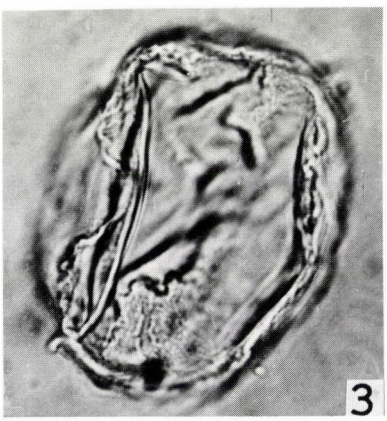

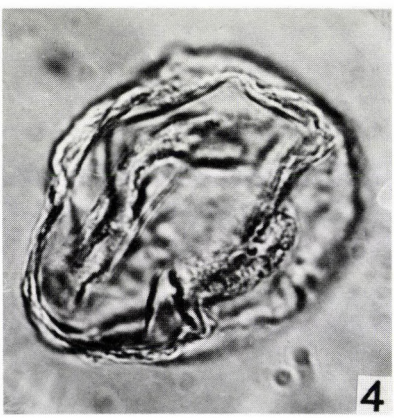

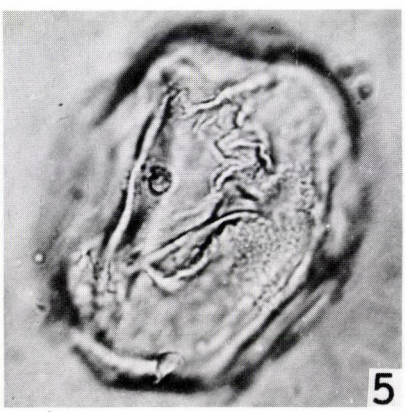

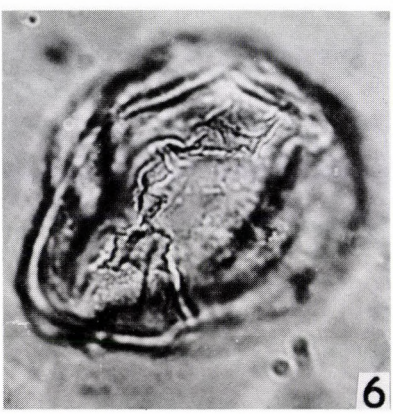

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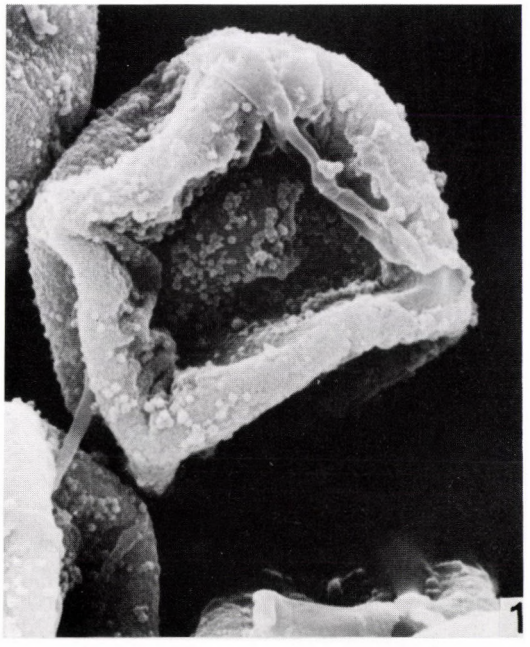
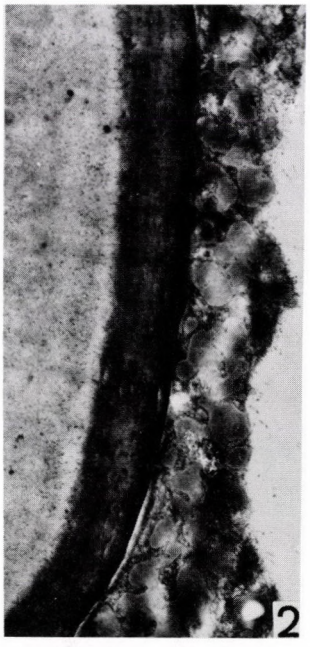
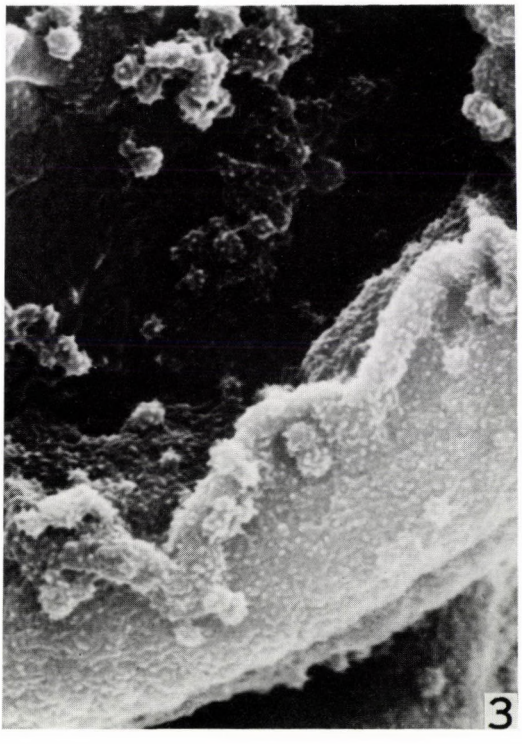






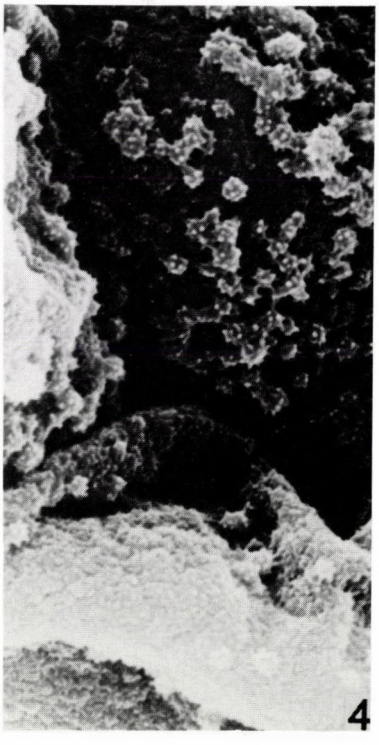
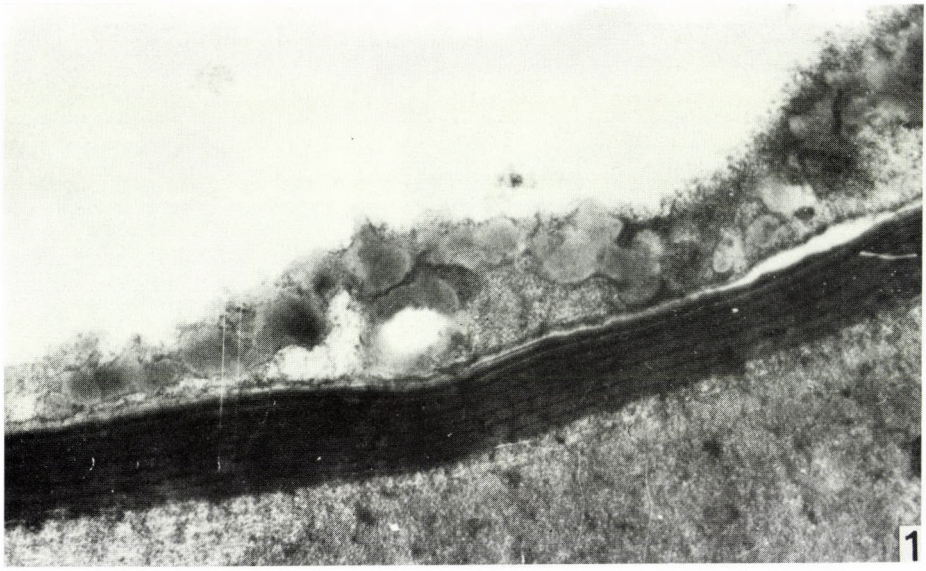
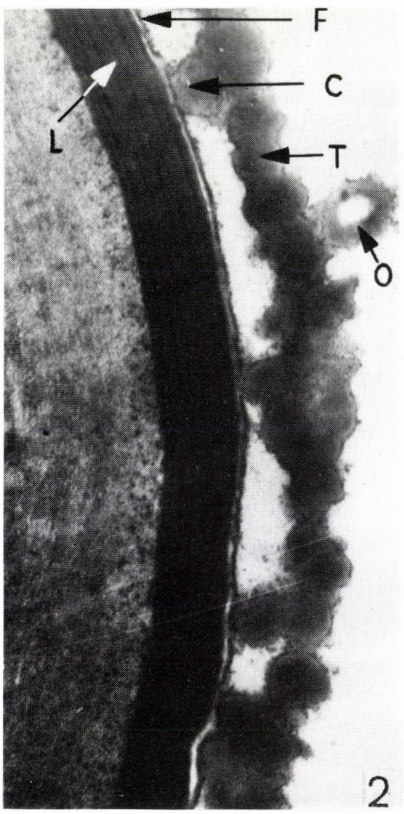


Plate VI





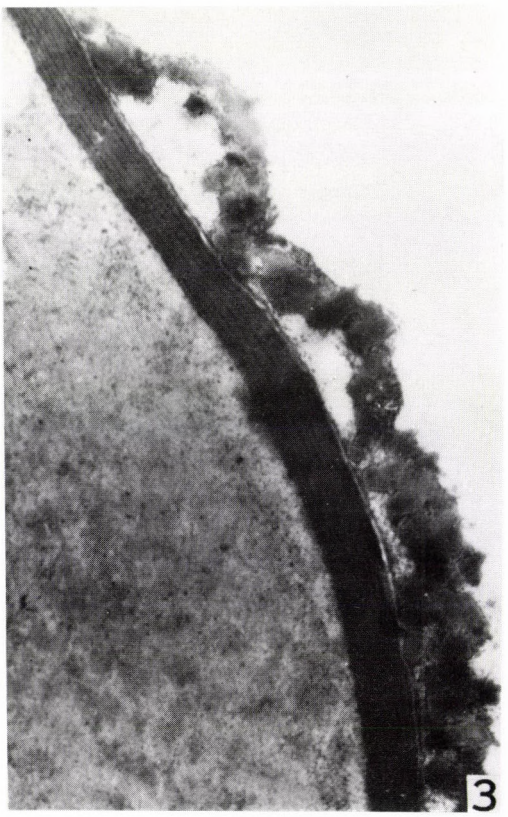
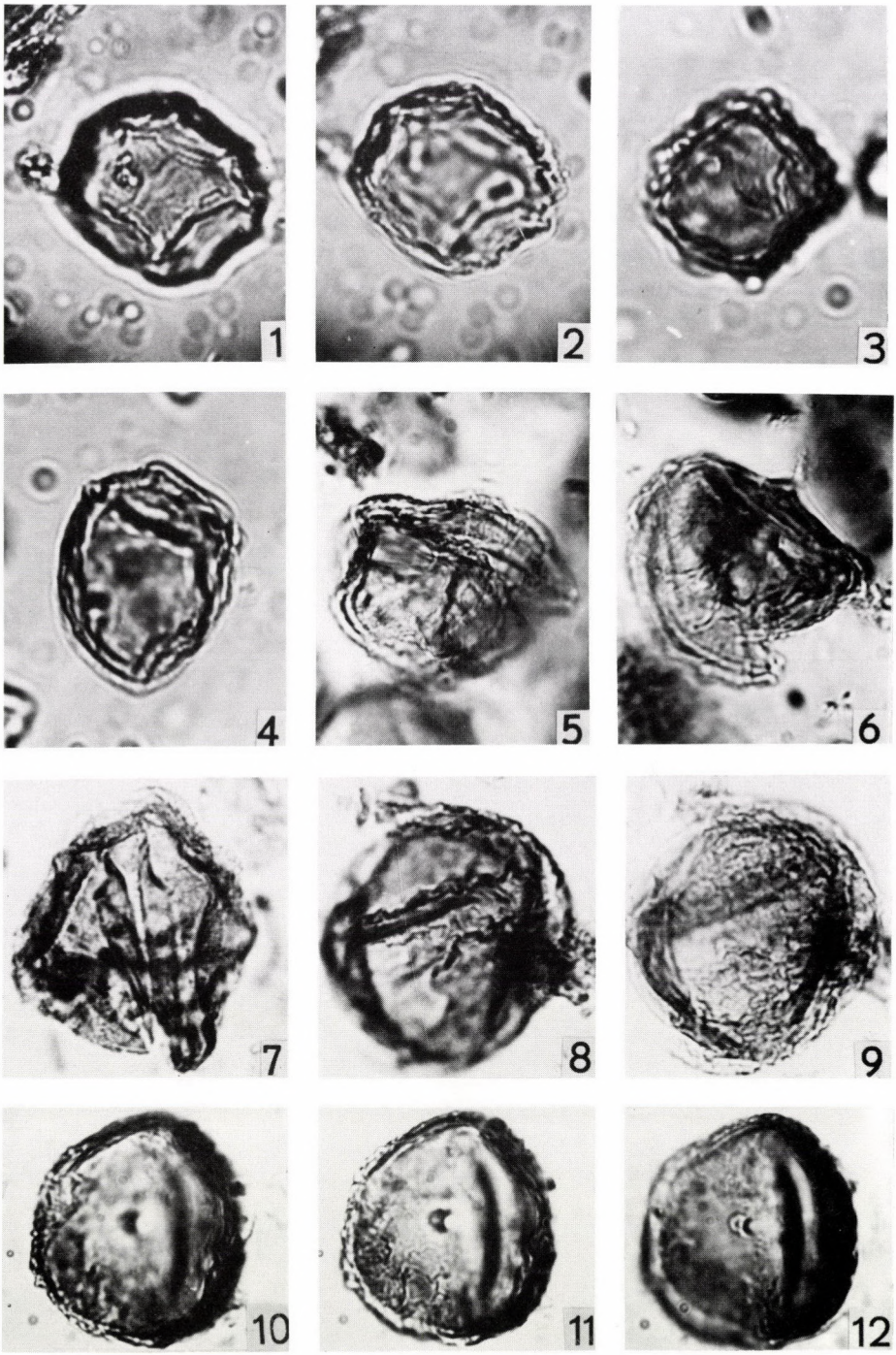


Plate VII



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LIGHT AND SCANNING ELECTRON MICROSCOPE STUDY OF LACTUCA L. AND CICHORIUM L. POLLEN (COMPOSITAE: LACTUCEAE)

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Cichorium intybus L., Lactuca serriola L. and three cultivars of Lactuca sativa L. were used to study the pollen morphology of two related genera of the same tribe (Lactuceae), two close species of the same genus and three cultivars of the same species in Light Microscopy and Scanning Electron Microscopy. Details of pollen features observed in LM and SEM were compared. Evidence from pollen morphology reinforces the close tie between the above-mentioned two genera and no significant variation between the two close species of Lactuca L. Minor differences between the cultivars of L. sativa L. were observable.

Keywords: Pollen — Lactuca L. — Cichorium L. — Cultivars

Introduction

Pollen features are considered of immense importance in taxonomy. They have been used in classifying and identification of species, genera, related tribes or sub-tribes.

Studies of STIX (1960), SKVARLA and LARSON (1965), SKVARLA and TURNER (1966), TOMB, LARSON and SKVARLA (1974), TOMB (1975), SKVARLA, TURNER, PATEL and TOMB (1977) and BLACKMORE (1981) have established the validity of using pollen features in Compositae.

In the present work the objectives were:

i) to obtain details of the pollen grain features under light and Scanning electron microscopes;

ii) to compare pollen features of Lactuca L. and Cichorium L., two genera of the same tribe;

iii) Lactuca serriola L. and Lactuca sativa L., two species of the same genus, the wild species and its close relatives, the cultivars;

Akadémiai Kiadó, Budapest

iv) three cultivars of L. sativa L., Webbs Wonderful, Tom Thumb and Lobjoit's Green Cos (henceforward referred to as Webbs, Tom and Cos).

These plants are all diploid with 2n = 18 (HAQUE and GODWARD 1985).

Material and Method

1. Description of materials

The external morphology of the pollen grains of Lactuca L. and Cichorium L. is illustrated in Figs 2—6 and 10. The following description is based on WODEHOUSE (1935), and these features are in part illustrated in Spencer Tomb's paper on Lactuceae (Fig. 30, Lactuca lessertiana, page 87, Grana 15, 1975) and Blackmore's paper on Compositae (Cichorium intybus, Figs 9 and 10, page 8. Bot. J. Linn. Soc. 82, 1981). Figures are quoted so that the description can be followed.

Each pollen grain is globular. There are three germ pores, hence the pollen grains are described as tricolporate. These 3 germ pores through each of which bulges a broad round papilla, are equally spaced around the equator of the grain and give it a trimerous character (Figs 2a and 4). The exine of the pollen grain is provided with prominent thickened ridges which enclose 15 variously shaped lacunae. The ridges are thick, minutely porous (Fig. 6) and carry conical spines, the echinolophate condition.

The 3 lacunae which enclose the 3 germ pores are hexagonal in form, bounded by 6 ridges and are called poral lacunae. Each poral lacuna has 2 narrow gaps, one in each of 2 opposite ridges, the interlacunar gaps (Figs 2c and 6).

In polar view (Fig. 4) the germ pores appear in profile on the edge of the pollen grain, and altogether 5 lacunae are seen, 3 of which are near to poral lacuna and are called abporal lacunae. Each abporal lacuna is bounded by 5 ridges and hence is pentagonal in form and communicates with its adjacent poral lacuna through its interlacunar gap. Between the abporal lacunae alternating with the pores, are the 3 paraporal lacunae.

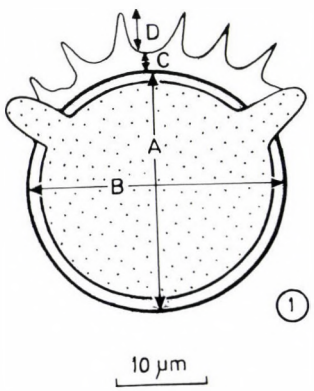


Fig. 1. Diagram of the pollen grain of Lactuca L.

A: internal diameter, B: internal diameter, C: thickness of the ridge, D: spine length x 1500

Figures 2—6. SEM micrographs of Lactuca L.

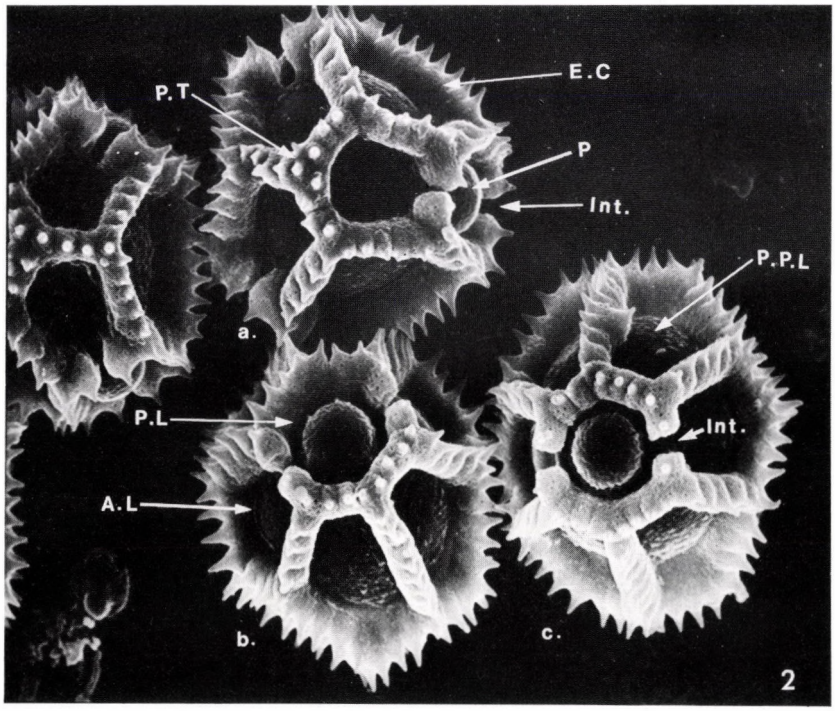


Fig. 2. Pollen grains of Lactuca sativa cultivar Webbs, in different views: P: Germ pore (colpus) with papilla, E. C.: Equatorial crest, A. L.: Abporal lacuna, P. L.: Poral lacuna, P. P. L.: Paraporal lacuna, Int.: Interlacunar gap, P. T.: Polar thickening 3000 x

a. Polar view (slightly oblique) showing the polar thickening with spines and thick ridges with minute pores and spines, equatorial crest, lacunae and interlacunar gap; b. Equatorial view, slightly oblique; c. Equatorial (apertural) view, showing colpus with papilla and interlacunar gaps

Each paraporal lacuna is broad and bounded by 5 ridges giving it a pentagonal form. The ridge which traverses the equator from one poral lacuna to another is called the equatorial crest (Figs 2a and 5). Over each pole of the pollen grain, at the junction of 3 ridges, the point of junction becomes expanded to form a triradiate area covered with spines, called the polar thickening. This is characteristic of Cichorium where it is always present. It may be present in Lactuca (Fig. 2a) although one of the differences between the two genera is stated to be the absence of the polar thickening in Lactuca (WODEHOUSE 1935). In both Lactuca and Cichorium the size of the polar thickening and number of spines on it is somewhat variable (Table 2). Another difference between these two genera is the smaller size of the pollen grains of Lactuca (Table 1).

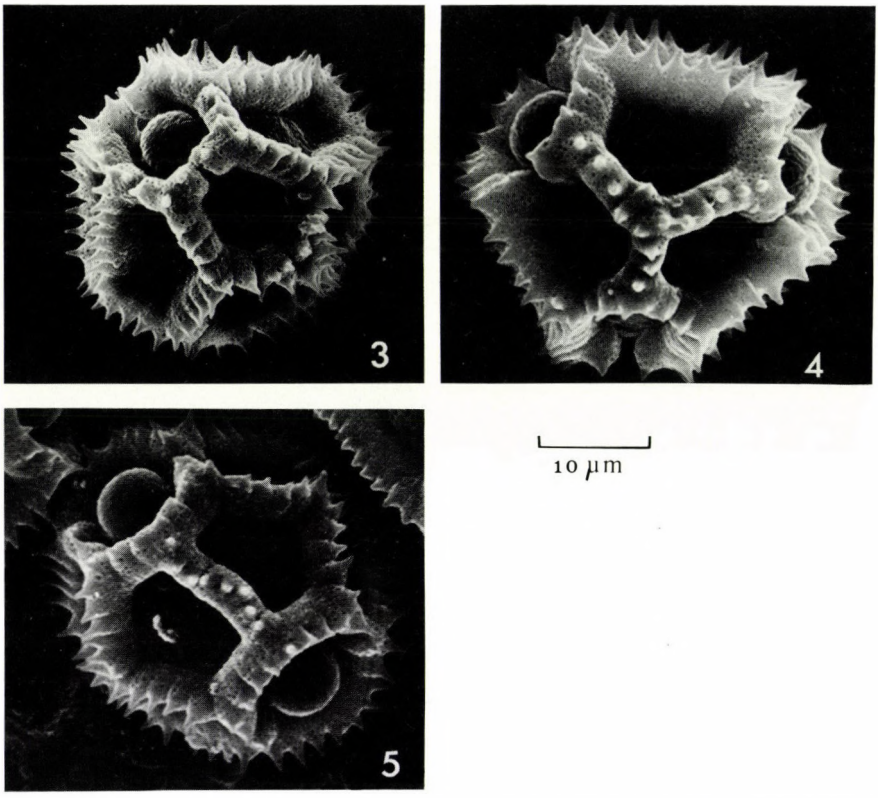


Fig. 3. Pollen grain of L. sativa L. cultivar Tom, equatorial view (slightly oblique), showing colpus with papilla, interlacunar gaps, lacunae and ridges with spines and minute pores. 1790 x

Fig. 4. Pollen grain of L. sativa L. cultivar Cos, polar view (slightly oblique), showing the polar thickening with few spines. 1790 x

Fig. 5. Pollen grain of L. serriola L., equatorial view, showing two colpi and the equatorial crest. 1790 x

2. Methods of preparation

Seeds of Lactuca sativa L. (cultivars Webbs Wonderful, Lobjoits Green Cos, and Tom Thumb), Cichorium intybus (Witloof Chicory) obtained from Samuel Dobie & Sons Ltd., of Clwyd, and Lactuca serriola L. collected from the field in Essex. They were germinated and the plants grown on to flowering.

For SEM study unopened florets with mature anthers fixed in 1:3 acetic-alcohol were washed with distilled water three or four times in a small watch glass to remove the fixative. Then a single floret was transferred to a microslide followed by a large drop of distilled water. The floret was dissected with a pair of needles under the dissecting microscope to take out the anthers. The anthers were teased and pressed with the needles to release pollen grains. Debris was removed and then pollen grains were mounted on brass stubs using a pasteur pipette. The stubs with pollen grains were left to become air-dry in a covered petri dish. When dry the

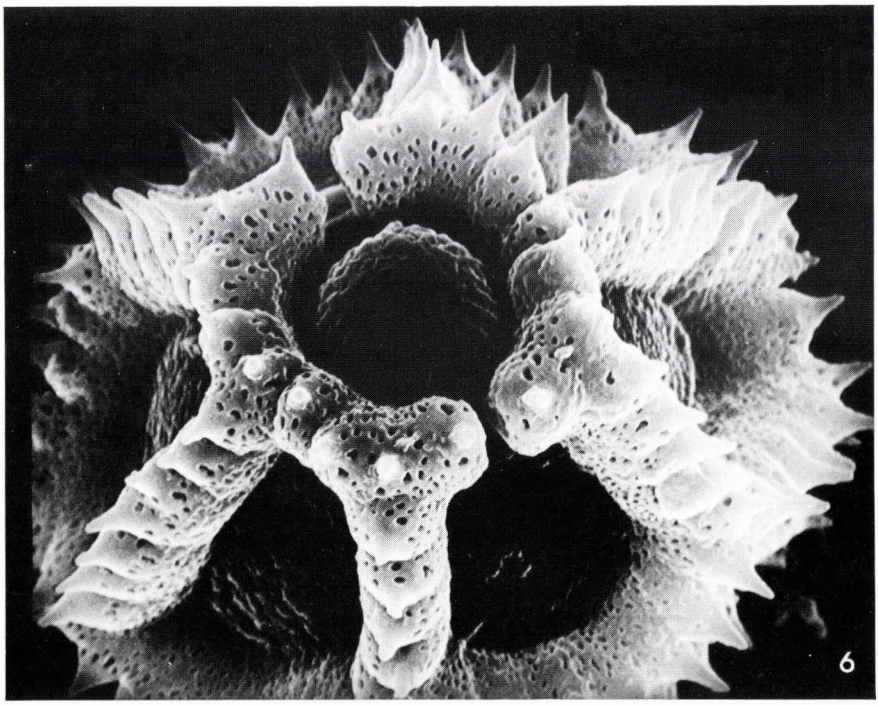


Fig. 6. Pollen grain of L. sativa L. cultivar Tom. View of poral lacuna with papilla, showing irregularly-shaped minute pores in the ridges and spines projecting from them. The surface of the papilla is seen to be minutely ridged. The two interlacunar gaps are clearly seen. 6590 x

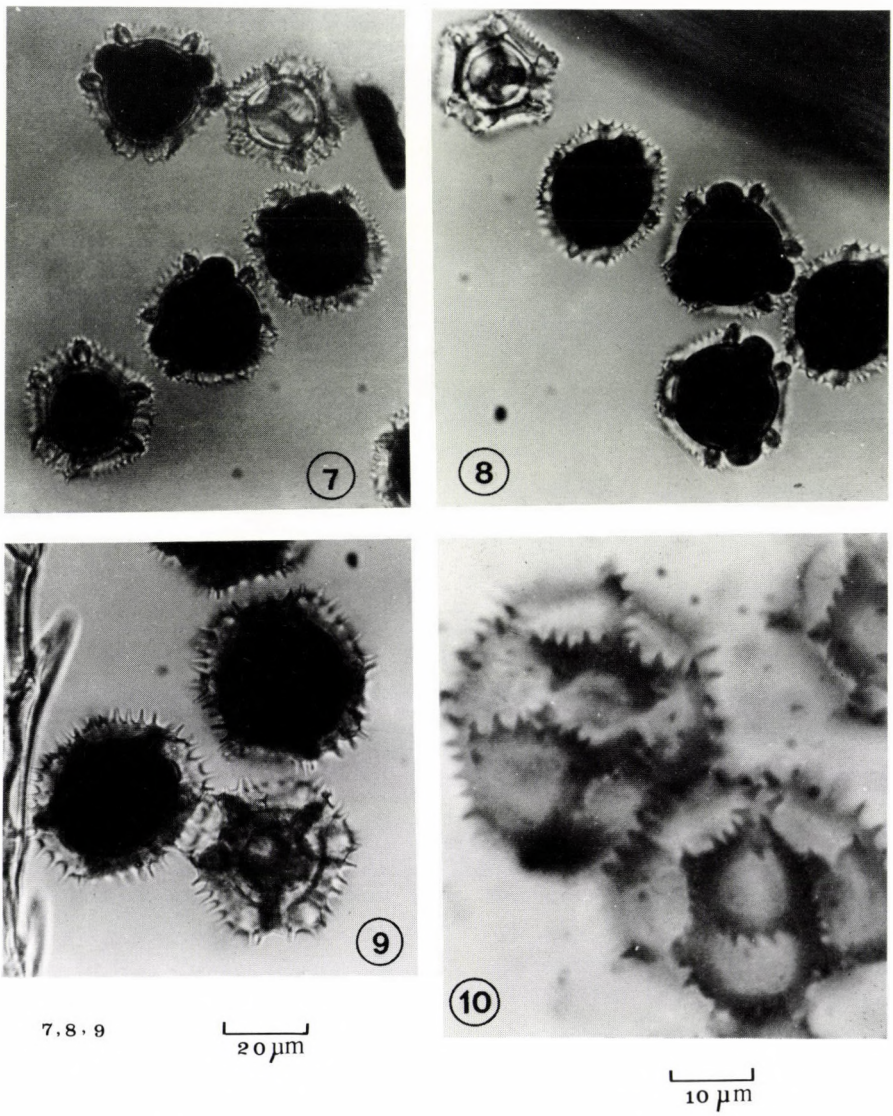
stubs were put under vacuum, subsequently argon, and coated with gold-palladium; then observed and photographed using a Jeol JSM-35 Scanning Electron microscope operating at 15 KV. 149 photographs have been studied, the magnification of prints varying from 792 x to 7920 x.

For light microscope study pollen was stained with aceto-carmine on a microslide, then observed and photographed under high power and oil immersion lenses.

Measurement of pollen grains and exine features

Pollen grains were measured with the visual light microscope. The accompanying diagram (Fig. 1) shows where the internal diameter was measured. A mean of the two measurements A and B was taken. The exine thickness was measured along the line C; this thickness represents the ridge. The spine length was measured along the line D. The photographs (Figs 7—10) illustrate the images obtained. Only stained (presumably fertile) pollen grains were measured.

Using the SEM photographs, numbers of spines on the ridges and number of spines on polar thickenings, and also the numbers of spines round the lacunae have been counted, assuming that the limits of each ridge and of the areas of polar thickenings are distinguishable. The appellations used for particular ridges and lacunae are shown in Fig. 1, page 425 Vol. XXV — No. 3—4, Pollen et Spores, HAQUE and GODWARD (1983).



Figures 7—10. Pollen grains of Lactuca L. and Cichorium L., photographed in light microscopy

Fig. 7. Pollen grains of L. sativa L. cultivar Tom, in different views showing papillae, ridges with small spines and lacunae. 640 x

Fig. 8. Pollen grains of Lactuca serriola L., wild species, in different views showing papillae, ridges with spines and lacunae. 640 x

Fig. 9. Pollen grains of Cichorium intybus L., showing papillae and ridges with long slender spines. 640 x

Fig. 10. Pollen grains of C. intybus L. in equatorial and equatorial lateral views, showing ridges with spines, interlacunar gaps, equatorial crest and lacunae. 1300 x

Results

The data for the various features of the pollen grains are presented in Tables 1 and 2. Table 1 summarizes the LM-measurements and Table 2 summarizes the data obtained from the study of the SEM.

It is seen that there is a considerable amount of variation as to pollen grain diameter, thickness of the ridges, length of spines, and numbers of spines around lacunae and on ridges, even within the single species. Similar variation occurs in each cultivar.

Table 1

Range of measurements in LM.
Sample size = 100 pollen grains from 10 plants (10 pollen grains from each plant), in each cultivar and species

Species or Cultivars	Diameter of fertile pollen grains in µm Range	Thickness of the ridges of the pollen in µm Range	Length of spines on pollen grains in µm Range
C. intybus	34.61—39.10	4.73—5.67	2.43—3.04
L. sativa			
Tom	21.79—26.28	2.57—2.97	2.03—2.43
Webbs	23.07—26.92	2.57—3.24	2.03—2.43
Cos	23.07—25.64	2.43—2.97	2.03—2.43
L. serriola	21.79—25.64	2.57—2.97	2.03—2.43

The detailed data for Diameter of fertile pollen grains, Thickness of the ridges of the pollen and Length of spines on pollen grains with standard deviations and statistical significance were published in Pollen et Spores, HAQUE and GODWARD (1983), but the range was not there given.

Table 2

Range of measurements found using the Scanning Electron Micrographs.
Sample size = 20 pollen grains in each cultivar and species

Species or Cultivars	No. of spines round the			No. of spines on the		
	Poral Lacuna Range	Abporal Lacuna Range	Paraporal Lacuna Range	Equatorial Crest Range	Poral Thickening Range	The ridges forming Polar Thickening Range
C. intybus	14—16	16—18	21—24	6—7	8—11	16—18
L. sativa cultivar						
Tom	16—17	19—21	24—26	8—9	6— 8	11—14
Webbs	16—18	18—20	23—26	7—9	3— 6	9—12
Cos	17—19	19—20	24—26	7—9	2— 4	9—11
L. serriola	17—19	19—20	23—26	7—9	4— 6	12—14

The detailed data for No. of spines round the Poral Lacuna, Abporal Lacuna, Paraporal Lacuna, and on the Equatorial Crest, Polar Thickening, the ridges forming the Polar Thickening with standard deviations and statistical significance were published in Pollen et Spores, HAQUE and GODWARD (1983), but the range was not there given.

Conclusion and Discussion

There are obvious differences only between the genera Cichorium L. and Lactuca L., but minor differences are present between the cultivar Tom Thumb and the other two cultivars of L. sativa L. plus L. serriola L. in the case of numbers of spines, and between Tom Thumb plus Webbs on the one hand, and Cos plus L. serriola L. on the other, in the case of diameter of the pollen grain.

Such differences have never previously been recorded between cultivars of the same species, although within genus differences have been observed (KEELEY and JONES, 1977).

It is of interest that observable differences, although perhaps minor, have arisen between cultivars. In the minds of many workers, diameter of the pollen grain, and numbers of spines, would not necessarily be regarded as minor; and might be considered to rank as species differences. It is of interest that the genomes of the single species, L. sativa L. is the one case and L. serriola L. is the other, contain the variability that could give rise to such differences in the cultivars. The extent of these differences appears only when the range is given, and is not available from means.

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ANÁLISIS POLÍNICO DE SEDIMENTOS MARINOS DEL OCCIDENTE DE LA ISLA DE LA JUVENTUD (CUBA)

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(Llegado: 25 Junio 1990)

Pollen analysis of marine sediments resulting of samples cores from the northwestern and southwestern regions of Isla de la Juventud were carried out. Some cores were taken in the northwestern region at a distance of 1-1.5 kms off the coast. In the southwestern region the cores arise from B.' de San Pedro. The sediments consist of peat, clay and peat with intercalations of marine mollusks as well as sandy clay. In the northwestern region the samples of sediments and palynomorphs evidence that the area was emerged with different types of communities and habitats; this reveals part of the Pleistocene's history in the region. The existence of a coastal plain in the region during the Pleistocene when the sea level was lower is pointed out. This plain may have served as a route or bridge between Pinar del Río and Isla de la Juventud.

By the results of pollen and spores the peat studied belongs to two different periods: Pleistocene and Holocene. The presence of Podocarpus angustifolius pollen in sediments of B. de San Pedro suggests climatic conditions different to that of today.

Introduccion

El análisis de polen constituye una técnica de la investigación muy valiosa para el estudio de la historia de la vegetación, la reconstrucción del pasado y del clima, de ahí que represente una base importante de datos relacionados con el ambiente en el cual una biocenosis se desarrolló y los cambios experimentados como consecuencia de los acontecimientos geológicos.

En nuestro país, estas investigaciones en sedimentos marinos son escasas o prácticamente no existen, conociéndose solamente la realizada por la compañía NEDECO (1959), la cual realizó la determinación del polen en turba de la Ciénaga de Zapata, así como el fechado de la misma.

Akadémiai Kiadó, Budapest

El trabajo realizado tiene dos objetivos: 1) Conocer las especies de plantas que han intervenido o intervienen en la composición de la turba; 2) Interpretar los resultados de polen de sedimentos obtenidos a 1 km de la costa hacia el mar en el área comprendida entre estero El Soldado y Punta de Buenavista, así como la zona interior de la Bahía de San Pedro, con el fin de lograr una pre-reconstrucción botánica de la historia del área como una necesidad planteada en el conocimiento del Antropógeno en Cuba.

Materiales y Metodos

Las muestras para el espectro palinológico proceden de diferentes pozos realizados en el área de la Bahía de San Pedro y la región noroccidental de la Isla de la Juventud (Fig. 1). De cada pozo se tomó sólo una muestra para un estudio preliminar; no obstante, las mismas han contribuido considerablemente para un análisis de polen por la abundancia y el buen estado de conservación de los mismos.

Las muestras fueron preparadas para análisis con KOH 10%, H_2O_2 y acetolisis de ERDTMAN (1966). La separación de los palinomorfos se realizó en una solución de bromoformo-alcohol (p. e. 2,0) y posteriormente montados en glicerina-gelatina.

La determinación de las especies de moluscos marinos fue realizada gentilmente por el DR. A. DE LA TORRE. La identificación de los palinomorfos se realizó, en algunos casos, por la comparación con los siguientes textos: ERDTMAN (1966); STUCHLIK y MONCADA (1983); HOOGHIEMSTRA (1984); GRAHAM (1985b); así como con la palinoteca del Instituto de Ecología y Sistemática de la Academia de Ciencias de Cuba.

Resultados

Características de la turba y de la vegetación

A través de los resultados obtenidos de palinomorfos en las turbas procedentes del SO del estero El Soldado y la región de la Bahía de San Pedro, puede considerarse que la turba se ha originado en dos ambientes diferentes, por lo que en su constitución participan diferentes plantas.

Una turba de la vegetación de manglares, propia de costas bajas y pantanos costeros de Rhizophora mangle, la cual se originó en condiciones de salinidad debido a las infiltraciones del agua de mar, como lo indica el incremento del polen de Rhizophora y Chenopodiaceae (Fig. 2). Esta turba que yace a unos 13,0-14,0 m (Pozo JD-12) de profundidad y 10 m debajo del nivel del agua procede del SO del estero El Soldado, es más antigua y debe corresponder al Pleistoceno Superior. La abundancia del polen de Rhizophora sugiere que la misma constituyó en el pasado bosques densos que cubrió el área de la denominada antigua línea de costa.

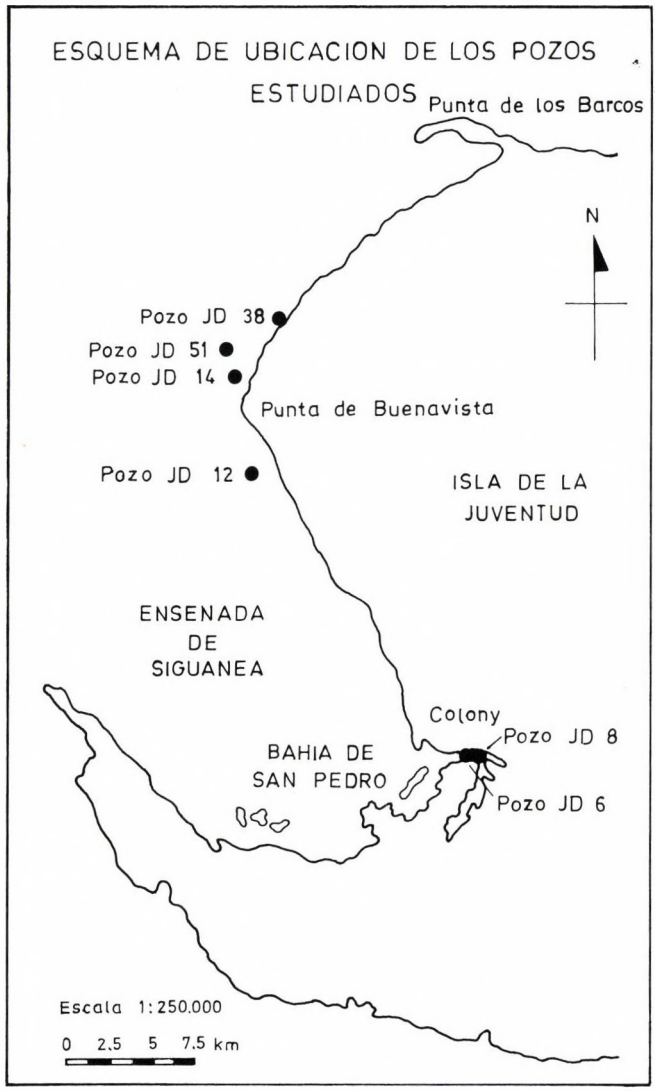

Fig. 1. Mape mostrando la porción occidental de la I. de la Juventud y las áreas de estudio

Por otro lado, en la región de la Bahía de San Pedro, la turba ha venido desarrollándose en un ambiente de agua salobre a dulce. Esta turba (Pozo JD-6) es más joven (Holoceno) y en su composición participan plantas acuáticas, helechos, y otras hierbas. En las Figs 2 y 3 se presenta la relación de los distintos grupos de pólenes cuyas plantas participaron en la

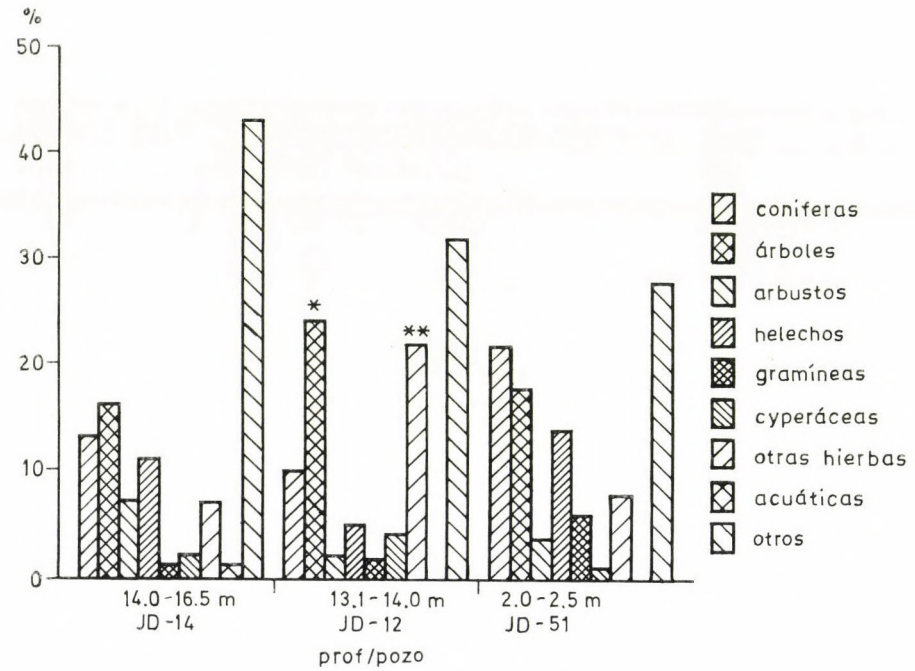

Fig. 2. Porcentaja de los grupos de palinomorfos encontrados en sedimentos marinos de la región noroccidental. Grupos de palinomorfos en cada pozo: JD-12, con dominancia del polen de *Rhizophora mangle y **Chenopodiaceae JD-14, bosque de ciénaga. JD-51, sedimento terrígeno, bosque semideciduo

composición de la turba, y en las listas 1-5 se relacionan los palinomorfos identificados.

Estas diferencias en el origen de la turba aparecieron también en la Ciénaga de Zapata. La compañía holandesa NEDECO (1959) determinó la edad de la turba en la Ciénaga de Zapata y dió un aproximado de 11 000 años para la turba más antigua, en la región occidental. De acuerdo a las determinaciones del polen, reportaron dicha turba como de Rhizophora. Hacia la región oriental de la Ciénaga la turba se ha originado en un ambiente de agua dulce; su edad fue estimada en 3300 años, hallándose que la misma coincide con la turba de los Everglades, en la Florida.

Los resultados palinológicos de los sedimentos estudiados han permitido obtener algunos datos relacionados con los tipos de vegetación y permiten realizar algunas pre-reconstrucciones de esas paleocomunidades.

Considerando los resultados del análisis de polen del pozo JD-12, la vegetación que dió origen a la turba en el estero El Soldado fue un bosque pioneril de Rhizophora mangle y especies de Chenopodiaceae. La presencia de los pólenes de Nymphaea y Nymphoides, a pesar de que aparecen en pequeñas cantidades, sugiere que se habían desarrollado lagunas. FRANCO (inédito) plantea: "mientras un mar somero cubría la llanura meridional, el área de la Ensenada de la Siguanea constituía un área parcialmente emergida ocupada por lagunas y pantanos, donde se depositaron sedimentos, acarreados por ríos de escaso caudal que llegaban al mar tal como se observa hoy en día". El harbazal de ciénaga estuvo representado por gramíneas que crecen en los lugares húmedos, así como también Typha domingensis, Cladium jamaicense, Cyperus sp., Caperonia palustris y helechos de los que se identificaron esporas del tipo Blechnum y tipo trilete; es decir, especies que se desarrollan bien en ambientes donde la salinidad no representa un factor limitande. El análisis de polen también aportó elementos emergentes del bosque de ciénaga con polen de palma del tipo Sabal, Acoelorraphe; Myrica, Xylopia e Ilex. También se hallaron granos de polen de Cuphea, Rauvolfia, y esporas de Lygodium, los que fueron determinados del pozo JD-14 que además aportó otros elementos del bosque semideciduo con polen de Ambrosia, Tribulus, Bursera simaruba, Sapotaceae, Malpighiaceae, Myrtaceae y Malvaceae. En el espectro de polen de todas las muestras, los granos de polen de Pinus tropicalis y P. caribaea fueron abundantes.

La muestra del pozo JD-51 evidencia otra situación diferente, pues el análisis de polen ha revelado algunas paleocomunidades que caracterizaron a la región: 1/ Bosque de manglar con Rhizophora mangle, cuyos pólenes, conjuntamente con los de Chenopodiaceae, estuvieron bien representados en la muestra; 2/ El herbazal, con Cladium jamaicense, gramíneas y comunidades de agua dulce con Utricularia; 3/ El bosque de ciénaga, con vegetación emergente de Sabal parviflora, Colpothrinax wrightii, Myrica cerifera, Xylopia aromatica. Las esporas de helechos identificadas correspondieron a: Selaginella, Pteris, Blechnum, Polypodium, y esporas de gran tamaño del tipo trilete-estriado (cf. Anemia); 4/ Bosque semideciduo mesofítico con: Metopium, Spondias, Ficus, Casearia, Cedrela, Eugenia, Cissus, Ipomoea, Bucida/ Conocarpus, Borreria, Bursera simaruba, Sapotaceae, Malpighiaceae, Bromeliaceae. Así como polen del tipo Ambrosia, Bombacaceae (Bombax), Ericaceae, Umbelliferae, Evolvulus, Aster, Pinus caribaea, P. tropicalis, Heliotropium antillanum y Acrocomia. En esta región, el suelo pudo haber constituido un

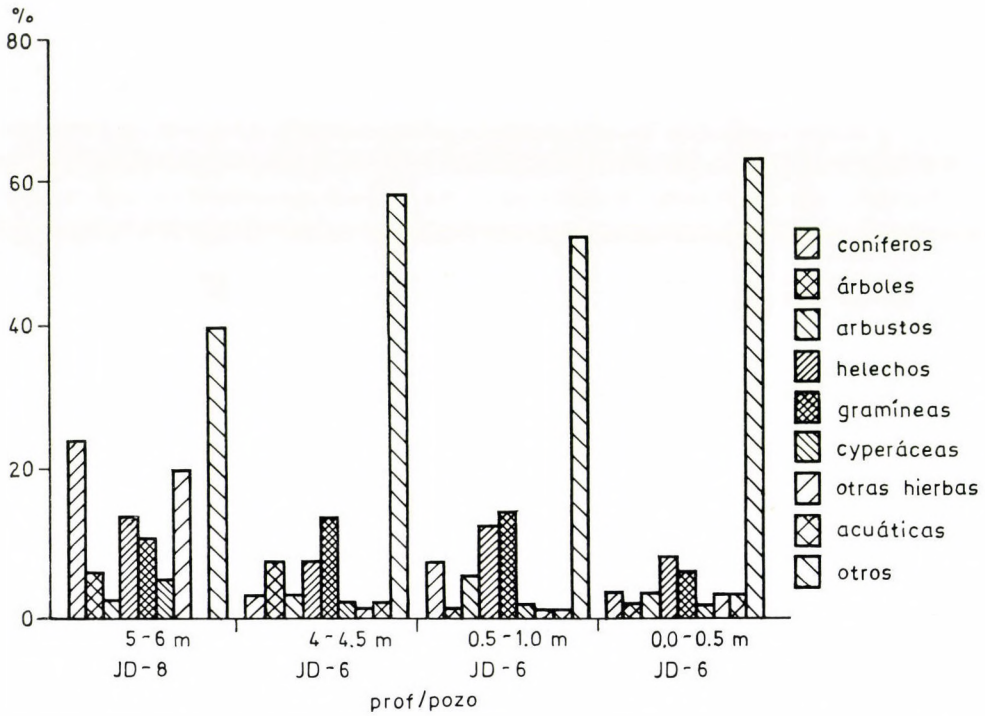

Fig. 3. Porcentaj de los grupos de palinomorfos encontrados en sedimentos marinos de la Bahía de San Pedro. Comparación de los grupos de polen hallados en los pozos JD-6 turba, y JD-8 arcilla aleurítica

factor importante para el desarrollo de la vegetación y de los diferentes habitats, según la evidencia de palinomorfos.

Contrariamente al noroccidente de la Isla de la Juventud, la vegetación que ha dado origen a la turba en la Bahía de San Pedro, corresponde a la vegetación que ha venido desarrollándose en condiciones de baja salinidad debido entonces a la gran influencia del agua dulce en la región durante el Holoceno. Los datos obtenidos proceden de las muestras de los pozos JD-6 y JD-8.

La composición del polen presentó cambios en el pozo JD-8, y esto es un hecho interesante para la interpreteción de los resultados (Fig. 3).

Los tipos de vegetación que caracterizaron la zona corresponden a: bosque de manglar, herbazal, comunidades acuáticas, bosque de ciénaga, bosque de galería y bosque semideciduo.

Herbazal representado por: Cladium jamaicense, Typha domingensis, Cyperus sp., gramíneas, Acrostichum aureum, Blechnum sp., Polypodium.

Comunidades acuáticas con Nymphaea, Nymphoides, Eichhornia y Utricularia. También se identificaron polen de Hydrocotyle, Caperonia palustris y Onagraceae.

El bosque de ciénaga se determinó por la presencia de los pólenes de: Myrica cerifera, Ilex cassine, Sabal parviflora, y el tipo de Bucida/Conocarpus. Bosque semideciduo, determinado por el polen de: Bursera simaruba, Eugenia sp., Celtis sp., Picrodendron macrocarpum, Metopium, Spondias, Guettarda calyptrata, Bunchosia, Faramea, Corchorus, Allophylus, Ipomoea, Gouania, Meliaceae.

Bosque de galería representado por el polen de Cyrilla racemiflora, Protium cubense, Melastomataceae y Podocarpus angustifolius, determinado del pozo JD-8.

Discusion

Los sedimentos de turba, arcilla aleurítica y arena con restos de materia orgánica provenientes de muestras recuperadas durante la perforación de los pozos (Tabla 1), representaron un material idóneo para el análisis palinológico. Las muestras, que se caracterizaron por la abundancia de palinomorfos, hicieron posible la pre-reconstrucción de algunas paleocomunidades, mediante la determinación del polen de familias, géneros y en ocasiones especies que estuvieron presentes en los sedimentos estudiados, de áreas que reflejan por su contenido de polen y esporas terrenos que en el pasado estuvieron emergidos y donde se desarrolló una vegetación de bosques. La región suroccidental y noroccidental de la Isla de la Juventud constituye un ejemplo donde huellas de la vegetación en la región (turba, polen, esporas) han permanecido como testigo, en diferentes sedimentos, hoy cubiertos por el mar. Esas comunidades vegetales estuvieron sometidas a las fluctuaciones del nivel del mar durante los períodos de transgresión-regresión marina del Pleistoceno Superior, los que también (FRANCO, inédito) tuvieron lugar en el Plioceno.

Conchas de moluscos marinos se hallaron en la zona correspondiente al bosque de ciénaga, procedentes del pozo JD-14. Este hecho es un indicio que implica una regresión marina que debe corresponder a la etapa Würm-Wisconsin del Pleistoceno Superior (A. DE LA TORRE, comun. pers.). Fueron identificadas: Chione cancellata, Borbatia, Bulla y Turridae.

Aunque se ha obtenido información preliminar de terrenos emergidos que durante el Pleistoceno Superior sustentaron comunidades vegetales, sólo

Tabla 1

Descripción del material

Pozo/prof. en metros	Prof. del agua de mar en metros	Caracteres de los sedimentos y localidades
JD-6 0.0—0.50 0.5—1.00 4.0—4.50 5.0—6.00	3.0	Turba en descomposición, color negro, moluscos presentes. La base arcillosa arenosa de color gris, debajo un incremento del material clástico cuarzoso intermezclado con aleurita.
JD-8 5.0—6.0 6.0—7.0	5.0—6.0 5.0	Interior de la Bahía de San Pedro.
JD-12 13.0—14.0	10.0	Fango aleurítico arcilloso con restos de moluscos con intercalaciones de turba. SO del estero El Soldado, 1.5 km de la costa al mar.
JD-14 14.0—16.5	10.0	Fango aleurítico con intercalaciones de turba y conchas de moluscos. NO de Pta. de Buenavista, 1 km de la costa.
JD-51 2.0—2.5	2.0	Arena gruesa con intercalaciones de arcilla aleurítica; con raíces de Thalassia. NO de Pta. de Buenavista, 1 km de la costa.
JD-38 3.0—4.50	10.0	Aleurita y arena cuarzosa con intercalaciones de turba y moluscos. Desembocadura estero del Pinar, a 200 m.

investigaciones posteriores de un estudio detallado de la secuencia estratigráfica geológica/palinológica harán posible una reconstrucción histórica de la vegetación y del clima en base a un perfil. No obstante, las muestras de sedimentos provenientes de los pozos JD-12, JD-14, JD-38 y JD-51 (Fig. 1) manifestaron asociaciones de polen que dan indicaciones de comunidades vegetales que ocuparon el área de estudio. Zonas costeras, pantanos, lagunas, ciénagas de agua salada o salobres con elementos de: bosque de manglar de Rhizophora mangle; herbazal de ciénaga con Blechnum, Typha, Cladium, Cyperus, gramíneas; bosque de ciénaga con Sabal, Acoelorraphe, Myrica, Cuphea; comunidades acuáticas con Nymphaea, Nymphoides. Así como elementos de bosques semideciduos y manigua con: Rauvolfia, Ambrosia, Tribulus, Sapotaceae, Myrtaceae y Burseraceae. El polen anemófilo de Pinus caribaea y

P. tropicalis estuvo presente en todas las muestras. La evidencia de estas zonas de vegetación fueron obtenidas de los pozos JD-12, JD-14 y JD-38. Entre otros microorganismos encontrados en las muestras de los pozos JD-14 y JD-51 están los Hystrichosphaeridae (Dinoflagellatae). ROSSIGNOL (1964) los ha mencionado en sedimentos del Pleistoceno del Mediterráneo Oriental y excluye la hipótesis de redeposición.

De los resultados de polen se deduce que hacia el occidente de la Isla de la Juventud la abundancia del polen de Rhizophora mangle y Chenopodiaceae indica que en el pasado la localidad de la perforación (Pozo JD-12) fue ocupada por una vegetación pionera de ambiente salino que estuvo influenciada por las oscilaciones del nivel del mar. En la Fig. 2 se observa que los porcentajes mayores de granos de polen, corresponden a árboles, destacándose el polen de Rhizophora mangle y, entre otras hierbas, el polen de Chenopodiaceae. STUCHLIK (1964) ha señalado que Chenopodiaceae aparece en sedimentos marinos conjuntamente con Hystrichosphaeridae y constituye un elemento pionero después de una retirada del mar. Resultados similares de Rhizophora y Chenopodiaceae han sido reportados por VAN DER HAMMEN (1984) de la Ciénaga Grande de Santa Marta, en Colombia.

Cabe señalar que el sedimento procedente del pozo JD-12 se caracterizó por la poca representación del polen de gramíneas y de otros tipos, lo cual puede tener exlicación en alguna afectación ecológica en la región, como a una menor afluencia del agua de los ríos en la región, así como además al ambiente salino en la misma. NEDECO (1959) mencionó la afluencia del agua de mar hacia la parte occidental de la Ciénaga de Zapata, donde hallaron turba de mangle, Rhizophora, y una vegetación poco desarrollada.

La composición de polen en el sedimento del pozo JD-14 demuestra un origen de zonas pantanosas por lo que refleja la vegetación del bosque de ciénaga y sus alrededores; es decir, el límite de ciénaga. La abundancia de palinomorfos correspondieron a palmas (Acoelorraphe) y a las esporas de helechos, aunque éstos no fueron muy variados. Entre otros pólenes presentes en bajas proporciones, se identificaron: Myrica cerifera, Caperonia palustris, Ilex, y Cuphea. La vegetación de los alrededores, es decir, el bosque semideciduo, estuvo representado por el polen de: Trema, Bursera simaruba, Myrtaceae y Anacardiaceae.

La muestra del pozo JD-51, por las características del sedimento, así como por el contenido de polen, sugiere haber estado cubierta por plantas de diferentes comunidades y habitats, todo lo cual implica cambios de las condiciones edáficas del sedimento y de la humedad del suelo probablemente

debido a alguna afluencia activa de agua dulce en la región que hizo propicio un ambiente favorable a la vegetación que allí se desarrolló. El sedimento se corresponde con lo que denominamos planicie costera, llanura o sabana, que en el Pleistoceno parece haber ocupado una gran extensión en el occidente de Cuba, extendiéndose desde el NO de la Isla de la Juventud hasta Pinar del Río inclusive, durante el tiempo en que el nivel del mar estuvo bajo.

Este sedimento, compuesto de arena gruesa, arcilla aleurítica, minerales y fragmentos pequeños de rocas, se relaciona, por sus elementos, a un material terrígeno. La vegetación que sustentó el área, según el análisis de polen, se corresponde en gran medida con la vegetación que en la actualidad presentan las áreas vecinas (La Coloma, Guane, Sabanalamar, Los Indios, Punta de Buenavista). Las características edáficas de este sedimento marino hacen pensar en este planteamiento, de donde se infiere que la antigue planicie costera tuvo su continuidad en la llanura costera actual del occidente de Cuba (sur de Pinar del Río y región noroccidental de la Isla de la Juventud). FRANCO (inédito) en relación a la región, manifestó: "en el ángulo noroccidental de la Isla de la Juventud participan los materiales derivados de vulcanitas y rocas asociadas que se encuentran en la denominada zona tectónica de Sabana Grande. Este macizo está formado por rocas terrígeno-carbonáticas metamorfizadas y plegadas más o menos completamente". A. DE LA TORRE (1972) manifestó resultados similares de la región.

La asociación de polen evidencia zonas de manglar, herbazal, comunidades de agua dulce, bosque de ciénaga, bosque semideciduo mesofítico, bosque de pinares, así como elementos de la planicie o sabana. Todo lo cual evidencia las distintas paleocomunidades existentes en la región durante el Pleistoceno.

En la muestra JD-51, Rhizophora y Chenopodiaceae mostraron un ligero aumento, lo que implica que la zona estuvo sometida a la influencia marina (transgresión). Las esporas de los helechos estuvieron bien representadas en cantidad y número de especirs, así como también las gramíneas. Este aumento podría estar relacionado con un incremento de la humedad, según sugiere la presencia de Botryococcus en el sedimento. Esto se opone a lo encontrado en las muestras de los pozos JD-12 y JD-14, en las que los granos de polen de las gramíneas y las esporas de helechos descendieron, situación ésta que podría tener conexión con un clima menos favorable en el Pleistoceno. Por otra parte, la abundancia del polen de Pinus, no obstante a que el mismo es

transportado por el aire, hace pensar en el desarrollo del bosque de pinares en el área de la planicie, hoy ocupada por un mar somero.

En el área de las perforaciones realizadas en la Bahía de San Pedro y en contraste con la región noroccidental, la región sur debe haber permanecido sumergida hasta el Pleistoceno Superior, período en el cual comienzan a manifestarse algunas áreas de terrenos emergidos, los que emergieron en la totalidad del área insular durante el Holoceno; así lo ha considerado G. FRANCO (inédito).

En la Fig. 3 (Pozo JD-6) la composición de polen refleja la vegetación de la ciénaga y sus alrededores. Los pólenes de gramíneas y esporas de helechos en la muestra de los pozos JD-16 y JD-8 se incremantaron (Fig. 3) en comparación con lo observado en la turba del pozo JD-12 así como en la muestra del pozo JD-14; los de Chenopodiaceae descendieron. Este cambio en la composición de los palinomorfos es algo significativo que podría tener su explicación en un clima menos favorable durante el período de la deposición de la turba del pozo JD-12 en el Pleistoceno Superior, y un clima más favorable (húmedo) que puede haber determinado una abundancia de gramíneas y helechos como se comprobó en las muestras de los pozos JD-6 y JD-8 las que se corresponden probablemente con distintas etapas del Holoceno. Los pólenes de gramíneas observados en las muestras tomadas a mayores profundidades se caracterizaron por su gran tamano.

La muestra del pozo JD-8 a unos 5.0--6.0 m de profundidad, muestra algunos elementos de Podocarpus angustifolius que aunque no aparece hoy en la actualidad, sugiere que el género estuvo presente en la Isla en la etapa Pleistoceno-Holoceno, todo lo cual hace pensar acerca de las condiciones ambientales que, al parecer, no fueron similares a las de hoy en día; posiblemente el clima fue frío y húmedo. Por otro lado, la muestra indica, además, un buen desarrollo del bosque al inicio del Holoceno, según se infiere de la composicón de polen de los pozos JD-8 y JD-6 (4.0--4.50 m de profundidad).

Conclusiones

1. Por el análisis de polen los sedimentos marinos estudiados de turba, arena, fango y arcilla aleurítica evidenciaron zonas que durante el Pleistoceno constituyeron terrenos emergidos. Durante el período de las regresiones marinas, el bosque de Rhizophora mangle marcó la posición de la denominada antigua línea de costa en el occidente de la Isla de la Juventud.

2. En la parte occidental de la Isla de la Juventud, según la evidencia geológica y palinológica, el área NO de Punta de Buenavista estuvo emergida durante algunas de las etapas del Pleistoceno (Würm-Wisconsin). Esta área parece corresponder y tener su continuidad en la llanura costera en la región sur de Pinar del Río, teniendo un contacto en La Coloma, y con la región NO de la Isla de la Juventud.

3. Esto hace suponer que la unión entre Pinar del Río y la Isla de la Juventud tuvo lugar durante el tiempo en que la planicie costera emergida facilitó una unión directa entre ambas regiones. Esta planicie tuvo como límite hacia el Este un mar poco profundo, considerando que hacia la región occidental de la Ciénaga de Zapata (Ensenada de la Broa) la NEDECO (1959) encontró turba muy antigua, la cual fecharon como del Pleistoceno.

4. Esta área que yace bajo el mar, pudo funcionar en el Pleistoceno como puente "corredor" que permitió el intercambio de varias ondas de migraciones en el Pleistoceno.

5. La igualdad florística entre el sur de Pinar del Río y el occidente de la Isla de la Juventud, indudablemente se dió durante la existencia de una planicie costera o llanura que pudo servir de zona receptiva de frutos y semillas y la que a su vez sostuvo comunidades similares a ambas regiones.

6. El ambiente terrestre y acuático, en el Pleistoceno, sostuvo comunidades vegetales equivalentes a las actuales del territorio pinero.

7. La presencia de Podocarpus en la Isla de la Juventud tuvo que requerir condiciones climáticas diferentes a las de hoy en día.

8. En la Bahía de San Pedro (Isla de la Juventud), al igual que en la Ciénaga de Zapata, en la Composición de la turba participan especies comunes a las que aparecen en las comunidades pantanosas de los Everglades de la Florida.

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Lista 1

Relación de los diferentes palinomorfos identificados del analisis polínico de sedimentos marinos. Pozo JD-6

Helechos

Acrostichum aureum
Blechnum serrulatum
Polypodium

Gimnospermas

Pinus caribaea
Pinus tropicalis

Angiospermas

Amaranthus
Ambrosia
Bucida/Conocarpus
Caperonia palustris
Cladium jamaicense
Cyperus
Cyrilla racemiflora
Chenopodiaceae
Eichhornia crassipes
Gramíneas
Ilex
Labiatae
Malpighiaceae
Melastomataceae
Metopium
Myrica cerifera
Myrtaceae
Nymphaea
Nymphoides grayanum
Palmae
Picrodendrum macrocarpum
Rhizophora mangle
Sapotaceae
Typha domingensis
Trema
Utricularia

Lista 2

Pozo JD-8

Helechos

Gimnospermas

Pinus caribaea
Pinus tropicalis
Podocarpus angustifolius

Angiospermas

Allophylus
Amaranthus/Chenopodiaceae
Ambrosia
Borreria
Bunchosia
Colpothrinax wrightii
Catostemma (?)*
Corchorus
Cyperus
Daphnopsis
Faramea occidentalis
Gouania
Gramíneas
Guettarda calyptrata
Heliotropium
Ilex
Ipomoea
Malvaceae
Melastomataceae
Meliaceae
Moraceae
Myrica cerifera
Myrtaceae
Onagraceae
Protium cubense
Rhizophora mangle
Sabal parviflora
Sapotaceae
Spondias

*Tipo Catostemma (30 m de diámetro; 6-porado, con los márgenes engrosados; espinulados).

Lista 3

Pozo JD-12

Helechos

Blechnum
Tipo trilete

Gimnospermas

Pinus caribaea
Pinus tropicalis

Angiospermas

Ambrosia
Bucida/Conocarpus
Cladium jamaicense
Cyperus
Chenopodiaceae
Gramíneas
Malvaceae
Myrica cerifera
Myrtaceae
Nymphaea
Nymphoides grayanum
Palmae
Rauvolfia
Rhizophora mangle
Typha domingensis
Tribulus
Trema

Lista 4

Pozo JD-14

Dinofíceas

Hystrichosphaera

Helechos

Blechnum
Lygodium
Polypodium
Tipo trilete

Gimnospermas

Pinus caribaea
Pinus tropicalis

Angiospermas

Acoelorraphe wrightii
Ambrosia
Anacardiaceae
Bucida/Conocarpus
Bursera simaruba
Caperonia palustris
Cuphea
Cyperaceae
Chenopodiaceae
Gramíneae
Ilex
Jacaranda coerulea
Malpighiaceae
Myrica cerifera
Myrtaceae
Nymphaea
Nymphoides
Rhizophora mangle
Sapotaceae
Trema
Typha domingensis
Xylopia aromatica

Lista 5

Pozo JD-51

Algas

Botryococcus

Dinofíceas

Tipo Hystrichosphaera

Helechos

cf. Anemia
Blechnum
Polypodium
Pteris
Selaginella

Gimnospermas

Pinus caribaea
Pinus tropicalis

Angiospermas

Acrocomia
Amaranthus/Chenopodiaceae
Ambrosia
Tipo Bombax
Borreria
Bromeliaceae
Bucida/Conocarpus
Casearia
Cissus
Cladium jamaicense
Colpothrinax wrightii
Ericaceae
Evolvulus
Ficus
Gramíneae
Ipomoea
Malpighiaceae
Meliaceae
Metopium
Myrica cerifera
Myrtaceae
Rhizophora mangle
Sapotaceae
Spondias
Umbelliferae (Apiaceae)
Utricularia
Xylopia aromatica

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OCCURRENCE OF ANABAENOPSIS RACIBORSKII WOLOSZ. IN THE POND TÓMALOM NEAR SOPRON, HUNGARY

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A dense population of the N_2-fixing blue-green alga Anabaenopsis raciborskii Wolosz. was found in the eutrophic pond Tómalom, west Hungary. Trichomes: 50-150 μm long, straight or slightly curved rarely up to half-circle; vegetative cells: 1.8-2.2 x 5.5-10.0 μm, cylindrical, slightly constricted at cross walle, with small granules, terminal vegetative cells are growing narrow towards the end of the trichomes; heterocysts: 2.2-2.5 x 4.0-9.0 μm, always in the end of the trichomes, definitely drop-like shape; spores: only one spore was found in the whole material, 4 x 16 μm, long-oval shape, large granules inside. A continuous overlap was observed between the typical A. raciborskii and A. raciborskii var. serians (Presc.) Hamar. The accumulation of records on A. raciborskii suggests that a blomm-forming blue-green alga is expanding in Europe.

Anabaenopsis (Cylindrospermopsis) raciborskii has been a widespread N_2-fixing blue-green alga. It is common in the subtropical lakes, in the reservoirs in southern Sovietunion and also reported in Brasilia and in the temperate regions of North America.

In Central Europe the species has been found in Austria, Czechoslovakia and Romania (HORECKA and KOMÁREK 1979).

SZALAI (1942) found the alga first in Hungary in river Kőrös, however, based on the drawing in the cited paper it should be questioned whether the given species belongs to A. raciborskii or not. More than 30 years later the Hungarian A. raciborskii data have begun to accumulate. SCHMIDT (1977) found it in a southern Hungarian lake, while HORECKA and KOMÁREK (1979) in middle Hungary. In 1975 it was found in considerable amount ($3.6\ 10^6$ trichomes 1^{-1}) in the Tokaj section of river Tisza (HAMAR 1977).

A. raciborskii appeared in Lake Balaton in 1979 (OLÁH et al. 1981). In late summer and autumn of 1982 a heavy bloom of A. raciborskii swept through the lake, the bloom peaked at 10^8 trichomes 1^{-1} in the Tihany region of the lake (PADISÁK et al. 1984; G.-TÓTH and PADISÁK 1986).

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Fig. 1

On 21 June 1984 Dr. Tamás TAKÁTS collected scooped samples in the eutrophic pond Tómalom near Sopron (western part of Hungary). Among 42 others (blue-greens 8, diatoms 5, coccal greens 29) A. raciborskii was the dominant species.

Morphological description (Fig. 1):

Trichomes: 50-150 μm long, straight or slightly curved rarely up to half-circle;

Vegetative cells: 1.8-2.2 x 5.5-10.0 μm, cylindrical, slightly constricted at cross walls, with small granules, terminal vegetative cells are growing narrow towards the end of the trichomes;

Heterocysts: 2.2-2.5 x 4.0-9.0 μm, always in the end of the trichomes, definitely drop-like shape;

Spores: only one spore was found in the whole material, 4 x 16 μm, long-oval shape, large granules inside.

A continuous overlap was observed between the typical A. raciborskii and A. raciborskii var. serians (Presc.) Hamar.

The accumulation of records on A. racibroskii suggests, that a bloom-forming blue-green alga is expanding in Europe.

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CHANGES IN NUTRIENT CONTENTS OF TERMITOMYCES ROBUSTUS (BEELI) HEIM AND LENTINUS SUBNUDUS BERK DURING SPOROPHORE DEVELOPMENT

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Samples of mature, young and very young sporophores of Termitomyces robustus (Beeli) Heim and Lentinus subnudus Berk were analysed for their nutrient contents. The amino acid, protein, glycogen, lipid, sugar, ascorbic acid and ash contents were found to increase from very young to mature sporophores. These macronutrients except the crude fibre were more abundant in the pilei than the stipes. In contrast, the micronutrients did not show any definite trend in the sporophores investigated. However potassium was the most abundant followed by phosphorous and magnesium in that decreasing order. Manganese was the least abundant in the sporophores. It is clear from this study that T. robustus and L. subnudus would compare favourably with other well-known nutritious mushrooms.

Introduction

Mushrooms as food and delicacies are now assuming greater importance in human diets worldwide. Edible mushrooms are highly nutritive and compare favourably with meat, egg and milk. They are known to contain a lot of water, proteins, lipids, sugars, amino acids, glycogen, vitamins (B, C, D), and mineral elements (Ca, K, Na, P, Fe, Cu, Mn, S, Mg) (MUKIIBI 1973; HINNERI 1975; PARENT and THOEN 1977; MOORE et al. 1979; GRUEN and WONG 1982; ZAKHARY et al. 1983). Apart from their nutritive values, mushrooms also have potential medical benefits especially as antitumor (LUCAS et al. 1957) and hypocholestromic agents (SUZUKI and OSHIMA 1976).

Few studies have been carried out on the changes in nutrient contents of mushrooms during sporophore development. Some of those carried out were done by HINNERI (1975), ROBERT (1977), MOORE et al. (1979), GRUEN and WONG (1982) and ZAKHARY et al. (1983). There is presently no published work on any Nigerian mushroom on the changes of nutrient contents in sporophores during development. The present study was therefore an attempt at investigating changes in nutrient contents during sporophore development of two

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Nigerian edible mushrooms, Termitomyces robustus (Beeli) Heim and Lentinus subnudus Berk. It is hoped that this study will provide adequate information on the best developmental stage for harvesting their sporophores.

Material and Methods

Termitomyces robustus samples were collected from the field while Lentinus subnudus samples were harvested from daily-watered logs of wood at very young, young and mature stages (Table 1). These were divided into pilei and stipes, dried for 2 days at 70 °C, powdered in a Moulinex blender and sieved through a 250 μm sieve. The residue was repeatedly reground and resieved and the final powdered samples were employed in the following proximate analyses:

Ash: Three grams of powdered sample were ashed in a Gallenkamp furnace in a previously ignited crucible of known weight at 550 °C for 6 hours. Fairly cooled crucibles were put in desiccators and later weighed (PARENT and THOEN 1977; OSBORNE and VOOGT 1978).

Ethanol-Soluble Sugars: One gram of powdered sample was extracted for 6 hours in a Soxhlet extractor with 30 ml boiling 80% ethanol. The amount of ethanol-soluble sugars in 1 ml of the extract was determined using the phenol-sulphuric acid method of DUBOIS et al. (1956).

Free amino acids: One hundred milligrams of powdered sample were extracted with 15 ml distilled water in a water-bath at 75 °C for 10 minutes. The supernatant obtained after centrifuging was used for the amino acid assay using the ninhydrin method of YEMM and COCKING (1955) as modified by ROSEN (1957).

Protein: Five hundred milligrams of powdered sample were extracted with 50 ml 2% NaCl in a water-bath at 60 °C for 1 hour. The saline extract was filtered and 50 ml of 3% copper acetate monohydrate were added to the filtrate to precipitate proteins (OSBORNE and VOOGT 1978). The precipitated proteins were centrifuged out, washed with 50 ml cold distilled water and dissolved in 50 ml 0.1 M NaOH. The quantity of protein in the alkaline solution was estimated using folin-phenol method of LOWRY et al. (1951) with casein as the standard protein.

Total lipids: Total lipid quantities present in the powdered samples were determined using the petroleum ether method as described by MUKIIBI (1973), PARENT and THOEN (1977).

Mineral elements: One gram of each powdered sample was assayed for phosphorous, calcium, magnesium, potassium, sodium, manganese, iron, copper and zinc at International Institute for Tropical Agriculture (I.I.T.A.), Ibadan, Nigeria. This was carried out with the aid of flame photometer and automated atomic absorption spectrophotometer after wet digestion.

Ascorbic acid: This was determined using the 2,6-dichloroindophenol titration method as described by LAMBERT and MUIR (1976).

Glycogen: This was determined using the anthrone method of CARROLL et al. (1956).

Crude fibre: This was determined using the A.O.A.C. method of 1980.

Moisture content: Freshly harvested very young, young and mature mushrooms of each species were divided into pilei and stipes, dried for 2 days at 70 °C and weighed.

Table 1

Sizes of T. robustus and L. subnudus sporophores employed as mature, young and very young mushrooms

Mushroom species	Pileus dimensions		
	Mature stage	Young stage	Very young stage
T. robustus	6—13 cm	3—5.9 cm	less than 3 cm
L. subnudus	4— 6 cm	2—3.9 cm	less than 2 cm

Table 2

Moisture, dry matter and Macronutrients contents of various stages of T. robustus and L. subnudus.
Values are means of three replicates calculated as % dry weight except A and B that were calculated as % fresh weight

Mushroom species	Moisture content (A)	Dry matter content (B)	Ethanol-soluble sugar	Glycogen	Crude fibre	Ash	Total lipids	Ascorbic acid	Protein	Free amino acids
T. robustus										
Mature pileus	86.03a	13.97c	7.93a	6.13a	7.11abc	9.67a	4.61a	0.073a	16.56a	9.87a
Mature stipe	85.07a	14.93ac	6.47b	4.93bc	8.65a	8.68b	3.48b	0.049dc	13.60b	5.40b
Young pileus	85.30a	14.71c	6.40b	5.20ab	6.40bc	7.38c	3.93b	0.062bc	13.35b	6.60b
Young stipe	84.75a	15.25ac	4.87cd	4.00cd	7.28ab	5.70d	2.71c	0.042dce	10.43c	3.87c
Very young pileus	83.12a	16.88ac	5.17c	3.00de	5.58c	6.61c	2.55c	0.052c	10.79c	3.60c
Very young stipe	82.30a	17.70a	3.87d	2.13e	6.22bc	3.57e	1.93d	0.036e	8.60d	2.10d
L. subnudus										
Mature pileus	92.64a	7.36c	6.47a	8.60a	15.57b	12.00a	3.58a	0.067a	19.05a	6.88a
Mature stipe	88.02abc	11.98c	4.23bc	6.87bc	22.08a	9.74c	2.49bc	0.049b	15.27ce	5.49ab
Young pileus	90.27ab	9.73c	5.13b	7.13b	10.74d	10.57b	2.67b	0.063a	17.23b	4.50bd
Young stipe	85.87bc	14.13b	3.77cd	6.20bc	13.41c	8.31d	1.75d	0.036c	11.35d	3.02cde
Very young pileus	89.98ab	10.02b	4.76c	6.00c	6.25e	8.63d	2.05cd	0.046b	15.47e	3.24de
Very young stipe	84.92c	15.08a	3.10d	4.67d	9.42d	6.25e	1.68d	0.024d	9.47f	2.60e

Means followed by the same letter (s) within any mushroom group are not significantly different at P = 0.01 by Duncan's multiple range test.

Results

In the pilei and stipes of both T. robustus and L. subnudus, the ash, ascorbic acid, amino acid, crude fibre, glycogen, lipid, moisture, protein and sugar contents increased with sporophore size. These macronutrients, except the crude fibre, were obtained in greater preponderance in the pilei (Table 2). In contrast, the dry matter contents decreased with sporophore size from the very young to mature stage (Table 2).

The pilei of the different sporophore sizes contained greater amounts of magnesium, phosphorous and potassium than their corresponding stipes (Table 3). In the case of calcium the converse was true (Table 3). With regard to the other mineral elements assayed, no such comparable pattern was found in the two mushroom species. For example, the pilei of T. robustus and L. subnudus contained lower amounts of manganese and iron, respectively, while the stipes of T. robustus at the different sporophore stages contained greater amounts of copper (Table 3).

The dominant macronutrient in the two mushrooms was found to be protein while the dominant micronutrient in them was potassium (Tables 2 and 3). L. subnudus contained greater amounts of ash, calcium copper, crude fibre, glycogen and magnesium than T. robustus did while T. robustus contained more amino acids, lipids, sugars, iron, manganese, phosphorous and potassium (Tables 2 and 3).

Table 3

Micronutrient contents of the various stages of T. robustus and L. subnudus. Values are for one replicate calculated as mg/g dry weight

Mushroom species	P	Ca	Mg	K	Na	Mn	Fe	Cu	Zn
T. robustus									
Mature pileus	8.686	0.069	1.204	27.637	2.926	0.030	0.992	0.064	0.116
Mature stipe	3.956	0.058	1.050	24.604	3.819	0.049	1.312	0.009	0.067
Young pileus	8.570	0.081	1.367	26.983	3.564	0.024	0.617	0.032	0.099
Young stipe	4.291	0.107	0.811	23.376	2.799	0.095	4.119	0.016	0.077
Very young pileus	8.669	0.079	1.365	25.759	3.084	0.029	0.935	0.034	0.092
Very young stipe	5.426	0.106	0.847	23.184	2.557	0.081	0.054	0.017	0.099
L. subnudus									
Mature pileus	5.828	0.642	2.174	21.657	2.521	0.024	0.272	0.065	0.105
Mature stipe	4.160	0.579	2.058	16.880	3.902	0.036	0.574	0.126	0.076
Young pileus	3.472	0.977	2.233	20.480	2.551	0.030	0.417	0.099	0.103
Young stipe	1.395	2.909	1.940	19.770	2.367	0.027	0.702	0.024	0.081
Very young pileus	4.090	1.051	2.221	20.099	2.636	0.025	0.244	0.073	0.106
Very young stipe	1.531	2.690	1.766	14.805	1.980	0.025	0.560	0.047	0.071

Discussion

In Lentinus subnudus and Termitomyces robustus, the ash, ascorbic acid, amino acid, crude fibre, glycogen, lipid, moisture, protein and sugar contents increased with sporophore development (Table 2). ZAKHARY et al. (1983) working with Agaricus rodmani and A. campsteris recorded a similar increase in lipid, carbohydrate and crude fibre contents but a slight decrease in protein and ascorbic acid contents. The continuous increase of macronutrients obtained in the present study shows that the sporophere of L. subnudus and T. robustus is an active sink during development.

With the exception of crude fibre, the pileus accumulated more macronutrients than the stipes (Table 2). This finding is in agreement with those of MOORE et al. (1979), JANDALK and THIANGA (1981) and GRUEN and WONG (1982), who found the pileus to be a more active sink than the stipe in Coprinus lagopus, Macrolepiota procera and Flammulina velutipes. On the contrary, the greater crude fibre contents found in the stipes may be due to its mechanical role of support for the sporophore.

In both L. subnudus and T. robustus, protein was found to be the most predominant macronutrient while potassium was the most predominant micronutrient (Table 3). This is identical with the results obtained by JANDALK and THIANGA (1981) but differs from those of HINNERI (1975) and ZAKHARY et al. (1983), who recorded magnesium and iron as the predominant micronutrients, respectively. The non-definite trend in the distribution of micronutrients in the various sporophore stages of L. subnudus and T. robustus is not strange. HINNERI (1975) who studied the micronutrient distribution in the juvenile and mature sporophores of some mushrooms got a similar result. The higher calcium content in the stipes of both mushrooms is not surprising because the stipe supports the full weight of the heavier pileus.

From the present study, it is clear that the most nutritive sporophore stage in both L. subnudus and T. robustus is the mature stage. Coincidentally this is the stage at which field mushrooms are harvested for the table in Nigeria. At this stage, the sporophore is more nutritious in protein than cowpea, groundnut, cowmeat, turkey, fish, milk and fresh egg (CYENUGA 1968; OSBORNE and VOOGT 1978).

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THE MAIN CONSTITUENTS AND NUTRITIVE VALUE OF PODAXIS PISTILLARIS

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Edible mushroom Podaxis pistillaris growing in the wild in various regions of Saudi Arabia were collected to determine their nutritive value for human consumption. Chemical constituents determined were moisture content, total nitrogen, total protein, true protein, total carbohydrates, total lipids and ash content. Minarals such as, K, Na, Fe, Mg, Mn, Ca, Zn, Pb and Cd and seventeen essential amino acids were also determined. These constituents are discussed in terms of their relative importance as a source of nutrients for human consumptions.

Introduction

Podaxis is an edible mushroom which grows in sandy and sandy loam soil in areas with a long dry spell, around the world between 40^{O} N and 40^{O} S latitude (MORSE 1933). The genus was reported by MORSE (1933) to be monotypic with a single species, Podaxis pistillaris (L. ex Pers) Fr. However, it has been divided recently into more than one species (MCKNIGHT 1985). The fruiting bodies appear after rains, generally in the spring and early summer (KHAN and KHAN 1979). The fruiting body of P. pistillaris has a tall erect stalk, and an oval elongate dry head 6-10 cm long, 10-15 mm thick which in the early stages of development is smooth, but in later stages is covered with large ragged scales (MILLER 1972).

In Saudi Arabia, large quantities of Podaxis pistillaris are collected by nomades from the vast plains and deserts during the rainy season. The fruiting bodies are used both as food either alone or in combination with other food ingredients for flavour.

Nutritionally, mushrooms are referred to as "vegetable beef steak" by some authors, while others consider them to be of no significance. RAMASAMY and KANDASWAMY (1978) have reported that P. pistillaris is rich in proteins containing all the essential amino acids. However, research to evaluate the

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nutritional characteristics of the mushrooms has not been carried out intensively.

The present study was undertaken to evaluate the nutritive value of wild mushroom growing in various regions of Saudi Arabia as source of human food.

Material and Method

The fruiting bodies of P. pistillaris growing under natural condition were collected from Qaseem, Hail and Jouf regions of Saudi Arabia. First, they were dried in an oven at 70 °C for 72 h and finally ground to a powder.

For amino acid determination, the Podaxis were washed with dionized water to remove surface contaminants sliced into 5 mm thickness and freeze-dried.

The percentage of moisture content and ash were estimated by the standard methods of analysis using the A.O.A.C. procedure (1965). Total nitrogen and total protein was determined using Kel-Foss automatic 16200 (from A/S N FOSS ELECTRIC, Denmark). The true protein content of P. pistillaris cells was determined by the Robinson—Hogdon—Biuret method described by HERBART et al. (1971), using a Pye Unicam SP 8-400 UV VIS spectrophotometer.

The qualitative and quantitative determinations of amino acids present in P. pistillaris were made using an amino acid analyzer (L.K.B 4400). Total carbohydrates were determined by the phenol reaction (HERBART et al. 1971), and total lipids were determined by extraction of total crude lipids described by BLIGH and DYER (1951). The amount of potassium, sodium, lead, iron, cadmium, magnesium, manganese, calcium and zinc were determined in the same samples (CHAPMAN and PRAT 1961), using a Perkin—Elmer 305 B Atomic Absoprtion Spectrophotometer. The mean value of each constituent was calculated on dry weight basis.

Results and Discussion

The results (Table 2) shows that P. pistillaris contains approximately 71% moisture which is less than the moisture content of A. bisporus (90.2%) (ABOU-HEILAH et al. 1986). Fresh specimens of truffles (Tuber spp.) generally contain about 90% moisture (BARBERO 1969). Since P. pistillaris has a leathery texture it is expected to have a lower moisture content compared to other mushrooms. Failure to take into consideration the high moisture content of fresh or dehydrated mushrooms will lead to inflated estimates of their nutritive value.

The protein content of P. pistillaris (Table 1) compares favourably with that of other mushrooms such as Volvariella diplasia (BANO et al. 1971) and Agaricus bisporus (CHANG 1972). The protein content of P. pistillaris changes with the advances in phenological stages and increase with an increase in the age of the sporophore (2.3%--26.5%). This is in agreement with the results of RAMASAMY and KANDASWAMY (1978), however, CHANG (1972) reported that protein content of V. volvacea decreased with an increase in

Table 1

Total protein content in fresh fruiting bodies of *P. pistillaris* using different conversion factors

Factors	Percent (%)*
(T.N X 4.38)**	21.81
(T.N X 6.25)	31.13
(T.N X 8.48)	37.4

*Values are the mean of three replicates

**Podaxis varies in total protein content from as low as 2.3% to as high as 26.5% (In edible stages averaged 21.81%)

Table 2

Main chemical constituents of fresh fruiting bodies of *P. pistillaris* (on dry weight basis)

Constituents	Percent (%)*
Moisture content	76.00
Total nitrogen	04.98
True protein	34.90
Total carbohydrates	18.50
Total lipids	02.25
Ash content	12.37

*Values are the mean of three measurements.

the age of the sporophore. The percentage protein in *P. pistillaris* (21.81%) is lower than that in *A. bisporus* (38.2%) (ABOU-HEILAH *et al.* 1986), but is comparable to those in dried peas (24.2%), beans (24.0%) and corn flour (7.8%) (FLEGG and MAW 1976).

Studies on total mushroom protein (N X 625), however, suggest that only 34-89% of the protein is digestible (LINTZEL 1943). Other studies indicate a probable digestibility of 60-70% (GILBERT and ROBINSON 1957). The reduced digestibility of mushroom protein can be due to the presence of large amount of nonprotein nitrogen in their chitinous cell walls, and thus nitrogen is calculated as total protein by standard nitrogen analysis.

A closer approximation of total protein of mushrooms can be obtained using a conversion factor (70% N X 6.25) or (N X 4.38). Although the use of this conversion factor may not be appropriate to correct total protein

Table 3

Amino acids content of fresh fruiting bodies of P. pistillaris (on dry weight basis)

Amino Acids	gm/100 gm*	%
Alanine	1.630	6.5
Arginine	1.772	7.4
Aspartic acid	3.203	13.2
Cystine	0.272	1.1
Glutamic acid	4.714	19.3
Gylcine	1.366	5.7
Histidine	1.199	4.9
Isoleucine	1.339	5.4
Leucine	1.759	7.3
Lysine	1.277	5.4
Methionine	0.168	0.6
Phenylalanine	1.098	4.5
Proline	0.845	3.4
Serine	1.410	5.7
Threonine	1.182	4.8
Tyrosine	0.327	1.3
Valine	0.847	3.4

*Values are the mean of three measurements.

content in all species of mushrooms, it is applicable in estimation of total proteins in P. pistillaris.

The total proteins calculated as (N X 4.38) may show that mushrooms have a high nutritive value but it is a less accurate indicator for "true protein". Using (N x 4.38) factor we found total protein in P. pistillaris to be 21.81% (Table 1) while true protein which was determined colormetrically averaged 34.90% (Table 2).

The corrected total protein content of mushrooms show extreme variation even among different samples of a given species. Protein content may vary from as low as 4.9% for species of Auricularia to as high as 44% for Agaricus (CRISAN and SANDS 1978). However, our analysis indicates variation in protein content of Podaxis from as low as 2.3% to as high as 26.5%, while in aged palatable stages it averaged to 21.81%. CHANG (1972), has also found little difference in the crude protein content of Volvariella volvacea cultivated on different composts.

Using the factor (N X 4.38), our average results for total proteins in mushrooms fall within the range of analysis of mushroom strains 24-44% reported by CRISAN and SANDS (1978).

Table 4

Analysis of minerals in fresh fruiting bodies of P. pistillaris (on dry weight basis)

Mineral constituents	(ppm)*
Cadmium	Nill
Calcium	11.82
Iron	00.45
Lead	00.06
Magnesium	05.29
Manganese	00.03
Potassium	16.45
Sodium	05.80
Zinc	00.68

*Values are the mean of three measurements.

To solve the confusion concerning mushroom protein, FITZPATRICK et al. (1946) found that a purified mushroom protein isolate, contained 11.79% N rather than the expected 16%. Based on their data, a conversion factor of (N X 8.48) would be more appropriate for estimating mushroom proteins. This factor seems to be accurate based on our results of total protein and true protein. Since our total N content (4.98%) (Table 2) is far below the presumption of 16% we believe, according to our findings of total N (Table 2), total protein (N X 8.48), (Table 1) and true protein (Table 2), that a conversion factor of (N X 8.48) is the most accurate factor when compared with (N X 6.25) and (N X 4.38) factors, because total protein should always be higher that true protein as it contains conjugated "true protein" in addition to free protein.

The qualitative analysis of P. pistillaris indicated the presence of seventeen amino acids including all the essential amino acids listed by GOPALAN et al. (1974), all fairly in good amounts (Table 3). Comparison of the amino acid compositions of mushroom proteins with other protein sources. It appears that mushroom proteins are relatively high in lysine (5.4%) which is comparable to the lysine in Pleurotus cystidiosus (MISRA et al. 1983). Mushrooms can be used in human diet to supplement lysine deficiency due to deficiency or lack in cereal and vegetable proteins.

The method described by BLIGH and DYER (1951) was used to determine the crude lipids in P. pistillaris. The lipid content of mushrooms on dry weight basis was found to be 2.25% shown in (Table 1).

Fresh mushrooms contain relatively large amounts of carbohydrates (3-28%), and ash (8-23%) (CRISAN and SANDS 1978). The carbohydrates content may consist of a large variety of compounds. Carbohydrate content of P. pistillaris was analysed and found to be 18.5% and ash was 12.37% (Table 1). These percentages fall within the range reported by CRISAN and SANDS (1978) who worked on A. bisporus.

Mineral analysis of mushrooms showed that potassium (16.45 ppm) and calcium (11.82 ppm) rank high in comparison with other elements (Table 4). Trace amount of iron (0.45 ppm) was found which is in agreement with the findings of CRISAN and SANDS (1978). Manganese (0.03 ppm) was also present in trace amount. The undesirable minerals such as lead was found to be (0.06 ppm) which is below the range reported by THOMAS et al. (1972), for A. bisporus (0.36 ppm).

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CULTIVATION AND NUTRITIVE VALUE OF LEUCOCOPRINUS BIRNBAUMII

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Leucocoprinus birnbaumii an edible mushroom was analysed chemically for its crude protein, amino acids, sugar and mineral content. It was also cultivated artificially on chopped paddy straw at 25 °C.

Introduction

During a survey of agaric flora of Lucknow and nearby areas we collected an edible mushrooms, Leucocoprinus birnbaumii. Because of its rarity, this mushroom has remained neglected and there is no report of its chemical analysis. To find out its nutritive value it was analysed for its crude protein, amino acid, sugar and mineral content and tried to cultivate it artificially under controlled conditions.

Material and Method

Chemical analysis

Sporophores were dried in an oven at 40 °C. Total nitrogen was determined by Micro-kjeldahl method as described by A.O.A.C. (1960). Crude protein was calculated by using the conversion factor (N X 6.25). For estimation of amino acids approximately 20 mg of powdered material was hydrolysed by 6N HCl in vacuum sealed tubes for 24 h at 100 °C. The vacuum dried hydrolysate was finally dissolved in sodium citrate buffer of 2.2 pH, centrifuged, filtered and applied on LKB 4101 amino acid analyser column after necessary dilution.

Total sugar and reducing sugars were estimated by ANTHRONE Somogyi's (1945) (MORRIS 1948) methods, respectively. Qualitative estimation of sugars was done by means of unidirectional descending paper chromatography using upper phase of acetic acid—n-butanol—water (1:4:5) as solvent and n-aniline phthalate as developer.

Sodium and potassium were estimated by flame photometer and calcium, magnesium, iron, zinc, manganese and nickel were estimated by atomic absorption spectrophotometer.

Cultivation

The culture of L. birnbaumii was obtained from young sporophore tissue on potato dextrose agar slants under aseptic conditions. Mycelium grew rapidly at 25 °C. Its spawn was

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Fig. 1

prepared on the wheat grains and paddy straw was chosen as the substrate. Paddy straw was chopped and soaked overnight in water. Excess water was drained off and then sterilized at 15 lb/in^2 steam pressure for two hours. Straw was filled in shallow trays and spawning was done in 2-3 tiers. After spawning trays were covered with polythene sheets and kept at 25 ^{o}C. After about 30-40 days primordium formation started and mature sporophores developed after 2-3 days (Fig. 1).

Results and Discussion

Total nitrogen and crude protein are 6.35% and 39.69%, respectively, on dry weight basis. Nitrogen content is higher as compared to several other mushrooms viz. Tricholoma flavovirens (1.83--4.07%) (MOTSKUS 1977a), Lactarius torminosus (2.2--5.6%) (MOTSKUS 1977b) and Paxillus involutus (2.24--4.2%) (MOTSKUS 1977c). Protein content is higher than many Pleurotus spp. such as P. eous (25.0%), P. florida (27.0%) (BANO et al. 1981a), P. cystidiosus (24.2%) (MISRA et al. 1983) and Agaricus bisporus (35.0%) (FLEGG and MAW 1976) but lower than Macrolepiota rachodes (41.47%) (GUPTA et al. 1982).

Table 1

Amino acid content of L. birnbaumii
(g/100 g of protein)

Arginine	4.82
*Lysine	2.30
Histidine	2.20
*Phenylalanine	4.33
Tyrosine	0.86
*Leucine	6.88
*Isoleucine	4.25
*Methionine	11.22
*Valine	4.85
Cystine	0.59
Alanine	7.93
Glycine	10.11
Proline	5.27
Glutamic Acid	9.49
Serine	5.31
*Threonine	4.77
Aspartic Acid	9.54
*Total essential amino acid	38.60

Amino acid analysis shows the presence of seventeen amino acids including all the essential ones (Table 1). Essential amino acid content is quite high (38.6%), higher than in many Pleurotus spp. viz. P. flabellatus (31.0%) (BANO et al. 1963), P. ostreatus (24.93%) (JANDAIK and KAPOOR 1976), P. eous (35.98%) and P. florida (32.61%) (BANO et al. 1981a), Agaricus bisporus (20.66%) (KALBERER and KUNSCH 1974) and Macrolepiota rachodes (31.6%) (GUPTA et al. 1982).

Sugar analysis shows that L. birnbaumii is poor in its sugar content. It is only 1.197% of which 1.08% is reducing sugar. It is lower as compared to many mushrooms such as Agaricus spp. (3.5%), Boletus edulis (5.2%), Lactarius deliciousus (3.0%) (SINGER 1961), Macrolepiota rachodes (16.49% reducing and 3.57% non reducing sugar) (GUPTA et al. 1982) but higher than Pleurotus sajor-caju (0.285%) (JANDAIK and KAPOOR 1975).

Qualitative analysis of sugars revealed the presence of glucose only. However, many workers (McCONNELL and ESSELEN 1947; HUGHES et al. 1958; HOLTZ 1971; PARRISH et al. 1971) have found xylose, ribose, rhamnose, fucose,

Table 2

Mineral content of L. birnbaumii
(g/100 g of dry weight)

Na	0.100
K	12.44
Ca	0.993
Mg	0.280
Fe	0.1597
Zn	0.0642
Mn	0.0773
Ni	0.0505

glucose, galactose, mannose, sucrose, mannitol, inositol, galacturonic acid and glucuronic acid as sugar component in Agaricus bisporus.

L. birnbaumii contains fairly good amount of different minerals (Table 2). Potassium is quite high (12.44%) while zinc, manganese and nickel are in very low quantity.

ANDERSON and FELLERS (1942) found high amounts of potassium, phosphorus, copper and calcium in Agaricus campestris. JANDAIK and KAPOOR (1975) reported phosphorus 1.62%, potassium 2.58%, calcium 0.04%, magnesium 0.16%, sodium 0.20%, copper 0.02%, zinc 0.0025%, manganese 0.0042% and iron 0.010% on dry weight basis in Pleurotus sajor-caju. DRABAL et al. (1975a, b) reported iron 70—1530 mg/kg, manganese 9—100 mg/kg and copper 8—179 mg/kg in fifteen different mushrooms. BANO et al. (1981b) found high content of potassium, phosphorus and magnesium in four species of Pleurotus, namely P. eous, P. florida, P. flabellatus and P. sajor-caju.

Thus it is evident that L. birnbaumii is also a good source of protein, amino acids and minerals.

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THE REPRODUCTIVE BIOLOGY AND THE DEGENERATION PATTERNS OF TWO LICHEN POPULATIONS: LECANORA CARPINEA AND LECIDELLA ELAEOCHROMA

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Production of spores of two lichen populations were investigated between 1979 and 1988 in differently polluted zone of West Hungary. The whole and the reproductive area (A and A_r) of the populations were also measured in a polluted area of Szombathely city. Apothecia have fallen out of the thalli of L. carpinea and not a single was produced during the 10 years period. In the case of L. elaeochroma pieces of thalli in different size have fallen out of the boles, together or without apothecia. The ratio of $\frac{A}{A_r}$ shed light on the characteristic pattern of the degeneration of the species.

Statistical examinations showed significant negative correlations between the spore production of the two lichen species and the SO_2 concentrations. Significant positive correlation was found between the spore production of L. carpinea and the acidity of the rain. It was suggested that only long-term investigations serve reliable results in the field of bioindication.

Introduction

The main purpose of this study to give new informations about the reproductive biology of two lichen species, Lecanora carpinea and Lecidella elaeochroma on the basis of 10 years investigation.

Some examples are given of the "performance" (SEAWARD 1976) and "environmentally induced modification..." (POELT 1974) of lichen thalli, but there were no long term investigations made about the reproductive biology and degeneration pattern of lichen populations.

Lichens are growing very slowly so we need more than one or two years research to reach exact results in the field of ecology and coenology. In consequence of this fact it is impossible to work on the level of autecology, and the studies with the conception of "one thalli -- one ecological factor" have no morphological and/or taxonomical backgrounds.

Measurements on the growth rate of one colony or examinations on the degeneration of a thallus serve no reliable results neither of the direction

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nor about the rigour of environmental pollution. Using one species for the bioindication of an area leads astray, too. The epiphytic microcoenosis of the lichens must be find out again, but only with the application of strict methods, reflecting to the particular physiology and morphology of these organisms (e.g. KISS 1986; KUPFER-WESELY and TÜRK 1987).

One of the new possible ways is the application of the life-strategy concept (KISS 1985, 1987), including multidimensional approaches and well defined disciplinar roots (KISS 1988).

This paper consist of a little part of an extensive material which was elaborated between 1979 and 1988.

Methods

One of the approaches studying reproductive biology is the estimation of the spore production, applying light microscope. Samples of apothecia were taken from the middle part of the colonies. It was thought that these apothecia are neither the oldest nor the youngest in their "ages". Samplings were carried out annually, always in the April, "...because the amount of rainfall undoubtedly influences the reproduction of lichens" (PYATT 1974), and the spring and/or the autumn were considered as optimal seasons of the spore production (VERSEGHY 1965).

Thalli in the diameter between 1.5 and 3.0 cm were selected for examination. Collections were made from the following sites:

1. near Kőszeg (little town), in the valley called "King", and in orchards.
2. Olad (little village), in an orchard.
3. Szombathely (bigger, polluted city), in an avenue.

Substrata were Juglans regia trees at the age between 30 and 50. Colonies living on the smooth surfaces of the bark were investigated between the height of 0.5 and 1.0 meter.

Longitudinal sections were made from the apothecia with the use of freezing microtome. Twenty apothecia were investigated from the above-mentioned localities every year. Exceptions were made with the thalli of L. elaeochroma in the polluted region of Szombathely. In this area the production of the apothecia is not as common as in less polluted zone.

The estimation of the spore production based on the conception of VERSEGHY (1965), but the interval of the scale used by the author was broader; usually between 25, 50, 75 and 90%.

Results were represented with the use of column diagrams. The numbers above the columns show, how many apothecia fall under an estimated per cent (%) of asci containing spores.

Statistical examinations were made in searching of the connections between the spore production and the acidification of the rain, and between the spore production and changing SO_2 pollution, during the past 10 years. Statistical comparisons were made between the spore production of the two lichen species in differently polluted zone, too. Linear regression — r — were applied for statistical examinations.

Another important aspect of the researches of the reproductive biology of the lichen thalli was the yearly measurement of the whole- and the so-called reproductive-area (A and A_r) of the populations evaluating by computer, type HP 9620. The ration of $\frac{A}{A_r}$ served interesting results about the type of the pattern of degeneration of the species.

To the tracing of lichen thalli sheets of cellophane were pinned up with steel needles to the surface of the bark. Beside these drawings transitional schemes (KISS 1988) were also used for the interpretation of the performance of different life-strategy types into each other.

Results

Spore production

Figures 1, 2, and 3 show the changes of the reproductive biology of L. carpinea. Trens are unambiguous; spore production were continually decreased in all three localities. Otherwise these performances took place in very different "levels". This species is near to the extinction in Szombathely. Only 10 per cent of asci contained spores in the investigated apothecia between 1985 and 1988. The number of apothecia which were contained spores were also reduced, especially in the past four year. The column diagram of Kőszeg and Olad seems more balanced but on the basis of this kind of interpretation some very interesting "hidden" phenomena ar also observable:

a/ In the orchard of Olad, asci with 50% of spores were found already in 1979 and with a break in 1981 it was continued until 1988. 1985 was also a very interesting year: asci with 25% of spores were dominated. Asci with 10% of spores were occurred at first in 1985 with 6 apothecia and in 1987 with 11 apothecia. These signs represented the unambiguous decrease of the spore production.

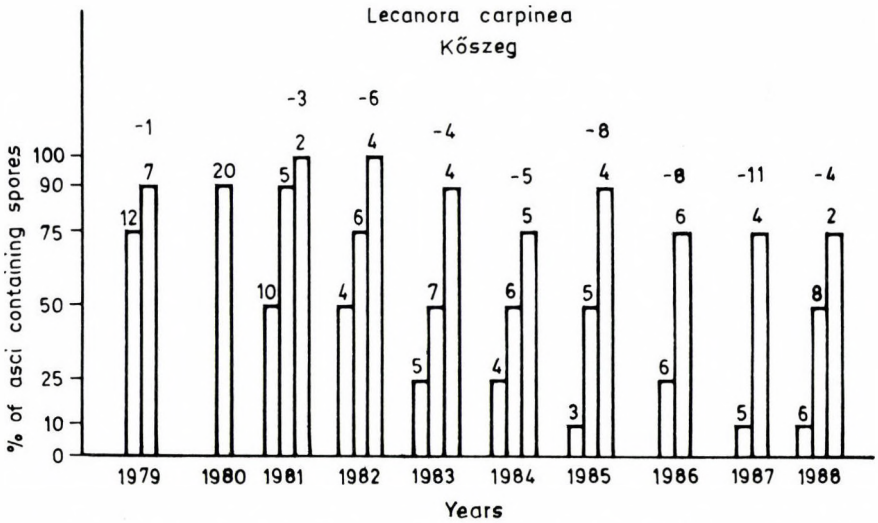

Fig. 1. The reproductive behaviour of Lecanora carpinea in an unpolluted region of Kőszeg ("King Valley").
Positive numbers express the number of apothecia contained spores. Negative numbers show the number of apothecia without spores

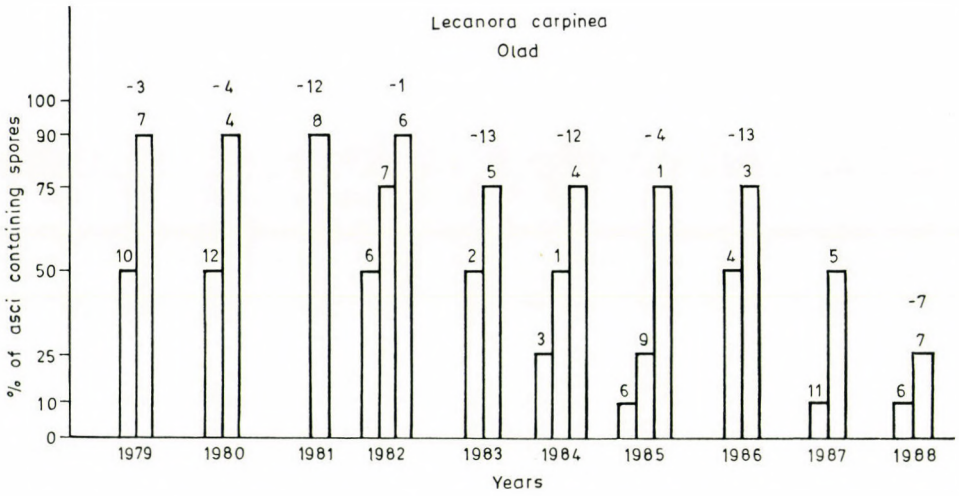

Fig. 2. The reproductive behaviour of Lecanora carpinea in the orchard of Olad

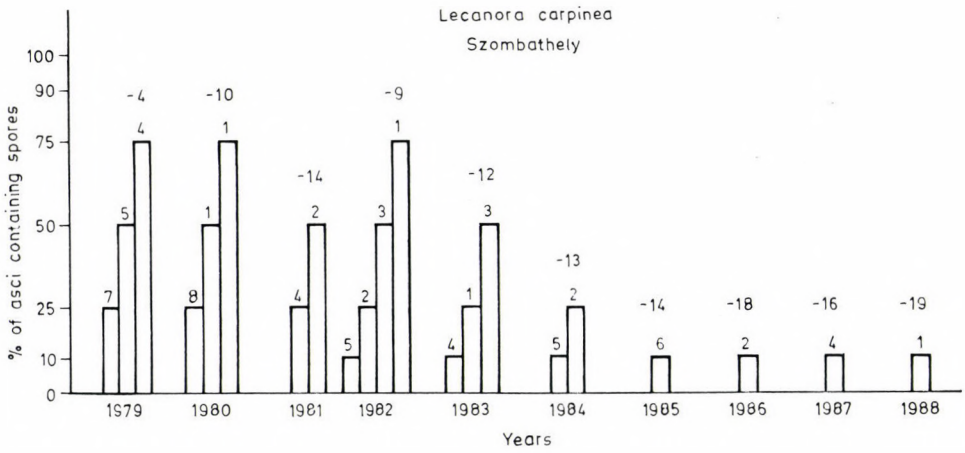

Fig. 3. The reproductive behaviour of Lecanora carpinea in a polluted region of Szombathely

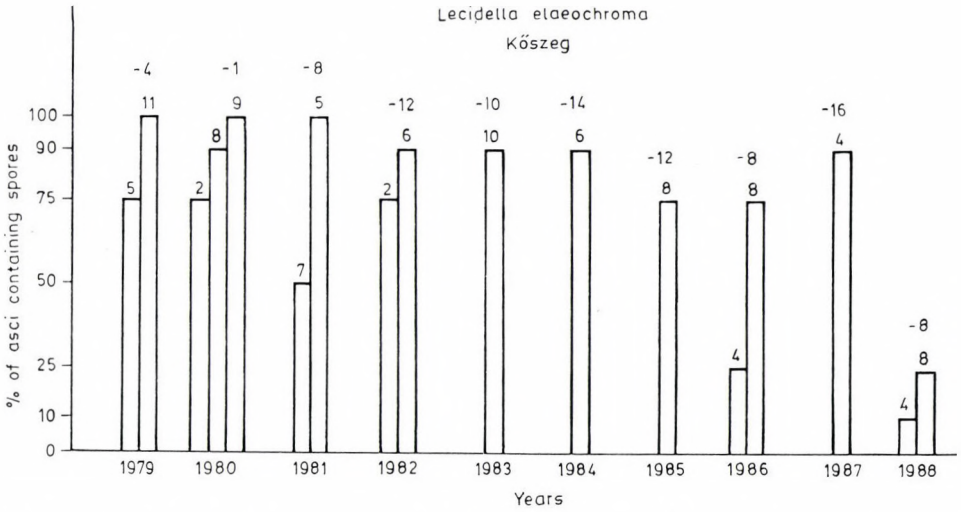

Fig. 4. The reproductive behaviour of Lecidella elaeochroma in an unpolluted region of Kőszeg ("King Valley")

b/ Trends were very similar in the "King Valley" and in the orchards of Kőzseg but the asci with 50% of spores were detected at first in 1981 (Fig. 1).

Asci with 25% of spores occured at first in 1983, but asci with 75% of spores were found in 1988, too.

Figures 4, 5, and 6 shows the sopre production of Lecidella elaeochroma.

The sudden tumble of the spore production in 1988 (Fig. 4) seems unexpected at first sight. This type of decrease appears characteristic to this species. In the orchard of Olad this phenomenon was also observed in 1985 (Fig. 5). However, the production of spores is more uniform in Kőszeg than in the orchard of Olad. The thalli of L. elaeochroma produce fewer apothecia than L. carpinea but at the same time the efficiency of the spore production is higher within the asci of L. elaeochroma (Figs 1 and 4). For example in 1979, 1982, 1983, 1984 and in the year of 1987 the per cent of asci with spores were always in a higher level in the apothecia of L. elaeochroma. But this situation is valid only for the unpolluted area of Kőszeg.

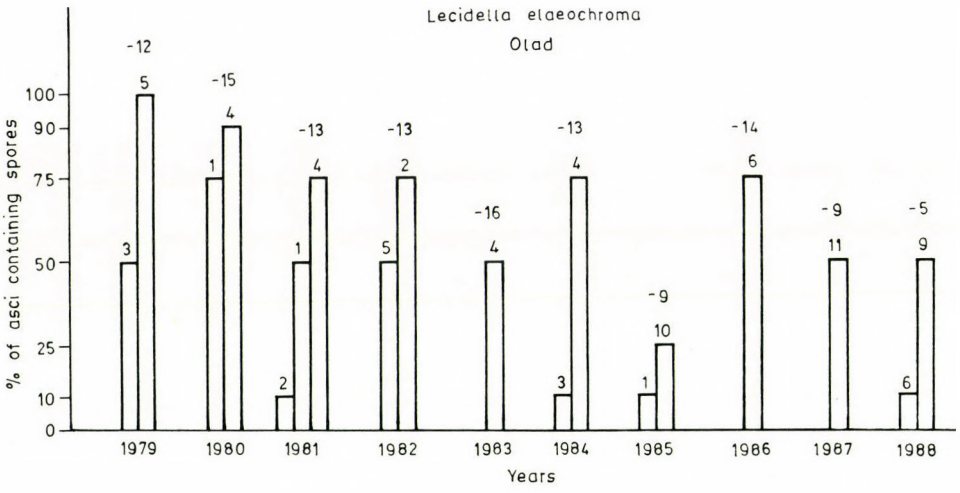

Fig. 5. The reproductive behaviour of Lecidella elaeochroma in the orchard of Olad

The reverse was found in the orchard of Olad. The spore production of L. elaeochroma was highly irregular. The per cent of asci with spores was in a permanent change from year to year (Fig. 5). If we make a comparison between these two species within the orchard of Olad, we have to recognize big differences (Figs 2 and 5). It reflects the necessity of long time investigations.

To tell the truth, long term studies were neglected from the field of lichen ecology and coenology and we have no long-term studies about the reproductive biology of lichens.

Let's see the Fig. 1 on the basis of the so-called "short time" investigations. Working with 3 years periods we will obtain very different and unreliable results.

First period: 1979—1981: The per cent of asci with spores were ever increasing during this interval. It seems that the air pollution did not rise between 1979 and 1981.

Second period: 1982—1984: This situation is the opposite of the previous one. The per cent of asci with spores were decreasing. Conclusion: the air pollution were increased during this period...

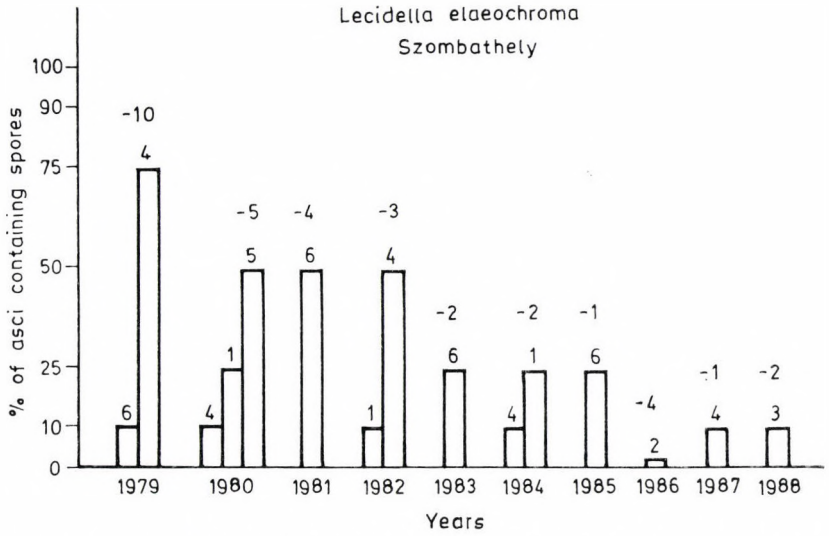

Fig. 6. The reproductive behaviour of Lecidella elaeochroma in a polluted region of Szombathely

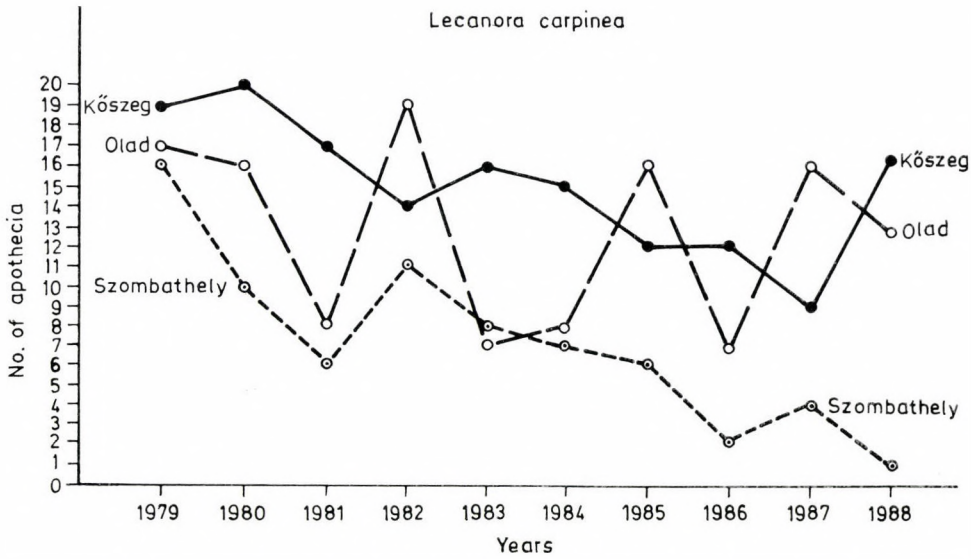

Fig. 7. The number of apothecia of Lecanora carpinea contained spores between 1979 and 1988

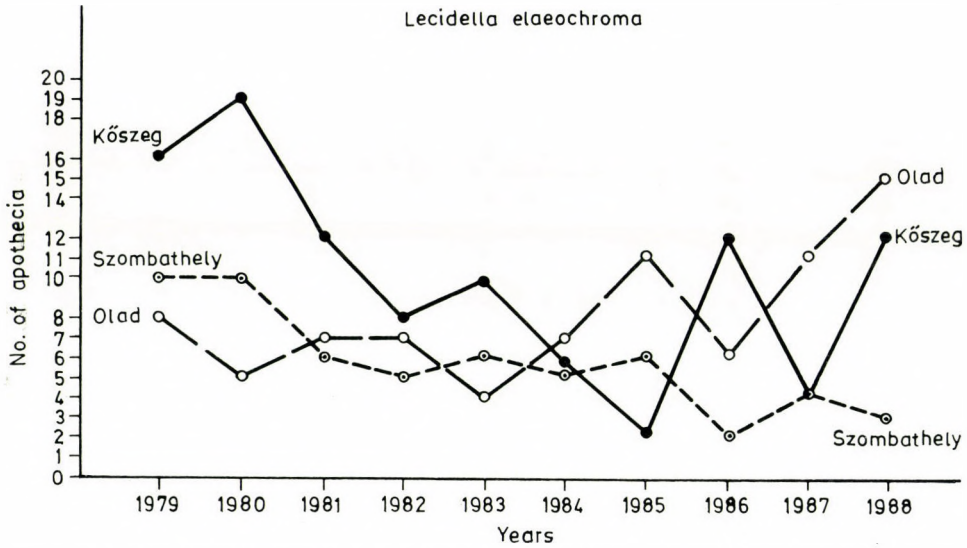

Fig. 8. The number of apothecia of Lecidella elaeochroma contained spores between 1979 and 1988

Third period: 1985--1987: The tendency is about the same as in the preceding case but there are some "irregularities" exist. For instance: the occurence of the 10% of asci with spores in 1985 and 1987, and the 25% of asci with 6 apothecia in 1986. It should be clear that these kind of short term investigations have no reality.

Let us see two other diagrams (Figs 7 and 8) about the number of apothecia contained spores. Table 1 help us to make a survey through the large number of data and gives some informations about the fertility of the apothecia.

The curves -- Figs 7 and 8 -- and Table 1 show big irregularities, especially in the orchard of Olad and in the "King Valley" of Kőszeg. Trends seem unambiguous only in the city of Szombathely, where the air pollution was continually increased between 1979 and 1988. Table 2 recalls this tendency.

Table 1

The number of apothecia contained spores during the past 10 years period in three different localities
20 apothecia were investigated in each sites from year to year

Taxa and Localities	Years 1	2	3	4	5	6	7	8	9	10	Σ	$\bar{x}$	s
L.c., K.	19	20	17	14	16	15	12	12	9	16	150	15	3.36
L.c., O.	17	16	8	19	7	8	16	7	16	13	127	12.7	4.71
L.c., SZ.	16	10	6	11	8	7	6	2	4	1	71	7.1	4.46
L.e., K.	16	19	12	8	10	6	2	12	4	12	101	10.1	5.26
L.e., O.	8	5	7	7	4	7	11	6	11	15	81	8.1	3.31
L.e., SZ.	10	10	6	5	6	5	6	2	4	3	57	5.7	2.63

Abbreviations: K: Kőszeg, O: Olad, SZ: Szombathely
L.c.: Lecanora carpinea, L.e.: Lecidella elaeochroma
Years are symbolized by numbers. For example; 1979: 1, 1980: 2, etc. ...
Σ: sum of the apothecia contained spores between 1979 and 1988
$\bar{x}$: annual mean values, s: standard deviation

Table 2

The annual mean values of SO_2 and NO_2 in Szombathely, between 1979 and 1988

Years	SO_2 $\mu g\ m^{-3}$	NO_2 $\mu g\ m^{-3}$
1979	50	28
1980	60	25.5
1981	75	26.2
1982	80	25
1983	85	27
1984	88	30
1985	88	32
1986	89	35
1987	89	35.6

In the case of the rain - pH the values showed marked decrease during the past 10 years (from 5.02 until 3.14) and at the same time the values of standard deviation -- s — changed irregularly.

Table 3

The annual mean values of the rain ($\bar{x}$) and the values of standard deviation (s) between 1979 and 1988

Rain - pH: Szombathely

Years	$\bar{x}$	s
1979	5.02	0.890
1980	4.35	0.921
1981	3.81	0.957
1982	3.60	0.850
1983	3.58	0.822
1984	3.26	0.902
1985	3.24	0.817
1986	3.20	0.789
1987	3.14	0.709

In consequence of these facts the correlation coefficient (r) was calculated only for the locality of Szombathely.

Table 4

The results of the statistical examinations between the spore production of the lichen species — L.c. and L.e. — and some external ecological factors as SO_2 and rain-pH. r = correlation coefficient, p = level of significance

Taxa, SO_2, rain-pH	r	p (%)
L.c. — rain pH	0.882	0.1
L.e. — rain pH	0.622	5
L.c. — SO_2	-0.823	1
L.e. — SO_2	-0.888	0.1
L.c. — L.e.	0.844	0.1

The correlation between the spore production of L. carpinea and the acidity of rain is considerable high. The thalli of this species are usually full of apothecia thus the raindrops are infiltrating directly into these reproductive organs.

The level of significance between the acidity of the rain and L. elaeochroma is lover. The thalli of this species produce apothecia in fewer number thus higher amount of polluted raindrops are infiltrating into the vegetative parts of the thalli. Therefore first of all the vegetative parts of the thalli are dying by the air pollution. The negative correlation between the fertility of L. elaeochroma and the level of SO_2 is also high. It seems that L. elaeochroma is more sensitive to the air pollution than L. carpinea. One of the possible reasons of this are the more extensive vegetative surfaces of the thalli of this species, adsorbing and/or absorbing huge amount of pollutants by dry or/and wet deposition.

The level of significance between the spore production of these species, in the polluted region of Szombathely, reflects to the similarities of their life-strategies; both includes into the SP_{EpCr}-type. It means that these colonies are disseminated by spores (Sp), they are living on the

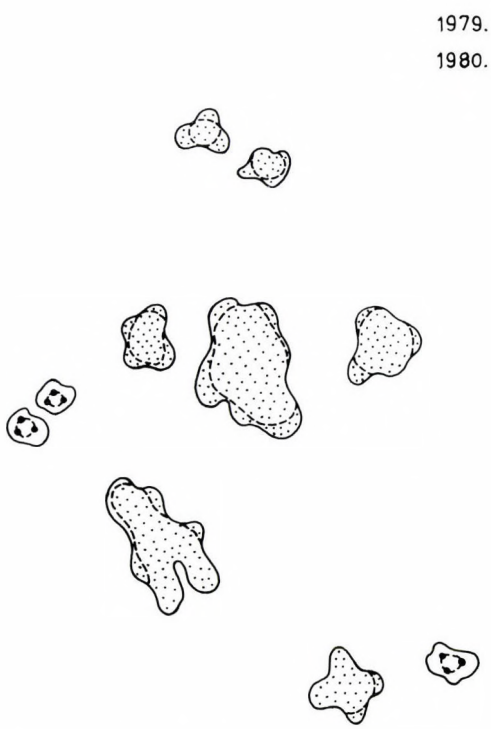

Fig. 9. The pattern of a crustose — Sp_{EpCr} — "community" from the polluted zone of Szombathely. The reproductive area — A_r — of the colonies are marked with dotted line

1988.

Fig. 10. Ten year's after... Dead and dying colonies on the bark. Apothecia of Lecanora carpinea were perished by air pollution. Lecidella elaeochroma is represented only with a little piece of a colony...

surface of the bark (Ep = epiphytic), and the form of the colonies is crustose (Cr).

Degenaration patterns

Figures 9 and 10 show another aspect of reproductive biology, namely; the change of the reproductive area of the lichen populations. The change of the pattern during the past 10 years was obvious. Apothecia of L. carpinea have fallen out of the thalli, and not a single was produced within the above mentioned period. In this case the life-strategy type of Sp_{EpCr} changed into Tf_{EpCr} type; $Sp_{EpCr} \longrightarrow Tf_{EpCr} \longrightarrow$ Extinction.

In the case of L. elaeochroma pieces of thalli in different size have fallen out of the boles, together or without apothecia. The possible transitional schemes:

a) $Sp_{EpCr} \longrightarrow Tf_{EpCr} \longrightarrow$ Extinction

b) $Sp_{EpCr} \longrightarrow$ Extinction

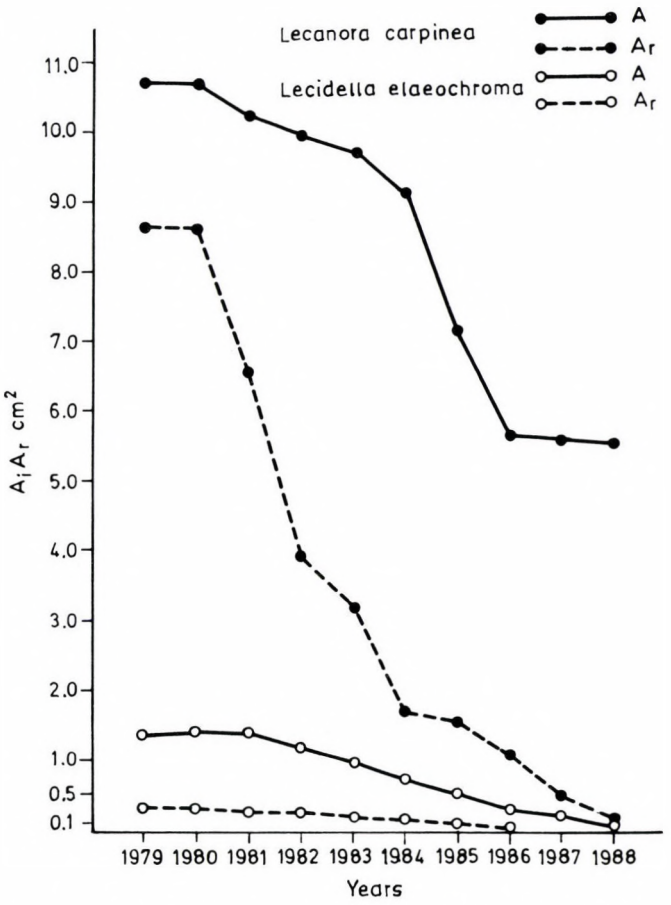

Fig. 11. The change of the whole (A_r) and the reproductive area (A_r) of the colonies between 1979 and 1988, in a polluted zone of Szombathely. The decrease of A_r in the case of Lecanora carpinea is very expressive

The form of b) is characteristic to this species. Therefore there are big differences between the degeneration patterns of these species.

The strong degeneration of the apothecia of L. carpinea is well observable on Fig. 11. The slope of the curve of A_r is more steeper than the another one which reflects to the changes of the whole area of the population. The situation is quite different by the thalli of L. elaeochroma. These differences become more perceptible on Fig. 12. If the degeneration of the vegetative zone -- A -- of a population is less serious than that of the reproductive one -- A_r --, the ratio of $\frac{A}{A_r}$ shows an increasing trend. This is the curve of L. carpinea (Fig. 12).

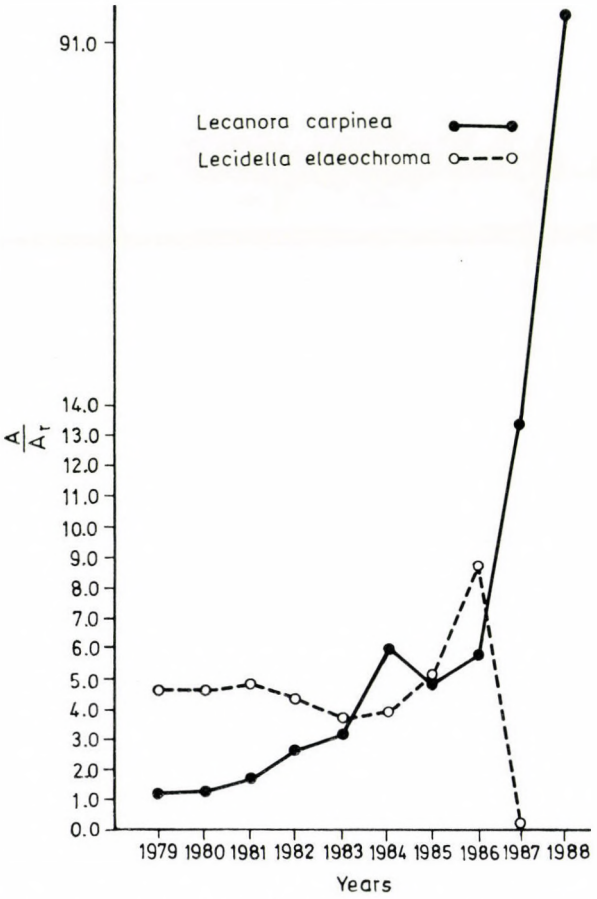

Fig. 12. The values of the ratio of $\frac{A}{A_r}$. There are big differences between the species

The curve of L. elaeochroma (also on Fig. 12) shows an irregular shape. The degeneration of A and A_r are nearly at the same level in this species, because the apothecia are fallen out of the boles together with little or bigger parts of the thalli.

Conclusion

1. On the basis of 10 years researches it seems that only the long-term investigations serve exact results in the field of bioindication using epiphytic lichens.

2. Each species -- higher or lower plant or aminal -- needs an observational time; an "optimal" observational time of its own.

3. The minimal observational time must be equal with the time of the complete life-cycle of the observed organism.

4. Lichens are growing very slowly and they are able to live for many hundred years. So the 10 years observational time seems also very short!

5. Each life-strategy type require observational time of different lengths (KISS 1986). The Sp_{EpCr} type needs the longest one, because the growth rate of this type is the slowest.

6. It is nearly impossible to determinate the exact age of the apothecia within a thallus. For example the thalli of L. carpinea consist of areoles. Apothecia are developing on the areoles. Each apothecium develops in different time, according to the age of its areola. So, one thallus of L. carpinea has plenty of pieces -- I mean areoles and apothecia -- at different age!

Only one thing is sure: the oldest apothecia are situated in the middle part of the colony. The extinction of the apothecia is independent from their age. The fewer the apothecia within a thallus the higher the differences between the age of them.

7. Spore production and fertility are highly affected by the acidification of the rain and by the increasing level of SO_2. This conclusion if valid first of all to the thalli living in the polluted area of Szombathely city.

8. The degeneration pattern of the populations give us well observable and useful key to the estimation of the level of the air pollution.

9. The changes of the reproductive area of the species (A_r) have crucial importance in the Sp_{EpCr} strategy-type because spores are the only dissemination propagules. The main reason of the extinction of L. carpinea and L. elaeochroma is the decay of apothecia.

10. The ratio of $\frac{A}{A_r}$ gives the specific signs of the degeneration or in other cases the specific characters of the development of the thalli.

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CHANGES IN NUCLEIC ACID LEVEL OF CELL NUCLEI AND HORMONE CONTENT IN SHOOT TIPS OF APPLE TREES TREATED BY PACLOBUTRAZOL (CULTAR)

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Repeated paclobutrazol (375 ppm a.i.) treatments were carried out to young apple trees of cv. Gloster. Growing elongated shoots were sampled on the 2nd and 4th day after the second application of this potent growth regulator.

Using Avena coleoptil bioassay and lettuce hypocotyl test to investigate hormon-like activity in the diffusate of shoot tips, it has been found that due to the treatments there are much lower auxin-like activity and diffusible gibberellin content.

According to microscope-photometrically measurements of DNA, and DNA+RNA level in cell nuclei of apices of shoot tips, it was proved a more uniformly higher nucleic acid level in the shoot apex of trees treated.

Keywords: apex, apple, auxin-like activity, Cultar, cytochemistry, DNA, gibberellin, growth retardant, paclobutrazol, RNA

Introduction

The really potent growth regulator Cultar (formerly known as PP 333) contains paclobutrazol which exerts its effect by interacting antagonistically with endogenous gibberellins (QUINLAN 1981a). Paclobutrazol proved to be an inhibitor of gibberellin biosynthesis (STEFFENS et al. 1983) and, because its effect can be reversed by gibberellins, it should be taken for antigibberellin (QUINLAN and WEBSTER 1982; WAMPLE and CULVER 1983).

Very soon after the first report written on paclobutrazol trials with apple (QUINLAN 1981b), more and more paper have been published presenting results of paclobutrazol application in a wide range of fruit species, listed in our paper (BUBÁN 1986b). Having some promising experiences by use of Cultar in young apple trees (BUBÁN 1986a), we have tried to get further informations about the mode of action of the paclobutrazol.

Akadémiai Kiadó, Budapest

Material and Method

Young apple trees of cv. Gloster were sprayed with Cultar and Alar-85 (daminozide) resp., at the green-cluster stage and/or 3 weeks after petal fall (Table 1). Actively growing shoot tips were sampled on the 2nd and 4th day after the second spraying.

Table 1

Shoot growth in apple trees of cv. Gloster

Treatments	Date recorded at 5th and 7th of June*		
	shoot length, cm	number of internodia	length of internodia, mm
Cultar 375 ppm a.i. 9th Apr. and 3rd June	18.7	13.2	14.2
Alar-85 2550 ppm a.i. 3rd June	30.4	14.8	19.9
Untreated	30.8	15.1	20.4

*Sampling shoot tips for laboratory investigations at the same time

Diffusate of these shoots tips obtained by centrifugation in 20% ethanol at 2000 g for 45 minutes (ROBITAILLE and CARLSON 1976) was investigated for gibberellin content and auxin-like activity using bioassay of lettuce hypocotyl and Avena coleoptyl (FRANKLAND and WAREING 1960; MILBORROW 1970; NAGY and TABI 1982, and GOLDSCHMIDT and MONSELISE 1968; KNEGT and BRUINSMA 1973; HEMBERG and TILLBERG 1980; NAGY and TABI 1983; resp.).

Histological sections of apices of shoot tips were prepared after embedding in paraffin. Staining sections for microscopic photometry (by MFV 4001, Zeiss) was carried out according to a modified Feulgen-procedure (HESEMANN and BUBÁN 1973; BUBÁN and HESEMANN 1979) for staining DNA of cell nuclei. As described elsewhere (MITCHELL 1968) sections for DNA + RNA estimation were stained by gallocyanine-chromealaune (GCA).

In order to compare various regions within the apex, in each section the extinction value from pith-rib meristem was used as a standard, i.e. it was taken as a unity and data for other regions were related to this value (HESLOP-HARRISON and HESLOP-HARRISON 1970).

Results and Discussion

Due to the early treatment with Cultar at the green-cluster stage (9th of April) there was an obvious growth retardation by the end of the 3rd week after petal fall (Table 1). Because of the short time (2 to 4 days) between the Alar-85 application and sampling shoot tips, this chemical could not exert (of course) any growth inhibition in term of length of shoot and internodia, resp.

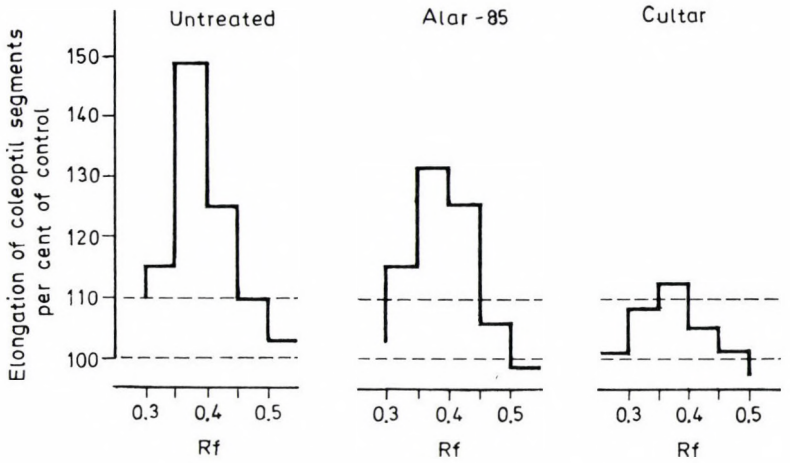


Fig. 1. Auxin-like activity in diffusate of shoot tips

Results of bioassay (Figs 1 and 2), however, show a dramatic decrease in endogenous hormone level of shoot tips received one of both growth retardants.

The lower gibberellin level caused by Cultar application can explain the enhanced flowering even in young trees treated by Cultar (BUBÁN 1986a,

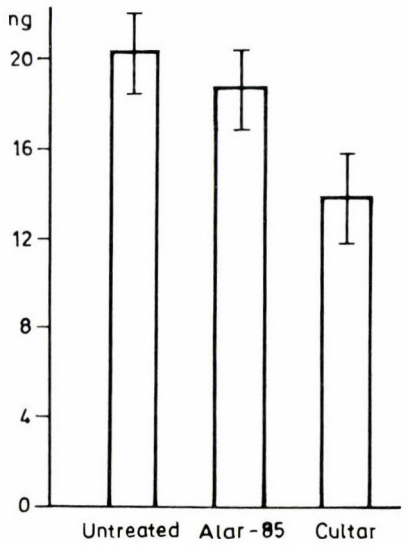
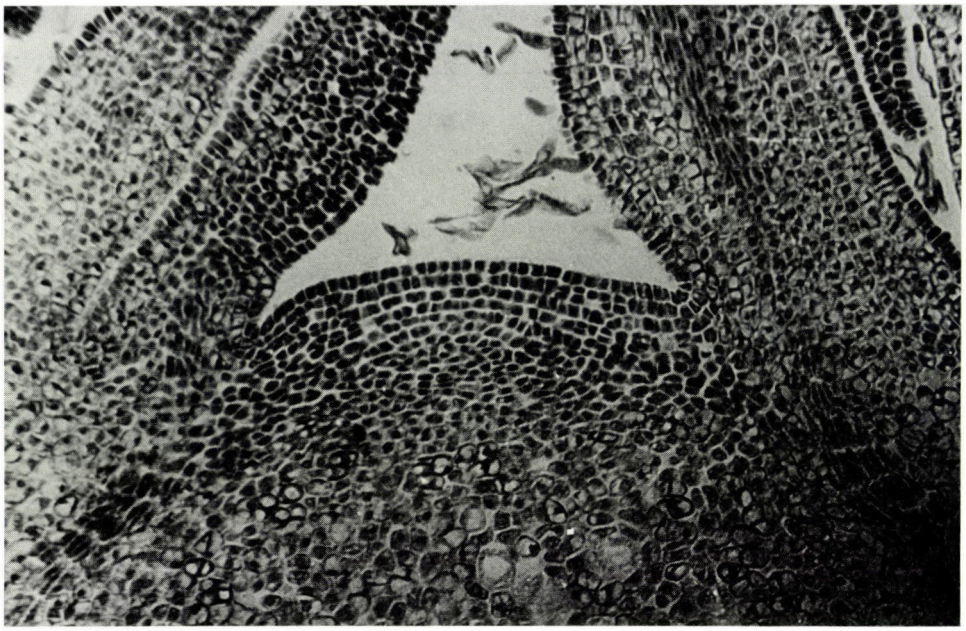
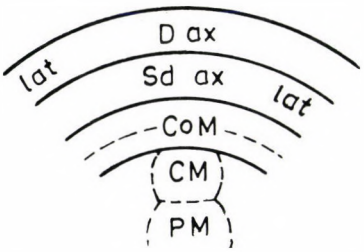


Fig. 2. Diffusible gibberellin content in GA_3 equivalents





D = Dermatogen
Sd = Subdermatogen
CoM = Cortical meristem
CM = Central meristem
PM = Pith-rib meristem

Fig. 3. Zones investigated within the apex
Measurements were made in both of the axial and lateral part of the D and Sd.
n = 32 to 34 (as an average) measurements taken in each zone of the apex

1986b). Namely, Alar, or Alar + ethephon treatments (being known to promote flower bud formation) are able to decrease gibberellin activity in shoots of apple (HOAD and MONSELISE 1976; GROCHOWSKA et al. 1984) and cherry trees, too (RYUGO et al. 1973). Furthermore, chemicals (bromouracil, gibberellic acid) used for inhibition of flower bud differentiation induce a higher level of endogenous gibberellins (EL-MAHDY et al. 1986).

Before discussing results of cytophotometrical investigations some details are needed regarding histological structure of the apex.

The diagram and a median longitudinal section (Fig. 3) represents the zonation of apices investigated. The terminology used for describing this type of apex organization is based upon a histogenic concept of GUTTENBERG (1960), HAGEMAN (1960) and KALBE (1962, cit. GIFFORD and CORSON 1971). Our decision for using this terminology is reasoned by the well-known relationship between the histogenic function and cytochemical characteristics of certain zones of the apex.

Below the dermatogen and the one-layered subdermatogen a cortical meristem can be recognized, usually consisting of two cell layers (depending on ontogenetic stage of the apex it can also be built by one to three layers of cells). This cortical histogen derived — at least partly — from the subdermatogen (GUTTENBERG 1960). Histological investigations carried out by others (HILKENBÄUMER and BUCHLOH 1954; MARRO and RICCI 1962) revealed quite commonly 4 (sometimes more) similar cell layers constituting the apical part of the apex even in apple trees.

Table 2

DNA staining (ext.) and its relative values (Q)

Zones within the apex	Cultar		Untreated	
	ext.	Q	ext.	Q
D ax	0.363	1.13	0.321	1.00
Sd ax	0.376	1.17	0.351	1.10
D lat	0.425	1.33	0.394	1.24
Sd lat	0.419	1.30	0.395	1.24
CoM	0.372	1.16	0.347	1.08
CM	0.350	1.10	0.347	1.08
PM	0.320	1.00	0.318	1.00

Ext.: extinction measured at 550 nm
Q = quotient related to the pith-rib meristem

Table 3

DNA+RNA staining (ext.) and its relative values (Q)

Zones within the apex	Cultar		Untreated	
	ext.	Q	ext.	Q
D ax	0.387	1.20	0.367	1.04
Sd ax	0.402	1.24	0.360	1.03
D lat	0.473	1.46	0.461	1.31
Sd lat	0.450	1.39	0.420	1.20
CoM	0.392	1.21	0.358	1.02
CM	0.317	0.98	0.361	1.03
PM	0.323	1.00	0.351	1.00

ext.: extinction measured at 525 nm
Q = quotient related to the pith-rib meristem

The central meristem (HILKENBÄUMER and BUCHLOH 1954) — characterized by cell division of undefined direction — has no histogenic function, however, it could be found in all of Rosaceae plants investigated by ROUFFA and GUNCKEL (1951). As the forerunner of the pith exists the meristem identified as pith-rib meristem by GIFFORD (1963), BERNIER (1971) and by many others cited by GIFFORD and CORSON (1971), it is rarely mentioned as pith meristem or rib meristem (HAGEMAN 1960, and HARA 1962 cit. by GIFFORD and CORSON 1971, resp.).

As for nuclear DNA and DNA + RNA estimations, measurements were undertaken in various zones of the apex (Fig. 3).

The presented results (Tables 2 and 3) demonstrate a more uniformly (and) higher nucleic acid level in the shoot apices from trees treated. This fact suggest that there is a higher frequency of cells being at the G_2 phase of the mitotic cycle, i.e. between the end of DNA replication and prophase. — Actually it is the same that a decreased mitotic activity was found in the shoot tips of apple trees treated by ALAR (WILDE and EDGERTON 1969).

It can not be excluded that this modified pattern of nucleic acid level within the apex may reflect some changes in reallocation of essential assimilates or other agents of importance playing a role in morphogenetic activity of the apex. This interpretation — based upon SACHS' (1977) ingenious suggestion — may be perhaps much more than a theoretical possibility.

There is hardly any possibility for comparing our results to that of others. The only one paper (reporting study of changes in DNA and RNA level

in buds of apple trees) pointed out the importance of RNA level es regards flower bud differentiation (XUEMING et al. 1990). Nevertheless, a paper written on cytochemical investigations of growing shoot tips in fruit trees is not available in the literature relating to the subject.

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NECTARY SURFACE OF PLUM VARIETIES

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Surface of the intrafloral, receptacular nectary in the plum varieties is covered by cuticle. Ornamentation of cuticle is characteristic to varieties or variety-groups. Structure of primary cuticle is basically of two types: striate and reticulate. Striate form is extremely common, this is characteristic to most varieties. Between the two basic types, there are intermediate forms. Cuticular striate are radially ordinated around the stomata of the nectarian epidermis covering the gland. Thin sulci acting as microcapillaries distribute the secreted nectar throughout the whole surface and retain it at the same time. Thick, striated cuticle has a good nectar retaining effect, while thin reticulum is less effective. Consequently, the former structure is more attractive for insects than the latter one.

Keywords: plum, nectary

Introduction

The epidermal surface of the plant organs is covered by a multilayered cuticle, which is a noncellular material composed of lipids, waxes and cutin (SITTE and RENNIER 1963; STACE 1965; SARGENT 1976; MARTIN and JUNIPER 1970; JUNIPER and COX 1973). Its outer layer, the primary cuticle forms striae, sulci and ridges. According to several authors, the cuticular ornamentation is suitable for differentiating taxa (STACE 1965; DUNN et al. 1965; SINCLAIR and SHARMA 1971).

Cuticular structure of the nectarian surface and its evolution was studied by DURKEE (1983).

Cuticular ornamentation of the nectary of some plum varieties and numerous Prunoideae taxa was described by OROSZ-KOVÁCS et al. (1989) and OROSZ-KOVÁCS (1989). However, only a small amount of data is available for having a more comprehensive knowledge about this topic.

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Material and Method

The studied flowers of nine plum varieties originated from the basic collection of the Experimental Station of the Fruit and Ornamental Plant Production and Development Corporation in Cegléd.

The living material collected for investigation was fixed in 0.2 mol glutaraldehide, and washed in 0.1 mol Na-cacodilate-buffer, then dehydrated in ethylene-series. After drying on the critical point, the material was prepared for SEM-study. SEM-micrographs were executed by an ASID-4 SEM adapted to a Yeol 100 C equipment.

Results

The floral nectary of the plum varieties is a receptacular one. The adaxial surface of the receptacle is covered on the inner side by the gland in the area extending between the ovary and the stamens. Between the tightly closed polygonal cells of the epidermis, acytic stomata are found. Stomata are situated on the same level as the epidermis cells, e.g. convar. "Korai Besztercei" (Plate II, Fig. 4). The sunken stomata occur usually on broadly extended dirk-shaped receptacles of xeromorphic character, but they are unsignificant for the tight tubular types.

The outer surface of the nectarian epidermis is covered by cuticle. The nectarian stomata are also covered by cuticle until the start of the secretion. The secreted material breaking up to the surface through the opening of the stomata, pushes up the cuticular membrane and breaks it up (Plate II, Fig. 4). Through the broken up cuticular slit the two bean-shaped guard-cells become conspicuous. In some varieties the outflow of the nectar is controlled by trichomas, e.g. the unicellular covering hairs of the cv. "Korai Besztercei".

Cuticle is produced by epidermal cells secreting liquid procutin, which migrates by transfusion through the cell wall and develops a continuous multilayered membrane on the outer surface of the epidermis. The outermost layer of the cuticle is the primary one, which has a characteristic ornamentation in the case of the studied varieties. The cuticular ornamentation may be classified into two types: striate and reticulate.

Striate type of cuticle occurs in some infraspecific taxa of the species Prunus domestica L., P. italica Borkh. em. Kárp. and P. salicaria Lindb. These kinds of nectary surfaces are characterized by a special ordination of the cuticular striae and sulci. They are usually directed parallelly with the long axe of the subtended cells, while they are radially oriented on the cells around the stomate (Plate I, Figs 1—4). The radiate

structure of the cuticular sulci acts as a system of microcapillaries, distributing the nectar breaking up to the surface. According to the capillar effect, the nectar remains for a longer time in the sulci of the striae. The more striated is the glandular cuticle, the more nectar can be retained by the flower of the plums, and the greater is the insect-attracting effect of the variety.

Glandular cuticle of the plums has in a greater part of the varieties a striated structure. Based on the cuticular ornamentation of the striae, wrinkles and sulci, the form of their folding the different varieties can be distinguished. On the outer surface of the epidermal cells, the cuticular striae are strongly undulated in the interstomatal space, while on the circumstomatal epidermal cell they have straight pattern. In the varieties "De Sota", "Zöld ringló" and "Ageni" cuticle of the cells adjacent to the stomate is strongly striated and undulated. On the top of the epidermal cells with papilla, the cuticle forms sharp crests, and the thin fissures indicate the place of the cell-boundaries (Plate I, Fig. 2).

In the cv. "Zöld ringló" epidermal cells are without papillae, so the cuticular striae cover evenly the cell surface, and the cell-boundary is hardly or not conspicuous below them. The cv. "Ageni" is recognizable by its sunken stomata and the radiated multilayered cuticular striae.

The nectary cuticle in the cv. "Beregi datolya" may be classified into the striate-fissurate type. Its epidermal cells have papillae on their anticlinal wall, and the cuticular ornamentation reveals the cell-boundaries. The direction of the striae is parellel with the longitudinal axis of the cells and no radial ornamentation is to be observed around the stomata either (Plate II, Figs 1, 2). Nectarian stomata are totally covered by the strongly undulate cuticular striae of the cuticle cells, stomata are indicated only by fine surface-interruptions formed by tubules running in the depth.

The cuticle-pattern of nectary-surface in the "Korai Besztercei" plum variety is reticulate. Thickenings of cuticle develop above the anticlinal walls of epidermal cells. Surface of cuticle is unfolded, ridges do not characterize this variety. Surface is not able to retain nectary, it evaporates quickly, that's why insect-attractivity of flowers of this variety doesn't last long (Plate II, Fig. 5).

As intermediate form between the striated and reticulated cuticle patterns the "Bódi" plum can be considered, whose nectary-surface is only a little striated, sulcated, but at the same time it is thickened above the

periclinal/radial wall of the epidermis-cells, that is reticular as well (Plate II, Fig. 3).

Based on the above-mentioned can be established, that the rhythmically produced nectar of the plums is kept back by the different nectary surfaces in a different manner. Hardly proportioned, striate types can keep back the nectar longer, more or less reticulated forms of unfolded surfaces keep back it for a shorter time. The varieties "Santa Rosa", "De Sota", "Zöld ringló", "Althann ringló", "Ageni" and "Beregi datolya" do attract pollinating insects for longer time, while the "Bódi" plum and "Korai Besztercei" do it only for shorter time.

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Plate I. Nectary surface of plum varieties

Fig. 1. Nectary surface of Prunus salicina Lindl. cv. "Santa Rosa" (1000 x)

Fig. 2. Cuticle on the nectary of Prunus domestica L. convar. "De Sota" plum variety (3000 x)

Fig. 3. Cuticle ornamentation of the nectary epidermis in the Prunus italica Borkh. em. Kárp. convar. claudiana Poiret "Zöld ringló" plum variety (3000 x)

Fig. 4. The sunken stoma of xeromorphic character with radial cuticle striation in the Prunus domestica L. "Ageni" plum variety (3000 x)

Plate II. Nectary surface of plum varieties

Fig. 1. Nectary surface of Prunus italica Borkh. em. Kárp. convar. mamillaris Schübl. et Mart. "Beregi datolya" plum variety (1000 x)

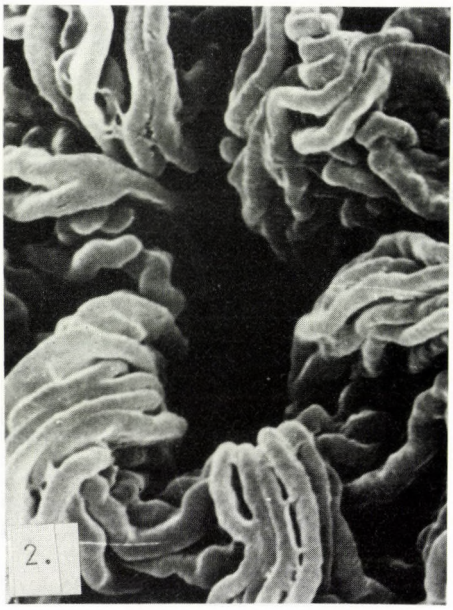
Fig. 2. The same with 3000 x

Fig. 3. Reticulate nectary cuticle of the Prunus insititia Jusl. cv. "Bódi szilva" plum variety (3000 x)

Fig. 4. Stoma of nectary with breaking up cuticle in Prunus domestica L. "Korai Besztercei" plum variety (3000 x)

Fig. 5. Reticulated nectary cuticle with unicellular hair in the "Korai Besztercei" plum variety (3000 x)

Plate I



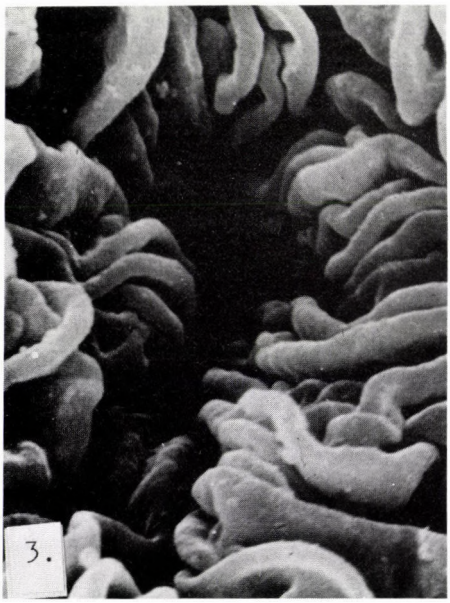
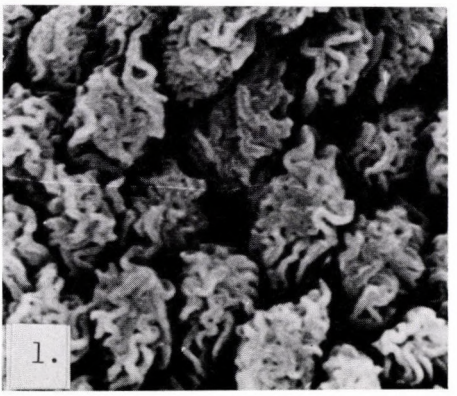
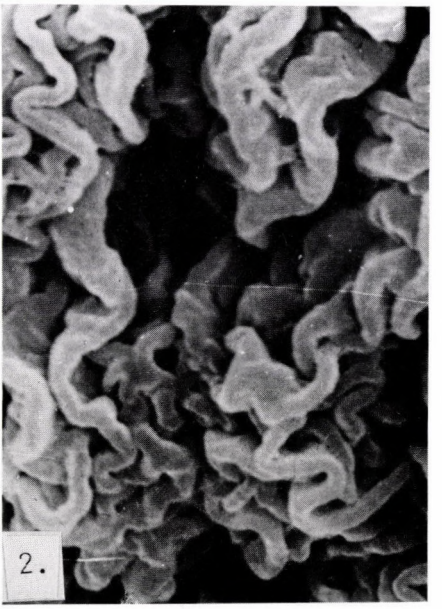
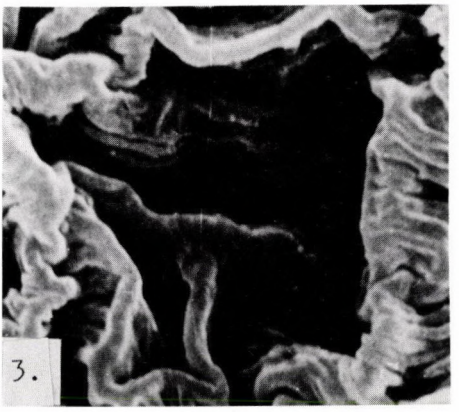
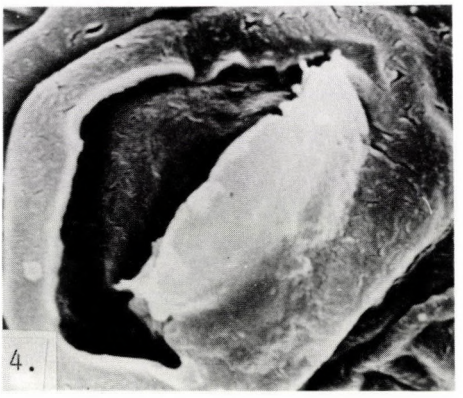


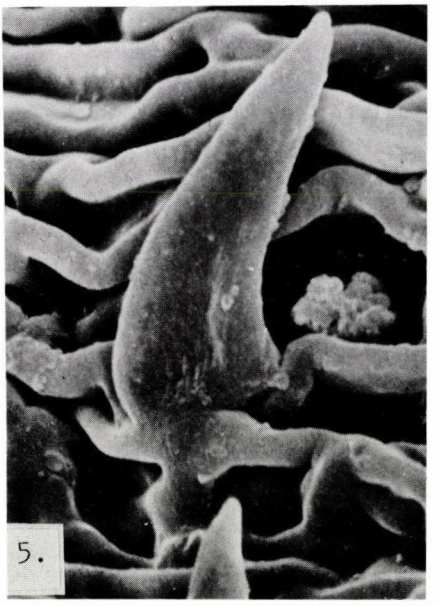
Plate II











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SCREENING OF SOME INDIAN LEGUMES FOR SEED PROTEIN

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Forty-six species of leguminous weeds have been screened for the contents of total N, protein N and protein in their seeds. Protein concentrates have been extracted from the seeds and analysed for nitrogen and protein contents. Extractabilities of total N, protein N and protein have been calculated and species showing promising potential of seed protein yield have been indicated.

Keywords: Wild Legumes, Seed Protein

Introduction

Seeds are a major source of protein for the human beings. The leguminous seeds are most important from this point of view and many of them are routinely consumed throughout the world (AYKROYD and DOUGHTY 1964). A large number of legume species grow wild as weeds. In tropical and subtropical areas they produce a considerably large amount of leaves and seeds. Although, some workers have investigated the possibilities of using legume weeds for obtaining leaf protein concentrates (PIRIE 1971, 1978; TELEK and GRAHAM 1983), little work has yet been done about obtaining the seed protein concentrate (SPC) from wild legumes (PANT and TULSIANI 1969). Protein concentrates of wild legume seeds have a promising potential of compensating the shortage of food and feed protein which prevails over a large part of the world, especially the developing regions (WOODHAM 1973; SHARMA 1987).

The present study has been aimed ad assessing the SPC yield potential of the seeds of some wild legumes from the flora of Gorakhpur. It includes estimation of nitrogen (N) and protein contents in seeds and SPC and also per cent extractabilities of SPC, total N and protein N. The results have been used to categorize the plants according to their SPC yield.

Akadémiai Kiadó, Budapest

Experimental

1. Seeds

Seed samples were collected from leguminous species growing in and around Gorakhpur, which has a subtropical climate with dense and luxurient vegetation. Mature seeds were collected, sun dried and stored. Before extracting the SPC, seeds were thoroughly washed.

2. Extraction of SPC

Ten g of seeds was homogenised with 100 ml double distilled water in a waring blender for 30 m and the homogenate was filtered through double layered muslin cloth. The SPC was precipitated upon acidifying the filtrate to pH 4.5 with 0.1 N HCl. The precipitated protein was separated by centrifugation at 3000 rpm for 20 m and washed with water before heating for coagulation at 60 ^{o}C for 20 m. The coagulated SPC was washed with acetone for removing most of the water and lypoids and was then dried at 60 ^{o}C under vacuum (MOLINA and BRESSANI 1973).

3. Analysis

Dry matter of seeds was determined by keeping the samples at 60 ^{o}C for 48 h and all the subsequent calculations have been made on dry weight basis. Analysis of total N and protein (TCA insoluble) N was done by the micro-digestion method of DONEEN (1932) followed by colorimetric estimation according to SNELL and SNELL (1953). Each estimation was repeated thrice and the mean value were recorded. The per cent extractabilities of SPC, total N and protein N were calculated by the following formulae (BYERS 1961):

$$\% \text{ Extractability of SPC} = \frac{\text{Dry weight of SPC}}{\text{Dry matter in seed samples used for extracting SPC}} \times 100$$

$$\% \text{ Extractability of Total N} = \frac{\% \text{ total N in SPC}}{\% \text{ total N in seed sample}} \times \% \text{ SPC extracted}$$

$$\% \text{ Extractability of Protein} = \frac{\% \text{ Protein in SPC}}{\% \text{ Total N in seed sample}} \times \% \text{ SPC extracted}$$

Results

Forty-six species belonging to the three sub-families of leguminosae (Papilionaceae, Caesalpinaceae and Mimoseae) were included in the present study. Data about the contents of dry matter, protein N, total N and protein in the seeds and the extractability of SPC from them, is recorded in Table 1.

The results of analysis of SPC for its total N, protein N and protein contents as well as the extractabilities of total N and protein N are presented in Table 2.

Tables 1 and 2 show that maximum extractability of total N (43.33%) was obtained with Acacia concinna and the minimum (6.37%) with Cassia auriculata. The percentage of protein N in SPC was highest (13.50%) in Acacia concinna and lowest (5.40%) in Bauhinia variegata and Desmodium heterocarpon.

On the basis of per cent extractabilities of protein (SPC) from the seed samples, the species have been grouped in four categories, as follows:

Category A (SPC-extractability 12-16%)

Only three plant species were found in this category, viz., Acacia concinna, Delonix regia and Uraria picta which yielded the maximum (12-16%) amount of SPC.

Category B (SPC-extractability 8-12%)

Thirteen species, viz. Crotalaria juncea, Caesalpinia pulcherrima, Albizia procera, Indigofera ennaphylla, Caesalpinia bonducella, Acacia arabica, Indigofera linifolia, Atylosia scarabaeoides, Abrus precatorius, Bauhinia variegata, Alysicarpus monilifer, Dalbergia sissoo and Cassia fistula yielded 8-12% SPC.

Category C (SPC-extractability 4-8%)

Twenty-five plant species, viz. Desmodium triflorum, D. gangeticum, Tephrosia purpurea, Tamarindus indica, Cassia tora, Crotalaria striata, Indigofera tinctoria, Medicago denticulata, Desmodium heterocarpon, Cysmopsis tetragonoloba, Zornia diphylla, Pithecellobium dulce, Mimosa rubicaulis, Crotalaria sericea, Bauhinia purpurea, Alysicarpus longifolius, Cassia absus, Cassia auriculata, Crotalaria medicagenia, C. calycina, Cassia obtusifolia, Calliandra brevipes, Alysicarpus vaginalis, Albizia lebbeck and Sesbania sesban yielded 4-8%.

Table 1

Analysis of total N, Protein N, Protein and SPC extractability of the seeds under survey

Sl. No.	Species	% Composition of seeds (dry weight basis)			% Extractability of SPC (dry weight basis)
		Total N	Protein N	Protein	
1	2	3	4	5	6
1.	Acacia arabica Willd.	4.92	4.20	26.25	9.83
2.	A. concinna DC.	5.40	4.32	27.00	16.25
3.	Alysicarpus monilifer DC.	5.52	4.56	28.50	8.56
4.	A. vaginalis DC.	3.00	2.76	17.25	4.00
5.	A. longifolius W & A.	4.16	4.44	27.75	4.53
6.	Albizia lebbeck Benth.	4.92	1.56	9.75	4.00
7.	A. procera Benth.	3.84	2.64	16.50	11.33
8.	Atylosia scarabaeoides Benth.	3.72	2.52	15.75	9.66
9.	Abrus precatorius L.	2.16	0.48	3.00	9.28
10.	Bauhinia variegata L.	4.40	3.72	23.25	9.20
11.	B. purpurea L.	4.92	2.18	13.50	4.50
12.	Cassia tora W & A.	5.40	1.80	11.25	6.75
13.	Cassia auriculata L.	7.32	3.36	21.00	4.51
14.	C. absus L.	2.25	1.56	9.75	4.50
15.	Cassia obtusifolia L.	4.56	3.36	21.00	4.33
16.	Cassia fistula L.	3.56	3.12	19.50	8.83
17.	C. occidentalis L.	3.72	2.04	12.75	5.73
18.	Crotalaria ferruginea Grah.	7.20	4.20	26.25	5.25
19.	C. striata DC.	5.04	4.20	26.25	6.70
20.	C. sericea Retz.	3.72	3.12	19.50	4.80
21.	C. calycina Schrank.	3.84	2.40	15.00	4.33
22.	C. medicagenia Lamk.	4.68	3.60	22.50	4.36
23.	C. juncea L.	5.76	3.72	23.25	11.57
24.	Caesalpinia pulcherrima SW.	6.50	4.70	29.37	11.45
25.	C. bonducella Flem.	5.16	4.92	30.75	10.16
26.	Cysmopsis tetragonoloba (L.) Taub.	2.76	2.16	13.50	6.00
27.	Calliandra brevipes Benth.	3.36	2.52	13.75	4.33
28.	Delonix regia (Boj) Raf.	6.96	5.04	31.50	13.53
29.	Dalbergia sissoo Roxb.	4.56	3.24	20.25	8.36
30.	Desmodium gangeticum DC.	3.00	2.16	13.50	7.16
31.	D. triflorum DC.	3.12	2.64	16.50	7.50
32.	D. heterocarpon DC.	2.52	1.80	11.25	6.00
33.	Indigofera tinctoria L.	4.68	3.12	19.50	6.33
34.	I. enneaphylla L.	3.60	3.12	19.50	10.50
35.	Indigofera linifolia Retz.	5.52	2.88	18.00	9.75
36.	I. hirsuta L.	2.52	1.68	10.50	5.83
37.	Mimosa rubicaulis Lamk.	2.52	2.18	13.62	5.16
38.	Medicago denticulata Willd.	3.84	2.50	15.62	6.33
39.	Melilotus indica All.	3.60	3.00	18.75	3.66
40.	Peltophorum inerme (Roxb.) Naves	4.42	3.72	23.25	2.53
41.	Pithecollobium dulce Benth.	4.92	3.24	20.25	5.16
42.	Sesbania sesban (L.) Merr.	5.76	2.16	13.50	4.50
43.	Tephrosia purpurea Pers.	4.08	3.43	21.75	7.16
44.	Tamarindus indica L.	2.76	2.28	14.25	6.91
45.	Uraria picta Desv.	4.68	3.72	23.25	12.70
46.	Zornia diphylla Pers.	3.24	2.88	18.00	6.00

Table 2

Analysis of Total N, Protein N and Protein in SPC and Extractabilities of Total N and Protein N

S. No.	Source of SPC	% composition of SPC (dry weight basis)			% Extractability	
		Total N	Protein N	Protein	Total N	Protein N
1	2	3	4	5	6	7
1.	Acacia arabica	13.80	11.40	71.25	27.57	22.77
2.	A. concinna	14.44	13.50	84.37	43.33	40.62
3.	Alysicarpus monilifer	11.30	9.40	58.75	17.52	14.57
4.	A. vaginalis	8.40	7.80	48.75	11.20	10.40
5.	A. longifolius	7.30	6.20	38.75	6.40	5.44
6.	Albizia lebbeck	12.90	8.60	53.75	10.48	6.99
7.	A. procera	10.80	7.40	46.25	31.86	21.83
8.	Atylosia scarabaeoides	10.20	8.60	53.75	26.48	22.33
9.	Abrus precatorius	6.90	5.70	35.62	29.64	24.48
10.	Bauhinia veriegata	7.20	5.40	33.75	15.05	11.50
11.	B. purpurea	13.20	9.00	56.25	12.28	8.37
12.	Cassia tora	11.30	9.30	58.12	11.30	11.62
13.	C. auriculata	10.35	8.10	50.62	6.37	4.99
14.	C. absus	8.40	6.00	37.50	15.00	10.71
15.	C. obtusifolia	7.80	6.30	39.37	7.40	5.98
16.	Cassia fistula	10.60	8.30	51.87	26.29	21.90
17.	C. occidentalis	13.30	9.80	61.25	18.94	15.09
18.	Crotalaria ferruginea	9.00	7.40	46.25	6.56	6.39
19.	C. striata	15.30	10.50	65.62	20.33	13.95
20.	C. sericea	10.80	7.40	46.25	13.93	9.54
21.	C. calycina	12.30	11.40	71.25	13.86	12.84
22.	C. medicagenia	11.40	9.30	58.12	10.68	8.66
23.	C. juncea	14.90	12.80	80.00	29.92	25.71
24.	Caesalpinia pulcherrima	13.70	11.80	73.75	24.13	20.79
25.	C. bonducella	13.80	12.90	80.62	27.17	25.40
26.	Cysmopsis tetragonoloba	8.40	7.62	47.62	18.26	16.56
27.	Calliandra brevipes	9.60	7.50	46.87	12.37	9.66
28.	Delonix regia	14.70	11.50	71.87	28.57	22.35
29.	Dalbergia sissoo	12.80	10.60	66.25	23.46	19.46
30.	Desmodium gangeticum	8.70	5.90	36.87	20.76	14.02
31.	D. triflorum	8.40	7.60	47.50	20.19	18.26
32.	D. heterocarpon	7.50	5.40	33.75	17.85	12.85
33.	Indigofera tinctoria	12.00	8.60	53.75	16.23	11.63
34.	I. enneaphylla	9.90	8.10	50.62	28.87	23.62
35.	I. linifolia	10.30	8.60	53.75	18.19	15.19
36.	I. hirsuta	12.00	8.10	50.62	27.76	18.73
37.	Mimosa rubicaulis	6.90	6.30	39.37	14.12	12.90
38.	Medicago denticulata	9.60	9.30	58.12	15.82	15.33
39.	Melilotus indica	9.60	8.10	50.62	9.76	8.23
40.	Peltophorum inerme	12.60	10.50	65.62	7.21	6.01
41.	Pithecellobium dulce	10.80	7.40	46.25	11.32	7.76
42.	Sesbania sesban	8.20	7.50	46.87	6.40	5.85
43.	Tephrosia purpurea	10.50	7.40	46.25	18.42	12.98
44.	Tamarindus indica	8.40	6.90	43.12	21.03	17.27
45.	Uraria picta	12.30	10.80	67.50	33.37	29.30
46.	Zornia diphylla	7.80	7.20	45.00	14.44	13.33

Table 3

Categorization of the species under survey

Species	Protein N* extracted	Total N* in SPC
Category IA (Extractability of protein N more than 20% and total N in SPC more than 12%)		
Acacia arabica	22.77	13.80
A. concinna	40.62	14.30
Crotalaria juncea	25.71	14.90
Caesalpinia pulcherrima	20.78	13.70
C. bonducella	25.40	13.80
Delonix regia	22.35	14.70
Uraria picta	29.30	12.30
Category IB (Extractability of Protein N more than 20% and total N in SPC 9-12%)		
Albizia procera	21.83	10.80
Atylosia scarabaeoides	22.33	10.20
Cassia fistula	21.90	10.60
Indigofera eneaphylla	23.62	9.90
Category IC (Extractability of Protein N more than 20% and total N in SPC 6-9%)		
Abrus precatorius	24.48	6.90
Category IIA (Extractability of Protein N 10-20% and total N in SPC more than 12%)		
Cassia occidentalis	15.09	12.30
Crotalaria striata	13.95	15.30
C. calycina	12.84	12.30
Delbergia sissoo	19.46	12.80
Category IIB (Extractability of Protein N 10-20% and total N in SPC 9-12%)		
Alysicarpus monilifer	14.57	11.30
Indigofera tinctoria	11.63	12.00
I. hirsuta	18.73	12.00
I. linifolia	15.19	10.30
Medicago denticulata	15.33	9.60
Tephrosia purpurea	12.98	10.50
Cassia tora	11.62	11.30
Category IIC (Extractability of Protein N 10-20% and total N in the SPC 6-9%)		
Alysicarpus vaginalis	10.40	8.40
Bauhinia variegata	11.50	7.20
Cassia absus	10.71	8.40
Cysmopsis tetragopoloba	16.56	8.40
Desmodium triflorum	18.26	8.40
D. heterocarpon	12.85	7.50
Mimosa rubicaulis	12.90	6.90

Table 3 (contd.)

Species	Protein N* extracted	Total N* in SPC
Tamarindus indica	17.27	8.40
Zornia diphylla	13.33	7.80
Desmodium gangeticum	14.02	8.70
Category IIIA (Extractability of Protein N below 10% and total N of SPC more than 12%)		
Albizia lebbeck	6.99	12.90
Bauhinia purpurea	8.37	13.20
Peltophorum inerme	6.01	12.60
Category IIIB (Extractability of Protein N* below 10% and total N in SPC 9-12%)		
Cassia auriculata	4.99	10.35
Crotolaria sericea	9.54	10.80
C. medicagenia	8.66	11.40
Calliandra brevipes	9.66	9.60
Melilotus indica	8.23	9.60
Pithecellobium dulce	7.76	10.80
Category IIIC (Extractability of Protein N below 10% and total N in SPC 6-9%)		
Alysicarpus longifolius	5.44	7.30
Cassia obtusifolia	5.98	7.80
Crotalaria ferruginea	5.39	9.00
Sesbania sesban	5.85	8.20

Category D (SPC-extractability below 4%)

In this category, two species viz., Melilotus indica and Peltophorum inerme yielded very low amount of SPC, i.e., below 4%.

The plant species under survey were further grouped into 9 categories on the basis of percentage extractability of protein N from seed samples and the total N present in SPC in Table 3.

Discussion

Legume seeds are one of the major conventional sources of food and feed protein. Importance of legume seeds in nutrition is well documented (AYKROYD and DOUGHTY 1964; OYENUGA 1966). However, most of the legumes consumed today are cultivated pulses. A large section of wild leguminous seeds has remained untapped for food, although, the use of seed protein concen-

trate has been often advocated for suplementing food and feed (ALTSCHUL 1965). The few studies done with wild leguminous seeds have proved their nutritional potential (PANT et al. 1974a,b).

The amount of seed protein concentrate obtained from per unit seed samples directly depends upon the extractability of protein, which in its turn, is affected by many factors such as plant species, nitrogen level of the substratum and the procedure involved in extracting the protein concentrate (ARKCOLL 1971; PIRIE 1978; LEXANDER et al. 1970).

In the present investigation it has been found that different legume species differ in the contents of protein N in their seeds. CROOKE (1946) has also concluded that plants belonging to the same family differ markedly in their protein content.

The extractability of SPC per unit seed sample is also influenced by the extraction procedure. Each step involved in the method of extraction, e.g. homogenization, extraction, precipitation, coagulation and drying may affect the extractability of SPC in its own way. In the present investigation, extraction was done with water and precipitation at pH 4.5, followed by heat coagulation and filtration. However, other methods such as precipitation by electrolytes, coagulation by heat and separation by differential solubility have also been employed for extracting protein concentrates from plant samples (PANT and TULSIANI 1969; PIRIE 1978).

Extractability of protein from a plant sample also depends on the degree of homogenization and disintegration of tissues of the sample. Elaborate machinery is used for large-scale extraction. However, for laboratory scale work, use of waring blender has been recommended (BYERS 1961; BOYD 1968; LEXANDER et al. 1970). In addition to the machinery and tissue disintegration, some other factors are also reported to affect the extractability of protein concentrate such as phenols and their oxidative products and mucilage (COHEN et al. 1956; LOOMISH and BATTAILE 1966).

In the present investigation phenolic interference was not noticed. However, mucilage was found to interfere in the extractability of SPC from some seeds.

In the present study, the extractability of seed protein and SPC yield varied considerably among the species tested (Tables 1 and 2). The maximum extractability of SPC was recorded in Acacia concinna (16.25%), Delonix regia (13.533%) and Uraria picta (12.70%). Some other species have satisfactory levels of SPC extractability e.g. Crotalaria juncea (11.57%), Caesalpinia pulcherrima (11.45%) and Albizia procera (11.33%). Very low

values of SPC yield were recorded for Peltophorum inerme (2.533%) and Melilotus indica (3.666%). Variation in the composition and extractability of seed protein from leguminous species have also been reported earlier (PANT et al. 1968; PANT and TULSIANI 1969).

In the surveys about the leaf protein concentrates plants have been classified in various ways. BYERS (1961) grouped the plants of her study into several categories on the basis of per cent extractable protein N and nitrogen content of protein isolate. VALLI DEVI et al. (1965) suggested six categories of plants following the criteria of BYERS (1961). LEXANDER et al. (1970), however, emphasized that protein produced per unit leaf area, i.e. per cent extractability of SPC should also be taken into consideration. In the present investigation, therefore, three criteria were adopted for categorization of species under survey, viz. (i) per cent extractability of SPC, (ii) per cent extractability of protein N and (iii) content of total N in SPC (Table 3). This categorization reveals that Delonix regia, Crotalaria juncea, Caesalpinia pulcherrima, Acacia concinna and Uraria picta have high potential of SPC yield. It is suggested that further studies about the nutritional characterization of SPC of these species should be done, before their use in food and feed supplementation.

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BOTANICAL IDENTIFICATION OF IPOMOEA TRICOLOR CAV. SEED SAMPLES FROM HUNGARY AND THIN-LAYER CHROMATOGRAPHIC EXAMINATION OF THEIR HALLUCINOGEN ERGOT ALKALOIDS

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Ground morning glory seed as a psychomimetic drug is used experimentally in Hungary, too. It is a fact known from the literature that the seed of Ipomoea tricolor Cav. contains hallucinogen ergot alkaloid more by at least one order of magnitude than the seeds of other Ipomoea and Quamoclit species.

Morphological examinations have pointed out that the fruit, seed and seedling of I. tricolor are characteristic and definitely differ from the phenotypic features of I. purpurea, an Ipomoea species similarly frequent in Hungary, as well as from those of other frequent (Colvolvulus tricolor L.) and less frequent (Quamoclit lobata (Llav. et Lex.) House and Q. coccinea (L.) Mnch.) ornamental taxa. The morphological examination of intact seed- and seedling samples is particularly suitable for identification.

The 3 thin-layer chromatographic development systems elaborated for the analysis of alkaloid contents can be used without modification under over-pressure (OPLC) development conditions, too. We carried out the screen examination of Ipomoea taxa widespread as ornamentals in Hungary using an easy to standardize method (densitometry following OPLC development on HPTLC layer) also suitable for the serial examination of a large number of samples.

Among the seed samples of Hungarian origin considerable amounts of ergot alkaloids could be detected in the seed extract of Ipomoea tricolor. The major components of the average total alkaloid content of 0.07 per cent are ergometrine (0.04%) and ergotamine (0.009%).

Keywords: Ipomoea, Quamoclit, hallucinogen ergot alkaloids, seed and seedling morphology, thin-layer chromatography (TLC, OPLC, HPTLC), densitometry

Introduction

In Hungary two frequent morning glory species are known, both of them are much liked ornamentals. Distinction between Ipomoea tricolor Cav. (syn.: I. violacea L. seu I. rubro-coerulea Hook) and I. purpurea (L.) Roth (syn.: Pharbitis purpurea Voigt.) as well as other similarly known ornamental taxa of the family Convolvulaceae cultivable in Hungary, such as e.g. Convolvulus

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tricolor L., Quamoclit lobata (Llav. et Lex.) House, Quamoclit coccinea (L.) Mnch. (JÁVORKA and CSAPODY 1962; SOÓ 1968) is made easy by morphological differences, too.

According to RÁCZ (1985) in narcomaniac subway gatherings experiments with ground morning glory seed are carried out in Hungary too, though for the time being only in a narrow range. There are abundant literary data concerning the occurrence of ergot-alkaloids as particularly characteristic of certain tribus of the family Convolvulaceae (Ipomoeae, Argyreiae). Rivea corymbosa (L.) Hall.f. (= Turbina corymbosa (L.) Raf.), Ipomoea tricolor Cav. and the Argyreia species excel above all with their relatively high ergot-alkaloid contents (CHAO and DER MARDEROSIAN 1973).

A typical example of the hallucinogen plants is the white funneliform "ololiuqui" (Rivea corymbosa), once a magic plant of the Mexican Aztec-Zapotec indians; first of all the round brown seed of its berry was used to prepare magic brew for their cultic ceremonies (SCHULTES 1941, 1961; HOFMANN 1961; HOFFER et al. 1967; FARNSWORTH 1968), but tea was also made from the green shoot ("coatlxoxouhqui"), according to the descriptions of the Spanish conquerors (SCHULTES and HOFMANN 1987). The seed of Ipomoea tricolor ("badoh negra") was used for the same purpose by the Zapotec indians (FARNSWORTH 1968).

The main active ingredients of the Rivea corymbosa seed are d-lysergic acid amide (ergine) and d-lysergic methylcarbinolamide, in addition chanoclavine, elymoclavine, lysergol and ergometrine are also contained in it (ROTH et al. 1984).

Among the Ipomoea species I. tricolor contains significantly more ergot-alkaloid than other Ipomoea or Quamoclit taxa (DER MARDEROSIAN 1967). The Hagers Encyclopaedia — summarizing the earlier results — gives the following average data: 0.035% (+) — lysergic acid amine (ergine), 0.005% (+) — iso-lysergic acid amide (iso-ergine), 0.005% chanoclavine, 0.005% elymoclavine and 0.005% ergometrine (ergonovine) characterize the seed of I. tricolor. Its total alkaloid content is about 0.06%. HOFMANN (1970) also reported the presence of cycloclavine. STAUFFACHER et al. (1965) isolated the peptid-type ergosine/ergosinine alkaloid pair from the seed of I. argyrophylla.

Within the seed of Rivea corymbosa the clavine and lysergic acid alkaloids are mostly concentrated in the embryo (TABER and HEACOCK 1962), their presence is characteristic first of all of the seed in the case of morning glory species as well (TABER and VINING 1963). Of the elymoclavine-

penniclavine transformation taking place in the leaf of Ipomoea tricolor GRÖGER (1963) gave an account. Ergot alkaloids can also be detected in leaf and stalk, but their presence mainly can be proved with biochemical results concerning the seed. In the immature seed chanoclavine can be found in the first place; in the course of maturing its quantity decreases, while that of lysergic acid increase (GENEST 1966).

According to recent American reports Ipomoea and Quamoclit species present as weed plants mainly in soya crops may represent potential danger, because when wingled in the fodder or in seeds to be processed may cause poisoning (WILKINSON et al. 1986); moreover, the morning glory seeds contain a considerable amount of chlorogenic acid whereby they further reduce the nutritive value of the cultivated plant (FRIEDMAN et al. 1989). Analyses by WILKINSON et al. (1987) have proved that the seeds of the wild (weed) species of Ipomoea and Quamoclit contain 10-15 times less total alkaloid than the seed of Ipomoea tricolor which is no weed plant and is known as an ornamental, so — in our opinion — they cannot cause any problem in the practice.

Knowing these facts we wished to obtain confirmative data from Hungary concerning whether the I. tricolor (= I. violacea) known and cultivated as an ornamental in Hungary really contained ergot alkaloids, whether they could be determined with a simple method, and the "dangerous" seeds botanically distinguished from other Ipomoea and Quamoclit species not dangerous according to the literature.

Material and Method

The seed samples true to species providing a basis four our comparative examinations were placed at our disposal by the Ecological and Botanical Research Institute of the Hungarian Academy of Sciences, Vácrátót, for which thanks are due in this place, too. The seed samples obtained by further propagation were compared with the original samples, and in the case of Ipomoea and Convulvulus taxa with several garden seed samples coming from different places of Hungary (Pécs, Villány, Nagyatád). The reproduction of Quamoclit species was but partly successful — supposedly because of their peculiar ecological demands. Qu. hederifolia while developing capsule produced no seed owing to its late flowering. Of the seeds and capsules Erzsébet PAPP prepared drawings, we thank for her work again.

Taking the morphological and phytochemical comparative study of DER MARDEROSIAN et al. (1964) as a model we used the seed map of SCHERMANN (1966) for the seed identification and the seedling map of Vera CSAPODY (1968) for the seedling identification. In respect of general morphology we characterized the habit after JÁVORKA and CSAPODY (1962).

The isolation and measuring of the ergot alkaloids were carried out with the experiences of NIWAGUCHI and INOUE (1969) and WILKINSON et al. (1986, 1987) taken into consideration and the method of BOTZ et al. (1990) used. The test materials were placed at our disposal by the G. Richter Chemical Works, for which we express here our thanks.

Five g of ground seed was extracted with a 7:3 ratio mixture of acetone and tartaric acid (with an amount of 3 x 150 ml, for 20 minutes each). After centrifuging and filtering the acetone was distilled in vacuum, then shaken with a 3:1 ratio mixture of dichloro-methane and isopropanol. The acidic-aqueous solution thus purified was alkalified with sodium-bicarbonate to pH 8-9, then shaken with a 3:1 ratio mixture of dichloro-methane and isopropanol (3 x 30 ml), finally the organic phase was concentrated dry by vacuum distillation. The concentrated residue was taken up with 2 ml methanol, from this stock solution were the thin-layer chromatographic separations carried out.

By the application of the "PRISMA" optimization model we employed three eluants of different composition for the OPLC separations carried out on HPTLC layer (BOTZ et al. 1990):

1. ethanol + tetrahydrofuran + ethyl acetate (1:1:8) (Fig. 5, A. densitogram),
2. water + ethanol + diethyl ether (5:35:60) (Fig. 5, B. densitogram),
3. acetonitrile + ethanol + toluene (85:10:5) (Fig. 5, C. densitogram).

Data of chromatography:
Layer: Kieselgel 60 F_{254} (TLC, HPTLC), Merck (Darmstadt)
Apparatus: CHROMPRES 10 (Labor MIM, Budapest)
Eluant inlet pressure: 1.5 bar
Vapour pressure: 11 bar
Flow velocity: 0.8 cm/min
Temperature of chamber and eluant: 20-22 °C
Development distance: 6; 17 cm
Detection: UV: 250 nm
VIS: Van Urk reagent: 450 nm
Densitometer: Shimadzu CS-930 (Kyoto, Japan)
CAMAG TLC Scanner II (Muttenz, Switzerland)

Result and Evaluation

The Ipomoea-, Quamoclit- and Convolvulus-species well known and mostly cultivable in Hungary are all annual ornamentals easy to propagate from seed. Owing to their characteristic habit they are readily recognized, especially in flowering state (JÁVORKA and CSAPODY 1962).

The foliar leaf of the fully developed plant is characteristic of the species (Fig. 1). The sessile leaves of Convolvulus tricolor are oval-lanceolate (Fig. 1/5), totally different in shape compared to the other taxa examined.

The whole shoot system of Ipomoea tricolor is glabrous, the leaf is broad, cordate-oval, with widely sinuate leaf base (Fig. 1/1). I. purpurea is a hirsute plant, the leaf is broad, cordate, the leaf shoulders are pointed with a narrow sinus (Fig. 1/2). A Dutch cultivar (Royal Sluis), the scarlet "rubricoerulea" commercially available in Hungary, that we take for I. purpurea, is characterized by trifid leaves (Fig. 1/3). Here we note that this cultivar does not reach maturity in Hungary. Considering its original seed lot it does not belong to I. tricolor either morphologically or from the point of view of ergot-alkaloid content (its name may be misleading), in spite of the fact that the size of its seed (but not the shape of seed) is

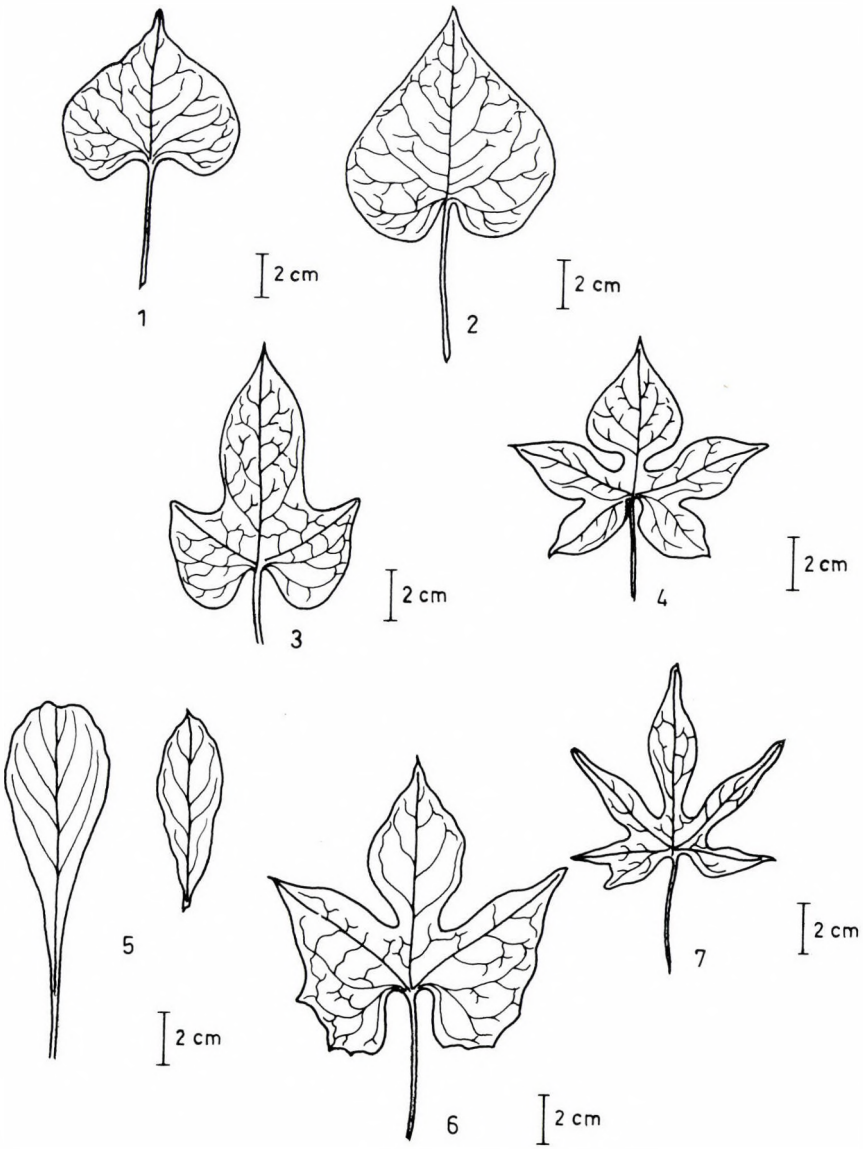


Fig. 1. Foliar leaf forms (Drawing by E. Papp).
1: Ipomoea tricolor, 2: I. purpurea, 3: I. purpurea cf. "rubricoerulea", 4: Quamoclit hederacea, 5: Convulvulus tricolor, 6: Quamoclit lobata, 7: Q. coccinea

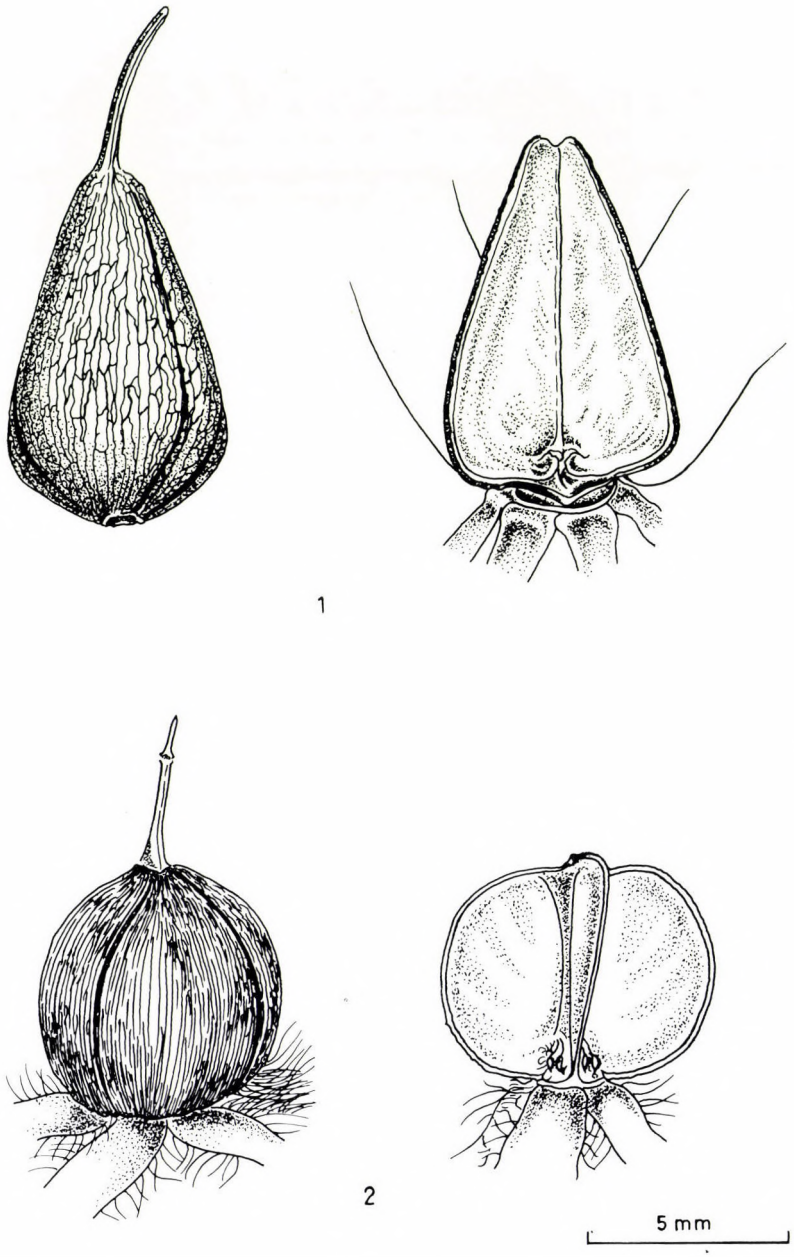


Fig. 2. Fruit (left) and its contents (right) (Drawing by E. Papp).
1: Ipomoea tricolor, 2: I. purpurea, 3: Quamoclit coccinea, 4: Q. lobata, 5: Convolvulus tricolor

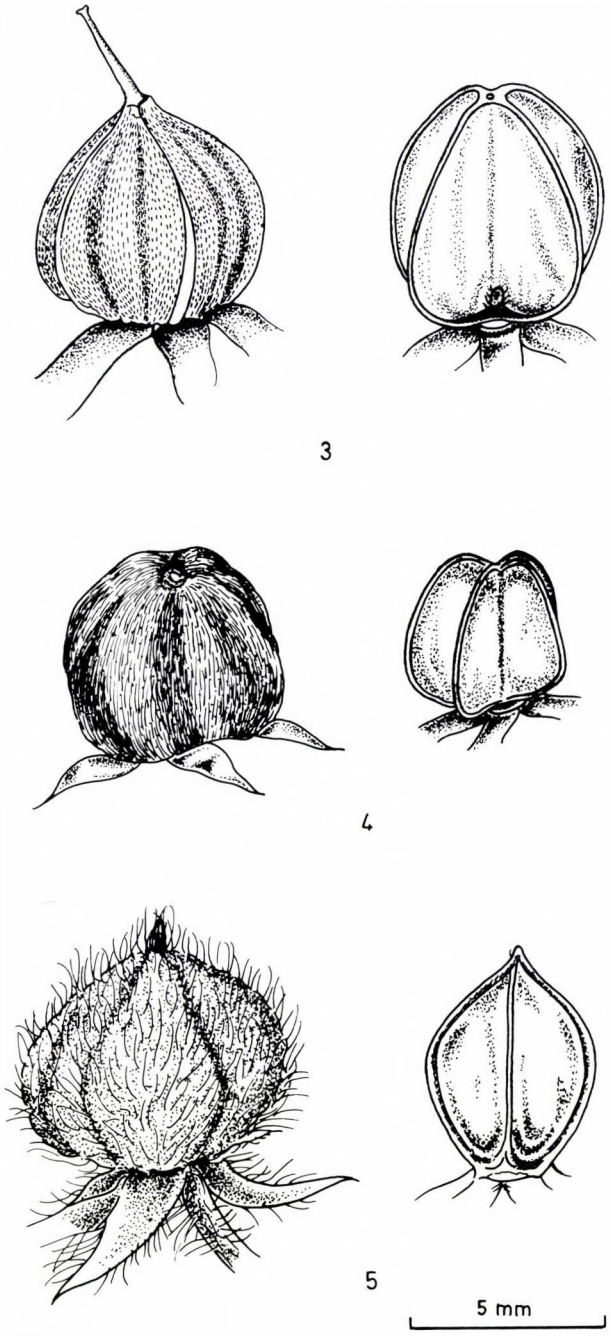


Fig. 2

close to the relatively largest seed measurements of Ipomoea tricolor. (It might be tetraploid I. purpurea.)

The leaf of Quamoclit hederacea, a species not reaching maturity in Hungary, resembles most of all the leaf of ivy (Hedera helix) (Fig. 1/4). The leaf of the scarlet-flowered Quamoclit coccinea usually is five-cleft, cordate shouldered (Fig. 1/7), but may also be cordate, pointed and whole (JÁVORKA and CSAPODY 1962). Qu. lobata, a species with flowers red in bud stage, then orange and ultimately yellowish white, has cordate shouldered, three-cleft leaves (Fig. 1/6).

The fruit is also of characteristic shape (Fig. 2). The mostly four-seeded capsule of I. tricolor is oblong, slightly bent at the top ending in a relatively long arista, while the capsules of all the other taxa are spherical or nearly so. The capsule of I. purpurea has three loculi with 2 seeds in each, is finely costate and ends in a narrower arista. Q. coccinea has a smaller capsule. The capsule of Q. lobata is a somewhat flattened sphere with a mostly broken tip; the capsules joining each other on one side form a characteristic raceme. The capsule of C. tricolor is bilocular with four seeds, its surface is comose differing thereby from the former ones.

The seed of I. tricolor is the longest of all (6-7 mm), dark brown or blackish when mature. As a further major characteristic, its hilar part is wider, while the opposite side gradually becomes narrower. The dorsal side of the seed is rugose, its ventral edge is straight (Fig. 3).

The seed of I. purpurea (Fig. 3) is only 4-5 mm long, with slightly glaucous surface, its shape resembles a segment of orange. The surface of seed from Q. coccinea and Q. hederifolia is mottled; Q. lobata has a small and usually slightly hairy seed. The seed of C. tricolor is spherical and papillate, totally different from the former ones. (The commercial, and sometimes even officially used Hungarian name "dwarf morning glory" is therefore incorrect.)

As to the seedlings, the shape of the cotyledon of I. tricolor is the most characteristic of all (Fig. 4) by its deeply bifid blade it can be reliably identified. (The seeds germinate well in dark in 2-3 weeks, but owing to their hard-coatedness germination may be protracted. When planted in pure sand after germination, it can be further raised.)

To be able to characterize the extracts made from the ground seed we had to carry out the thin-layer chromatographic examination and densitometric evaluation of the test materials, the authentically identical ergot-

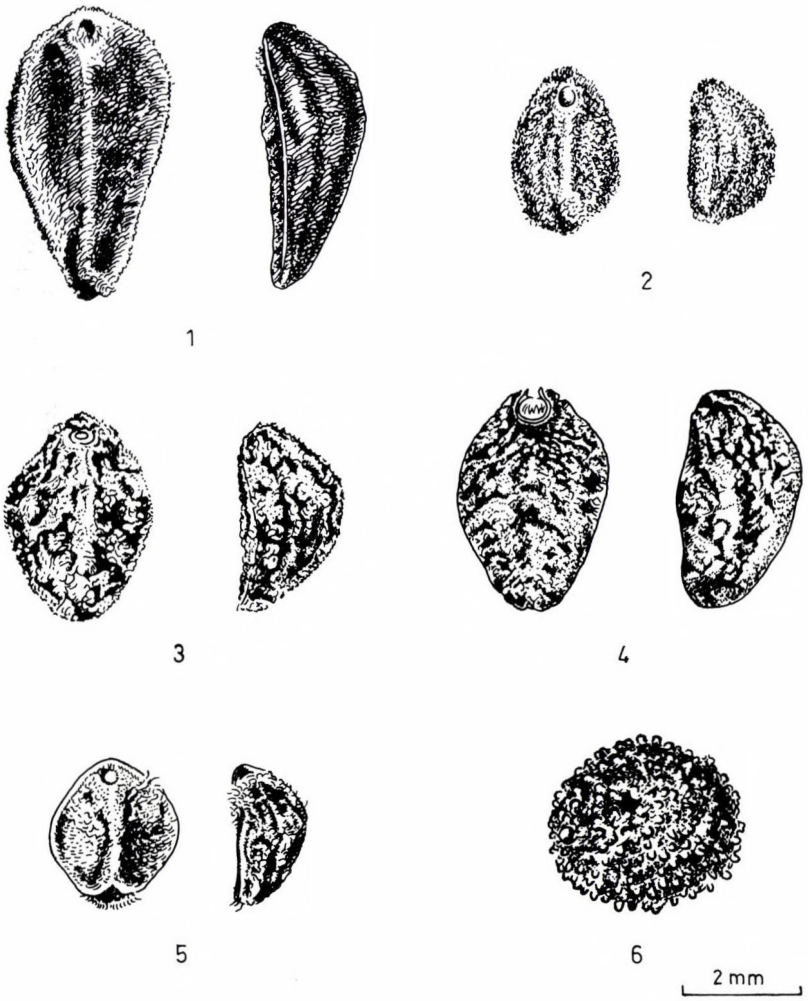


Fig. 3. Seed in hilar- (left) and side view (right) (Drawing by E. Papp).
1: Ipomoea tricolor, 2: I. purpurea, 3: Quamoclit coccinea, 4: Q. hederifolia, 5. Q. lobata, 6. Convolvulus tricolor

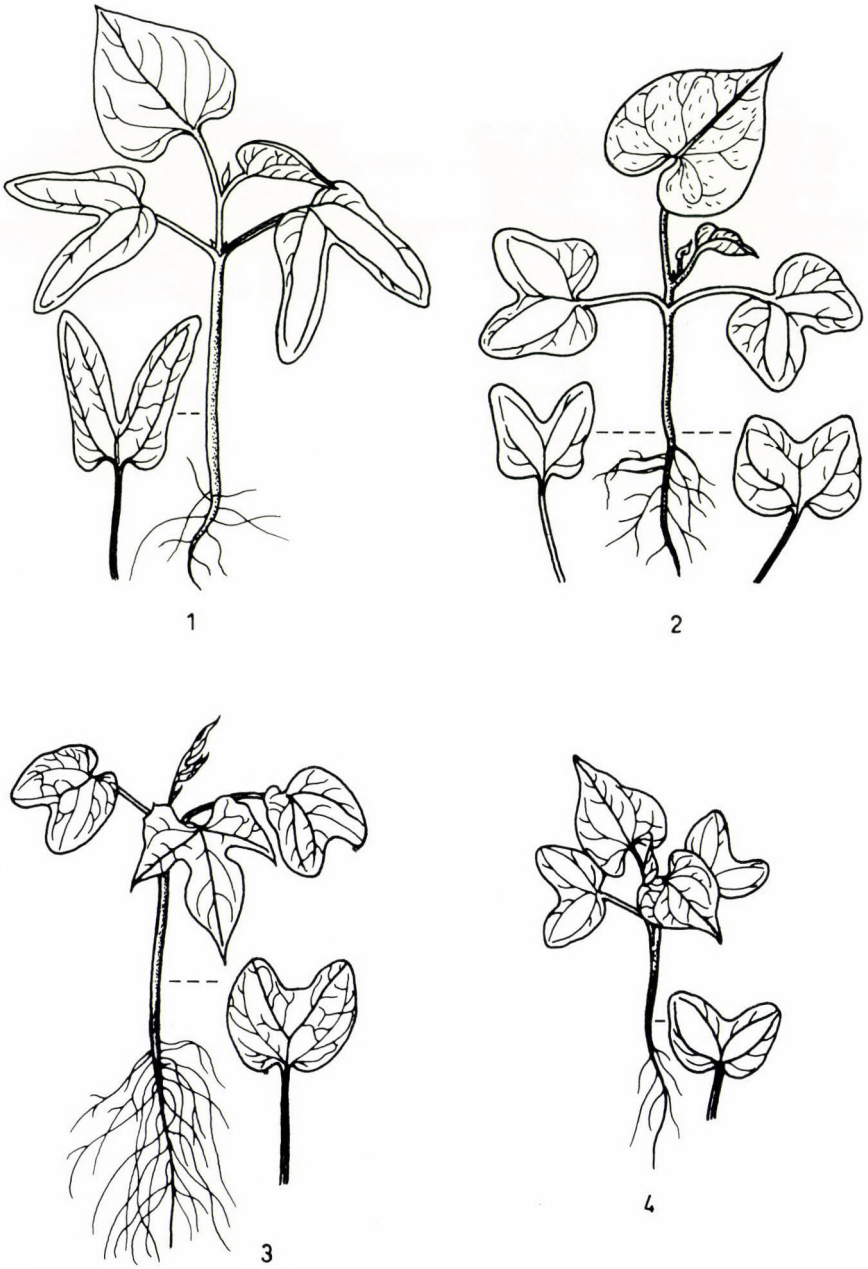


Fig. 4. Seedling with cotyledon (After V. CSAPODY 1968).
1: Ipomoea tricolor, 2: I. purpurea, 3: Quamoclit lobata, 4: Q. coccinea

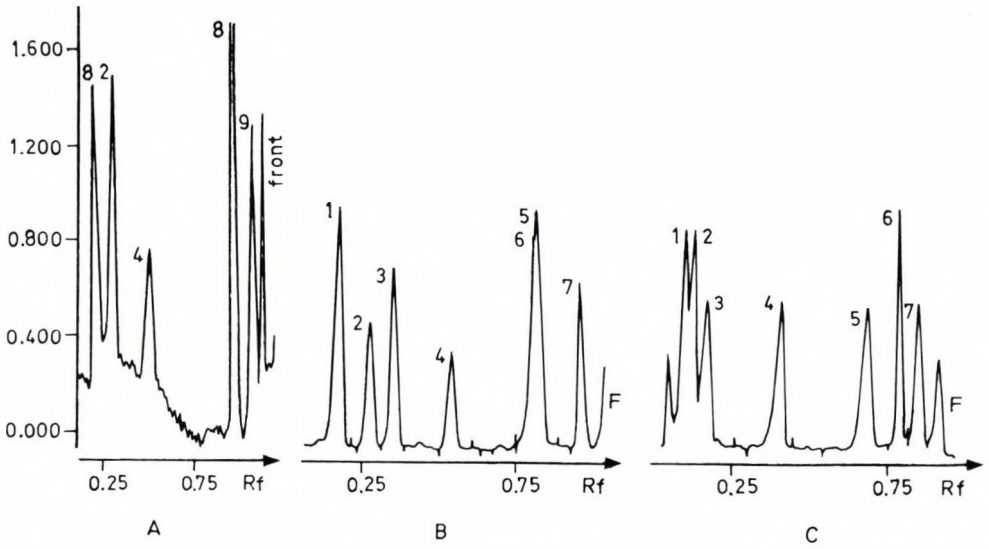


Fig. 5. Densitometric evaluation of ergot-alkaloid separation (dissolved in a 1 + 1 mixture of methanol-chloroform, from each standard alkaloid: 2 μg/μl).

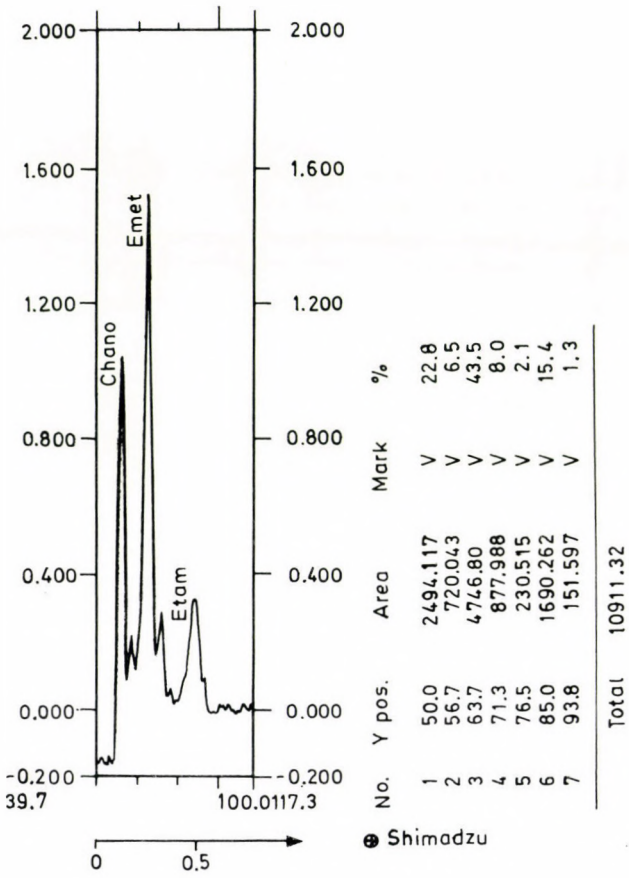
Eluant: A: ethanol + tetrahydrofuran + ethyl acetate (1:1:8); B: water + ethanol + diethyl ether (5:35:60); C: acetonitrile + ethanol + toluene (85:10:5).

Symbols: 1: lysergol, 2: ergometrine, 3: agroclavine, 4: ergotamine, 5: ergocristine, 6: ergotaminine, 7: ergocristinine, 8: chanoclavine, 9: ergometrinine

alkaloids too. After the optimization all three eluants are suitably used (Fig. 5/A, B, C).

The densitogram for ergot-alkaloids characteristic of the seed of I. tricolor (Fig. 6) shows that in the seed extract ergometrin is present in the largest amount, though ergotamin and chanoclavin are also found in it. Our quantitative calculations are summarized in Table 1. According to the data of the table the alcoholic-aqueous extract (20 g ground seed soaked in 100 ml 50% ethylalcohol for 1 week) contains about one-seventh of the total alkaloid content of the intact seed.

Our phytochemical analyses, in agreement with the results published in the literature prove that out of the ornamentals known in Hungary from the family Convolvulaceae only Ipomoea tricolor may represent a danger in the case of drug addiction. The seed and seedling of this plant have marked phenotypic characters which make its reliable identification possible. And through the rapid and simple phytochemical analysis applied, the ground seed can be identified by the quantitative and qualitative examination of the ergot-alkaloids.



No.	Y pos.	Area	Mark	%
1	50.0	2494.117	V	22.8
2	56.7	720.043	V	6.5
3	63.7	4746.80	V	43.5
4	71.3	877.988	V	8.0
5	76.5	230.515	V	2.1
6	85.0	1690.262	V	15.4
7	93.8	151.597	V	1.3
		Total 10911.32		

Fig. 6. Densitogram for Ipomoea tricolor seed (HPTLC, OPLC, VIS).
Symbols: EMET = ergometrine, ETAM = ergotamine, CHANO = chanoclavine

Table 1

Quantities of ergot-alkaloids in the seed and extract of Ipomoea tricolor

	Seed		Alcoholic-aqueous seed extract	
Total alkaloid content (expressed in ergotamine tartrate)	0.070 ± 0.008%		0.010 ± 0.003%	
	Alkaloid content %	As a % of total alkaloid content	Alkaloid content %	As a % of total alkaloid content
Ergometrine D-lysergic acid methyl-carbinolamide iso-lysergol agroclavine	0.0421 ± 0.0051	46—59	0.0061 ± 0.0011	42—61
Ergotamine ergostine	0.0091 ± 0.0017	9—13	0.0012 ± 0.0004	7—15
Chanoclavine α-dihydrolysergol noragroclavine	—	14—21	—	22—31
Ergometrinine	0.00042 ± 0.00018	1— 6	—	—

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We are indebted to Mrs. O. FRANCZKE special assistant, who with her conscientious work and continued effort contributed to the completion of our paper; thanks are also due to dr. N. NAGYIDAI, head of section for his calling our attention to the data of Ipomoea abuse in Hungary.

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ORGANIZATION OF TISSUES IN THE DEVELOPING AND MATURE PERICARP OF CARISSA CARANDAS L. (APOCYNACEAE)

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The fruit of Carissa is a two loculed berry developing from a bicarpellary, syncarpous superior ovary. Ovules appear to be borne on axile placenta at the base of the ovary and on parietal placenta towards the tip of the ovary. Stomata are present on the epidermis of ovary and fruit but hairs are totally absent. Anatomically pericarp can be distinguished into epicarp, mesocarp and endocarp. Epidermis and few layered collenchymatous zone below it form the exocarp. Mesocarp is bulky and is parenchymatous throughout the development and at maturity of the fruit. Endocarp is multilayered and parenchymatous but the innermost layer shows lignification on its inner tangential walls.

Introduction

Family Apocynaceae is characterized by the presence of laticiferous cells and follicular fruits developing from an apocarpous or subapocarpous pistil, even though a great diversity in fruit type such as capsule, berry and drupe can be seen in this family. Fruit of Carissa in an indehiscent berry with persistent calyx. The ripe fruits are dark brown in colour and have a sweet sour taste (PICHON 1953).

Very few developmental studies in berries have been done (LAWRENCE 1960; WAZYNSKA 1967; DAVE et al. 1975, 1979, 1980, 1982a, b, 1985). According to FAHN (1967) the juicy pericarp of berry have three zones; the outer-zone which usually contains pigments of the fruit (exocarp), a relatively bulky zone below it (mesocarp) and the membranous inner zone (endocarp). Anatomically pericarp of Averrhoa (DAVE et al. 1975) and tomato (DAVE et al. 1982b) are divisible into multilayered epicarp, fleshly mesocarp and parenchymatous endocarp. This paper deals with the detailed development and organization of tissue layers in the berry of Carissa carandas.

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Material and Method

The flowers and different developmental stages of fruits of Carissa carandas L. were collected from Sardar Patel University botanical garden and fixed in F.A.A. Materials were processed for paraffin sectioning (SASS 1958) and sections were stained with Safranine Fast green. Presence of tannin was detected by tannic acid—ferric chloride (JOHANSEN 1940) and starch by PAS (JENSEN 1962). SEM study of the fruit has been done using S-10 Cambridge Stereoscan Microscope placed at ATIRA (Ahmedabad).

Table showing the size of the ovary and fruits fixed at different stages of their development.

Stage	Length (cm)	Breadth (cm)	Remarks
1	0.3	0.2	Ovary from flower bud and open flower
2	0.5	0.3	Young fruit
3	0.8	0.5	
4	1.1	0.8	Developing fruit
5	1.6	1.1	
6	1.8	1.1	Mature brown fruit

Results

Carissa is a thorny shrub having bicarpellary, syncarpous two-chambered superior ovary. Ovules are borne on axile placenta at the base and on parietal placenta towards the tip of the ovary. Ovary is somewhat globose in shape.

Ovary wall

It is 16-18 layered thick (Figs 2, 3). Outermost layer is the epidermis, formed of radially elongated rectangular cells with abundant cytoplasm and centrally placed prominent nuclei; covered externally with a thin cuticle. Stomata are frequently present but hairs are totally absent on the ovary (Figs 2, 3).

Below the epidermis is the mesodermis or ground tissue formed of parenchyma cells with small intercellular spaces and show both anticlinal and periclinal divisions. Outermost 2-3 layers of ground tissue and septal zone also show tanniniferous cells (Fig. 2). The 34-36 vascular bundles embedded in the mesoderm tissue show different stages of their development because of their branched nature. Bundles are conjoint, collateral or bicollateral with more phloem elements than xylem tissues. Laticifers are highly

branched and are seen nearer to the vascular bundles, rarely associated with vascular elements. Outer mesoderm tissue shows druses of crystals.

Inner epidermis is 2-3 layered consisting of tangentially elongated parenchymatous cells. Cells are filled with abundant cytoplasm and have prominent nuclei. Stomata and hairs are totally absent on the inner epidermis.

Septal zone is formed of 10-12 layers of cells including the inner epidermal layers binding on either side of the septa. Vascular elements present in this zone are feebly developed. In the lower region of the ovary the placenta appears axile and towards the tip it is parietal. This is because of incomplete carpel fusion from middle part of the ovary to the tip (Fig. 2). Extreme centre of the placenta shows inward growth (Fig. 4 at arrow), but is not reaching upto the inner epidermis to make it tetralocular or multilocular.

Developing pericarp

After pollination ovary develops into fruit, which is an indehiscent berry (Fig. 1) with persistent calyx. Anatomically fruit wall can be distinguished into three zones; epicarp; mesocarp and endocarp (Figs 17, 18).

Epicarp is multilayered covered externally with a cuticle. At maturity of the fruit cuticle becomes thick and shows intrusions (Figs 5, 18), but are not reaching upto hypodermal layers. In surface view epicarpic cells are polygonal with thick cuticular walls (Fig. 20). Stomata are present frequently on the epicarp (Figs 6, 19) and are anomocytic with 4 or 5 subsidiary cells, but hairs are totally absent. A zone of 4-5 layers of hypodermal cells become collenchymatous (Fig. 5). The collenchymatous hypodermis together with epidermis constitute the multilayered epicarp. Epicarp is separated as a rind of the fruit on its peeling. Hypodermal cells show coloured pigments at maturity of the fruit.

Mesocarp consists of 25-35 layers of cells when the fruit measures 0.5-0.8 cm in diameter. A mature fruit have 55-65 layered mesocarp. Towards the centre of the mesocarp small and large cells are intermingled to form a network of parenchyma cells (Fig. 7). Starch grains and druses of crystals are present in the outer mesocarpic cells (Figs 8, 9, 18). There are 30-34 vascular bundles embedded in the developing mesocarp whereas 60-65 in the mature mesocarp. Vascular bundles are conjoint, collateral, bicollateral or concentric and are scattered in the mesocarpic cells. Bundles show difference in their stages of development because of ramification. Laticifers

are highly branched (Fig. 10) and are rarely associated with vascular elements. Some of the laticifers are reaching upto the endocarp.

Endocarp is 4-6 layered and consists of tangentially elongated parenchyma cells. Cells are devoid of crystals and sclereids but at a later stage inner tangential wall of the inner endocarp shows lignification (Figs 11, 18). On the endocarp stomata and hairs are totally absent.

Septal zone is very thin in the developing fruit stages. Mature fruit has a 24-26 layered bulky septa showing the presence of abundant tannin contents and vascular strands (Fig. 12). In mature fruit extreme centre of the septa shows a constriction lined with small parenchyma cells, extending from the middle of fruit to the apex indicating incomplete fusion of the carpels (Figs 12, 13 at arrows). A portion of the style is persisting along with the fruit and the cells adjacent to it are found to be filled with tannin contents (Figs. 14).

Placenta is very small and parenchymatous throughout its development. Seeds receive their vascular supply from the placental bundles (Fig. 15). Extreme base and tip portions of the seed chamber are devoid of seeds. Sometimes development of seed can be seen only in a single locule and the other locule is empty (Fig. 16).

Discussion

In berries, especially in the members of Solanaceae, a collenchymatous hypodermis fails to develop because of the persistent and protective calyx (ROTH 1977); also DAVE et al. (1980, 1982a, 1985). But in Carissa, persistent calyx is present at the base of the fruit and has been observed with collenchymatous hypodermis. Because of the lack of mechanical tissues in berries, epicarp with thick cuticle and collenchymatous hypodermis gives mechanical support. Single layered epidermis with a zone of collenchymatous hypodermis constitute the multilayered epicarp, covered externally with a thick cuticle showing strong intrusions in the fruit of Carissa. In berries stomata are not frequent where the epidermis is thick and leathery (ROTH 1977). But the intensity of stomata in Carissa is more and are of anomocytic types. As in the berries of Solanaceae (ROTH 1977) and Averrhoa (DAVE et al. 1975), calcium oxalate crystals are present in Carissa.

Mesocarp is fleshy and formed of both large and small parenchymatous cells with intercellular spaces. Laticiferous tissues are present throughout the mesocarp. SOLEREDER (1986) observed the presence of mucilage cells in

the vegetative parts of Carissa and in the fruit of Tabernaemontana. In our observation mucilage cells in the fruit of Carissa are found to be totally absent. Vascular bundles are conjoint, collateral or bicollateral and are scattered in the mesocarp. Endocarp is multilatered in early stages but their outer tangential walls of the innermost layer become lignified at maturity. The endocarps of Solanum, Withania, Lycopersicon and Physalis berries are parenchymatous (DAVE and MENON 1986), whereas the single layered endocarp of Capsicum is formed of alternately arranged parenchyma cells and the sclereids (DAVE 1986). DAVE et al. (1975) observed that the endocarp of Averrhoa is multilayered parenchymatous and devoid of sclereids.

According to FALLEN (1985) the primitive tribe Carisseae is characterized by the presence of a completely syncarpous ovary and berry fruit. WOODSON (1930) has opined that the most advanced character in family Apocynaceae is the syncarpous unilocular condition derived from apocarpous bilocular ovary through a syncarpous bilocular intermediate form. On the basis of this hypothesis, Carisseae have a syncarpous bilocular ovary and such genera have a clear epidermal lining where the two carpels are fused. According to RAO and GANGULI (1963), Carissa have a single ovary of two carpels, which is bilocular with axile placentation. In our observations, it is seen that there is a distinct line of the union of two carpels in the centre which makes the two locules distinct. But we also found that on the same line there is partial or incomplete separation of the two carpels at a mature stage of the fruit. Carissa have a syncarpous bilocular ovary and the ovules appear to be borne on the axile placenta at the base of the ovary and on the parietal placenta towards the tip of the ovary. The ovary of Capsicum also shows axile placentation in basal and middle region and parietal apically (LAWRENCE 1960; DAVE et al. 1979).

Acknowledgement

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Plate I

Fig. 1. Mature berry with persistent calyx (K). 4 x

Fig. 2. Transection of ovary showing stomata (arrows), ovary wall (OW), ovules (O) and septal zone (arrow head). 32 x

Fig. 3. A portion of the ovary wall showing stomata (arrow), outer epidermis (OE), vascular bundles (VB), mesoderm (ME) and inner epidermis (IE). 183 x

Fig. 4. Placental outgrowth (at narrow) in the ovary stage, which is not reaching up to the endocarp. Septa (SP), placeta (PL) and ovule (O). 125 x

Fig. 5. In mature fruit cuticle is very thick (arrow) and extent up to the hypodermis. Collenchyma (CO). 226 x

Fig. 6. SEM photograph of a stomata (ST). 350 x

Fig. 7. Mesocarp of mature berry is formed of parenchyma cells with different shapes. 105 x

Fig. 8. A starch grain under polarized light. 280 x

Fig. 9. Druses of crystals are present on the outermesocarp. 125 x

Plate II

Fig. 10. Laticifers (L) are of branched type and vascular strands are seen nearer to it. 95 x

Fig. 11. Endocarpic cell showing lignification on its inner tangential walls (at arrows). Mesocarp (MC). 103 x

Figs 12, 13. In mature fruit extreme centre of the septa is constricted (arrow), which extends from middle to tip region of the fruit. Septa (SP). 98 x

Fig. 14. A portion of the style (SY) is persisting along with the fruit. 36 x

Fig. 15. Seeds (SE) receive their vascular supply (VS) from the placental bundles. 130 x

Fig. 16. Transection of a mature fruit showing the development of seeds (SE) in one chamber and the other is empty. Septa (SP), Seed chamber (SC). 16 x

Plate III

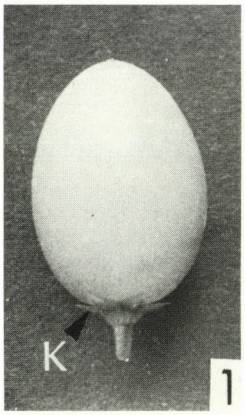
Fig. 17. Diagramatic and cellular diagram showing different pericarpic zones and seeds. 7 x

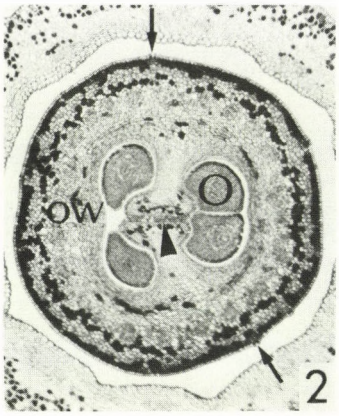
Fig. 18. Epicarp (EC), Mesocarp (MC), Crystal (C), Vascular bundle (VB), Endocarp (ED), Septa (SP), Seed (SE), Seed chamber (SC). 195 x

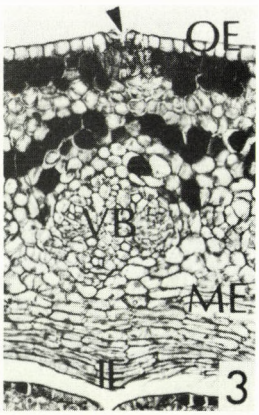
Fig. 19. Developing fruit showing stomata on the epicarp. 140 x

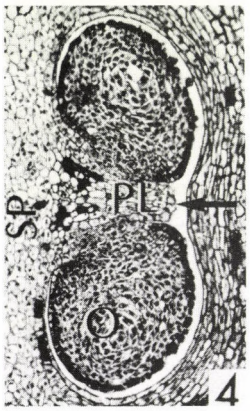
Fig. 20. Surface view of epicarpic cells from a mature fruit. 220 x

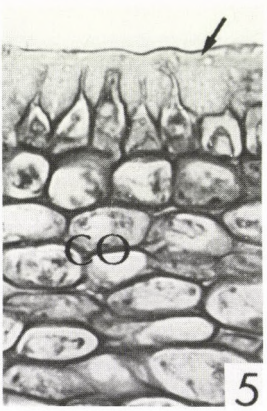
Plate I

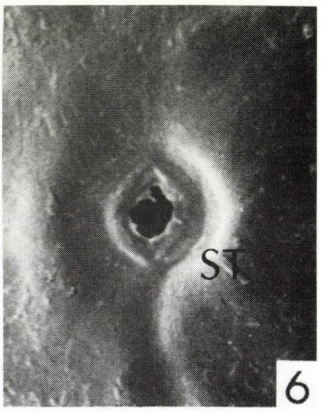


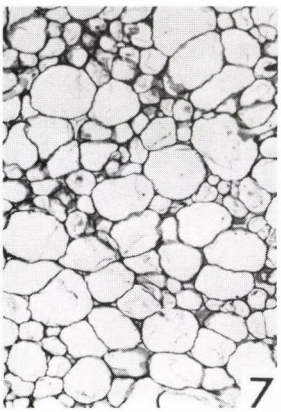


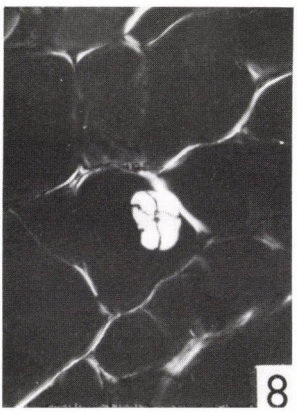


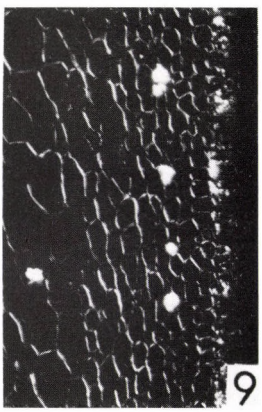












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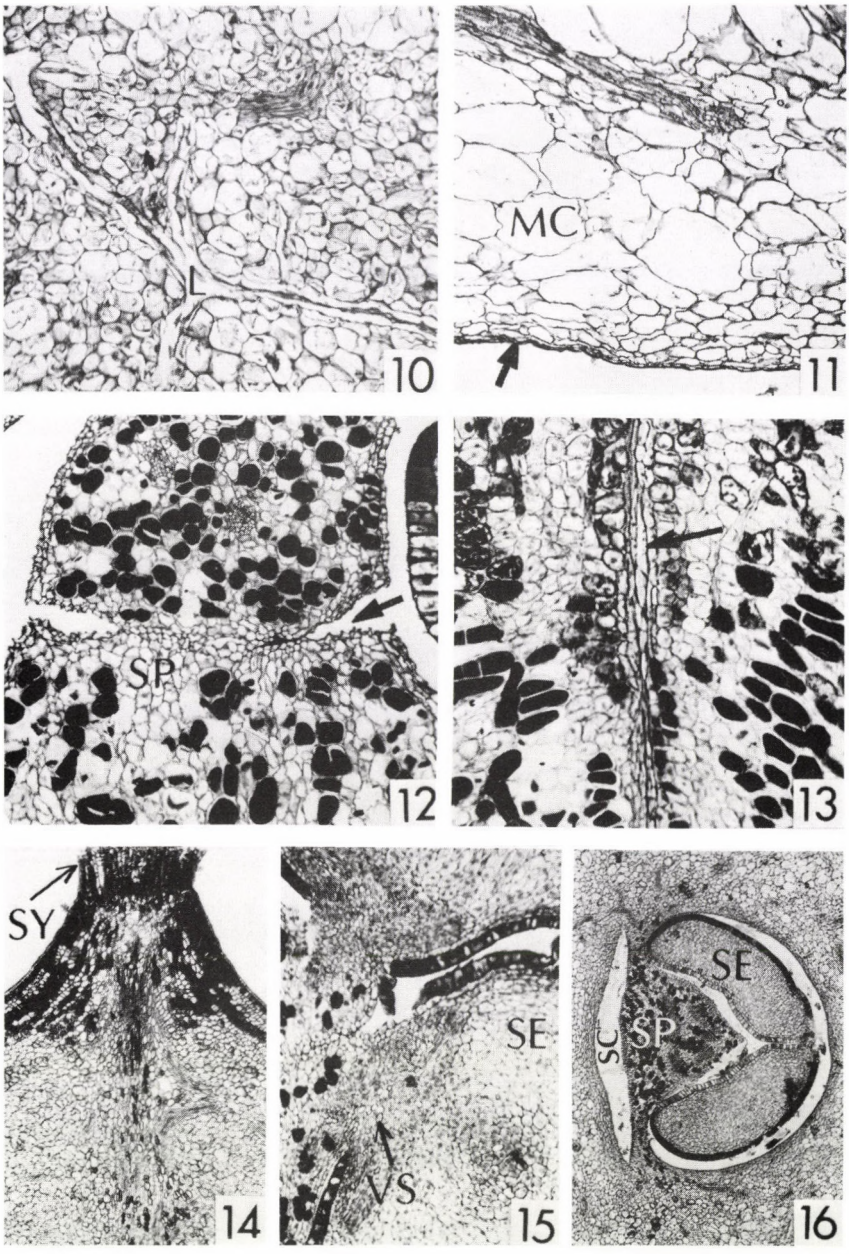

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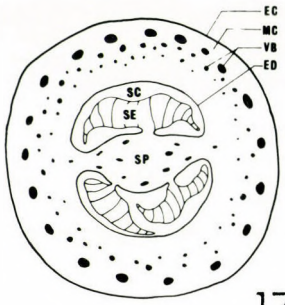

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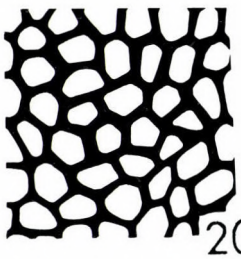

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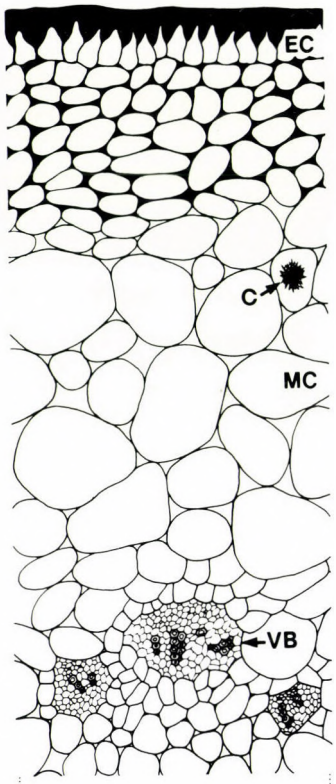

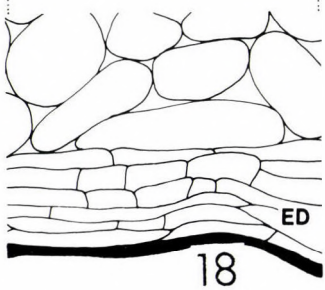

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CARACTERISTICAS DE LA EPIDERMIS FOLIAR DE GOETZEACEAE

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(LLegado: 25 Junio 1990)

The comparative study of the foliar epidermis of 4 genera of Goetzeaceae (Goetzea, Espadaea, Bissea (Henoonia), and Coeloneurum) showed the stability of the cellular features in all genera such as: anomocytic stomatal complex; sinuous to slightly curved anticlinal epidermal walls, uniseriate trichomes with a larger distal cell and verrucose walls. The resemble of the searched anatomical features in this family suggest great affinities with the Solanaceae.

Introduccion

La posición sistemática de los géneros Goetzea WYDLER; Espadaea A. Richard in Sagra; Bissea Fuentes y Coeloneurum Radlkofer, ha sido motivo de discusión desde el establecimiento de los mismos. Si bien es cierto que entre ellos existe una fuerte relación morfológica no menos lo es el hecho de haber sido referidos separadamente a diferentes familias.

Goetzea fue descrito originalmente como un representante de Ebenaceae (WYDLER, 1830). ENDLICHER (1843) colocó el género en Styracaceae, pero como él incluyó Symplocaceae en esta familia, es dificil saber a que grupo lo atribuyó. WETTSTEIN (1891) lo colocó en Solanaceae, Cestreae-Goetzeinae.

Espadaea A. Richard in Sagra ha sido referido a Verbenaceae (RICHARD, 1850; BENTHAM y HOOKER, 1873--1876) y Solanaceae. Cestreae-Goetzeinae (WETTSTEIN, 1891) donde es mantenida por AMSHOFF (1957).

Bissea Fuentes (1985), fue originalmente descrito como Henoonia Grisebach para Sapotaceae (GRISEBACH, 1866), a pesar de que el autor encontró afinidades con Myrsinaceae. Este criterio, aunque con reparos es mantenido por BENTHAM y HOOKER (1873--1876). WETTSTEIN (1891) transfiere el género a Solanaceae, Cestreae-Goetzeinae. Según KRAMER (1939), la estructura de la madera de Henoonia angustifolia Urban, pertence a la familia Sapotaceae, y es muy afin al género Bumelia Sw. AMSHOFF (1957) sin embargo mantuvo el género en Solanaceae.

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Coeloneurum fue descrito por RADLKOFER (1888) para Solanaceae, y mantenido en ella (Cestreae-Goetzeinae por WETTSTEIN (1891) y MOSCOSO (1943).

Sólo MIERS (1869) y AIRY SHAW (1965) plantearon el establecimiento de estos cuatro géneros como una familia independiente: Goetzeaceae.

El presente trabajo pretende contribuir al conocimiento de la posición sistemática de estos géneros, mediante el estudio de la epidermis foliar; asi como brindar una mejor comprensión de las afinidades y delimitaciones del grupo.

Materiales y Metodos

Se estudiaron hojas de los géneros que comprende Goetzeaceae, para lo cual se obtuvieron muestras de los Herbarios del Instituto de Ecologia y Sistemática de la Academia de Ciencias de Cuba (HAC); del Jardin Botánico Nacional (HAJB); y de la Universidad Friedrich Schiller de Jena (J).

Las epidermis fueron obtenidas de la porción central de hojas adultas mediante maceración del mesófilo en una mezcla de peróxido de hidrógeno 30% y ácido acético glacial en relación 1:1, la que se calentó en un baño de agua a 60 ^{o}C hasta la separación de ambas epidermis. Se eliminó el resto de mesófilo de las superficies interiores con ayuda de un pincel y se montaron las muestras en portaobjetos en medio de Ferrant. Para cada taxon se realizaron 15 mediciones del largo y ancho de los estomas y del largo de los pelos.

Los datos del material investigado en cada especie aparecen al final de cada descripción.

Las observaciones con el microscopio electrónico de barrido se relizaron en un MEB Hitachi 300 después de metalizar las muestras con oro.

Las láminas permanentes se encuentran depositadas en el Dpto. de Botánica del Instituto de Ecologia y Sistemática de la Academia de Ciencias de Cuba.

Resultados y Discusion

En los cuatro géneros estudiados, las hojas constituyen órganos de gran variabilidad morfológia y biométrica. Los caracteres morfológicos de las hojas se aprecian en la Tabla 1.

Tabla 1

Principales caracteres morfológicos de las hojas de los representantes de Goetzeaceae

Taxon	Forma	Base	Apice
Goetzea elegans	obovada	cuneada	agudo
Espadaea amoena	lineal obovada	cuneada	redondeado apiculado emarginado
Bissea myrtifolia	oblonga obovada	aguda obtusa cordada	agudo acuminado mucronado
Coeloneurum eggersii	eliptica	cuneada	agudo
Coeloneurum ferrugineum	eliptica	aguda cuneada	agudo pungente

Descripciones anatómicas de las epidermis

Goetzea Wydler

Hojas hipostomáticas, pelos simples, solo presentes en la superficie abaxial, uniseriados, mayormente de 2 a 3 células, base on paredes engrosadas; la célula apical mas aguzada y con pequeñas vesiculas. Células epidérmicas de la base de los tricomas dispuestas en forma de roseta y con paredes mas o menos sinuosas.

Células epidérmicas de la superficie adaxial poligonales, con paredes anticlinales muy sinuosas y paredes periclinales finamente reticuladas cuando se observan con microscopia a tras luz (Fig. 1). Las fotografias realizadas en el MEB evidencian que estos reticulos son pequeñas protuberancias redondeadas que se agrupan sobre esta pared (Fig. 2).

Las células de la superficie abaxial presentan similares caracteristicas a las de la adaxial pero con dimensiones menores (Fig. 3). Sobre los nervios las células se presentan de formas rectangulares, manteniendo la sinuosidad de sus paredes.

Estomas de tipo anomocitico, y ligeramente hundidos (Fig. 4), con dimensiones promedio de 23 y 18 µm de largo y ancho respectivamente.

Material estudiado: Goetzea elegans Wydl. Flora von Westindien No. 1133. Puerto Rico; Sierra de Luquillo 9-10 m alt. Abril 1883. Leg. EGGERS.

Espadaea A. Richard in Sagra

Hojas hipostomáticas con pelos simples uniseriados, con abundantes verrugas en sus superficies; constituidos por 2-3 células, aguzados en el extremo distal, raras veces unicelulares y sólo presentes en la superficie abaxial, con una longitud promedio de 76 µm.

Células epidérmicas poligonales e isodiamétricas, con paredes anticlinales gruesas y sinuosas. En la superficie adaxial las paredes periclinales presentan áreas mas delgadas a modo de fisuras perpendiculares a la pared anticlinal (Fig. 5). Las células que recubren los nervios son rectangulares, dispuestas en varias filas y con paredes menos sinuoses. La observación topográfica con el MEB permite ver el relieve de esta superficie donde las paredes anticlinales resultan ligeramente elevadas, asi como también la presencia de ceras epicuticulares (Fig. 6). Las paredes periclinales de las células epidérmicas de la superficie adaxial finamente reticuladas.

Superficie abaxial con estomas de tipo anomocitico, con valores promedio de 25 y 19 µm de largo y ancho respectivamentes Las células epidér-

micas de esta superficie presentan similares caracteristicas a las de la superior pero con dimensiones menores (Figs 7 y 8).

Material estudiado: Espadaea amoena A. Richard in Sagra HAJB No. 4694 Prov. Villa Clara. Sierra del Escambray, camino de la costa a loma del Burro. Col. J. BISSE y L. ROJAS. Nov. 1967.

Bissea Fuentes

Hojas hipostomáticas con pelos simples mayormente unicelulares y confinados a la superficie abaxial, con paredes gruesas; en ocasiones también formados por 2-3 células con paredes mas finas. Se observan inclusiones amarillas en el interior de algunos tricomas, los que poseen una longitud promedio de 164 µm.

Células epidérmicas poligonales e isodiamétricas. Las de la superficie adaxial con paredes anticlinales rectas a ligeramente curvas y gruesas, con extensiones de estos engrosamientos hacia las superficies periclinales, dejando un área reducida de pared fina en la periclinal (Fig. 9). Las células epidérmicas de la superficie abaxial con paredes anticlinales ligeramente gruesas y curvas y en ocasiones algo sinuosas (Fig. 10).

Estomas de tipo anomocitico, ligeramente hundidos (Fig. 11), con valores promedios de 27 y 20 µm de largo y ancho respectivamente. En ocasiones cubiertos por algunas células epidérmicas.

Material estudiado: Bissea myrtifolia (Griseb.)Fuentes, HAJB No. 25944. Prov. Holguin. Monta seco a una loma de serpentina 3 km al oeste de Playa Guardalavaca. Col. J. BISSE, A. ARECES y Lutgarda GONZÁLEZ. Abril 1975.

Coeloneurum Radlk.

Hojas hipostomáticas, pelos simples, uniseriados con superficies ligeramente verrugosas, de 2-3 células, la del extremo distal mas aguzada; raras veces unicelulares y solo presentes en la superficie abaxial, con longitud promedio de 66,4 µm. Los tricomas están ausentes en C. eggersii Radlk.

Células epidérmicas poligonales e isodiamétricas con paredes anticlinales sinuosas con reforzamientos (Figs 12 y 13). Sobre los nervios las células se presentan alargadas con paredes menos sinuosas y dispuestas en filas. Las células de la superficie adaxial poseen áreas mas finas hacia la pared periclinal. Paredes periclinales finamente reticuladas y elevadas (Fig. 14). Las células de la superficie adaxial con mayores dimensiones que las de la abaxial.

Estomas de tipo anomocitico con valores de 23,8 y 18,1 µm de largo y ancho respectivamente en C. ferrugineum (Spr.) Urb. y de 20,8 y 16,8 µm en C. eggersii Radlk. (Figs 15 y 16).

Table 2

Principales caracteres de la epidermis foliar observados en Goetzeaceae.
Leyenda: NG = Tricoma no glandular; anomoc = anomocitico

Especies	Estomas		Caract. celulas epider. no especializadas		Tricomas	Otras
	Tipo	Dimensiones en µm	Sup. inferior	Sup. superior		
Goetzea elegans	anomoc	23 x 18	sinuosas	sinuosas	NG abaxial	Paredes periclinales sup. adaxial reticulao con protuberancias
Espadea amoena	anomoc	25 x 18	sinuosas	sinuosas	NG abaxial	Presencia de zonas mas finas en las paredes periclinales y finamente reticuladas en la cara adaxial
Bissea mytifolia	anomoc	24 x 18	curvas a sinuosas	rectas	NG abaxial	Paredes anticlinales sup. adaxial engrosadas
Coeloneurum eggersii	anomoc	21 x 17	sinuosas	sinuosas	ausentes	—
C. ferrugineum	anomoc	24 x 18	sinuosas	sinuosas	NG abaxial	—

El estudio al MEB de esta especie mostró que los tricomas presentan pequeñas protuberancias que en ocasiones asemejan estrias (Fig. 17). Las paredes periclinales de las células epidérmicas tienden a ser ligeramente abultadas en la cara adaxial, mientras que en la abaxial presentan ornamentos similares a verrugas diminutas y los estomas ligeramente hundidos (Fig. 18).

Material estudiado: Coeloneurum eggersii Ex Herbarium Krug et Urb. No. 2366 leg. Eggers in Santo Domingo prope Santiago in Cuesta Piedra 6-VI-1887 Coeloneurum ferrugineum N.Y. Bot. Garden No. 2618 Dupl. Leg. L. R. HOLDRIGE; Morne a Cabrits 26 Abril 1942.

Las caracteristicas de la epidermis foliar de Goetzeaceae son en nuestro criterio poco variables. Los principales caracteres presentes en las hojas de los cuatro géneros que conforman la familia se incluyen en la Tabla 2 como son estomas de tipo anomocitico confinados exclusivamente a la superficie abaxial; células epidérmicas no especializadas con paredes anticinales sinuosas (Goetzea, Espadaea y Coeloneurum) a ligeramente curvas (Bissea) y paredes periclinales mayormente con un fino reticulo; y pelos simples uniseriados compuestos por hasta 3 células de las cuales la apical presenta mayores dimensiones del largo. Muchos de los caracteres antes señalados han sido reprotados por METCALFE y CHALK (1950) y AHMAD (1964) para Solanaceae, sin embargo estos autores ofrecen una mayor diversidad de caracteres en este grupo taxonómico y no de forma tan estable como se encuentran en los representantes de Goetzeaceae. AHMAD (1964) por su parte destacó también como caracter muy interesante en Solanaceae la presencia de estriaciones cuticulares en todos los géneros estudiados por él, caracter que no fue observado en el transcurso de nuestra investigación en ninguno de los representantes.

Nuestros resultados concuerdan también con los reportados por ZONA (1989) con la diferencia que los ejemplares de Bissea estudiados aqui presentaron las paredes anticlinales de las células epidérmicas adaxiales ligeramente curvas como se observa en la Fig. 9.

En nuestro criterio, la estabilidad de los caracteres de los géneros estudiados refuerzan la opinión de la existencia de Goetzaceae, aunque tomando en cuenta las semejanzas en las caracteristicas de la epidermis foliar, este grupo debe estar muy próximo a Solanaceae, como ha sido señalado por AIRY SHAW (1965), HUNZIKER (9179), CARLQUIST (1988) y ZONA (1989).

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Plate I

Fig. 1. Goetzea elegans: Superficie del epidermis foliar adaxial, 400 x

Fig. 2. Goetzea elegans: Superficie foliar adaxial con MEB, 500 x

Fig. 3. Goetzea elegans: Superficie del epidermis foliar abaxial, 400 x

Fig. 4. Goetzea elegans: Superficie foliar abaxial con MEB, 500 x

Fig. 5. Espadaea amoena: Superficie del epidermis foliar adaxial, 400 x

Fig. 6. Espadaea amoena: Superficie foliar adaxial con MEB, 500 x

Plate II

Fig. 7. Espadaea amoena: Superficie del epidermis foliar abaxial, 160 x

Fig. 8. Espadaea amoena: Superficie foliar abaxial con MEB, 300 x

Fig. 9. Bissea myrtifolia: Superficie del epidermis foliar adaxial, 400 x

Fig. 10. Bissea myrtifolia: Superficie del epidermis foliar abaxial, 200 x

Fig. 11. Bissea myrtifolia: Superficie foliar abaxial con MEB, 450 x

Fig. 12. Coeloneurum ferrugineum: Superficie del epidermis foliar adaxial, 500 x

Plate III

Fig. 13. Coeloneurum ferrugineum: Superficie foliar adaxial, 500 x

Fig. 14. Coeloneurum ferrugineum: Superficie foliar adaxial con MEB, 600 x

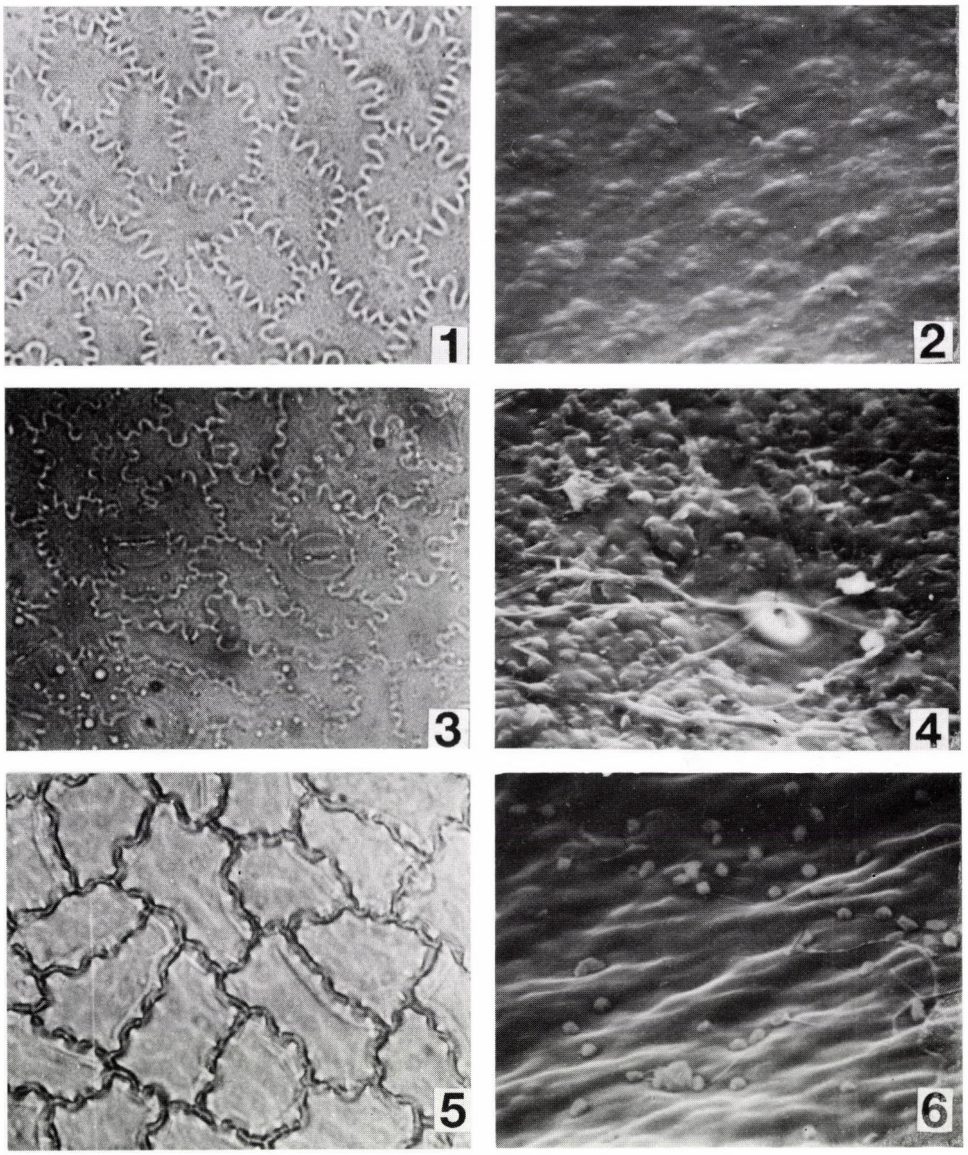
Fig. 15. Coeloneurum ferrugineum: Superficie del epidermis foliar abaxial, 140 x

Fig. 16. Coeloneurum ferrugineum: Superficie foliar abaxial, 500 x

Fig. 17. Coeloneurum ferrugineum: Superficie del epidermis foliar abaxial con MEB, 700 x

Fig. 18. Coeloneurum ferrugineum: Superficie foliar abaxial con MEB, 1000 x

Plate I



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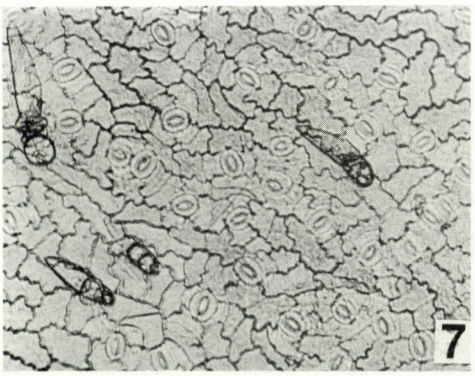

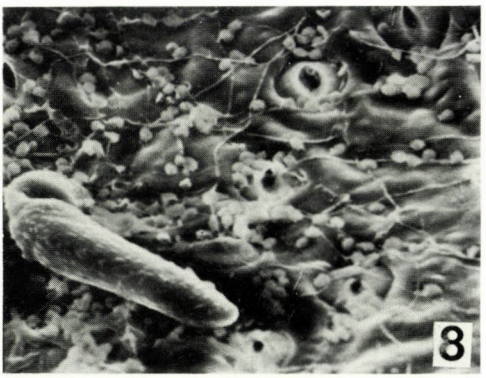

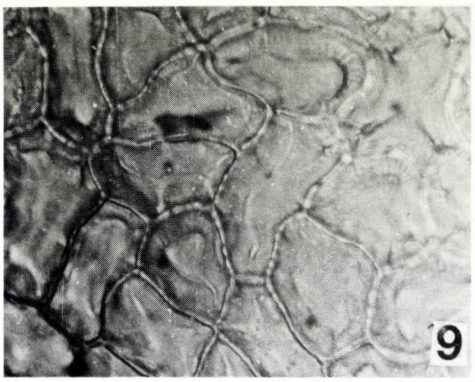

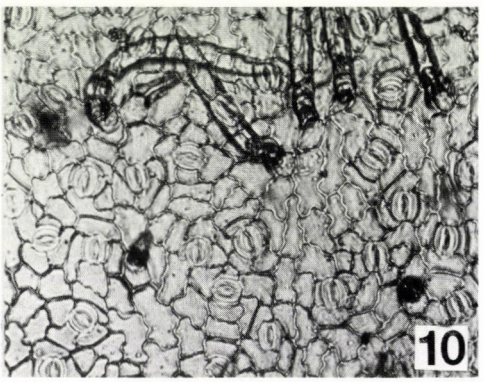

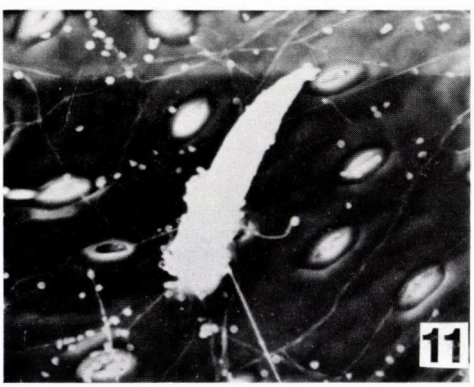

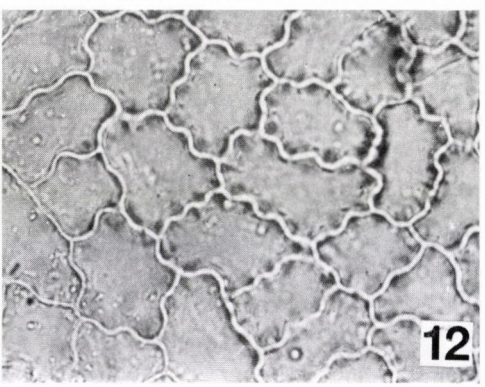

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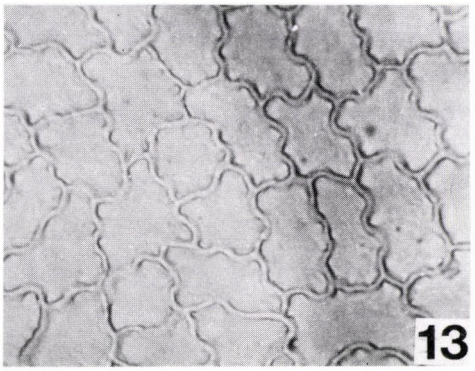

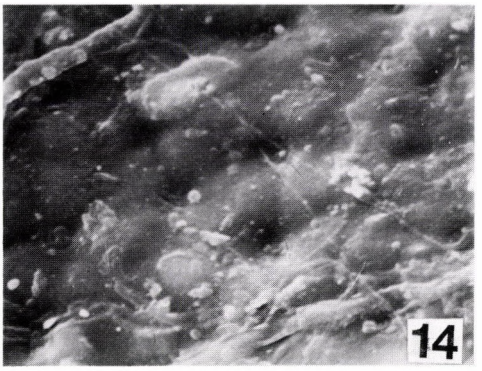

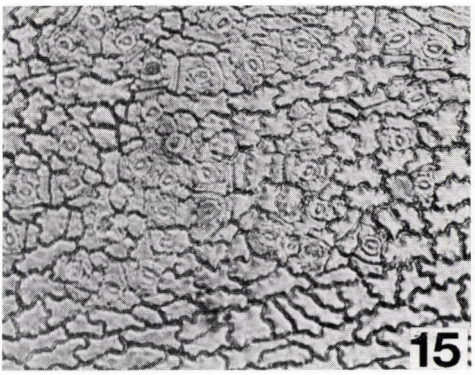

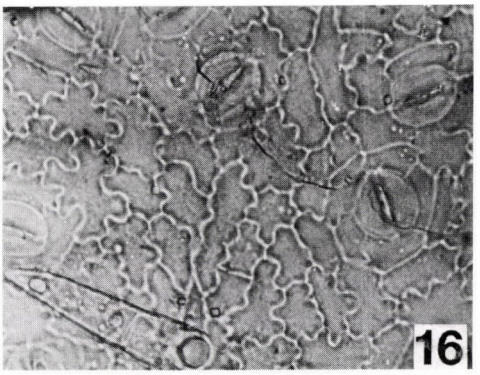

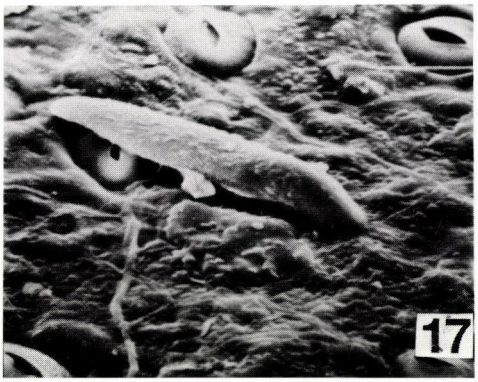

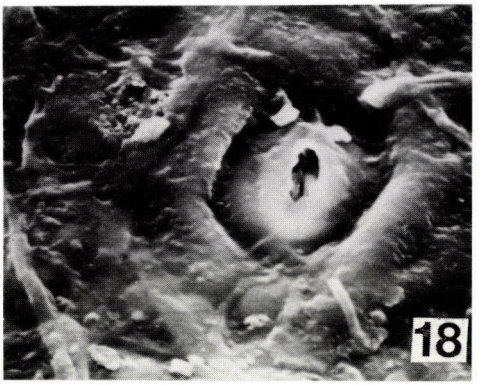

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DRY MATTER YIELD, EVAPOTRANSPIRATION AND WATER UTILIZATION OF RICE VARIETIES AT TWO SOIL MOISTURE AND NITROGEN LEVELS

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An experiment was carried out in a growth-house with three varieties of rice. The effects of different water supplies and nitrogen rates on dry matter yield, evapotranspiration and water utilization were studied.

Lower soil moisture tension at both nitrogen rates increased the grain yield in all three varieties. The higher nitrogen rates decreased the grain yields at both soil moisture levels. There was a high correlation between the grain sterility means of the varieties and treatments and grain yield. At lower nitrogen rate the raised soil moisture significantly increased the total dry matter yield in all varieties.

While the raised water supply at panicle initiation only slightly increased the total evapotranspiration, the grain yield increase was significantly affected by the higher soil moisture.

In the NPK-treatment the evapotranspiration coefficients calculated for the total plants of the three varieties were more favourable at higher soil moisture. In the N_2PK-treatments opposite results were obtained. Evapotranspiration coefficients calculated for grain at both nitrogen rates were lower at higher soil moisture.

Introduction

Though in Hungary rice cultivation takes place on a relatively small area (14 000 ha) it is indispensable to obtain more information about the main factors that greatly affect the profitable rice production also under our climatic conditions. VAMADEVAN (1972) suggested that the evapotranspiration rate of rice is influenced mainly by meteorological factors. This is why the evapotranspiration values of rice are so differing in various countries, but also even in the same sites in consecutive years depending on the changing meteorological conditions. So far only paddy rice has been cultivated in Hungary. Rice cultivation under flooded conditions requires more water than under irrigated upland (from now on irrigated) practices. Since the water is limited such cultivation practices should be introduced which even with less water give reasonable yields.

A number of experiments have been carried out in various countries to examine how the rice reacts to sprinkling irrigation. The experimental

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results indicate that the morphologically non-aquatic rice plant thrives best in submerged soil (CLARK et al. 1957; CHAUDRY and McLEAN 1963; VLAMIS and DAVIS 1944). PATRIK and FONTENOT (1976) found that the reduced soil conditions caused by either low soil moisture tension or low soil oxygen content enhanced the early vegetative growth of lowland rice.

Higher yield brought about by waterlogging may be explained partly by the increased availability of some plant nutrients under reduced conditions (SHAPIRO 1958). Also, standing water regulates the microclimate, controls the weeds and may enhance nitrogen fixation by blue-green algae and other organisms. This may be important where soil fertility limits production (SENEWIRATNE and MIKHELSEN 1961).

We began our growth-house experiments with rice varieties including their water consumption in 1983. In 1984 we had examined the daily course of meteorological factors and their effect on rice evapotranspiration and found that the meteorological factors similarly affected the evapotranspiration of both, the flooded and irrigated rice plants, though the evapotranspiration of irrigated plants compared to those of flooded ones was only 62.38%.

Higher irrigation rates and more frequent irrigations increase evapotranspiration therefore if the higher soil moisture can be restricted to a later developmental stage water utilization could be likely increased.

This is why our aim of the work reported here was to examine the effect of increased soil moisture at panicle initiation stage at two nitrogen rates on rice yield and water utilization of three rice varieties.

With high nitrogen rates we tried to get some information about the differential reaction of the tested varieties.

Material and Method

Three candidate rice varieties (Karmina, Viola, Krista) were selected for irrigation study. The seeds were planted on 12 May 1987. The pots containing the rice plants were placed on carts running on tracks and moved every morning into a space enclosed by a wire net. During the night or raining the carts stayed under a glass roof.

In the pots for 7 kg absolute dry soil, air dry alluvial-meadow plow layer soil (Szarvas—Bikazug) was placed. The maximum waterholding capacity of the soil was determined in laboratory and a value of 49.6 was obtained (expressed in weight % of absolute dry soil). Other main characteristics of the soil used in the experiment: pH (H_2O): 6.69, pH (KCl): 5.65, total salt %: 0.06, humus %: 2.02, total N %: 0.14.

P_2O_5 Al-P	method	64.4 ppm
K_2O Al-K		181.0 ppm
K_A/soil plasticity index		44.50

The soil on which the three rice varieties developed was filled with water to 70% of its maximum waterholding capacity at daily waterings. At the panicle initiation stage a higher soil water regime also was introduced. Half of the plants continued their development at increased soil moisture, 90% of the maximum waterholding capacity of soil.

The active ingredients per pot were as follows: N: 2.1 and 3.15 g (ammonium sulfate), P_2O_5: 1.4 g (superphosphate), K_2O: 1.4 g (potassium sulfate). Both nitrogen treatments received the same amount of phosphorus and potassium.

Twenty seeds were sown in each pot. After emergence the seedlings were thinned to fifteen. The treatments were replicated four times. The amount of evapotranspired water was restored by daily waterings. The plants were harvested at full ripening stage. At the end of the experiment, after counting the filled and sterile grains, the grain was stripped off by hand and the roots were washed out of the pot soil. The plant parts were dried at an oven temperature of 60 ^{o}C. The drying continued until there was no more change in the subsequent weight measurements.

For data evaluations statistical methods adapted by SVÁB (1973) were used.

Results and Discussion

Though roots play a very important role in the dry matter (DM) production and transpiration of plants, under our experimental conditions neither the different nitrogen supply nor the raised soil moisture at panicle initiation produced significant difference in root DM yield among the examined three rice varieties (Table 1). No significant differences were observed between treatment means of rice varieties either.

In our calculations straw includes all aerial plant parts except grain. In the examined varieties at lower soil moisture the DM yield of straw was significantly higher in the N_2PK-treatment than in the NPK one. For the Karmina and Viola varieties the raised nitrogen and water supply significantly increased the DM yield compared to the NPK-treatment at lower soil moisture. Taking the treatment mean of varieties the Viola variety gave a significantly ($P < 0.05$) higher yield than the Karmina and Krista varieties.

Examining the aerial parts, the DM yield of Karmina variety in the NPK-treatment at lower soil moisture level was significantly lower than in the other three treatments. DM yield of Viola variety was significantly higher in the NPK-treatment at lower soil moisture tension than in the other three treatments. For the Krista variety there were significant differences in DM yield between the NPK-treated plants developed at lower soil moisture and the plants of the two raised soil moisture levels.

DM yield of the whole plant of the three rice varieties was lowest in the NPK-treatment at the higher soil moisture tension. The raised water supply significantly increased the yield in the NPK-treatment. Only Karmina variety showed significant yield increase in the N_2PK-treatment at both soil

Table 1

Effect of nitrogen treatment and water supply on dry matter weight ($g.pot^{-1}$) of three rice varieties

Plant parts		Roots			Straw			Grain			Aerial parts			Whole plant		
Treatment								Varieties								
Nutrient	Water	Karmina	Viola	Krista	Karmina	Viola	Krista	Karmina	Viola	Krista	Karmina	Viola	Krista	Karmina	Viola	Krista
NPK	70%	28.88	25.75	28.03	89.08	93.15	89.40	17.25	15.50	19.73	106.33	107.15	109.13	135.20	132.90	137.15
NPK	90%	33.95	23.68	28.75	93.83	105.28	96.43	24.63	26.13	25.70	118.45	131.40	122.13	152.40	155.08	150.88
N_2PK	70%	29.45	31.95	35.50	104.23	104.43	104.00	15.03	5.65	10.93	119.25	110.08	114.93	148.70	142.03	150.43
N_2PK	90%	31.43	25.23	24.65	99.23	106.38	97.38	18.83	9.03	23.18	118.05	115.40	120.55	149.48	140.63	145.20
LSD	0.1%								11.83			17.43				
	1.0%		NS			11.94			8.88			13.09				
	5.0%					8.84			6.58			9.70			13.41	
Variety mean		30.93	26.65	28.29	96.59	102.31	96.80	18.93	14.08	19.88	115.52	116.01	116.68	146.44	142.66	145.20
LSD	0.1%															
	1.0%		NS						4.44			NS			NS	
	5.0%					4.42			3.29							

Table 2

Effect of raised water supply on grain yield at two nitrogen levels

Nutrient	Grain yield increase (in %) related to the lower water supply		
	Karmina	Viola	Krista
NPK	143	169	130
N_2PK	125	160	212

moisture levels related to the lowest yield. TÓTH (1983) citing the results of other workers writes that rice leaves are sensitive to their water saturation deficit. If this deficit reaches 15% the leaves droop. At soya beans this drooping occurs only when the water saturation deficit reaches a value of 25%. The reduced water supply under our experimental conditions might had contributed to the grain yield decrease.

In all three rice varieties at both nitrogen levels the higher water supply increased the yield of the most important plant part, the grain (Table 2). In the NPK-treatment this difference was statistically significant at Karmina and Viola varieties (Table 1). The higher nitrogen rate unfavourably affected the grain yield at both soil moisture levels.

Though nitrogen plays the most important role in the rice yield increase, at high nitrogen application rates ammonium ion is accumulated in plant cells resulting limits in protein synthesis (SIMON 1983). In our experiment at the higher nitrogen level very likely the increased ammonium ions also contributed to the grain yield decrease by promoting grain sterility. Especially the Viola variety reacted adversely in grain yield to the higher nitrogen level. This variety was less adaptable to lower soil moisture at both nitrogen rates than the other two varieties examined. The mean grain yield of treatments of the Viola variety was significantly lower than of the other two varieties. While there were no significant differences between the total plant yield of rice varieties in various treatments (Fig. 1), at higher nitrogen rate significant differences were observed in grain yield at both soil moisture levels (Fig. 2).

Though the root DM yield was not influenced significantly by treatments in the three rice varieties, the root qualities might have contributed to decreased grain yields at higher soil moisture tension. HSIAO et al. (1980) pointed out that restricted root activity under dry conditions ad-

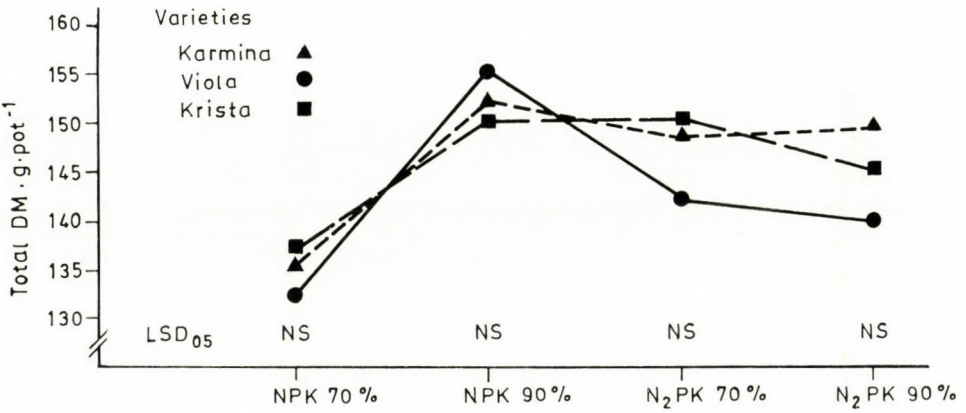

Fig. 1. Effect of nutrient and water supply on total (including roots) dry matter production of three rice varieties

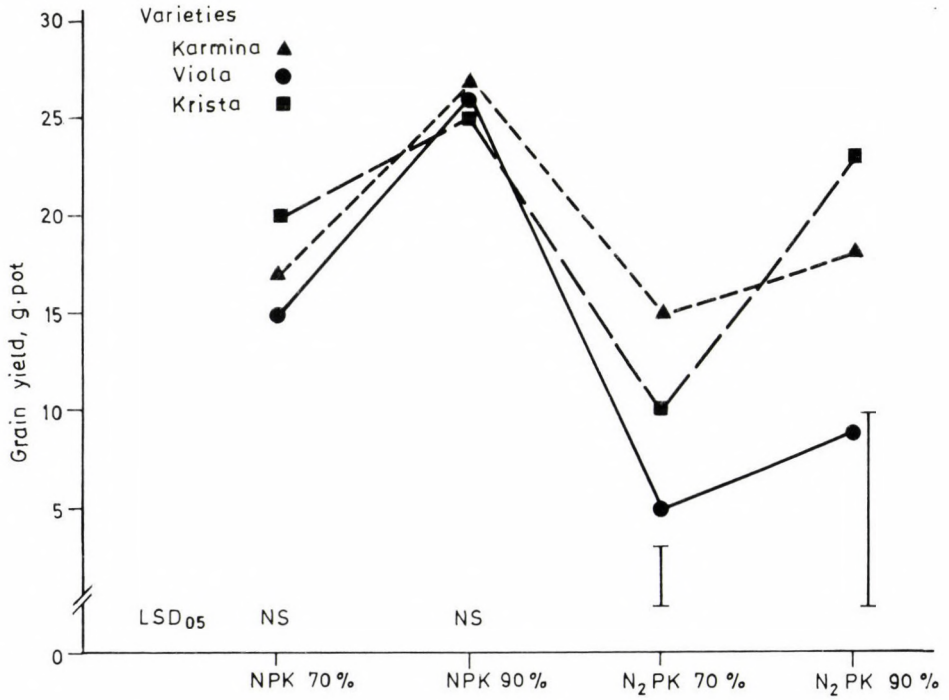

Fig. 2. Effect of nutrient and water supply on rice grain yield

Table 3

Effect of nitrogen treatment and water supply on grain sterility and grain yield

Treatment		Varieties					
		Karmina		Viola		Krista	
Nutrient	Water	Sterility %	Grain g	Sterility %	Grain g	Sterility %	Grain g
NPK	70%	36.73	17.25	40.73	15.50	29.04	19.73
	90%	24.65	24.63	35.11	26.13	29.90	25.70
N_2PK	70%	37.30	15.03	57.76	5.65	54.85	10.93
	90%	27.47	18.83	51.12	9.03	30.99	23.18

versely affects rice yield. In our experiment very likely the grain yield was mainly influenced by grain sterility. With one exception at Krista variety, at higher water supply decreased grain sterility and in accordance with this the grain yield increased (Table 3).

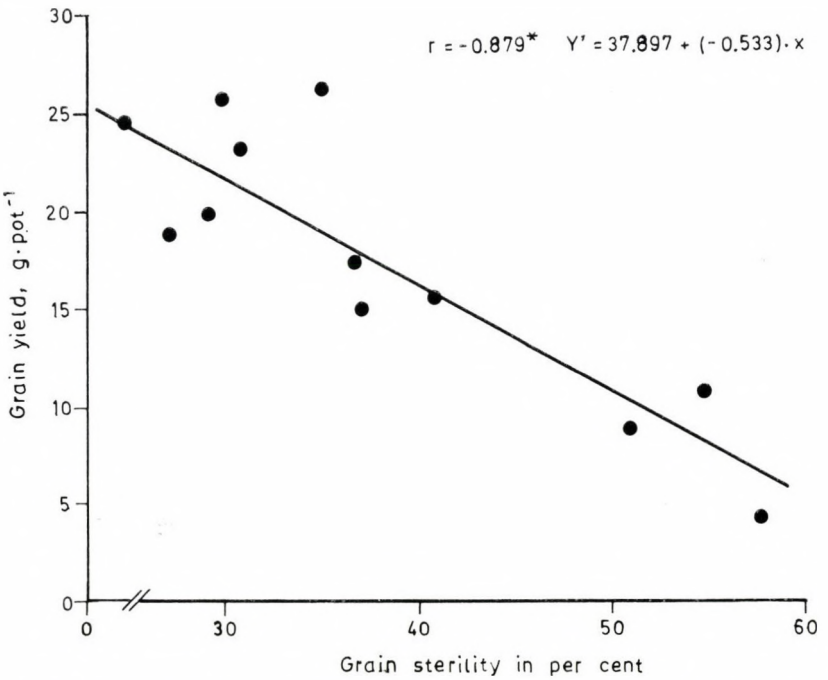

Fig. 3. Correlation between grain sterility and grain yield calculated from the mean values of three rice varieties and of different treatments

Table 4

Correlation between grain sterility (%) and grain yield

Treatment		Varieties	Main parameters		
Nutrient	Water		r	a	b
NPK	70%	Karmina, Viola, Krista	- 0.729**	27.246	- 0.275
	90%	Karmina, Viola, Krista	- 0.607*	36.553	- 0.370
N_2PK	70%	Karmina, Viola, Krista	- 0.773**	23.985	- 0.269
	90%	Karmina, Viola, Krista	- 0.617*	29.104	- 0.331
Mean values of all treatments		Karmina	- 0.520*	31.284	- 0.392
		Viola	- 0.802***	37.155	- 0.499
		Krista	- 0.740**	33.868	- 0.386

*, **, ***Significant at 5%, 1% and 0.1%, respectively

In the mean of treatments the highest sterility was observed at the Viola variety and the mean grain yield was also here the lowest among the varieties tested.

A linear negative correlation exists between grain sterility per cent and grain yield. Figure 3 shows the ratio between grain sterility and grain yield in the average of three rice varieties and of different treatments.

As seen in Table 4 in the average of the three rice varieties the highest correlation between grain sterility and grain yield was obtained in the N_2PK-treatment at higher soil moisture tension. The lowest ratio was observed in the NPK-treatment at lower soil moisture tension. Taking the mean values of all treatments highest correlation between grains sterility and grain yield was calculated at the Viola variety. The lowest ratio was obtained at the variety of Karmina. All "r" values were significant at least at 5% level.

Rice evapotranspiration is greatly influenced by meteorological factors. Earlier we had studied the effect of meteorological factors on evapotranspiration of irrigated rice (DEFLANDRE and SZLOVÁK 1984). Daily course of meteorological factors and of irrigated rice evapotranspiration is presented in Fig. 4. Figure 5 shows that the highest correlation was calculated between solar radiation and rice evapotranspiration. The relative humidity of air showed the lowest though still highly significant correlation ($P < 0.001$) with rice evapotranspiration. PETRASOVITS (1968) also emphasized

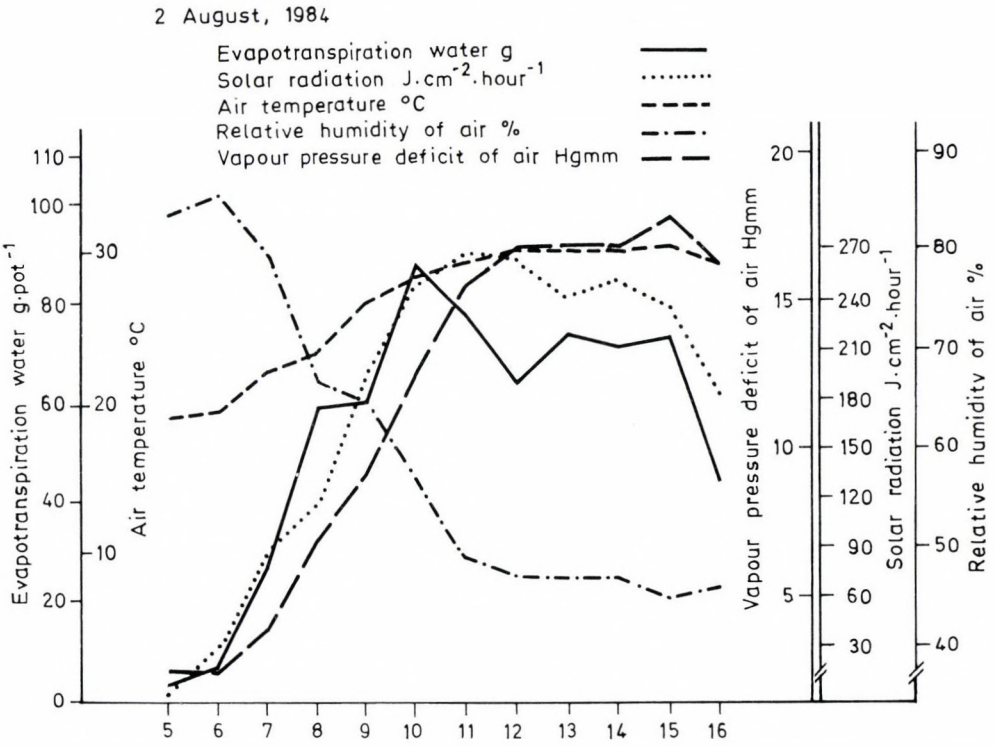

Fig. 4. Daily course of meteorological factors and of irrigated rice evapotranspiration

the great effect of solar radiation and air temperature on rice evapotranspiration.

At both nitrogen doses the higher soil moisture increased the evapotranspiration of all three rice varieties (Table 5). In maize experiments the higher water supply also increased the transpiration rates (SZLOVÁK 1967, 1972, 1973, 1974). In our rice experiment the evapotranspiration was highest in the N_2PK-treatment at lower soil moisture tension. The variety mean of treatments shows that the evapotranspiration of Viola variety was significantly higher than of the other two ones.

There were certain differences among rice varieties and treatments concerning the water utilization. With the exception in the N_2PK-treatment of Karmina variety, the evapotranspiration coefficient calculated for aerial parts was lower, that is more favourable at higher soil moisture level. Like the evapotranspiration, at 5 per cent significant level the variety mean

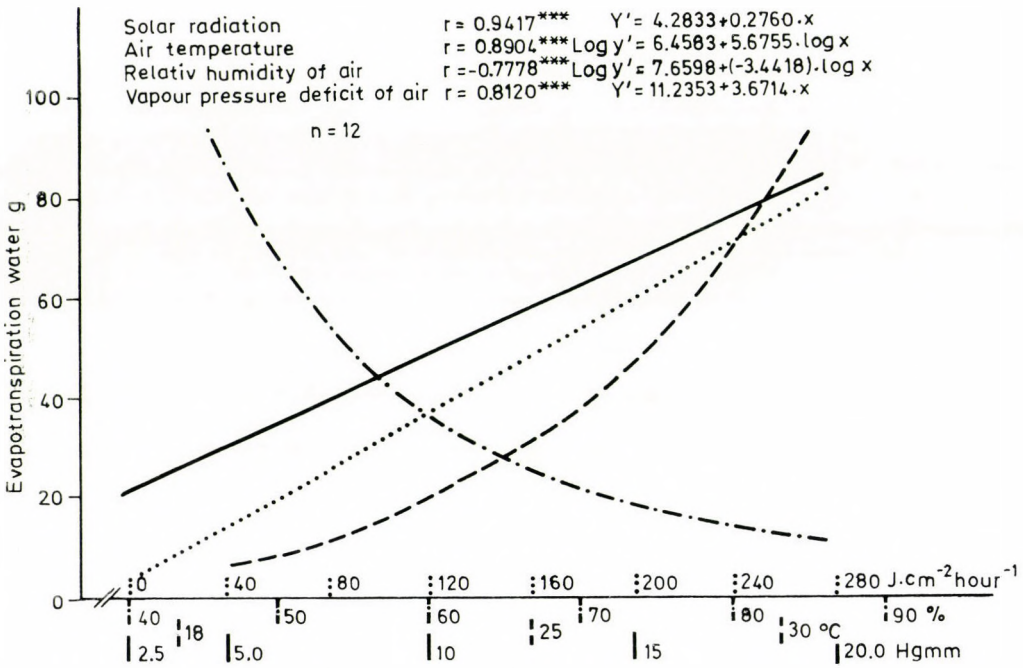

Fig. 5. Correlation between meteorological factors and irrigated rice evapotranspiration

evapotranspiration coefficient of Viola variety was higher than of the other two varieties.

Though the higher soil moisture introduced at penicle initiation only slightly increased the total evapotranspiration (6% in the mean of the three varieties in the NPK-treatment, and 3% in the N_2PK-treatment), the grain yields at both nitrogen levels were greatly increased (47% in the NPK- and 66% in the N_2PK-treatment).

Only in the NPK-treatment was the evapotranspiration coefficient, calculated for the whole plant, lower at the higher soil moisture level. In the N_2PK-treatment opposite results were obtained. The variety mean of all treatments was lower for Karmina and Krista varieties than for Viola. This difference was highly significant ($P < 0.001$).

Evapotranspiration coefficients calculated for grain were more favourable at higher soil moisture. Transpiration coefficients calculated for maize grain were also lower at plants that developed at higher soil moisture (SZLOVÁK 1983). Similar results were obtained when the variety means were compared.

Table 5

Evapotranspiration and water utilization of three rice varieties under different nitrogen and water treatments

Treatment		Evapotranspiration g			Evapotranspiration coefficient calculated for aerial parts			Evapotranspiration coefficient calculated for the whole plant			Evapotranspiration coefficient calculated for grain		
		Varieties											
Nutrient	Water	Karmina	Viola	Krista	Karmina	Viola	Krista	Karmina	Viola	Krista	Karmina	Viola	Krista
NPK	70%	59823	61606	61084	563.0	576.0	559.8	443.0	464.4	445.4	3580	4018	3123
NPK	90%	63021	66748	63460	532.0	508.6	520.0	413.4	431.1	421.6	2616	2726	2592
N_2PK	70%	62289	65893	63227	522.3	598.6	549.6	418.8	463.8	420.4	4183	13752	6096
N_2PK	90%	65076	67733	64499	553.6	589.4	536.3	436.7	483.1	445.8	3805	8161	2885
LSD	0.1%											5947	
	1.0%		NS			54.3			36.6			4465	
	5.0%					40.2			27.1			3307	
Variety mean		62552	65495	63068	542.7	568.1	541.4	428.0	460.6	433.3	3546	7164	3674
LSD	0.1%								24.4			2973	
	1.0%								18.3			2233	
	5.0%		2271			20.1			13.5			1654	

The experimental results clearly indicate that while the higher soil moisture increased the evapotranspiration of rice, the water utilization was generally more favourable at lower soil moisture tension.

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A CONTRIBUTION TO THE EMBRYOLOGY OF YOUNGIA JAPONICA (L.) DC. (ASTERACEAE)

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In Youngia japonica anthers are tetrasporangiate, but progressive sterilisation on the adaxial side of the anther leads to the formation of bi- and trisporangiate anthers. Anther tapetum is of the Periplasmodial type and its cells are multinucleate and polyploid. Microspore tetrads are either tetrahedral or decussate. Pollen grains are 3-celled when shed. The ovule is anatropous, unitegmic and tenuinucellate. Embryo sac development is of the Polygonum type. Endosperm is ab initio cellular and embryo development conforms to the Senecio variation of Asterad type.

Introduction

Embryological investigations in the tribe Cichoreae of the family Asteraceae are quite extensive and these have been reviewed by PULLAIAH (1984). Some of the papers that appeared since then are PULLAIAH and SWARAJYA LAKSHMI (1983, 1984) and SOOD and THAKUR (1985). A review of the literature reveals that embryology of Youngia japonica has not been reported except for an abstract by KAUL (1973). Hence the present investigation has been undertaken and the present report deals with the embryology of Youngia japonica (L.) DC.

Material and Method

Heads at different stages of development were collected from Pulney hills of Tamilnadu, India and fixed in formalin-acetic acid-alcohol (F.A.A.). Usual methods of dehydration, embedding and sectioning were followed. The sections were stained in Delafield's haematoxylin.

Akadémiai Kiadó, Budapest

Observations

Microsporangium, microsporogenesis and male gametophyte

Anthers in Youngia japonica showed much variation. In some cases anthers are tetrasporangiate (Fig. 2A) while in other cases they are bi- or trisporangiate (Fig. 1 A--C, E, F). In some other cases three well-developed sporangia and one hypertrophied sporangium is met with (Fig. 1D). All these conditions lead us to the conclusion that progressive sterilisation on the adaxial side of the anthers lead to the tri- or bisporangiate structures.

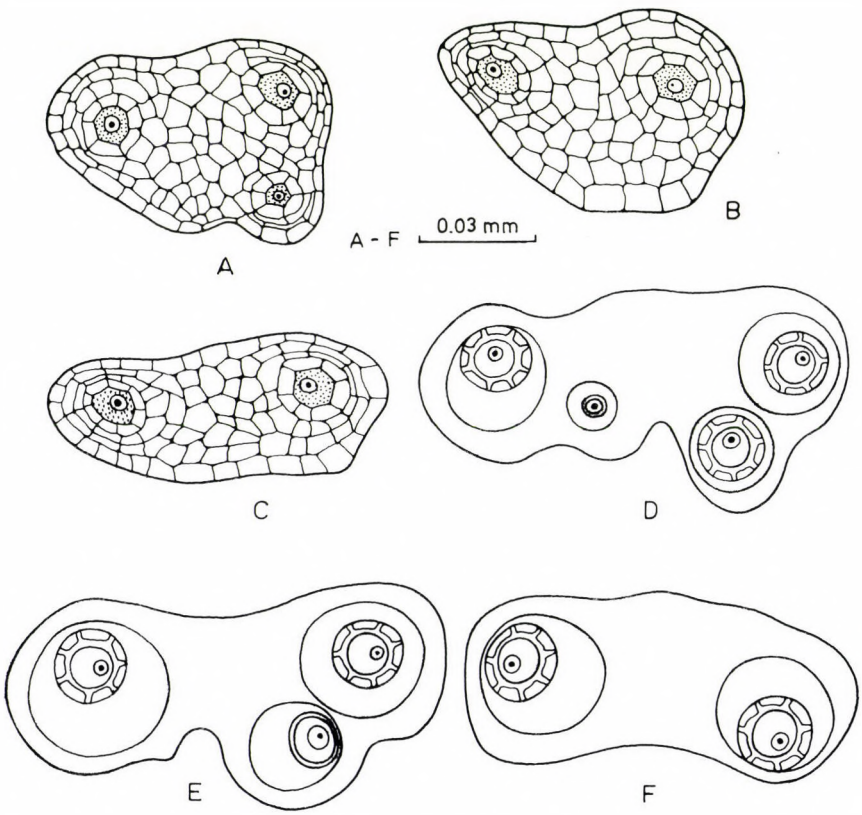


Fig. 1. Youngia japonica.
A/ T.s. of trisporangiate anther
B, C/ T.s. of bisporangiate anthers
D/ T.s. of anther showing three well developed sporangia and one hypertrophied sporangium
E/ T.s. of trisporangiate anther
F/ T.s. of bisporangiate anther

Male archesporium is hypodermal and consists of single row of 4-5 cells (Fig. 2B). The archesporial cells divide periclinally (Fig. 2B) forming primary parietal layer and primary sporogenous layer. The primary parietal layer undergoes division in a Dicotyledonous type (DAVIS 1966) forming a hypodermal layer, a middle layer and tapetum (Fig. 2C, D). The epidermal cells at maturity get much stretched, elongated and flattened. The hypodermal cells develop fibrous bands and become fibrous endothecium (Fig. 2I, J). The middle layer gets crushed and degenerated at the time of meiosis of pollen mother cells. The tapetal cells may become 2-nucleate (Fig. 2E). Tapetal cells with multinucleate and polyploid nuclei are also met with (Fig. 2F). At the one-nucleate stage of the pollen grains, the walls of the tapetal cells break down and the cytoplasm flows into the anther locule and finally coalasce in the centre forming periplasmodium (Fig. 2G, H). The tapetum is completely absorbed by the developing pollen grains and no trace of it is left at the time when two-celled pollen grains are formed (Fig. 2I).

The primary sporogenous cells divide transversely and produce single row of pollen mother cells (Fig. 2D) which undergo meiosis (Fig. 2K, L) resulting in tetrahedral and decussate microspore tetrads (Fig. 2M--O). Cytokinesis is by furrowing. The pollen grain after liberation enlarges (Fig. 2P). The nucleus of the pollen grain divides resulting in 2-celled pollen grain. The smaller generative cell gets pinched off and moves into the vegetative cell. Pollen grains at the time of anthesis are 3-celled with 3 germ pores (Fig. 2R).

Ovary and ovule

The inferior ovary is bicarpellary, syncarpous and unilocular with a single, basal, anatropous, unitegmic and tenuinucellate ovule (Fig. 3A--C). An integumentary tapetum is differentiated at about the megaspore tetrad stage (Fig. 3F). It remains uniseriate with uninucleate cells (Fig. 3L, M) till it is absorbed by the growing embryo. At the time of the organised embryo sac stage, the cells of the funicular epidermic in the region of the micropyle, enlarge accumulate dense cytoplasm, become glandular and function as funicular obturator (Fig. 3C, D).

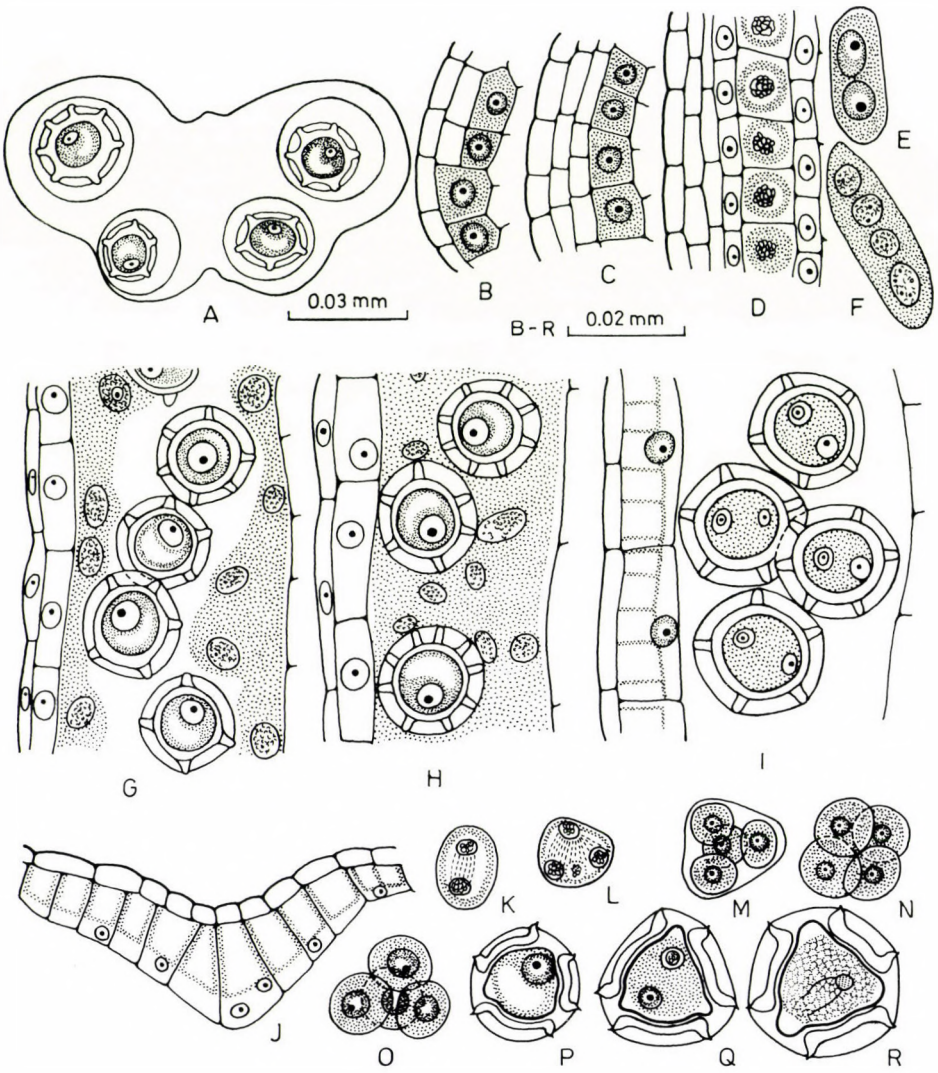


Fig. 2. Youngia japonica.

A/ T.s. of tetrasporangiate anther; B—D/ L.s. of part of anther lobe showing development of wall layers; E, F/ Anther tapetal cells; G, H/ L.s. of part of anther lobes showing periplasmodium and one-nucleate pollen grains; I/ L.s. of part of anther lobe showing endothecium and two-nucleate pollen grains; J/ Fibrous endothecium; K, L/ Pollen mother cell in meiosis; M, O/ Tetrahedral microspore tetrad; N/ Decussate microspore tetrad; P/ One-nucleate pollen grain; Q, R/ Two- and three-celled pollen grains

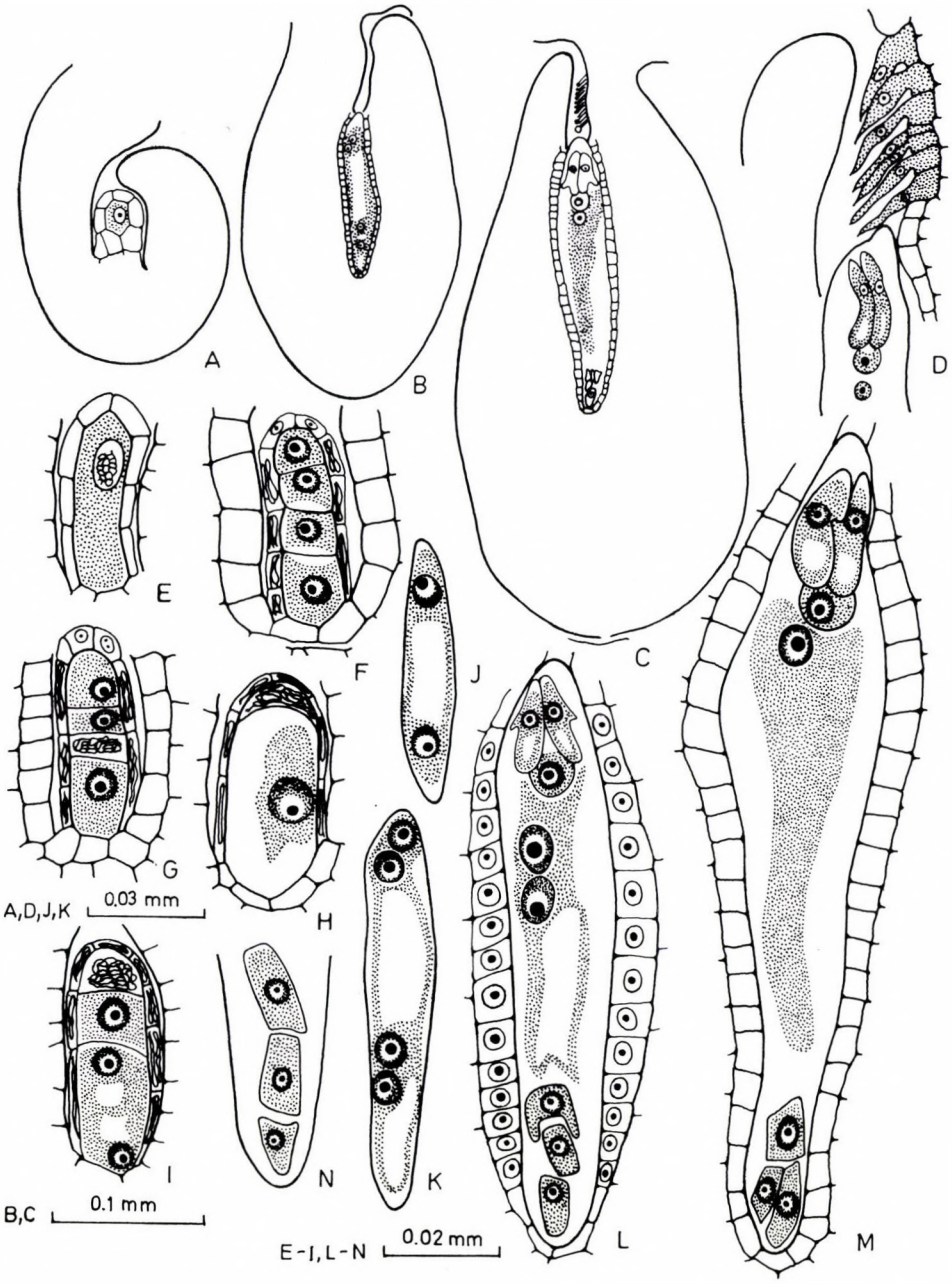


Fig. 3. Youngia japonica.

A—C/ Stages in the development of ovule; D/ L.s. of part of ovule showing obturator; E/ Megaspore mother cell; F, G, I/ Megaspore tetrads; H, J, K/ One-, two- and four-nucleate embryo sacs, respectively; L, M/ Young and mature organised embryo sacs, respectively; N/ Antipodals

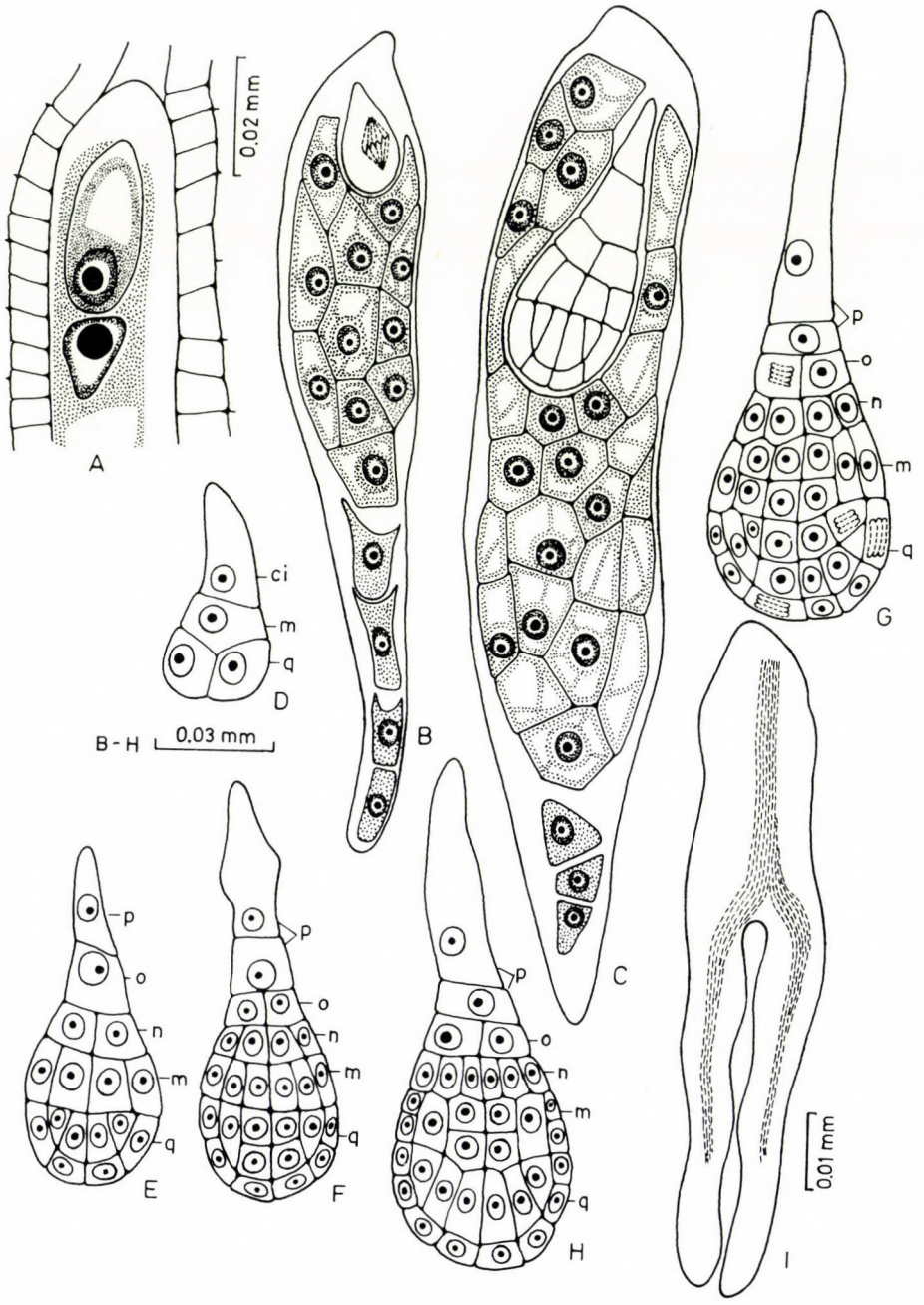


Fig. 4. Youngia japonica.

A/ Micropylar part of the embryo sac showing zygote and primary endosperm nucleus; B, C/ Stages in the development of endosperm; D—I/ Stages in the development of embryo

Megasporogenesis and female gametophyte

A hypodermal archesporial cell directly functions as the megaspore mother cell (Fig. 3E) which undergoes meiotic divisions resulting in a linear tetrad of megaspores (Fig. 3F, G). The chalazal megaspore is functional while the three micropylar megaspores degenerate (Fig. 3H). Rarely the subchalazal megaspore in addition to the chalazal megaspore is functional (Fig. 3I). The functional megaspore divides thrice mitotically to produce an 8-nucleate, 7-celled embryo sac of the Polygonum type (Fig. 3H--L). The synergids are hooked. The two polar nuclei fuse together to form a secondary nucleus (Fig. 3L, M). Antipodals are three in number and are arranged either in inverted 'T'-shaped manner or in a linear way (Fig. 3M, N). These cells increase in number up to four (Fig. 4B). Antipodals are persistent up to the globular embryo stage (Fig. 4B, C).

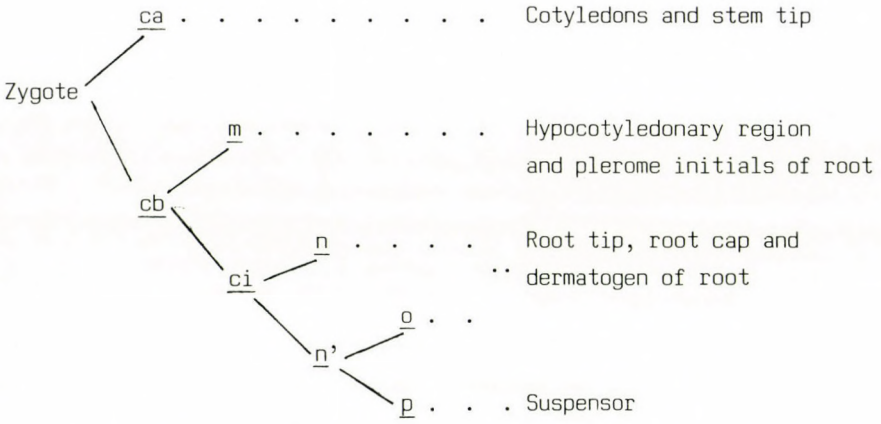
Fertilisation, endosperm and embryo

Fertilisation is porogamous. Syngamy and triple fusion occur more or less simultaneously (Fig. 4A).

The endosperm development is ab initio cellular. The primary endosperm nucleus divides much earlier than zygote and is accompanied by cytokinesis resulting in two cells. Later divisions occur in all planes forming in a massive cellular tissue (Fig. 4B, C). Endosperm is consumed by the growing embryo but for one or two layers.

Embryo development follows the Senecio variation of Asterad type. The zygote (Fig. 4A) after the resting period divides transversely giving rise to the terminal cell ca and the basal cell cb. The former undergoes vertical division forming two juxtaposed cells while the later undergoes a transverse division producing two superposed cells m and ci (Fig. 4D). Thus the four-celled 'T'-shaped proembryo is formed (Fig. 4D). The cell m also undergoes two vertical divisions at right angles to one another to produce quadrants. The cell ci divides by a transverse wall resulting in two cells n and n'. The cell n' after undergoing one more division produces two cells o and p (Fig. 4E). The cell p undergoes transverse division and produces 2-celled suspensor (Fig. 4F, G).

The destination of the individual cells of the proembryonic tetrad are given below in the schematic representation.



Thus, the embryo development follows the Asterad type of JOHANSEN (1950) and GRAND period I, Megarchetype II, series A, sub series A_2 in the first embryonic group of SOUÈGES (1939).

Discussion

The anthers are tetrasporangiate in Youngia japonica, but bi- or trisporangiate anthers are also met with. The present sutdy is in agreement with KAUL (1973) who reported that in Youngia japonica due to sterilisation of the anther lobes on the adaxial side bi- or trisporangiate are met with.

Anther tapetum in the family Asteraceae is of the Periplasmodial type which is also observed in Youngia japonica. However, in Sonchus oleraceus, S. asper (WALTER and KUTA 1971), Hypochoeris radicata and Youngia japonica (KAUL 1972, 1973), Tragopogon gracile (SINGH and KAUL 1974), Sonchus arvensis, S. asper (KAUL et al. 1975) and Prenanthes brunoniana (SOOD and THAKUR 1985) a glandular tapetum has been reported. A reinvestigation of the periplasmodium in Youngia japonica in the present study showed a periplasmodial tapetum which is short lived. As pointed by PULLAIAH (1984) Periplasmodial tapetum is a characteristic feature of the family Asteraceae and all those reports where a glandular tapetum has been reported appear to be erroneous.

In the present study it was observed that the pollen grains are 3-celled when shed. But WALTER and KUTA (1971) in Sonchus asper, KAUL (1972, 1973) in Hypochoeris radicata and Youngia japonica, SINGH and KAUL (1974) in Tragopogon gracile and KAUL et al. (1975) in Sonchus arvensis, S. asper reported that pollen grains are 2-celled at shedding stage. As majority of the species of Asteraceae (excluding the species which produce sterile

pollen grains) so far investigated show 3-celled pollen grains at the time of dehiscence (see also BREWBAKER 1967) and also because of the presence of 3-celled pollen grains in Youngia japonica it seems the observations of WALTER and KUTA (1971), KAUL (1972, 1973), SINGH and KAUL (1974) and KAUL et al. (1975) seem to be erroneus.

The presence of funicular obturator is an advanced feature and it has been reported in this tribe in Lactuca muralis (DAHLGREN 1920), Sonchus oleraceus (PULLAIAH 1976) and Youngia japonica (present study).

All the species that are investigated so far in this tribe except Taraxacum albidum (OSAWA 1913) show Polygonum type of embryo sac development (see PULLAIAH 1984) and the present species is no exception to this feature.

Both Cellular and Nuclear types of endosperm development are known to occur in this tribe, the former being more frequent that the latter. In the present study in Youngia japonica a Cellular type endosperm is met with.

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EFFECT OF NaCl SALINITY ON GROWTH AND NITRATE REDUCTION IN THE CULTIVARS OF SETARIA ITALICA (L.) P.B. DIFFERING IN SALT TOLERANCE

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The effect of NaCl salinity on growth, activities of NR and NiR and nitrate level in the leaves and roots of S. italica (L.) P.B. cultivars SIC-1 and CO-5 differing in salt tolerance has been investigated. It is found that in both the cultivars biomass (fresh weight) production is more under saline conditions except at 50 mM salinity level in the cultivar SIC-1. However, the dry weight of both the cultivars decreases due to salinity. The activity of NiR is maintained significantly higher than that of NR at all the salinity levels. This is well supported by very low amounts of nitrite-nitrogen in the tissue. NR activity decreases in the leaves while increases in roots of SIC-1 under saline conditions. In cultivar CO-5 however, NR activity increases in the young while decreases in the mature leaves and roots. On the other hand NiR activity in general increases with salinity in both the cultivars. Nitrate-nitrogen increases in the leaves of SIC-1 while in the roots of this cultivar and in the leaves and roots of cultivar CO-5 it decreases. It appears, therefore, that NR and NiR from CV CO-5 are some what stable to salinity and this may be considered as an adaptive feature of this cultivar towards salinity tolerance.

Introduction

Utilisation of saline soils is most essential because about 25% earth's surface is considered to be saline and each year more and more land is becoming saline. About 12 million hectares of land in India is affected by salinity or alkalinity (SHARMA and GUPTA 1986) and hence it is essential to develop salt tolerant crops. The tolerance of plants to salinity differs from species to species and therefore it is essential to screen the present day plants for their salt tolerance.

Millets are considered as highly nutritive, drought and temperature tolerant, hardy cereals. However, they have received scant attention as regards their improvement and physiology of salt tolerance, Setaria is the most neglected genus among the millets. In the present investigation, therefore, an attempt has been made to study the influence of salinity on growth and nitrate reduction in the two cultivars of Setaria italica (L.), differ-

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ing in salt tolerance to throw some light on the mechanism of salt tolerance in plants.

Material and Methods

Improved cultivars of S. italica (L.), SIC-1 and CO-5 were raised in soil from their seeds. After stabilizing the plants under natural conditions for one month, salt treatments were commenced. The plants were treated with increasing levels of NaCl (50, 100 and 200 mM) twice a week, alternating with watering the plants with equal amount of water to avoid salt accumulation and to check the loss of water due to evapotranspiration. Control plants received only water. After about one month 20 plants, from each treatment were carefully uprooted and analysed for biomass (fresh and dry weights) and moisture content. In order to determine activity of NR and NiR and to estimate NO_3-N and NO_2-N the material (fresh leaves and roots) was homogenized in extraction medium containing 3.3 mM dithiothreitol and 0.1 mM Na_2 EDTA in 3.3 mM Tris-HCl buffer, pH 7.2. The extract was filtered through double layered muslin cloth and the filtrate was centrifuged at 6000 x g for 40 min. The supernatant was stored at 0-4 ^{o}C and used as an enzyme source. Activity of NR (in vitro) was determined following the method of KAUFMAN et al. (1971). For determining the activity of NiR, 0.5 ml enzyme was incubated with 0.3 mM KNO_2, 0.2 M phosphate buffer pH 7 and 1.36 mM NaDH. The enzyme assay was done in light. The enzymatic reaction was terminated by adding sulfanilamide (1% in 1 M HCl) and NEEDA (0.02%) after 30 and 60 min in the leaves and roots, respectively. Standard curve of KNO_2 was prepared. Absorbance was read at 540 nm on double beam spectrophotometer (Shimadza, Japan). For estimation of NO_3-N: 0.5 ml enzyme preparation was mixed with 1.5 ml distilled water and 1.8 ml diphenylamine — H_2SO_4 reagent. After vigorous shaking the colour was allowed to develop for 10 min. Standard curve of KNO_3 was prepared and absorbance was recorded at 590 nm. For estimating NO_2-N, 0.5 ml of the same enzyme-preparation was mixed with 1.5 ml distilled water, 1 ml sulfanilamide and 1 ml NEEDA. After 15 min the optical density was read at 540 nm. Enzyme proteins were estimated following the method of LOWRY et al. (1951).

Results and Discussion

A/ Growth: Effect of salinity on fresh weight, dry weight and moisture content of S. italica cultivars SIC-1 and CO-5 (72 days growth with 32 days salt treatment) is shown in Table 1. It is evident that fresh weight in both the cultivars increases with increase in salinity level except a slight decrease at 50 mM NaCl in SIC-1. Dry weight in both the cultivars however, decreases with salinity. In CV SIC-1 the dry weight increases suddenly at 200 mM NaCl. The moisture % increases with salinity in both the cultivars.

Increase in fresh weight of Suaeda maritima grown in highly saline medium (340 mM NaCl) is attributed by YEO and FLOWERS (1981) to increased cell size. Similarly KARADGE and CHAVAN (1982) observed that salt stress increases biomass production in Sesbania aculeata and S. grandiflora, however a considerable decrease was observed only at the highest salt level (ECe 15 m S cm^{-1}). Inrease in dry weight under saline conditions is observed by AHMED et al. (1979) in oil producing crops and by OKUSANYA (1980) in

Table 1

Effect of NaCl salinity on fresh weight, dry weight and moisture content in the S. italica cultivars (SIC-1, CO-5) differing in salt tolerance

NaCl Treatment mM	SIC-1			CO-5		
	Fresh weight g plant^{-1}	Dry weight g plant^{-1}	Moisture %	Fresh weight g plant^{-1}	Dry weight g plant^{-1}	Moisture %
0 (Control)	2.15	0.7740	63.999	2.65	1.5324	42.175
50	1.72	0.5955	65.38	3.00	1.1091	63.03
100	2.60	0.7312	71.88	3.75	1.4674	60.869
200	3.30	1.1177	66.130	4.10	1.3003	68.285

Lavatera arborea. According to KINGSBURY and EPSTEIN (1986) the salt composition of external solution has little effect on the growth of salt resistant wheat line and superior compartmentation of Na^+ may be responsible for this. GAIKWAD et al. (1985) observed that salinity does not affect the growth in salt tolerant cultivars of Eleusine and Setaria.

The decrease in dry weight of S. italica cultivars, accompanied by an increase in moisture content and fresh weight may be due to accumulation of Na^+, Cl^- and water with increasing salinity. This is considered as an adaptive feature, a succulent character especially related to halophytes. Sudden increase in dry weight of CV SIC-1 plants at 200 mM NaCl however, cannot be explained. It appears that both the Setaria cultivars are salt tolerant and CV CO-5 appears to be more tolerant than CV SIC-1.

B/ Nitrate Reduction Enzymes:

From Table 2, it is clear that NRA (nitrate reductase activity) in the young leaves of SIC-1 is stimulated at 50 mM NaCl while inhibited by high salt concentrations (100 and 200 mM). In mature leaves it decreases at all the salt levels while in roots it increases. On the other hand, in CV CO-5, the NRA increases with salinity in young leaves only. Mature leaves and roots however, show a considerable decrease in it except a slight increase at 200 mM NaCl.

NiRA (NiR activity) is several fold higher than that of NR. NiRA increases both in the leaves and roots of SIC-1 except at 100 mM NaCl where it decreases slightly in the roots. In CV CO-5 it appears to be little bit stable to salinity in young leaves and roots, however, decreases in mature leaves and increases to some extent at 100 mM salt.

Table

Effect of NaCl salinity on the activity of nitrate reductase and nitrite reductase in the

NaCl Treatment mM	SIC-1											
	Young leaves				Mature leaves				Roots			
	NR	NiR	NO_3-N	NO_2-N	NR	NiR	NO_3-N	NO_2-N	NR	NiR	NO_3-N	NO_2-N
0 (Control)	7.265	112.6	101.4	0.509	8.519	106.2	90.8	0.0464	1.246	124.2	177.1	0.0378
50	8.529	136.3	107.1	0.505	6.189	120.0	97.0	0.0457	1.657	159.1	102.1	0.0404
100	6.920	123.7	115.2	0.0452	6.209	133.0	119.0	0.0406	1.279	116.0	137.7	0.0292
200	4.601	110.4	110.4	0.0360	7.426	114.9	120.2	0.0479	1.534	137.0	140.1	0.0358

NR = Activity is expressed as n moles of NO_2 liberated h^{-1} mg^{-1} protein.
NiR = Activity is expressed as n moles of NO_2 consumed h^{-1} mg^{-1} protein.
NO_3-N = is expressed as μg mg^{-1} protein.
NO_2-N = is expressed as μg mg^{-1} protein.
Each value is mean of three determinations.

BILLARD and BOUCAUD (1982) have suggested a noncompetitive inhibition of NR in Suaeda macrocarpa by salt. KABISHEVA et al. (1981) however, are of the opinion that dissociation of molybdofactor from enzyme apoprotein is responsible for decrease in NRA under saline conditions. SAFARALLIEV et al. (1981) feel that the decrease in NRA under salt stress may be due to dissociation of FAD and Mo.

NR is considered as a substrate inducible enzyme and its activity depends upon the concentration and rate of NO_3^- supply to the tissue. Substrate unavailability may also be responsible for inhibition of NR. In the present study the RNA decreases in the leaves of SIC-1 accompanied by a decrease in NO_2-N. NO_3-N level, however increases, indicating that substrate unavailability is not responsible for the observed inhibition of NRA. It appears, therefore, that the inhibition is probably due to partial dissociation of the enzyme protein or inhibition of its synthesis. An increase in NRA in the roots may be due to some disturbance in the translocation of NO_3^-.

In CV CO-5 NRA is stimulated in young leaves. In mature leaves, however, it decreases with decrease in NO_3-N. It appears that most of the NO_3^- absorbed is translocated and utilized in the young leaves. At higher salt concentration, however, NRA is recovered with increase in NO_3-N. The root NR appears to be stable to salinity.

2

leaves and roots of S. italica cultivars (SIC-1 and CO-5) differing in salt tolerance

CO-5											
Young leaves				Mature leaves				Roots			
NR	NiR	NO_3-N	NO_2-N	NR	NiR	NO_3-N	NO_2-N	NR	NiR	NO_3-N	NO_2-N
4.652	126.8	112.0	0.0321	7.765	129.9	100.1	0.0386	1.391	139.6	176.9	0.0349
5.717	125.5	99.8	0.0351	4.276	72.5	63.9	0.0292	1.190	146.9	122.8	0.0276
5.524	121.9	90.9	0.0374	6.445	149.3	115.9	0.0510	1.158	147.1	142.0	0.0291
5.878	109.2	100.5	0.0438	8.449	113.5	105.7	0.0511	1.472	124.1	182.3	0.0412

Inhibition of NiR due to salt stress is reported by HEUER and PLAUT (1979), RAJMANE (1984) and MURUMKAR (1986). Unavailability of substrate, disturbance in enzyme protein and decrease in supply of reducing power are some of the probable reasons for the inhibition. Chloroplastic location of NiR suggests that any change in photosynthetic activity is directly related to NiRa or vice a versa. Increase in NiRA in SIC-1 is associated with a decrease in NO_2-N which is well co-related with increased NRA in roots. In CV CO-5 in young leaves NRA increases but that of NiRA decreases slightly resulting in accumulation of NO_2^-. Decrease in reducing power may be responsible for this observed decrease. It appears that salinity affects the photosynthetic apparatus. Mature leaves, however, do not show any definite pattern. From the observations it appears that fluctuations are not related to substrate level but probably related to decreased supply of ferridoxin or disturbed enzyme protein. Root NiR appears to be stable to salt except a slight decrease at the highest salinity level.

Thus, in general it can be said that NO_3^- reduction process in S. italica cultivars is not much affected by salt. CV CO-5 appears to be more superior in this aspect. This clearly indicates the difference in varietal response given by these cultivars to salinity. NiR from both cultivars appears to be rather insensitive to salt. These may be considered as adaptive features of the cultivars when grown under saline conditions.

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COMPARISON OF PHOTOSYNTHETIC PERFORMANCES IN TWO GENOTYPES OF MAIZE DEEPOXIDATING VIOLAXANTHIN QUICKLY AS WELL AS SLOWLY

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We studied the differences in the performance (the dry matter production being connection with CO_2 assimilation, leaf area, ratio of photosynthetizing tissues, malate, sucrose, starch contents of the leaf) between the XQ and the XS genotypes (deepoxidating violaxanthin quickly (XQ) as well as slowly (XS) during the induction period of photosynthesis) of the C4 type maize. We observed the differences of the acclimation of the plants were grown at 3 light regimes: medium light (ML): 200 $\mu mol^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD) and 16 h — 8 h light dark period (LDP); low light (LL): 100 $\mu mol^{-2} \cdot s^{-1}$ PPFD and 16 h — 8 h LDP; short light (SL): 200 $\mu mol^{-2} \cdot s^{-1}$ PPFD and 30 min — 15 min LDP.

Dry matter reducing effects of the LL and SL treatments seemed to be connection with the low CO_2 assimilation rate measured at the PPFD where the plants were grown and with the low quantum yield. Comparing the XQ and XS genotypes of maize, we found that the malate, sucrose levels are lower and the starch level is higher in the leaves of the XQ than those of the XS genotypes.

Only in the XQ genotype comparing to the XS the decrease of ratio of sucrose is in positive correlation with the decrease of ratio of root dry mass and the increase of ratio of starch is in positive correlation with the increase of ratio of shoot. The quantum yield may be in positive correlation with chlorophyll a+b content and negative correlation with light compensation point.

Keywords: photosynthetic performance, violaxanthin cycle, CO_2 assimilation rate, malate, sucrose, starch, dry matter, maize genotypes

Introduction

The differences in the performances of the genotypes may be in close connection with photosynthetic pathways. For instance such as differences are shown by the variation found between the photosynthetic pathways of the C3 and C4 species of Panicum genus (DOWNTON 1975) and between the genotypes of Zea mays deepoxidating violaxanthin quickly (XQ) as well as slowly (XS) during the induction period of photosynthesis (MARÓTI 1986). Our aim was to study the differences in the performace (the dry matter production being connection with CO_2 assimilation, leaf area, ratio of photosynthetizing

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tissues, malate, sucrose, starch contents of the leaf) between the XQ and the XS genotypes of the C4 type maize. We observed the differences in the acclimation of the plants were grown in medium and low photosynthetic photon flux densities and short light dark period.

The carboxylating step is well characterized by the slope of the inital part of the curve of CO_2 assimilation rate versus incident photosynthetic photon flux density. This inital slope of the curve is the incident quantum yield efficiency that is lower in the C3 plants (0.052 mol $CO_2 \cdot mol^{-1}$ photon) than in the C4-NADP-ME plants (0.065 mol $CO_2 \cdot mol^{-1}$ photon) (EHLERINGER and PEARCY 1983). Since the optimal electrontransport needs equilibrium between production and consumption of the reduction power (NADPH) in the chloroplast therefore the export of the reduction power in the form of malate plays and important role in the acclimation to the changing conditions (SCHEIBE et al. 1986). The photosynthetic malate is precursor of the tricarboxylic acid cycle because the amino acids of the tricarboxylic acid cycle are synthetized from the photosynthetic CO_2 fixation (KENT 1979). Malate, exported from the bundle sheath chloroplast, carries reduction power to the peroxisoma, cytoplasm (TOLBART 1979), and mesophyll chloroplast (SLACK 1969). The increase of the ambient CO_2 concentration rises the malate level and reduces the nitrate level since the malate oxidation is the main NADH source for the nitrate reduction (NEYRA and HAGEMAN 1976; MARIGO et al. 1985). Certain plants can store malate in inverse quantity as nitrate in their vacuoli (GERHARDT and HELDT 1984; MARIGO et al. 1985).

The rate of the sucrose synthesis is in tight correlation with the rate of CO_2 assimilation through the fructose-2,6-bisphosphate regulation system (STITT et al. 1987). The vacuoli of the mesophyll cells are temporary pools of sucrose in the light if sucrose productivity surpasses the uptake capacity of phloem (KAISER and HEBER 1984). The excess sucrose synthesis gives a signal for the fructose-2,6-bisphosphate regulating system to start the starch synthesis (PREISS 1986).

The starch content of the leaves is in inverse ratio to the daily photosynthetic period (CHATTERON and SILVIUS 1979).

Genotype pairs were found in some plant species (maize, bean, sunflower) on the basis of leaf anatomy, chloroplast ultrastructure and physiological light acclimation. The rate of the deepoxidation of violaxanthin (i.e. the rate of developing of protongradient in thylakoids) quicker, the rate of quenching of chlorophyll-a fluorescence in the M-T period (i.e. start of the non-cyclic electrontransport) and the rate of oxygen evolution

is slower in the XQ genotypes than in the XS genotypes (PATAKY and MARÓTI 1985; WALKER 1985; MARÓTI 1986) during the induction period of photosynthesis. The XQ genotypes have lower dry matter, leaf thickness and growth rate, higher water content than the XS genotypes have (MARÓTI and MARGÓCZI 1984; MARGÓCZI and MARÓTI 1985). The quantity of appressed membrane in the chloroplasts of the XQ genotypes is greater, the number of grana, the sizes of the loculi are less than in the XS genotypes (PATAKY and MARÓTI 1985; MARÓTI 1986).

Material and Methods

The comparison of the photosynthetic performance was carried out on the XQ 'F2' line) and the XS ('P165' line) genotypes of maize (Zea mays L. 'Pioneer'). The maize plants were grown for 40 days in 600 cm^3 plastic pots in the mixture of sand-perlit (1:1) in phytotron where the CO_2 content in the air 330 $\mu mol \cdot mol^{-1}$, the saturation deficit of water vapour in the air 8.4 $mmol \cdot mol^{-1}$, the temperature 23 ^oC were. The 3 light treatments were: medium light (ML): 200 $\mu mol^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD) and 16 h — 8 h light dark period (LDP): low light (LL): 100 $\mu mol^{-2} \cdot s^{-1}$ PPFD and 16 h — 8 h LDP; short light dark period (SL): 200 $\mu mol^{-2} \cdot s^{-1}$ PPFD and 30 min — 15 min LDP. The light sources were fluorescent tubes (Tungsram F33 types).

The ML, the LL plants and SL plants were kept in darkness for 8 hours as well as 30 minutes then their totally developed 5. leaves were cut. The isolated leaves were illuminated with 800 $\mu mol^{-2} \cdot s^{-1}$ PPFD light in humid, 26 ^oC, 340 $\mu mol \cdot mol^{-1}$ CO_2 concentration air for 30 minutes, then they were fixed in liquid air and lyophilized.

The malate (HOHORST 1970), the sucrose and the starch contents (HANDEL 1968; DUBOIS et al. 1956) were determined. We measured the change of the rate of CO_2 assimilation with infrared gas analyser (VEB Junkalor: Infralyt 4). The calculations of the CO_2 assimilation rate, (incident quantum yield, light compensation point) were carried out after LONG and HALLGREEN (1985), JANAC et al. (1971), CAEMMERER and FARQUHAR (1981).

From the leaves we took samples for lyophilization, we made chlorophyll analysis (FRENCH 1960) and determined the ratio of the photosynthetic tissues after fixing with glutaraldehyde (KARNOVSKY 1965), imbedding in paraffin, making 15 μm thick cross sections.

Results

Dry matter production and its distribution (Figs 1, 2)

Dry mass of the total plant, the roots, the stem and the leaves decrease more intensively in the LL plants than in the SL comparint to the ML plants. The dry mass ratio of the leaves greater and that of the roots less at the LL and SL treatments than at the ML treatment.

Dry masses of the total plant, the roots, the leaves in the two genotypes do not differ but the mass of the stem and its mass ratio in the XQ genotypes greater than in the XS genotypes.

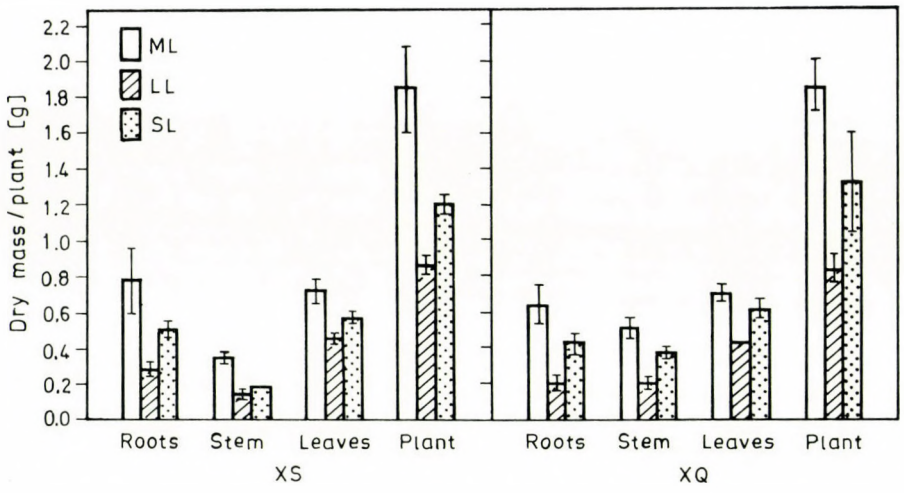

Fig. 1. Dry mass of the organs and the whole plant of the XS as well as the XQ maize genotypes grown at the ML, LL and SL light regimes

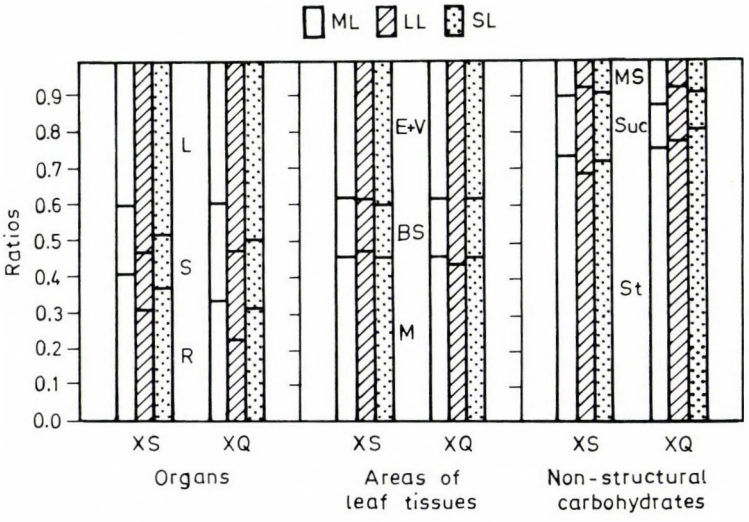

Fig. 2. Dry mass ratios of organs (L: leaves, S: stem, R: roots), ratios of tissue areas in leaf cross sections (E + V: epydermis and vascular tissues, BS: bundle sheath tissue, M: mesophyll tissue) and the ratios of the non-structural carbohydrates (MS: monosaccharides, Suc: sucrose, St: starch) in the XS as well as the XQ genotypes of maize grown at the ML, LL and SL light regimes

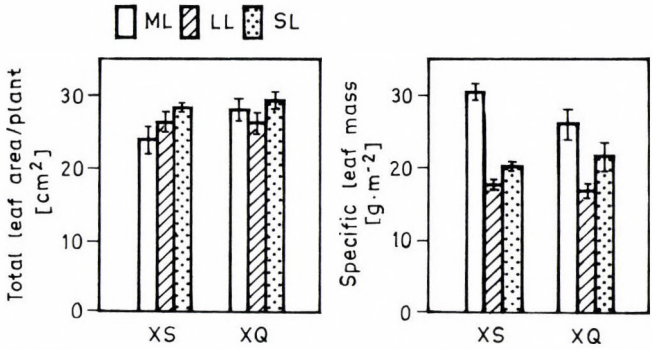

Fig. 3. Leaf area and specific leaf dry mass of the XS as well as the XQ genotypes of maize grown at the ML, LL and SL light regimes

Leaf area (Fig. 3)

The LL and SL treatments do not influence the total leaf area in maize genotypes. The leaf area of the XQ genotype is greater than that of the XS genotype grown at the ML. The leaf areas of the two genotypes are not different at either light treatment.

Specific leaf mass (Fig. 3)

The specific leaf mass of the LL and SL plants is lower than that of ML plants.

The specific leaf mass is lower in the XQ plants than in the XS plant only at the ML treatment. There are not any other genotypical differences.

Ratio of tissues in leaf cross-section (Fig. 2, Plate I)

There are not changes in the ratios of epidermis, mesophyll ground tissue and vascular tissue areas observed in cross section on the effects of the light treatments.

The ratios of bundle sheath tissue in the leaves of the XQ genotypes are greater than that of the XS genotypes grown at the LL and SL.

The rate of CO_2 assimilation after 60 minutes of illumination at the photosynthetic photon flux under the plants were grown, incident quantum yield and light compensation point (Fig. 4).

Significant differences cannot be observed in the CO_2 assimilation rate among the 3 light treatments and the 2 genotypes.

The quantum yield rises in the LL plants in both genotypes, but slightly declines in the leaves of the XS or does not change in the leaves

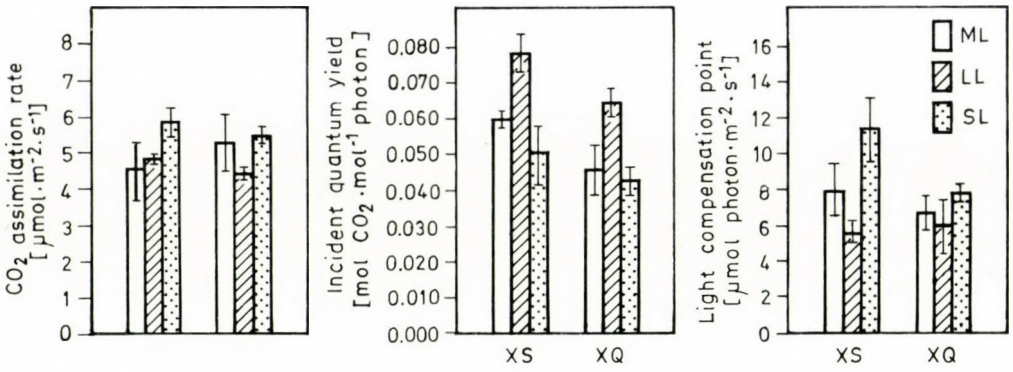

Fig. 4. CO_2 assimilation rate, incident quantum yield and light compensation point of the 5th totally developed leaves of the XS as well as the XQ maize genotypes grown at the ML, LL and SL light regimes

of the XQ genotypes grown at the SL comparing to the ML. Generally the quantum yield is lower in the XQ than in the XS genotypes in each light treatment except for the SL.

The light compensation point decreases in the XS or does not changes in the XQ genotypes grown at the LL but increases in each genotype grown at the SL comparing to the ML. Genotypical differences can be observed only in the plants grown at the SL where the light compensation point of the XQ is significantly lower than of the XS genotypes.

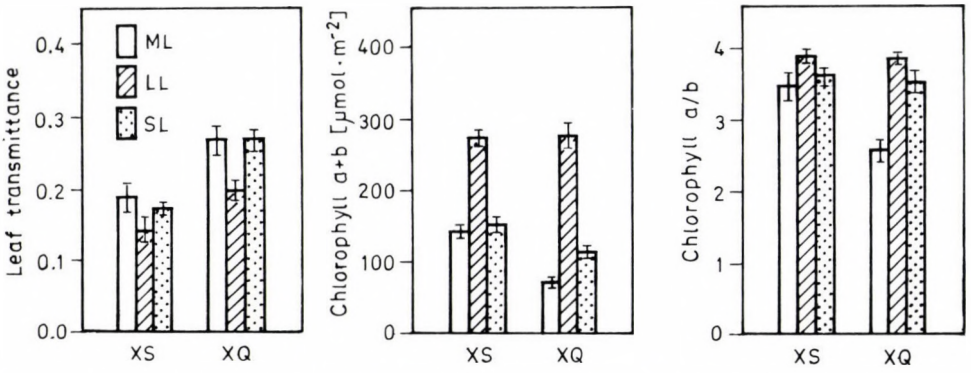

Fig. 5. Leaf transmittance, chlorophyll a + b content and chlorophyll a/b ratio in the 5th totally developed leaves of the XS as well as the XQ maize genotypes grown at the ML, LL and SL light regimes

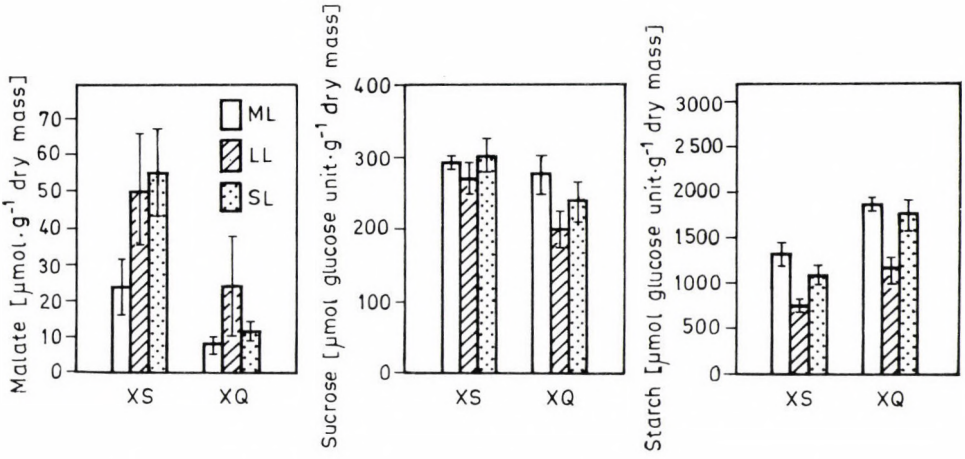

Fig. 6. Malate, sucrose and starch levels in the 5th totally developed leaves of the XQ as well as the XS genotypes of maize grown at the ML, LL and SL light regimes

Transmittances of leaves (Fig. 5)

Transmittances of the leaves measuring at 200 $\mu mol^{-2} \cdot s^{-1}$ PPFD are lower at the LL and similar in the SL plants than the ML plants. Generally the transmittances of leaves of the XS genotypes are less than that of the XQ genotypes.

Quantity of chlorophylls (Fig. 5)

The chlorophyll a/b ratio and the chlorophyll a + b content are significantly higher on the effect of the LL and slightly higher on the effect of the SL treatments than at the ML treatment. The chlorophyll a/b ratio and the chlorophyll a + b content of the XQ genotypes are lower than those of the XS genotypes with the exception of the LL plants.

Malate, sucrose and starch contents of leaves in the 30th minute of illumination (Figs 2, 6)

Malate content rises in the LL and SL plants comparing to the ML plants. In each treatment malate content is significantly lower in the XQ than in the XS genotypes.

Sucrose level is lower in the XQ genotypes grown at the LL and SL than in the XQ genotypes grown at the ML. There are not differences among light treatments in the XS genotypes. The ratio of sucrose within nonstructural

carbohydrate rises in the LL and unchangeable in the SL plants comparing to the ML plants. Sucrose ratio is lower in the XQ than in the XS genotypes in each treatment.

Starch level of the leaves decreases in the LL and unchangeable in the SL plants comparing to the ML. Level and ratio of starch are higher in the XQ than in the XS genotypes grown at each light treatment.

Discussion

The dry matter reducing effect of the LL and SL treatments may be the similar consequence of the differing changes in the membrane system of the chloroplasts (cf. LICHTENTHALER et al. 1981; MARÓTI and TAKÁCS 1983; WARD and WOOLHOUSE 1986).

The leaf area of the LL and SL plants are not significantly higher than that of the ML plants. The possible reason of this finding that the applied photon fluxes are far from the optimum values of the typical C4 light plant: maize (WARD and WOOLHOUSE 1986) for which the two applied PPFDs are shade conditions. This contributes to the unchangeable ratio of bundle sheath in the cross-section area of the leaves (Fig. 2).

Presumably there are not any correlations between CO_2 assimilation rate measured at the photon flux under the plants were grown and the dry matter production since the CO_2 may exist in the pools of several energy levels (e.g. hydrogencarbonate, oxalacetate, malate, aspartate, sucrose, starch (HEICHEL 1969). The low energy pools (e.g. malate) might support the acclimation to the LL and SL (MARÓTI 1986). The sizes and numbers of the low energy pools may be in positive correlation with the CO_2 assimilation rate and negative coorelation with the dry matter production. The greater quantum yield in the LL plants, where the dry matter is lower, showes that CO_2 fixation is more efficient here but the fixed carbon remains in the low energy compounds. The lower quantum yield in the SL plants indicates the weakly developed structure of the chloroplasts here. The quantum yield may be in positive correlation with chlorophyll a + b content and negative correlation with the light compensation point. The high chlorophyll a/b ratio supports the former conclusions, which showed weakly developed PS2 in the SL plants (MARÓTI 1982). But the increase of the chlorophyll a/b ratio in maize leaves grown at LL is very surprising (Fig. 5), because it is in contrast with the general observations (LICHTENTHALER 1981). In the leaves grown at the LL the rise of malate level, which is in contrast with findings

on the C3 type beech plant (Fagus silvatica) (LICHTENTHALER 1981), shows that low energy compounds accumulate for CO_2 transport (e.g. malate), and the quantitites of high energy compounds (e.g. starch) are reduced. Only in the XQ comparing to the XS genotype the decrease of sucrose ratio is in positive correlation with the decrease of roots ratio and the increase of starch ratio is in positive correlation with the increase of shoot ratio. These observations conforms the former results (MARGÓCZI 1985). But in plants grown at the LL and SL light regimes these correlation cannot be observed (Fig. 2). Comparing the XQ and XS genotypes of maize with those of bean (TÉCSI 1987) we found that the malate, sucrose levels are lower and the starch level is higher in the leaves of the XQ than those of the XS genotypes.

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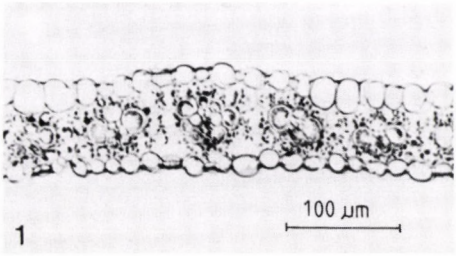
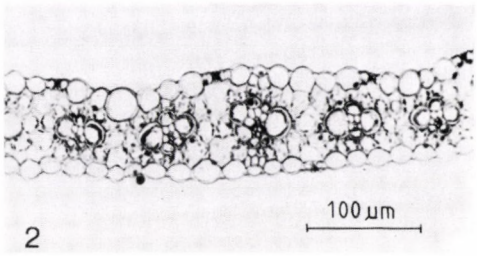
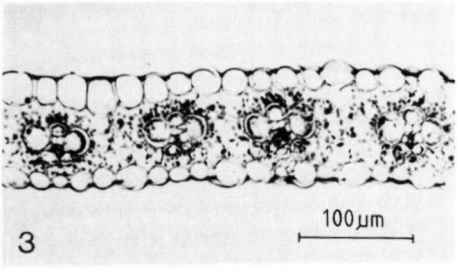
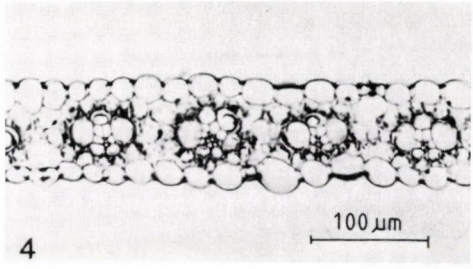
Plate I

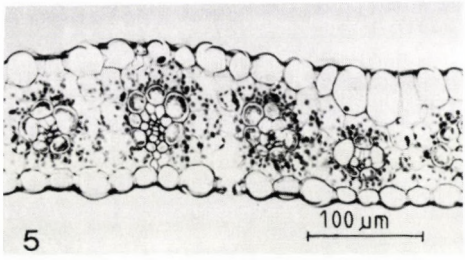
Cross sections from the 5th totally developed leaves of the XS and the XQ genotypes of maize grown at the ML, LL and SL light regimes











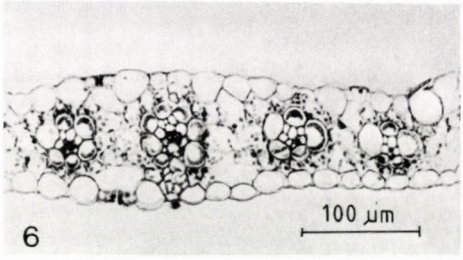


Fig. 1. XS genotype grown at ML

Fig. 2. XQ genotype grown at ML

Fig. 3. XS genotype grown at LL

Fig. 4. XQ genotype grown at LL

Fig. 5. XS genotype grown at SL

Fig. 6. XQ genotype grown at SL

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THE TYPICAL VEGETATION OF A PODZOLIZED SAND DUNE IN NORTHERN GERMANY AND ITS POSSIBLE RELATIONSHIP TO THE MINERAL NUTRITION OF PLANTS

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The vegetation of a typical podzolized sand dune in north-west Germany were presented. The macroelements (C, H, O, N, S, K, Ca and Mg) were quantitatively determined by different analytical techniques in leaves of Vaccinium vitis-idaea and needles of Pinus sylvestris growing on this soil. Both plants show high concentration factors of the investigated elements compared with the concentrations of elements found in the mineral soil. This seems to be a special adaptation of the plant species to the poor mineral soil on which they live.

Keywords: macro nutrients, podzol soil, Pinus sylvestris, Vaccinium vitis-idaea, multi-elemental analysis, adaptation, concentration factor

Introduction

In different vegetation zones, occurrence and growth of plants is mostly regulated by climatic conditions (LIETH and WHITTAKER 1975). Within the vegetation zones, the most important influence on the occurrence of plants seems to be the edaphic conditions. Besides the physical properties of soils such as water permeability, pore volumes, etc., the mineral nutrition of plants seems to play an important role in the distribution of different plants in various ecosystems (MARKERT 1987; MARKERT and JAYASEKERA 1987). In concurrence with other plant species the plants which have developed the best methods of existence will be promoted to settle in a specific area. These methods include, e.g. the developed of water storage tissues or adaptations of the metabolism (C_3-C_4-plants). Little is known about the direct role of minerals in the distribution of plants. We have only studied relatively extreme habitats. For example, we know that on soils which have naturally high heavy metal contents or which are polluted by man, only some plants are able to exist, because they have developed special resistance mechanisms against high heavy metal concentrations (WICKLAND 1983). In our

investigation we will give a short overview of a typical vegetation type on a podzol soil, found in different parts of Europe and make an attempt to discuss a possible relationship between macronutrients and existing plants.

Methods

The investigation area was located in Achmer, about 15 km to the north of Osnabrück, F.R.G. Samples were collected as carefully as possible to avoid contamination from the environment. Soil samples were taken randomly from the upper 60 cm of the soil profile, excluding the litter and any roots of the upper horizon visibly distinct. 60 cm represents the approximate rooting depth in the mineral soil. From the dominating plants Pinus sylvestris and Vaccinium vitis-idaea (red whortleberry) were selected for chemical analysis. Only the leaves of Vaccinium vitis-idaea and the needles of Pinus sylvestris were used for chemical analysis, since these are the most physiologically active parts of plants. After sampling the plants and soils were dried for 48 h at 105 °C and then homogenized using agate ball mills. After milling the samples were stored in plastic bags and aliquots were sent to the cooperating analysts, which are:

Dr. DE BRUIN, Delft, (NL) NAA* (Ca, K, Mg), Dr. EHMANN, Lexington, (US) NAA* (O), Dr. HOFFMANN, Munich, FRG, AES-ICP** (Ca, K, Mg, P, S), Dr. KRONER, Munich (FRG), EA (C, H, N), Dr. MARKERT, Osnabrück (FRG), AAS*** (Ca, K, Mg), Dr. SCHRAMEL, Munich (FRG), AES-ICP (Ca, K, Mg, P, S).

For AAS and AES-ICP analysis the samples were soluted by HNO_3, for INAA the samples were analysed without decomposition. The quality control of analytical date was checked by interlaboratory comparison of reference materials.

Results and Discussion

On the pleistocence quartz sand soils with less nutrients and a podzolic character, the oak-birch forest (Querceto-Betuletum roboris) is the characteristic vegetation type. The covering undergrowth of this forest is marked by azidophytic species (BURRICHTER 1983). This type of forest can be found in sandy uplands of the Netherlands, North Belgium and North West Germany. There are three subassociations (TÜXEN 1970; BURRICHTER 1986):

-- Querco-Betuletum typicum
-- Querco-Betuletum molinietosum
-- Querco-Betuletum alnetosum

*NAA: neutron activation analysis

**AES-ICP: Atomic emission spectrophotometry with inductively coupled plasma

***AAS: Atomic absorption spectrophotometry

Table 1

Querco-Betuletum typicum

No.	1	2	3	4	5	6
surface (m^2)	100	100	100	100	100	100
cover of trees (%)	75	70	70	60	60	60
cover of shrubs (%)	5	5	5	5	5	5
cover of herbs (%)	80	90	90	80	90	90
cover of mosses (%)	70	80	70	70	70	70
Number of species	9	10	12	13	8	8
AC: Querco-Betuletum typicum						
Polypodium vulgare	-	2	+	+	-	-
Populus tremula	-	-	+	1	-	-
VC: Quercion robori-petraeae						
Betula pendula	1	+	1	1	3	2
Melampyrum pratensis	-	+	-	+	-	-
Lonicera periclymenum	-	-	-	-	1	-
D1: Vaccinium vitis-idea	4	4	4	-	-	-
D2: Agrostis stricta	-	-	-	1	2	1
attendants:						
tree-layer:						
Quercus robur	4	3	3	3	3	3
shrub-layer:						
Frangula alnus	1	+	+	1	-	+
herb-layer:						
Avenella flexuosa	4	4	4	5	5	5
Calluna vulgaris	-	+	+	+	-	+
Rubus fruticosus agg.	+	-	+	+	-	-
Rubus idaeus	1	-	-	-	1	+
Pinus sylvestris (juvenil)	+	-	+	-	-	-
Epilobium angustifolium	-	-	-	+	-	-
Sorbus aucuparia (juvenil)	-	-	-	-	+	-
moss-layer:						
Pleurozium schreberi	3	-	1	3	3	3
Hypnum jutlandicum	-	3	2	1	-	-
Pohlia nutans	-	2	2	-	-	-

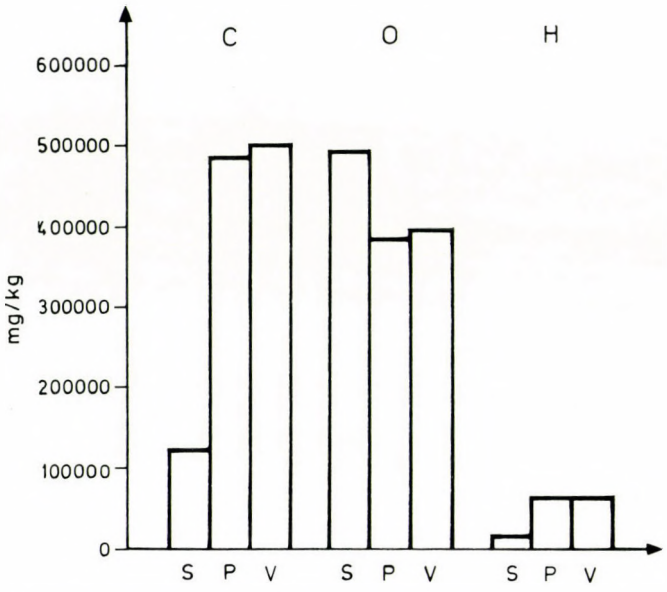

Fig. 1. C-, O- and H-concentrations of Vaccinium vitis-idaea (V) and Pinus sylvestris (P) and the corresponding podzolized soil (S) they grown. Analytical variances < 10%

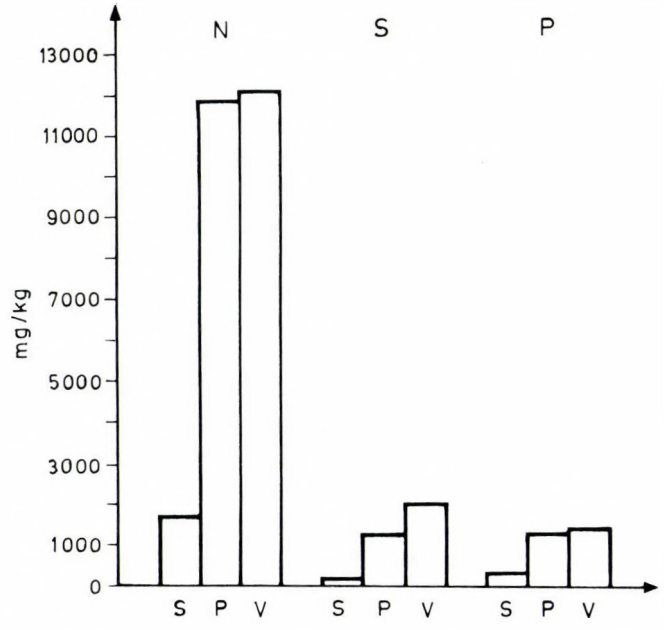

Fig. 2. N-, S- and P-concentrations of Vaccinium vitis-idaea (V) and Pinus sylvestris (P) and the corresponding podzolized soil (S) they grown. Analytical variances < 10%

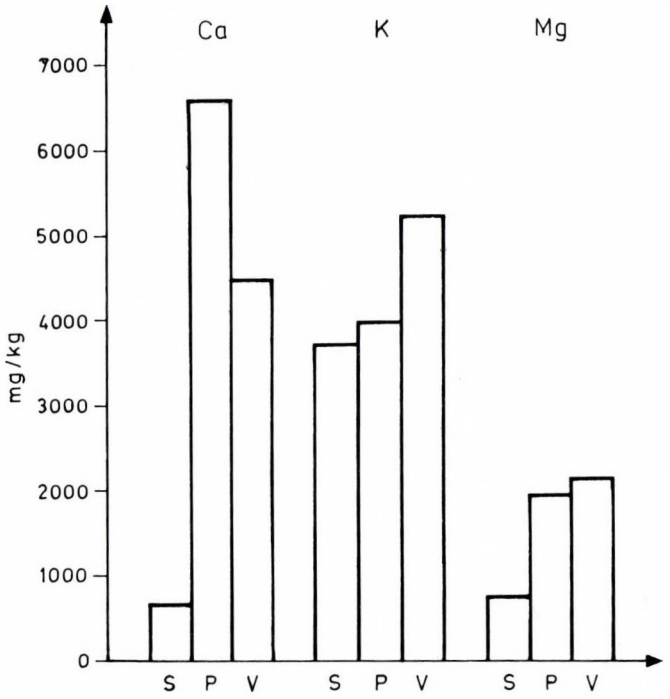

Fig. 3. Ca-, K- and Mg-concentrations of Vaccinium vitis-idaea (V) and Pinus sylvestris (P) and the corresponding podzolized soil (S) they grown. Analytical variances < 10%

In our study area we found only the typical association on dry sand-soils. As shown in Table 1, Quercus robur is dominating which represents a climax stage. Avenella flexuosa is the dominating plant in the undergrowth (WITTIG 1980). As characteristic species we have Polypodium vulgare and Lonicera periclymenum (BURRICHTER 1973) and as characteristic species of the alliance: Betula pendula, Melampyrum pratensis and Lonicera periclymenum.

This typical association is a mosaic of two different species: Vaccinium vitis-idaea (D1) and Agrostis stricta (D2). Agrostis stricta can be found only in lighter areas of the oak-birch forest. Vaccinium vitis-idaea is characteristic of slopes and inclines. Because of their high tolerance to cold this chaemaphyte can grow on these snowfree parts of the forest during winter. These parts are also exposed to wind. Avenella flexuosa will be less dominant in this part.

The results of the mineral analysis were presented in Figs 1--3. We can divide the macroelements in three groups according the kind of avail-

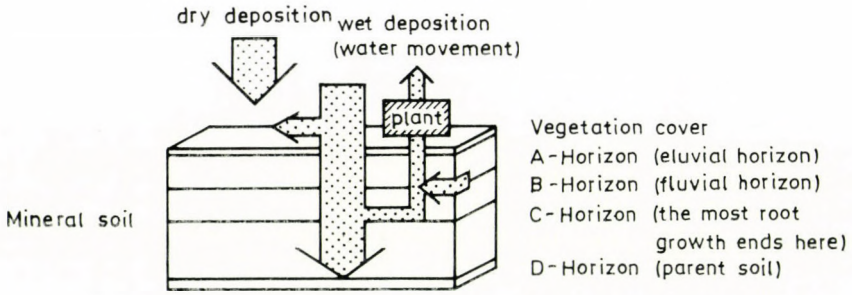

Fig. 4. The fluxes of water and minerals through a podzolized ecosystem

ability by plants. The elements C, H, O will be mainly taken up by plants in molecular forms such as H_2O and CO_2. N, P and S will be taken up mainly as anions (NO_3^-, $H_2PO_4^-$ and HSO_4^-) and Ca, K and Mg as cations (K^+, Ca^{2+} and Mg^{2+}).

The first three elements (C, H, O) are mainly needed by plants to build up structural elements like cellulose and other macromolecules. The source material (CO_2 and H_2O) should be available in sufficient amounts in the atmosphere and should not be a limiting factor for plants in this ecosystem. The other 6 elements (N, P, S, Ca, K and Mg) exist in very low concentration in the upper 60 cm of the investigated podzol soil, which is typical for this kind of soil (Fig. 4). For the genesis of this soil type,

Table 2

Enrichment factors (EF) of macronutrients by Vaccinium vitis-idaea and Pinus sylvestris. Enrichment factors were calculated by following equation:

$$EF = \frac{1 \cdot \bar{x}\ (\text{plant species})}{\bar{x}\ (\text{soil})}\ \text{(rounded results)}$$

Element	Pinus sylvestris	Vaccinium vitis-idaea
C	4	4
O	1	1
H	5	5
N	7	7
S	10	16
P	4	5
Ca	10	7
K	1	1
Mg	3	3

the following factors will be important: High average precipitation per year, low average temperature per year and rocks with low Ca- and Mg-content. If we compare the element concentrations of plants with these of soils, we see that the plant has become enriched with the elements to high concentrations. The concentration factors presented in Table 2 are between 1 (O and K) up to 16 for S in Vaccinium vitis-idaea.

From an ecological point of view it seems that plants which are growing on these poor soils have developed a special active uptake and concentration mechanism on a physiological level, to get enough of the essential elements. Because very little is known about the uptake mechanisms of minerals by plants, a clear-cut statement of the kind if uptake cannot be given here.

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WOOD PRODUCTION CAPACITY OF LOWLAND FOREST COMMUNITIES

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The size of wood production expected from afforestations possible at the sites of natural forest communities occurring in the forest-steppe climate belt (BORHIDI 1961) in the lowland regions of Hungary was to be determined. It was done by making use of earlier papers dealing with the relations of forest communities and types of site, so as collecting the numerical data expressing the wood production capacity from the wood charts. The data presented here are of informative nature, more exact data could be obtained by surveys made on the spot.

Introduction

Natural forest communities are always bound to definite site conditions, consequently, they can be used as indicators of the wood production capacity of the sites they occupy. This gave the idea (based on G. FEKETE's suggestion) to try to determine numerically the wood production capacity of various forest communities, that is, to express the result of forest management possible under the site conditions of the respective forest communities.

Material and Method

The task was solved by the following method. In an earlier paper (SZODFRIDT 1978) a survey was given of the relationship between the forest communities occurring in Hungary and their respective sites. Since the sylvicultural utilization of the different types of site had been earlier elaborated by JÁRÓ (1972), and it even appeared in the forest policy instructions (SZODFRIDT 1984), opportunity was given to determine numerically the wood production capacity of the types of site belonging to the different forest communities. The data given below indicate the total volume of wood produced up to the age of timber-harvest. Since the forest management works with the final felling ages varying with the species of tree, the felling ages of growing stocks suggested by JÁRÓ (1972) for the respective types of site are indicated in brackets after the figures expressing the wood production. The numerical values range between two limits for the reason that JÁRÓ divided in two yield classes the wood production possibly attained with the tree species on the suggested type of site. Therefore, when in JÁRÓ's work

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the wood production capacity is expressed by the term "good", this refers to yield classes I and II in the yield table set up for the tree species concerned. Thus, the wood production capacity based on the yield classes can be best defined with two data.

The data of the yield tables were copied out from SOPP's tables by SZÉLESSY to whom thanks are due for his kind efforts.

Results and Discussion

The data presented below are only of approximate accuracy, unsuitable for determining the actual wood production of a growing stock; they can be used for comparison and general information only. There are various reasons for this, some of which are listed below:

1. The yield tables contain the wood yield of forest stands in terms of unmixed forests. The Hungarian forests -- with few exceptions -- are actually mixed, so the calculation that only reckons with the tree species in question needs more or less corrections.

2. The data of wood production charts are mean values, that is, they are obtained by equilibrating more or less scattered data. Therefore the figures presented never are quite accurate, they reflect an average state. Thus, if we want to know the wood production capacity of a given forest stand, only the usual local survey and its internal evaluation can supply precise information.

3. The data presented below range between two extremes. Since a forest community may occur on more than one types of site, its wood production capacity may vary with the site conditions.

Wherever it was possible we tried to relate the given data to forest types, too, thus increasing the usefulness of our work.

The forest communities are discussed here in the order followed in the paper referred to (SZODFRIDT 1978). Out of them only those occurring on lowland areas have been worked up, forest communities of mountains and hill-countries will be discussed in a later work. The forest communities in the paper referred to were given names after SOÓ's work (1964--73), and they are discussed under the same names in this paper too, even if the names of some of them may have been modified. We do so with the purpose of making it easier to identify the data of this paper with those of the original work.

To make another remark before presenting the data: the Hungarian wood production research continuously produces new yield tables, the data of which are more accurate than those used in this paper. Therefore the data published here reflect the present conditions and relate to those forest

types and methods of forest tending to which the yield tables now in operation are adjusted. If there will be possibility to establish forests other than those discussed here, or with an advance of production techniques new methods increasing the volume of wood production appear, then the data presented here have to be modified.

The treatment follows JÁRÓ's categories of sylvicultural site evaluation and -system; instead of making them known here we refer to the description given in the above-mentioned paper (SZODFRIDT 1978).

Now we are going to discuss the wood production capacity for each lowland forest community. We have to mention a further point of view of the evaluation of data. The final felling age varies with the tree species, so the data had better be reduced to one-year increments of wood production, thus making them comparable. We intend to widen our work so as to deal with the financial result of management possible in the individual forest communities; this will provide a realistic basis for their evaluation.

1. Salicetum triandrae. It is a forest community first appearing on shallow islands of larger rivers. Its area is usually not large enough for regular forest management. Its only tree species that may supply useful wood is Salix alba; when felled in a 30-year rotation it gives a stand of medium wood production capacity with a total yield of 388-317 m^3. This capacity is only reached when the Salicetum triandrae has been sufficiently silted up to make plantation of Salix alba possible. In case of spontaneous occurrence the community is not considered suitable for any kind of afforestation, we must wait until the deposits of the rivers silt up the area to a height appropriate to Salix alba.

2. Salicetum albae-fragilis. A forest community of more advanced stage of siltation. In low sites periodically saturated alluvial soils only Salix alba can be taken into account; its felling age is 30 years, its wood production 388-317 m^3. In a more favourable case the "semihumid" forest type appears instead of the one called "humid"; then the site "periodically saturated site" becomes a site of permanent water influence. In this situation the volumes of attainable wood production are: Italian poplar "I 214": 369-298 m^3 (15), giant poplar "Robusta": 874-715 m^3 (25). These figures show the volumes of wood produced by stands cultivable on soils of loam physical properties. If the soil has the physical make-up of clay, the following tree species can be taken into account: early poplar "Marylandica": 583-477 m^3 (25), Salix alba 578-475 m^3 (30).

Table 1

Wood production in different biotops of alluvial hardwood forest community (Fraxino pannonicae-Ulmetum) growing in forest-steppe climate belt

Hydrology	Soil type	Physical structure	Depth of top soil	Tree species	Wood yield m^3/ha	Age year
Constant water stress	humous alluvium	sand and loam	medium deep and deep	Q. robur	1340—1161	(100)
				Italian poplar "I 214"	369—298	(15)
				giant poplar "Robusta"	874—715	(25)
	alluvial forest soil	sand and loam	medium deep	Q. robur	1340—1161	(100)
				Italian poplar "I 214"	369—298	(15)
				giant poplar "Robusta"	874—715	(15)
				white and grey poplar	634—493	(25)
		clay	medium deep	Q. robur	1340—1161	(100)
				early poplar "Marylandica"	583—477	(25)
				white and grey poplar	634—499	(25)
Periodical water stress	crude alluvium	sand	medium deep	Robinia	275—219	(25)
				white and grey poplar	502—397	(30)
			deep	Robinia	400—335	(25)
				Italian poplar "I 214"	369—298	(15)
				giant poplar "Robusta"	623—507	(20)
	humous alluvium	loam	shallow and medium deep	Q. robur	928—759	(90)
				Italian poplar "I 214"	242—195	(15)
				giant poplar "Robusta"	412—335	(20)
				black walnut	414—305	(80)
		clay	shallow and medium deep	Q. robur	694—572	(90)
				early poplar "Marylandica"	583—477	(25)
				white and grey poplar	392—307	(25)
	alluvial forest soil	sand and loam	medium deep	Q. robur	982—803	(100)
				Italian poplar "I 214"	242—195	(15)
				giant poplar "Robusta"	412—335	(20)

Table 1 (contd.)

Hydrology	Soil type	Physical structure	Depth of top soil	Tree species	Wood yield m^3/ha	Age year
			deep	Q. robur	982—803	(100)
				Italian poplar "I 214"	359—282	(15)
				giant poplar "Robusta"	623—507	(20)
		clay	medium deep	Q. robur	982—803	(100)
				early poplar "Marylandica"	583—477	(25)
			deep	Q. robur	1340—1161	(100)
				early poplar "Marylandica"	1087—892	(30)

3. Fraxino pannonicae-Alnetum. Found on fen soil with the hydrological category of temporary flooded site which only makes it possible to cultivate common alder (Alnus glutinosa); its wood production is 244-182 m^3 (50). The forest stands belonging here are usually of small extension, and owing to their valuable components that must be protected, sylvicultural utilization is neglected here. The best method is to try to maintain the groups of second growth trees mostly composed of Hungarian ash-trees appearing on tussocks in their natural state, and plant alder only of necessity.

4. Calamagrosti-Salicetum cinereae. Owing to the water cover only common alder can be reckoned with for afforestation; its wood production is 244-182 m^3 (50). The same can be said of this community as of the former one, it must be protected and kept in natural state, it is no place for business-like forest management.

5. Fraxino pannonicae-Ulmetum. Its wood production capacity is contained in Table 1 according to JÁRÓ's system of sylvicultural site types.

6. Querco robori-Carpinetum. Similarly to the former forest community the figures expressing its wood production capacity are shown in tabulated form (Table 2).

7. Aceri tatarico-Quercetum. On sandy rust coloured brown forest soil free from the effect of excess water the type of forest qualified dry is expected to yield the following volumes of wood: Scotch pine 642-535 m^3 (60), black locust 275-219 m^3 (25), pedunculate oak 549-391 m^3 (80). On a chernozem brown forest soil with the same hydrological category of water

Table 2

Wood production in different biotopes of the pedunculate oak hornbeam communities (Querco robori-Carpinetum s.l.) growing in forest-steppe climate belt

Hydrology	Soil type	Physical structure	Depth of top soil	Tree species	Wood yield m^3/ha	Age year
Tree from excess water	rust coloured forest soil and brown forest soil on sandy parent material	sand	deep + very deep	Scotch pine	1072—894	(80)
				Q. robur	863—706	(80)
				Robinia	463—387	(30)
	meadow forest soil	sand	deep + very deep	Scotch pine	1072—894	(80)
				Robinia	463—387	(30)
				giant poplar "Robusta"	412—335	(20)
Periodical water stress	meadow forest soil	sand	medium deep and deep	Q. robur	1340—1161	(100)
				giant poplar "Robusta"	583—477	(25)
	rust-coloured forest soil	sand	deep + very deep	Q. robur	1340—1161	(100)
Constant water stress	rust-coloured forest soil	sand	medium deep and deep	Q. robur	1340—1161	(100)
				Italian poplar "I 214"	242—195	(15)
				giant poplar "Robusta"	412—335	(20)
				common alder	806—642	(60)

losing site in the case of shallow top-soil a "very dry" type of forest may replace the former type; its utilization is possible with the following tree species and wood production: Austrian pine 557-459 m^3 (60), while in the "dry forest" type: Scotch pine 642-535 m^3 (60), black locust 275-219 m^3 (25), pedunculate oak 549-391 m^3 (80). We should like to note that the site is rather dry, so pedunculate oak in unmixed form should not be planted here, it is better to plant seedlings of some Quercus hybrid instead, grown from seeds collected from the existing stands. The community itself in a typical occurrence is today so rare that we can hardly speak of its sylvicultural utilization; the data concerning wood production are only given for the sake of completeness. The best we can do is to protect the existing stands of the community and use them for educational purposes.

8. Festuco-Quercetum. It is a forest community whose site conditions have not been fully explored so far, so its wood production capacity cannot be dealt with either. According to SOÓ (1964--1980) in the Danube--Tisza Interfluve it is the most widespread community. I myself -- though much in search of it -- have hardly encountered this community. Those stands as can be placed with it were mostly found north and east of the city of Kecskemét, on rust-coloured forest soil or multi-layer humous sand. There is urgent need of seeking out its existing stands and declare them protected, then making phytocenological and site evaluations, because in a short time this forest community -- found being very frequent by SOÓ -- will disappear without any trace. In this respect mainly the forests of Cegléd, Nagykőrös and Pusztavacs, and the one in the neighbourhood of Nyárlőrinc (nearby the so-called Erdő-Csárda) offer possibilities.

9. Junipero-Populetum albae. In its "very dry" types of forest the following tree species can be reckoned with: Scotch fir 442-368 m^3 (60), Austrian pine 557-459 m^3 (60), while for the site of its "dry" forest types the recommended species are: Scotch pine 701-585 m^3 (70). Planting white poplar at the site of this forest community cannot be safely suggested, since this tree species too demands a good water regime, it can hardly be grown in closed stands, the cost of afforestation will not be returned by the future wood yield, therefore this forest community can be maintained with its original composition of tree species only when nature conservancy aspects are to be considered. It is a thin, grove-like community which finds its conditions of existence first of all in depressions of sand-dunes or on lee sides. As a characteristic representative of the former lowland forest vegetation it demands protection wherever its typical composition stands can still be found.

10. Convallario-Quercetum. Since it may occur on many kinds of site, and consequently numerous tree species can be planted in its place, it had better be discussed in a tabulated form (Table 3).

11. Festuco-pseudovinae-Quercetum. In its forest type of "perehed water-table", on meadow forest soil with the physical properties of clay, in case of medium deep top-soil the following tree species can be reckoned with: pedunculate oak 928--759 m^3 (90), white poplar 392-307 m^3 (25), while in the case of periodical water influence the same community with its "semi-arid" forest types yields the following volumes of wood: pedunculate oak 928-759 m^3 (90), Italian poplar 242-195 m^3 (15), "Robusta" poplar 412-335 m^3 (20).

Table 3

Wood production in different biotopes of the mayflower pedunculate oak community (Convallario-Quercetum) in the forest-steppe climate belt

Hydrology	Soil type	Physical structure	Depth of top soil	Tree species	Wood yield m^3/ha	Age yield
Free from excess water	rust-coloured forest soil, and brown forest soil on sandy parent material	sand	deep	Scotch pine	1011—839	(70)
				Robinia	275—219	(25)
	meadow soil and its combinations	sand	deep	Q. robur	928—759	(90)
				Robinia	463—387	(30)
				Italian poplar "I 214"	242—195	(15)
				giant poplar "Robusta"	412—335	(20)
				white and grey poplar	392—307	(25)
Periodical water stress	humous alluvium	sandy loam	medium deep	Italian poplar "I 214'	242—195	(15)
				giant poplar "Robusta"	421—335	(20)
				white and grey poplar	392—307	(25)
				Q. robur	928—759	(90)
	rust-coloured forest soil, and brown forest soil on sandy parent material	sand	deep	Austrian pine	1011—839	(70)
				Robinia	463—387	(30)
				giant poplar "Robusta"	412—335	(20)
				Q. robur	863—706	(80)
			medium deep	Austrian pine	1011—839	(70)
				white and grey poplar	502—317	(30)
	meadow soil and its combinations	sand	medium deep	Q. robur	549—391	(80)
				white and grey poplar	502—397	(30)
			deep	Q. robur	1266—1098	(90)
				Italian poplar "I 214"	242—195	(15)
				giant poplar "Robusta"	623—507	(20)

Table 3 (contd.)

Hydrology	Soil type	Physical structure	Depth of top soil	Tree species	Wood yield m^3/ha	Age yield
Periodical water stress	meadow forest soil	sand	medium deep	Q. robur	928—759	(90)
				Italian poplar "I 214"	242—195	(15)
				giant poplar "Robusta"	412—335	(20)
				white and grey poplar	502—397	(30)
Constant water stress	meadow soil and its combinations	sand	medium deep and deep	Q. robur	1390—1161	(100)
				Italian poplar "I 214"	369—298	(15)
				giant poplar "Robusta"	874—715	(25)
	meadow forest soil	sand	medium deep and deep	Q. robur	1340—1161	(100)
				Italian poplar "I 214"	369—298	(15)
				giant poplar "Robusta"	874—715	(25)
				white and grey poplar	634—499	(25)

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THE EFFECT OF SO_2, SO_3^{2-} AND SO_4^{2-} ON PHOTOSYNTHETIC INTENSITY IN GAMETOPHORE GROWING UNDER CONDITIONS OF DIFFERENT CONCENTRATIONS OF INDUSTRIAL POLLUTION

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Long-term exposure to SO_2 at concentration of 0.3 $mg \cdot m^{-3}$ brought about changes of a two-stage pattern in photosynthesis of leaves. Chlorophyll content in the leaves dropped but then gradually increased finally was only insignificantly lower than that found in control cultures.

Sensitivity of Funaria hygrometrica to SO_2 in the dark exceeded that in the light if the photosynthetic intensity was used as the basis of comparison. The photosynthetic oxygen evolution was inhibited to a greater degree in gametophores of plants collected in pollution free environments than in those from the grounds of a metallurgic plant. Under the influence of SO_2, SO_3^{2-} and SO_4^{2-} the reduction of chlorophyll in moss leaves was not so pronounced as that of photosynthesis and its intensity was almost the same in gametophores grown up under conditions of different atmospheric pollution.

Introduction

Leaves of most moss species are composed of one layer of assimilative cells. Their physiological activity is closely associated with the occurrence of water in the liquid form in the environment (KRUPA 1974). Sulphur dioxide is easily soluble in water, therefore, the concentration of sulphur compounds in the surroundings of the green cell can cause changes much more pronounced than one might expect on the basis of the concentration of this gas in air. A close dependence of the physiological activity of mosses and lichens on water can be, among other factors, the reason of sensitivity of these plants to gaseous pollution of the atmosphere (RAO and LeBLANC 1966; SYRATT and WANSTALL 1969; NASH 1974; SMITH 1981).

Specific properties of mosses being taken into consideration, the effect of SO_2 and other sulphur compounds on the activity of gas exchange and on chlorophyll content in Funaria hygrometrica leaves was determined.

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Differences in the sensitivity of flowering plants to SO_2 applied in the light and in the dark are connected with the diffusion of this gas into the leaf (CARLSON 1979; BARTON et al. 1980; SMITH 1981; SAXE 1983). Simple anatomical structure of moss leaves allows to omit the problems resulting from the diffusion of gaseous pollution through stomata from the atmosphere, this making it possible to investigate direct effects of sulphur dioxide applied in the light or in the dark, on the photosynthetic apparatus.

The material used experiments grew in areas differing by the degree of air pollution. On this basis differences in the susceptibility to sulphur compounds and in the adaptation potential of mosses were discussed.

Material and Method

Gametophores of Funaria hygrometrica used in experiments were collected in their natural habitats. They were sampled in two places of different industrial pollution (KRUPA, TLAŁKA 1987). The plants were taken a thin layer of soil and planted in glass containers covered with a transparent plate, in a light thermostate. The plants were illuminated using 12-hour day and night period. As light sources, LRF type, 400 W lamps (prod. Polam) and 2 incadescent bulbs of 200 W were used. The intensity of radiation reaching the surface of the culture was 80 W/m^{-2}. In the light phase the temperature was 22 ^{o}C ($\pm$ 2 ^{o}C) and in the dark it was reduced to 15 ^{o}C to obtain the dew effect.

On the basis of measurements of gas exchange a ten-day adaptation period was established. After this time leaves from the upper part of stems were taken for experiments.

The detached leaves were placed in Petri dishes in 10 cm^{-3} of the Mohr medium and incubated during 12 hours in the atmosphere containing 0.3, 3, 5 and 10 mg $SO_2 \cdot m^{-3}$. The details of SO_2 preparation, solubility and methods of treatment were given in an earlier paper (KRUPA, TLAŁKA 1987).

In experiments including a 21-day exposure of gametophores to SO_2, a closed system composed of a vessel where SO_2 was released and a chamber with the plant material, was employed. The vessel with sulphuric acid was placed in a glass flask of 25 dm^3; the amount of Na_2SO_3 solution introduced through a glass tube to the vessel secured the concentration of sulphur dioxide in the system at the level of 0.3 $mg \cdot m^{-3}$. Liberated SO_2 with air passed through glass tubes to the chamber with gametophores. The rate of air flow in the closed system was 20 $dm^3 \cdot h^{-1}$. When the material for measurements was taken the system was ventillated and a new portion of SO_2 was liberated. Under identical conditions of light and temperature in a similar system the culture of gametophores in SO_2 free environment was carried out.

In the investigation on the influence of SO_3^{2-} and SO_4^{2-} ion, sodium sulphite or sodium sulphate were added to the medium in the amount which secured ion concentration of 3, 9, 15 or 30 $mmol \cdot dm^{-3}$. The intensity of photosynthesis in leaves was measured using a microrespirometric method in the light whose intensity exceeded the saturation point (KRUPA 1978). The results were statistically evaluated using the Student-Gosset test and the analysis of variance according to one-way classification was carried out.

Results

Intensity of gas exchange in leaves continuously exposed to SO_2

The leaves of Funaria hygrometrica gametophores collected in the habitat free of industrial pollution (marked with symbol A) showed net photosynthesis of 3.87 mm^3 O_2.10 min^{-1}·$mgdw^{-1}$. The intensity of respiration in the dark converted to the same unit of reference was 0.90 mm^3 O_2.10 min^{-1}. In gametophores from the habitat affected by strong emissions of industrial pollution (later referred to as habitat B) photosynthesis was slightly lower and its intensity was 3.44 mm^3 O_2.10 min.1 $mgdw^{-1}$. However, after the period of necessary adaptation the leaves from habitat B showed the intensity of respiration higher by almost 0.54 mm^3 O_2.10 min.1 $mgdw^{-1}$ than that of leaves from habitat A. The intensity of these processes was almost unchanged during 21 days, i.e., during the period of the experiment.

Already after a 24-hour treatment with sulphur dioxide at concentration of 0.3 $mg \cdot m^{-3}$ gametophores of mosses from habitat A showed decreased intensity of photosynthesis and respiration. The intensity of net photosynthesis of leaves was lower by almost 14% and that of respiration by 33% as compared to the values at the beginning of the experiment. When the treatment with the given compound was prolonged to 7 days the intensity of gas exchange did not significantly change. The drop in photosynthesis and respiration was not observed before the 14th day of the culture of gametophores in the atmosphere containing 0.3 mg $SO_2 \cdot m^{-3}$. After 21-day period of the experiment the final intensity of photosynthesis and respiration was by almost 50% lower than that of leaves incubated in the air free of sulphur dioxide (Fig. 1). Leaves from habitat B incubated in the atmosphere of 0.3 mg $SO_2 \cdot m^{-3}$ initially showed a decrease in the intensity of photosynthesis. After a 21-day culture the intensity of this process was lower by 32% as compared to control gametophores. The pattern of changes in the respiration of the leaves was similar to that of photosynthesis while after a 24-hour exposure of gametophores to SO_2 containing atmosphere, the intensity of respiration was lower by 10% only as compared to that found in control plants.

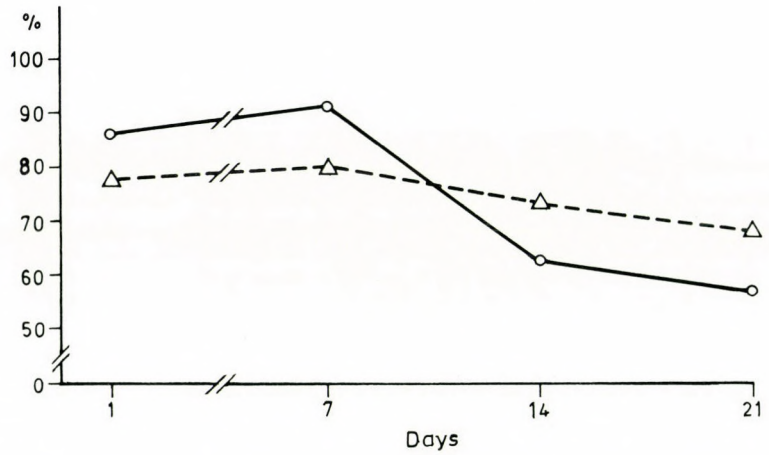

Fig. 1. Relative net photosynthesis rate in leaves of Funaria hygrometrica treated continuously with SO_2 at concentration 0.3 $mg \cdot m^{-3}$.
o——o — leaves from unpolluted and Δ----Δ — polluted habitat

The effect of SO_2 treatment in the light and in the dark on gas exchange

Data in the literature suggest that the damage to plants due to sulphur dioxide depends upon light. No data on such dependences in mosses having been quoted, the intenstiy of gas exchange in gametophores incubated during 12 hours in the atmosphere containing SO_2 at concentration of 3 $mg \cdot m^{-3}$, in the light and in the dark was measured.

The depressive action of SO_2 on photosynthesis was observed both in the light and in the dark. The intensity of net photosynthesis of leaves form plants sampled from habitat A and treated with SO_2 in the dark was by almost 50% lower than that in the control. In these leaves treated with SO_2 in the light the drop in the investigated process was less evident, reaching about 20% of the initial value. The dark respiration was reduced by 70% as compared to control plants. Similar dependences were found for gametophores collected from the habitat affected by industrial pollution, though, the depressive action of SO_2 on photosynthesis was slightly less significant. The drop of 11% was found in photosynthesis of gametophores treated with SO_2 in the light while in the dark this process was by 36% lower than that under control conditions. The difference in the intensity of real photosynthesis between the gametophores treated with SO_2 in the dark and in the light was in the same order of magnitude (21% and 18%) for the two habitats and was statistically significant ($t = 11.88$ and $t = 16.75$).

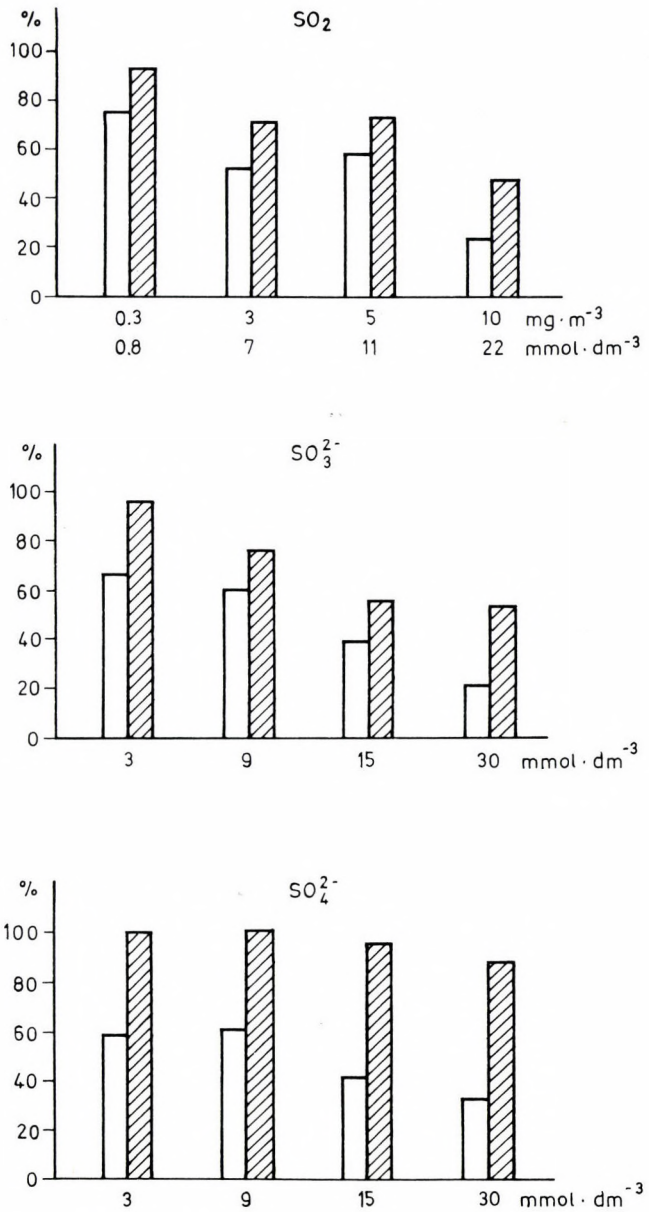

Fig. 2. Effect of SO_2, SO_3^{2-}, SO_4^{2-} on relative net photosynthesis of leaves of Funaria harvested from:

□ — unpolluted and ▥ — polluted areas. Time treatment 12 hours

The effect of sulphur compounds on gas exchange

The exposure of leaves from habitat A to the atmosphere containing 0.3 mg $SO_2 \cdot m^{-3}$ during 12 hours reduced the intensity of photosynthesis by about 20%. An increase in the concentration of the gas resulted in a further decrease in photosynthesis: in the leaves of Funaria hygrometrica treated with SO_2 at concentration of 10 $mg \cdot m^{-3}$ it was by 78% lower than in the leaves incubated in the atmosphere free of this gas. The same conditions depressed photosynthesis in leaves from habitat B to amuch less degree. The intensity of photosynthesis of these leaves decreased when the concentrations of SO_2 were elevated and finally, at the highest concentration applied, it dropped to the value lower by almost 50% than that found in the control. However, this value was by 30% higher than the intensity of photosynthesis in the leaves from habitat A (Fig. 2).

The exposure of leaves of the investigated moss species to a solution of sodium sulphite caused an evident inhibition of photosynthesis. The inhibition effect depended upon the concentration of sulphite ions in the environment of the leaf (Fig. 2). Leaves from the dumping ground of blast-furnace slag were much less sensitive to the investigated compound and the lowest concentration used (3 $mmol \cdot dm^{-3}$) did not affect the photosynthetic process. A more distinct dependence between the intensity of photosynthesis and the concentration of sulphite ions could be observed in leaves grown up in the habitat free of industrial pollution. Sulphite concentration of 3 $mmol \cdot dm^{-3}$ depressed the photosynthetic intensity by 30% as compared to the control. In the solution containing 30 mmol $SO_3^{2-} \cdot dm^{-3}$ photosynthesis of the leaves was by 75% lower than that measured in the control.

The respiration process of leaves to a small degree depended upon the influence of the investigated compound. The drop in the activity of respiration was only found in the case of leaves from habitat A incubated in the solution containing the highest sulphite concentration.

Changes similar to those observed in the photosynthetic intensity of Funaria leaves from the habitat lying far from the source of industrial pollution, under the influence of sulphite, appeared on sodium sulphate treatment. However, the effect of sulphate was lower by 5-10% on the average than that of sulphite. The effect of SO_4^{2-} was even lower in the case of leaves from the spoil heap in the metallurgic plant. The highest sulphate concentration employed in the experiment depressed photosynthesis by 10% only, as compared to intensities found in the control material (Fig. 3).

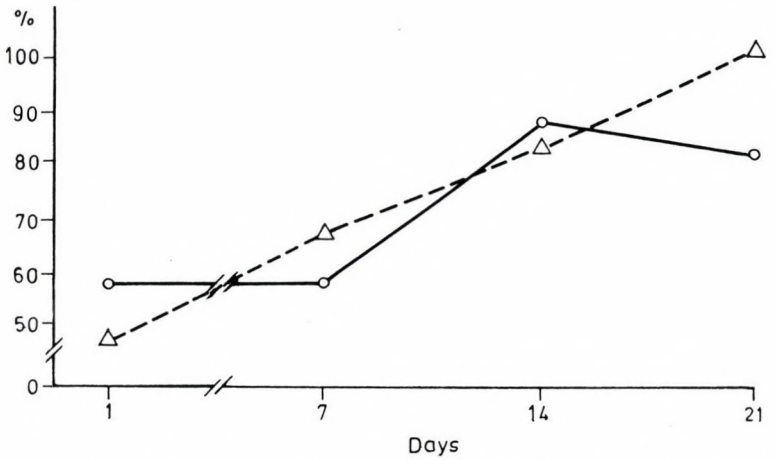

Fig. 3. Relative content of chlorophyll a+b in leaves of Funaria treated continuously with SO_2 at 0.3 $mg \cdot m^{-3}$ concentration. Description as in Fig. 1

Changes in chlorophyll concentration in the leaves continuously exposed to SO_2

A 24-hour incubation of gametophores from habitat A in the atmosphere containing 0.3 mg $SO_2 \cdot m^{-3}$ caused a 40% decrease in the content of chlorophyll a+b, as compared to the concentration of this pigment in leaves incubated in the atmosphere free of sulphur dioxide. The level of chlorophyll content in the leaves did not change during a 7-day treatment under these conditions. After this period the amount of chlorophyll increased and after 21 days of SO_2 action the final concentration of this pigment was lower by 15% only than that in control leaves (Fig. 3). In the leaves from habitat B the first 24 hours of exposure to 0.3 mg $SO_2 \cdot m^{-3}$ brought about a 50% decrease in the content of chlorophyll a+b as compared to the control. The prolongation of SO_2 treatment resulted in the elevated content of pigments in relation to the previously found value. After a 21-day incubation under these conditions the concentration of chlorophyll equalled that noted in plants growing in SO_2 free air (Fig. 3).

The effect of SO_2 treatment in the light and in the dark on chlorophyll content

The sulphur dioxide treatment in the light caused greater changes in the content of chlorophyll in the leaves of the investigated moss species

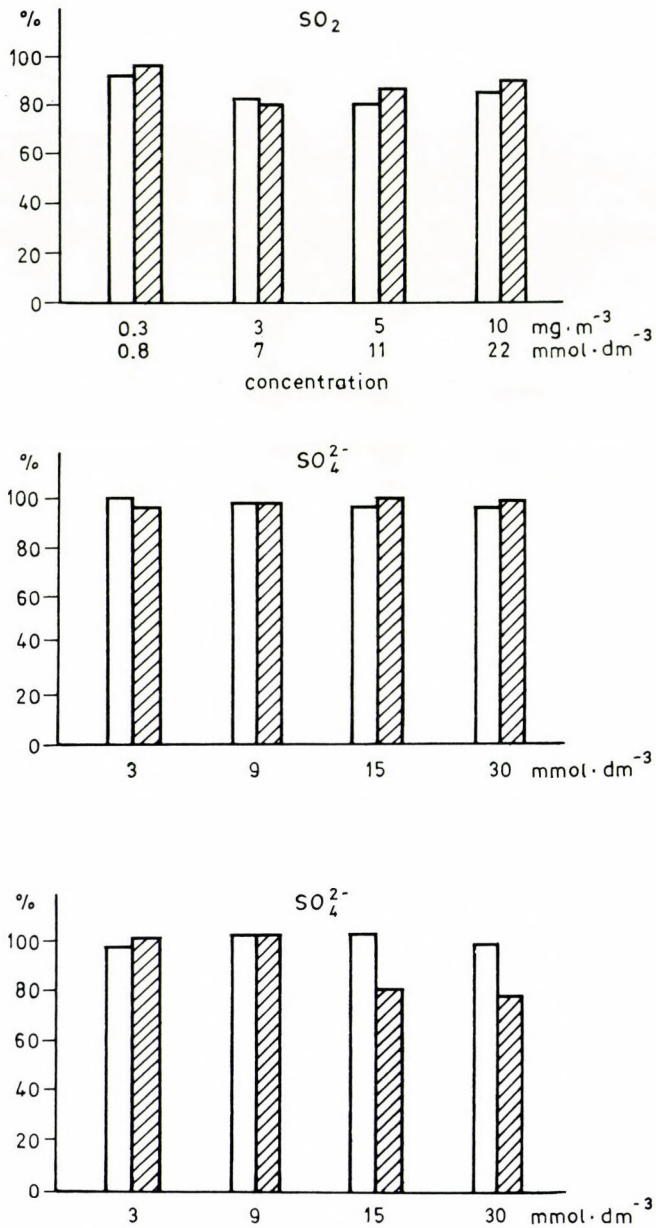

Fig. 4. Influence of different concentrations of SO_2, SO_3^{2-}, SO_4^{2-} on the relative chlorophyll content in leaves of Funaria hygrometrica.
Description as in Fig. 2

than in the dark. However, this dependence was only found in the case of leaves from habitat A. In leaves from habitat B sulphur dioxide reduced the concentration of chlorophyll by 20% as compared to the control, no significant differences being noted between the treatment in the light and in the dark.

The effect of sulphur compounds on chlorophyll content

A 12-hour incubation of leaves in air containing sulphur dioxide in the dark did not significantly affect the concentration of chlorophyll. A decrease in chlorophyll content was 20% on the average independently of the previous conditions of the leaf growth (Fig. 4).

Almost no changes were found in chlorophyll concentration in leaves treated with sodium sulphite for 12 hours (Fig. 4).

Neither did sodium sulphate decrease the concentration of chlorophyll in the leaves of Funaria; it was only in the range of the highest concentrations that the amount of pigments was reduced by 20% in the leaves taken from habitat B.

Discussion

Different moss species show different sensitivity to industrial pollution, particularly to sulphur dioxide. This differentiation can be associated with morphological and physiological traits of these plants. Gametophore of Funaria are relatively small and the leaves are built of one layer of assimilative cells.

In these plants the photosynthetic process which is particularly sensitive to the toxic action of air pollution, occurs at a full hydration of leaves. A 20% decrease in water content in the leaves causes a complete inhibition in photosynthesis (KRUPA 1977). This close connection with water can explain the specific response of mosses in natural conditions to air pollution, particularly to compounds readily soluble in water. The increased toxicity of SO_2 to flowering plants in the light is associated with the action of this compound on stomata (KONDO and SUGAHARA 1978; BISCOE et al. 1973; HEATH 1980; SAXE 1983). In the case of mosses the diffusion of sulphur dioxide occurs directly from the surface layer to the inside of the leaf cell.

Plants which were exposed to SO_2 fumigation in the light showed a lower reduction in photosynthesis than those treated in the dark. This

dependence could be found for gametophores taken as well from places free of pollution as from the spoil heap in the vicinity of the metallurgic plant. A difference of 34% between the treatments in the light and in the dark was found in the reduction in photosynthesis of gametophores from habitat A. The leaves from places of massive air pollution exposed to SO_2 in the dark showed a decrease in photosynthesis by 25% higher than in the light.

A decrease in photosynthesis of leaves treated with the pollutant during 12 hours in the dark depended upon the SO_2 concentration outside the leaf. The concentration of 10 $mg \cdot m^{-3}$ of this gas in air, this corresponding to SO_2 concentration in the solution of about 22 $mmol \cdot dm^{-3}$, caused an almost complete inhibition of photosynthesis.

Similarly, as in the case of protonema (KRUPA, TLAŁKA 1987) the toxicity of the investigated substance was more pronounced in leaves from pollution free habitats. The toxicity of sulphur dioxide depends on the duration of its action on the plant.

Under conditions of the long-term exposure of gametophores to this gas in air two phases of the decrease of photosynthesis can be differentiated. At first, significant reduction in the intensity of this process is found while no further decrease is noted before the 7th day of treatment. This two-stage pattern of changes can be associated with methods in treating gametophores but also with the accumulation of sulphur compounds both environment and inside the cell. It was found (GILBERT 1968) that plants in the vicinity of sources of sulphur dioxide emission contained considerably more sulphur than the plants growing at greater distances from the source of pollution. Plants transplanted from such places to the neighbourhood of the source of SO_2 emission showed enhanced symptoms of toxicity long before the sulphur concentration reached the level found in specimens grown up under conditions of strong air pollution (SAXE 1983).

The toxicity of SO_3^{2-} ions to photosynthesis is comparable to that of SO_2, the molar concentration of these substances in solutions which surround the moss leaves, being taken into consideration. The inhibition of the photosynthetic process by sulphite ions is evident in gametophores which grew in places of devoid industrial pollution. The toxicity of sulphate to photosynthesis is comparable to that of sulphur compounds discussed above, though the inhibition of the process is less pronounced.

A review of data on the effect of oxygen sulphur compounds on photosynthesis shows that none of the investigated substances was irrelevant to this process. In analysing the effect of the individual compounds on photo-

synthesis, the complex and multifarious action of pollutants of the natural environment must be taken into account. In the presented results changes in the photosynthetic activity were measured by the amount of liberated oxygen. If different mechanisms of the action of sulphur compounds on photosynthesis are taken into consideration, a detailed comparison of the present results with the data on CO_2 assimilation is rather difficult. The lack of close correlations between the amount of liberated oxygen and assimilated CO_2 is also caused by the occurrence of photorespiration found in mosses (DILKS 1976). However, it is unquestionable that in spite of different mechanisms of their action, sulphur dioxide and also sulphite and sulphate ions inhibit CO_2 assimilation and oxygen evolution (HILL 1971; PUCKETT et al. 1974; FERGUSON, LEE 1978).

Complex influences of sulphur compounds on the processes occurring in the cell are also suggested by changes in the intensity of respiration in the leaves of the moss species employed in the study. The intensity of this process was reduced when the sulphur dioxide treatment occurred in the light. This is also substantiated by the results of experiments with leaves exposed to the toxic substance in the light and in the dark. Yet, the respiration in the dark was not significantly changed if gametophores were exposed to SO_2 in the darkness.

Also the drop in the amount of chlorophyll in leaves treated with SO_2 in the light was greater than in the dark. However, no correlation was found between pigments in gametophores after a long-term treatment with sulphur dioxide. Initially the content of chlorophyll in these gametophores was lowered but afterwards it increased and its final value was only slightly lower than the initial one.

The reduction in chlorophyll content in the leaves treated with sulphur compounds in the dark was not large, reaching 20% at high SO_2 concentrations. The conversion of chlorophyll to pheophytin caused by SO_2 takes place when the pH of the solution was lower than 3 (RAO, LeBLANC 1966; SYRATT, WANSTALL 1969; MUDD, KOZLOWSKI 1975). However, changes in chlorophyll concentration caused by the discussed compounds were also observed at a less acidic reaction of the environment (MALHOTRA 1977; SOLDATINI et al. 1978; SAXE 1983). These results and particularly, the different reaction to sulphur dioxide in the light and in the dark suggest that their destructive effect on chlorophyll cannot be associated only with the changing pH of the cell inside. The process can be also related with the photooxidation of chlorophyll and can be enhanced by sulphur compounds. Because no distinct

differences were found between chlorophyll concentration in SO_2 treated leaves grown up under conditions of different atmospheric pollution, the differentiation in the tolerance of this gas cannot be due to the properties of photosynthetic pigments.

Plants which for a number of generations grew up under conditions of increased industrial pollution distinctly show the resistance to oxygen sulphur compounds. Therefore, it can be assumed that certain adaptation abilities were produced in these mosses, though the character and mechanisms of the resistance are not clear. It is probable that these morphologically simple plants can grow in the conditions toxic for other organisms owing to a complex of certain properties. Therefore, among other pioneer plants, mosses can settle areas degraded by the industry and play a significant role in the neutralization of different environmental pollutants.

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THE EFFECT OF SO_2, SO_3^{2-} AND SO_4^{2-} ON GAS EXCHANGE INTENSITY OF CAPSULES MATURING IN THE ENVIRONMENT OF VARIOUS DEGREES OF INDUSTRIAL POLLUTION

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Decreases in photosynthesis of Funaria hygrometrica capsules treated with SO_2 in the light and dark are almost the same. Prolonged action of SO_2 at concentration of 0.3 $mg \cdot m^{-3}$ did not significantly decrease the intensity of photosynthesis before the 14th day of treatment; the toxicity of the gas depended upon the concentration of industrial pollution previously affecting the sporogones. The sporogones which grew up under conditions of high concentrations of pullutants are also more tolerant of the toxic effect of SO_3^{2-} and SO_4^{2-} ions. Under the influence of sulphur compounds the changes in chlorophyll content were markedly lower than the decreases in photosynthesis, and to a small degree depended upon the conditions of plant growth. Decidedly different anatomical structure of the successive stages of Funaria development is not so distinctly correlated with the toxicity of sulphur compounds applied.

Introduction

The moss sporogones differ from gametophores by a different anatomical structure. The well developed assimilative tissue of the capsule shows a high photosynthetic activity (KRUPA 1969). The gas exchange between the inside of the capsule and the surrounding atmosphere occurs through stomata which function during a considerable part the life of sporogones (GARNER, PAOLILLO 1973). Therefore, the reaction of this moss generation to gaseous atmospheric pollution can be compared with that of higher plants. Diffusion of gaseous pollutants through stomata is decisive for the concentration of pollution inside the leaf, i.e. in the nearest surrounding of assimilative cells (UNSWORTH et al. 1972; BISCOE et al. 1973; KONDO, SUGAHARA 1978; BLACK 1979; SAXE 1983).

In previous papers the effect of SO_2, SO_3^{2-} and SO_4^{2-} on the photosynthetic intensity and chlorophyll content in the protonema (KRUPA, TLAŁKA 1986) and gametophores (KRUPA, TLAŁKA 1989) of Funaria hygrometrica was

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discussed. No data on the effect of these substances on processes occurring in sporogones which constitute an important generation in the moss development, have been available. In the present paper the effect of SO_2 and its derivatives on the intensity of photosynthesis and chlorophyll content in the capsules of Funaria hygrometrica was studied.

The morphological and anatomical differentiation of the separate developmental phases of mosses can present a model system used in the investigation of complex and multivarious effects of sulphur dioxide on plants. On the other hand, from the occurrence of the species in habitat differing by the degree of atmospheric pollution informations can be obtained on the adaptation ability of mosses.

Material and Method

Sporogones of Funaria hygrometrica were collected at habitats differing by the degree of atmospheric pollution. One station lies in the vicinity of the metallurgic plant on a spoil-heap of furnace slag and the other, in a forest far from source of industrial pollution (KRUPA, TLAŁKA 1986). The plants were adapted during 10 days under controlled conditions of temperature, humidity and light intensity (KRUPA, TLAŁKA 1986).

The capsules were cut off and placed in Petri dishes filled with the Mohr medium in the constant volume of 10 cm^3. The material was incubated during 12 hours in the dark at SO_2 concentrations of 0.3; 3; 5 and 10 $mg \cdot m^{-3}$. Because of their low specific gravity the capsules were always on the surface of the medium, only partly submerged in it.

The effect of SO_3^{2-} and SO_4^{2-} ions was determined by introduction the amounts of sodium sulphate or sodium sulphite to obtain the ion concentration of 3, 9, 15 and 30 $mmol \cdot dm^{-3}$ in the culture of capsules.

In experiments with a 21-day SO_2 treatment sporogones were incubated with gametophores in a chamber with a constant flow of air containing 0.3 mg $SO_2 \cdot m^{-3}$. The control material was incubated in an identical chamber but with the air flow without SO_2. The remaining conditions were the same as in the culture of gametophores, described in an earlier work (KRUPA, TLAŁKA 1989).

Photosynthetic oxygen evolution was measured using microrespirometric method in the light whose intensity exceeded the saturation point (KRUPA 1969). The concentration of chlorophyll was measured using a spectrophotometer, and computed in relation to the capsule dry weight. The capsules in the third stage of development (KRUPA 1969) were used in measurements. The results were statistically evaluated, the Student-Gosset test and analysis of variance being applied.

Results

The intensity of net photosynthesis of the capsules calculated per 1 g of dry weight, was about 10.8 $mm^3 \cdot h^{-1}$ and did not depend upon the conditions of their development. Sporogones which grew up under conditions of great concentration of industrial pollution, exposed to SO_2 at concentration of 3 $mg \cdot m^{-3}$ during 12 hours in the dark, showed a 15% decrease in photosyn-

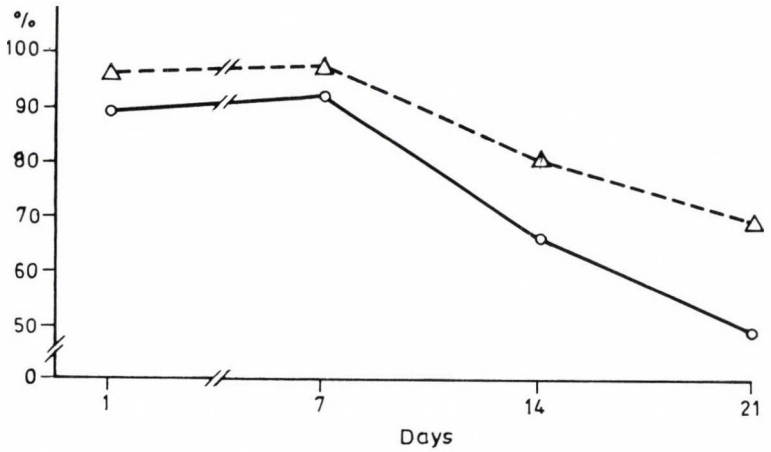

Fig. 1. Relative net photosynthesis rate in capsules of Funaria hygrometrica treated continuously with SO_2 at concentration 0.3 $mg \cdot m^{-3}$. Material from: o——o — unpolluted and Δ----Δ — polluted areas

thesis as compared with the control. Under the same conditions the capsules harvested from pollution-free environment showed a 50% decrease in the photosynthetic intensity. In both cases the toxic effect of SO_2 in the dark was higher by about 6% only than that (of SO_2 fumigation of capsules) in the light.

In sporogones of Funaria exposed to 0.3 mg $SO_2 \cdot m^{-3}$ in air during 12 hours, photosynthetic intensity was slightly lower than that in the control. No sooner than after a 14-day exposure under these conditions, a distinct decrease in photosynthetic oxygen evolution was found. Finally, after 21 days, the intensity of net photosynthesis was reduced by almost 50% in the capsules from habitat A (Fig. 1). Similar dependences could be found in the case of sporogones from habitat B, though, a lower decrease in gas exchange was observed.

Increased concentrations of SO_2 in the air surrounding sporogones during a 12-hour incubation in the dark brought about a decrease in photosynthesis. In the capsules from habitat A the intensity of photosynthesis was reduced by 70% after the SO_2 treatment at concentration of 10 $mg \cdot m^{-3}$, while the sporogones which grew in the conditions of industrial pollution were less sensitive and only the highest of the applied concentrations of this gas reduced the photosynthetic intensity almost by 60% (Fig. 2).

Similar dependences were observed in the capsules treated with sulphite. The reduction in photosynthesis of sporogones from habitat A, exposed

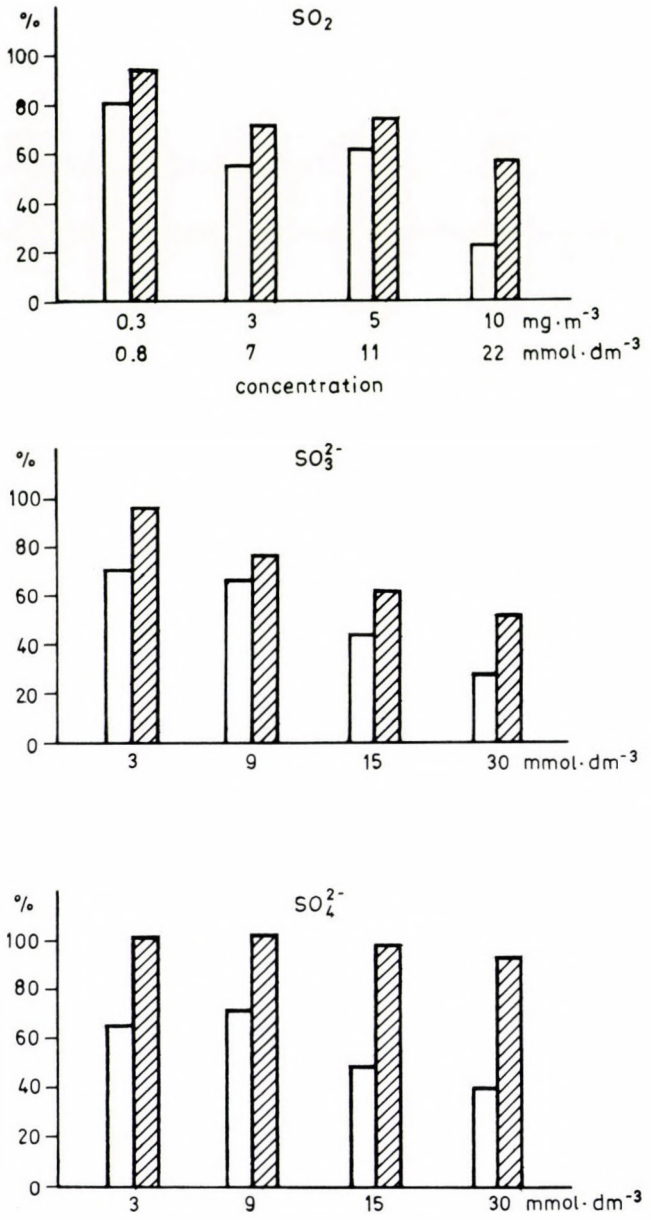

Fig. 2. The effect of different concentrations of SO_2, SO_3^{2-}, SO_4^{2-} and the rate of relative photosynthesis of capsules harvested from:
□ — unpolluted and ▨ — polluted areas. Time of treatment 12 hours

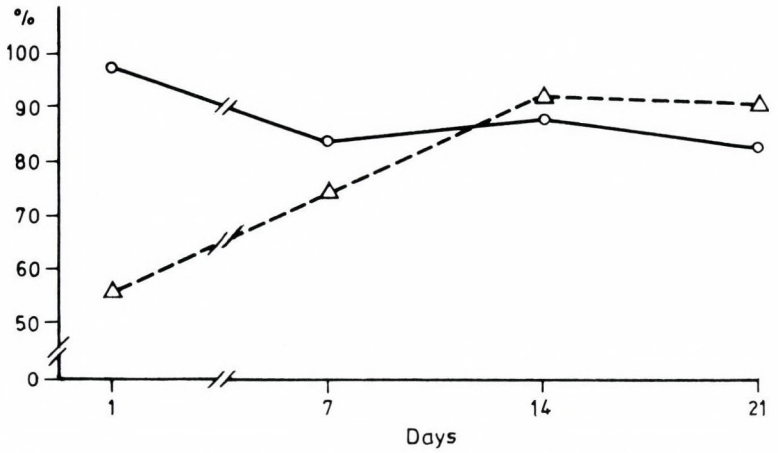

Fig. 3. Relative chlorophyll content in capsules treated continuously with 0.3 $mg \cdot m^{-3}$ SO_2. Description as in Fig. 1

to sulphite at concentration of 3 and 9 $mmol \cdot dm^{-3}$ was almost the same, amounting to about 40%. An increase in the concentration of SO_3^{2-} ions to 30 $mmol \cdot dm^{-3}$ brought about a further decrease in the intensity of the discussed process to about 60% of the control value. Sporogones from habitat B were less sensitive and the above-quoted concentration of sulphite ions reduced their photosynthesis by 40%.

The toxicity of SO_4^{2-} ions to photosynthesis of capsules from habitat A was similar to that of sulphite. Net photosynthesis was reduced by 60-70% when the concentration of sulphate ions varied within 9-30 $mmol \cdot dm^{-3}$. Sporogones from habitat B with a high level of industrial pollution were much less sensitive. Of the concentrations of SO_4^{2-} ions applied in the experiment, the highest one reduced photosynthesis by 20% only.

After the adaptation period the content of chlorophyll in relation to 1 g d.w. was the same independently of the habitat where the sporogenes grew up, its value amounting to about 5.70 mg.

When the capsules were exposed to SO_2 at concentration of 3 $mg \cdot m^{-3}$ during 12 hours in the light or dark, the content of chlorophyll decreased by 10% as compared with the control. Under the influence of SO_2 treatment in the light and dark differences in the concentration of the pigment were statistically insignificant.

Initially in sporogones from habitat A incubated in the atmosphere containing 0.3 mg $SO_2 \cdot m^{-3}$ no changes in chlorophyll content were observed.

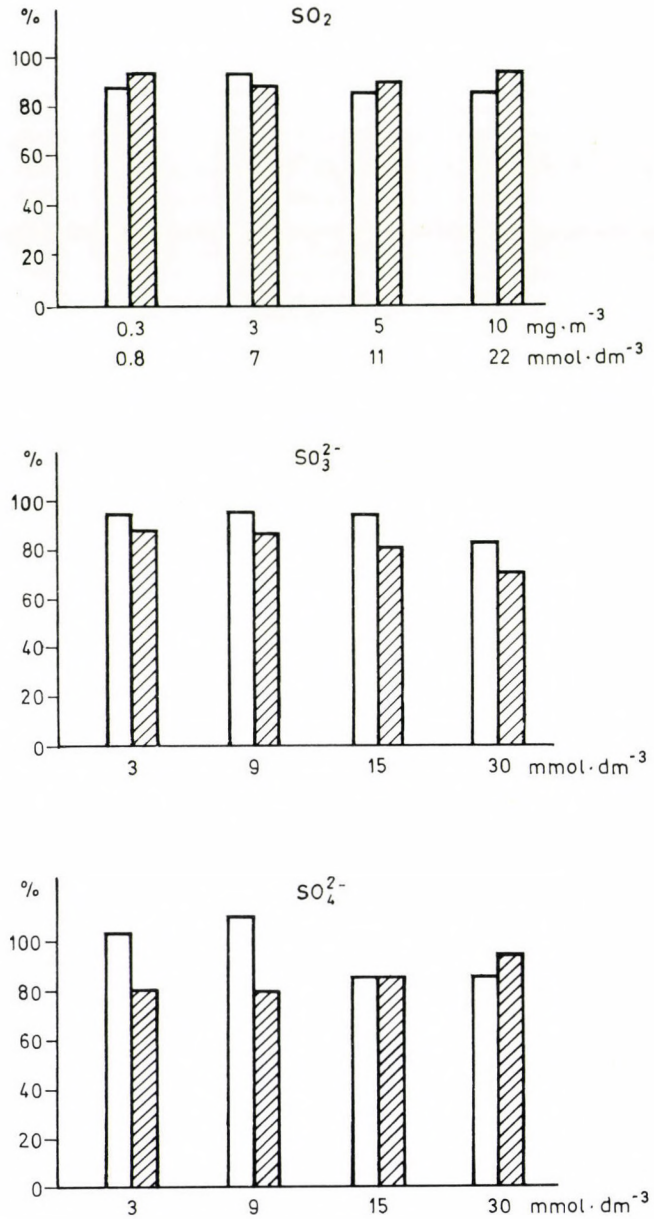

Fig. 4. Changes of relative chlorophyll a+b content in capsules at different concentrations of SO_2, SO_3^{2-}, SO_4^{2-}.
Description as in Fig. 2

Then, the total amount of the pigment gradually decreased, the final reduction of 15% as compared with the control being observed. This value did not significantly change to the 21st day of the experiment. Under the same conditions the pattern of the reaction was somewhat different in sporogones which grew up in habitat B. After a 24-hour exposure the fairly rapid drop in chlorophyll content amounted to 45% of the control value. As duration of SO_2 exposure increased, the amount of chlorophyll rose as compared with the former value. Finally only 10% of chlorophyll underwent decomposition as compared with values found in sporogones growing under control conditions (Fig. 3).

Sodium sulphite did not significantly change the concentration of chlorophyll in the capsules exposed to this substance during 12 hours in the dark. Only at the concentration of SO_3^{2-} ions amounting to 30 $mmol \cdot dm^{-3}$ the content of the pigment was reduced by 20% as compared with the control (Fig. 4).

The content of chlorophyll in the capsules which matured in habitat A, treated with lower concentrations of sulphate, was even slightly higher than in the control. An increase in the concentration of SO_4^{2-} ions to 30 $mmol \cdot dm^{-3}$ brought about a decrease in the initial chlorophyll content by 15-20%.

Discussion

In numerous species of mosses sporophytes are characterized by a distinct photosynthetic activity. The volume of photosynthetic production is significant in the total carbon balance of this moss generation (KRUPA 1969). The morphological structure and the appearance of stomata functioning for a longer part of the life of this generation (GARNEN and PAOLILLO 1973) contribute to a certain degree of independence of the sporogones from varying conditions of water supply. Gas exchange between the inside of the capsule and the outer environment occurs through stomata. Therefore, the effect of gaseous air pollution on the photosynthetic apparatus can be compared with that in flowering plants and the amount of toxic substances penetrating into the inside of the capsule is limited by diffusion resistance (MAJERNIK and MANSFIELD 1970, 1971; UNSWORTH et al. 1972; BISCOE et al. 1973; BLACK and BLACK 1979). The results of GARNER and PAOLILLO (1973) indicate that in the developmental stage of the capsules used in the present study only a part of stomatal apertures was functioning. In comparing the changes in the photosynthetic activity of leaves and capsules during a

prolonged SO_2 treatment, fairly significant differences in the inhibition of this process were observed at first. The toxicity of SO_2 to photosynthesis of the capsules was manifested somewhat later. However, after 21 days the final drop in the photosynthetic evolution of oxygen was similar. The role of stomata in limiting the toxic effect of SO_2 seems less significant. In comparing the intensity of photosynthesis of capsules exposed to SO_2 in the light and dark, the same inhibition of the process is noted. Similarly as in the case of leaves, the toxicity of SO_2 to the capsules was more drastic when the treatment was carried out in the dark. Changes in chlorophyll content in capsules fumigated in the light and dark were small and almost identical. On the other hand the toxicity of sulphur dioxide be measured by photosynthetic oxygen evolution. Mosses are among plants in which photorespiration was found (DILKS 1976). Disorders in the pattern of this process and in the respiration in the dark affect the intensity of gas exchange. It was found that a much greater drop in the intensity of the in-the-dark respiration of leaves and capsules was noted if the fumigation was carried out in the light.

The toxicity of sulphur dioxide to plants is associated with multivarious influences of this substance and its conversions occurring inside and outside the cell. The sulphite from produced in the aquatic environment is also toxic to the photosynthesis of capsules of the investigated moss species. Inhibition of photosynthesis caused by sulphite ions is comparable with effects of SO_2 in the separate ranges of concentrations. The toxicity of sulphate is particularly distinct if the capsules collected in habitat A were incubated in solutions containing 9-30 $mmol \cdot dm^{-3}$. The drop is photosynthetic intensity exceeded 60% of the control value. Sporogones from environments polluted with high concentrations of gases, were less sensitive to the applied sulphur compounds, this being particularly evident when sulphate was used in the treatment.

The above discussed results and other evidence suggest that the resistance of the investigated plants to different sulphur compounds is connected with the properties of cells where photosynthesis occurs. The lower sensitivity of plants which grew up in areas of high industrial pollution, is characteristic for gametophytes (protonema, leaves) and sporophytes. Both, the protonema and leaves have a simple anatomical structure, and therefore, their physiological activity is dependent upon water in the liquid form. Owing to the solubility of SO_2 in water, as easy penetration of the gas into the cell and a higher concentration of the compound in the

direct contact with it, can be assumed. In fact, the protonema and leaves of Funaria showed high sensitivity (the drop in photosynthesis from 50-80%), though the differences resulting from habitat conditions were evident (KRUPA, TLAŁKA 1986). This dependence was also found for sporogones, whose anatomical structure is more complex, as it was already mentioned here. Yet, the reaction to the sulphur forms applied was similar to that in the case of the gametophyte.

The differentiated sensitivity to SO_2, depending upon the degree of environmental pollution, makes it possible to perceive the basis of this reaction directly in cells which take part in photosynthesis. Changes in chlorophyll concentration are insignificant for photosynthesis, and the drop in its content is practically independent of habitat conditions. The applied concentrations of SO_2 and sulphur derivative forms indicate that Funaria hygrometrica belongs to resistant plants, though the character of the decreased sensibility is difficult to define. Certain traits of mosses, such as their small size, the close contact with the soil solution which can partly neutralize the toxicity of SO_2, or the dependence of the physiological activity upon the full hydration, can be partly responsible for decreased sensitivity, though they do not explain differences in the tolerance of industrial pollution.

Acknowledgement

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BOOK REVIEWS

editor: K. T. KISS

CHET, I. (ed.): Innovative Approaches to Plant Disease Control. — John Wiley and Sons, Inc., New York—Chichester—Brisbane—Toronto—Singapore, 1987. 372 pp.

Plant diseases are as old as agriculture itself and several controlling methods were developed based different pesticides mainly, but these xenobiotics mean potential hazards by their contamination and accumulation. These and other problems initialized more intensive research for new approaches to plant disease control. The international collective of authors are demonstrated the different viewpoints of the alternate control methods in this book presented in Wiley Series in Ecological and Applied Microbiology (Series editor: Ralph MITCHELL).

R. BAKER and F. M. SCHER (Chapter 1) discuss the possibilities of enhancing the activity of biocontrol agents, e.g. manipulation of environment, pathogens, hosts and the biocontrol agent itself. Connecting to this, the next chapter deal with the role of plant growth regulation with microorganisms, especially with fluorescents Pseudomonads, authorized by B. SCHIPPERS, B. LUGHTENBERG and P. J. WEISBEEK.

R. J. COOK and D. M. WELLER give a survey of management technics of take-all disease of cereals barley and wheat in Chapter 3. Connecting to this J. KATAN (Chapter 4) discuss the control potential of soil solarization against soil-borne pathogens. Some other aspects of control of pathogens can infect the roots or bulbs are also demonstrated. I. CHET (Chapter 6) reviews the application of Trichoderma as biocontrol agent, J. R. COLEY-SMITH presents alternative methods of controlling Sclerotium cepivorum — root of Allium, while N. C. SCHENK (Chapter 8) discuss the vesicular-arbuscular (VA) mycorrhizal fungi and the control of root diseases caused by fungi. Among of root-infecting pathogen bacteria, results of investigations on control of Agrobacterium with agrocin-producing bacteria are demonstrated by J. A. TOMLINSON in Chapter 10.

The potential of using fungal antagonists against diseases of aerial plant parts is discussed by B. DUBOS in Chapter 5. She demonstrates the microbial antagonisms including hyperparasitism of pathogens and the domestication of these agents in plant protection.

The biochemical background of pathogenesis or control of pathogens is also reviewed in this book by K. HAHLBROCK and D. SCHEEL (Chapter 11), and A. HÜTTERMANN and A. HAARS (Chapter 13) presenting the biochemical responses of plants to pathogens, and discussing the biochemical control of forest pathogens, respectively.

The complex nature of virus diseases of plants, and the perspectives in their control are treated in Chapter 11 by J. DUNEZ. He is showed the role of genetic manipulations discussed partly in other chapters, too, at different organisms. So, I. SELA's work (Chapter 15) presents genetic traits to be manipulated in plants used against viruses, while T. HASHIBA (Chapter 16) suggests using plasmids against fungus disease damping-off. On the other hand G. C. PAPAVIZAS (Chapter 9) and L. WILMITZER (Chapter 17) mention the potential of genetic manipulation in cases of biocontrol agents and host plants, respectively.

Because of complexity the book is usable well for researchers, students, biotechnologists and agronomists.

SIMAY, E. I.

JEFFREY, D. W. and MADDEN, B. (eds): Bioindicators and Environmental Management — Academic Press, London, San Diego, New York, Boston, Sydney, Tokyo, Toronto, 1991. 458 pp.

This book is a collection of materials presented at the 6th International Bioindicators Symposium held in Trinity College, Dublin, 23—28th September 1990. The volume consists of 35 papers. The aim of the symposium was "to promote the transfer of ideas regarding potential bioindicators, and originating in laboratories, into the harsher realities of field environmental monitoring". This effort has great importance because as in the introduction of the book it was mentioned that "although the concept of biomonitoring is ancient, its application to current monitoring problems is relatively slow to develop".

Papers dealing with problems of different ecosystems in several aspects were arranged under four subtitles.

The first one is "Bioindicators, Industry and Administration". This part contains 9 papers. Most of them are monitoring studies carried out in aquatic environment (marine, estuarine, freshwater) suffering from industrial pollution. There is an article in this chapter, which deals with an interesting, current theme; monitoring the effects of agricultural pesticides on wildlife.

In the second part of the book we can get information about "Environmental Radioactivity and Biomonitoring of the Chernobyl Accident" on the basis of 3 papers.

Under the third subtitle — Monitoring Long Term and Large Scale Environmental Trends — 5 papers can be read. Using the structure of animal communities, presence, abundance and distribution patterns of sensitive indicator plants as bioindicators of environmental changes were discussed. Provision for Areas of Outstanding Natural Beauty was also mentioned in this part.

The last chapter deals with "Basic Research in Biomonitoring". This is the most extensive part of the book. Animal behaviour, productivity, physiological, enzyme activity changes of test organisms can also be used to indicate environmental pollutants. Information about recent developments on this field can be available in more than dozen papers of this part.

This book is very useful for scientist working in environment and nature conservation. In the great variety of papers included in this book everybody can find valuable, interesting particulars relating to his own research field.

B. PAPP

KOLTIN, Y. and LEIBOWITZ, M. J. (eds): Viruses of Fungi and Simple Eukaryotes. — Marcel Dekker, Inc., New York and Basel, 1988. 434 pp.

Discussing the different aspects of viruses could infect fungi and some other eucaryotes, this book was published in Mycology Series edited by P. A. LEMKE under No. 7. This volume treats the results of researches in 19 chapters.

Viral diseases could cause medicinal or phytopathological problems were known for long time before the true nature of infection agents would be discovered. Similar to those, viruses of fungi and simple eucaryotes were also finding after the symptoms were known for years ago. One of these viral disease is an infection of mold fungus Penicillium stoloniferum. The prophylactic activity of its culture filtrate against some viral infections was observed in the early 1950s, but its viral nature was determined about ten years after, as it is reviewed in Chapter 1 by W. J. KLEINSCHMIDT.

The next three chapters present the results of work with retrovirus like Ty in Saccharomyces cerevisiae, including the description of viral nature of Ty by J. D. BOEKE and D. J. GARFINKEL (Chapter 2), results of investigations held on transcriptional regulation of Ty elements presented by F. WINSTON in Chapter 3, and a number of Ty- and δ-mediated recombination events in several regions of the genome described by S. W. LIEBMAN and S. PICOLOGLOU (Chapter 4).

An other part of the book deals also with S. cerevisiae, but an other type of viruses is discussed in it. These viruses contain dsRNA. T. FUJIMURA, R. ESTEBAN and R. B. WICKNER discuss the replication of dsRNA in Chapter 5, while J. A. BRUENN et al. (Chapter 6) give description of structure, transcription and replication of Killer Virus dsRNAs. There are informations about transcription and translation of KV genome in the next chapter authorized by M. J. LEIBOWITZ, I. HUSSAIN and T. L. WILLIAMS, suggesting the useful as model system for the study of viral gene expression in eucaryotic cells.

Some other chapters also discuss the killer systems of fungi, especially of yeasts as Chapter 8 (H. BUSSEY et al.) and Chapter 9 (S. L. STURLEY et al.). Y. KOLTIN (Chapter 10) describes the killer system of Ustilago maydis, a basidiomycete causing serious disease of corn, and J. S. KANDEL (Chapter 11) discuss the co-connections of killer systems described in different fungi and the pathogen yeasts.

A third type of extrachromosomal gene-holder is described in Chapter 12. by N. GUNGE. It is the linear DNA plasmid, known from different eucaryotes earlier, and from Kluyveromyces, too. The description with results of genetic studies are giving in this chapter.

The next part of the book gives some interest informations connecting with plant pathogenic fungi and the viruses of them. J. TARTAGLIA and D. L. NUSS (Chapter 13), and J. A. DODDS, R. A. VALVERDE and D. M. MATHEWS (Chapter 14) deal with structural properties of and detection and interpretation of dsRNAs, respectively. Some other works discuss viruses of serious pathogens Ophiostoma ulmi (Chapter 15 by H. J. ROGERS, K. W. BUCK and C. M. BRASIER), Helminthosporium victoriae (Chapter 16 by S. A. GHABRIAL), Endothia parasitica (Chapter 17 by N. K. van ALFEN) and Rhizoctonia solani (Chapter 18 by A. FINKLER, B.-S. BEN-ZVI and Y. KOLTIN).

Chapter 19 by J. L. Van ETTEN, A. M. SCHUSTER and R. H. MEINTS gives a review of viruses of Chlorella-like algae pointed out the less informations about them as they are about fungal viruses. However twenty-nine plaque forming viruses are mentioned in this chapter.

According to the fine data presented in this volume, the book may be essential for virologists, general mycologists, plant pathologists even for students.

SIMAY, E. I.

KRAMMER, K.—LANGE-BERTALOT, H.: Bacillariophyceae, 4. Teil: Achnanthaceae, Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema, Gesamtliteraturverzeichnis. In: ETTL, G., GÄRTNER, J., GERLOFF, J., HEYNIG, H., MOLLENHAUER, D. (eds): Süsswasserflora von Mitteleuropa, Band 2/4, G. Fischer Verlag, Stuttgart, Jena, 1991. 436 pp.

The book of diatoms is finally completed with this volume. Its structure is similar to that of the previous volumes. The editors' preface is followed by the determination key of the algal classes, the table of contents and a short introduction by the authors. Next we find a detailed description of the Achnantes and Cocconeis genera in a manner similar to that found in the previous volume (determination key and description of species and species groups, with many photos). It is followed by a supplement concerning the Navicula and Gomphonema genera, with 29 pages of micrographs. The bibliography includes works quoted in the previous volumes. This book ends with a list of species and corrections of figure legends from volumes 2/1 and 2/3 (e.g. bluegreen alga instead of Skeletonema potamos).

The publishing of the fourth volume completed the information about the family Naviculaceae, first of all about Navicula lineolatae group and some Gomphonemata forms. In contradiction to many other Pennatae taxa only a few new species was described in the 20th century from the Navicula lineolatae group. First of all the varieties of "old" classical species are distinguished. Although many of them would easily separated from the base form. There are many characteristic forms, among the varieties which would raise to species level on the basis of the comparison of large range of micrographs. The addenda about the Navicula lineolatae and Gomphonema contain the results of the investigation of material from the region of Alps and middle mountains. New species of the warmer climatic zones are published in the series Bibliotheca Diatomalogica. Unfortunately however the North American material is completely confused because of the low quality drawings and micrographs.

At the Gomphonema genus the taxonomical and nomenclatural information is insufficient. It is mainly caused because of the not clearly determined species of Ehrenberg. The holotypes are not known, so lectotypes are nearly impossible to select, the authors are forced to do a "second-hand" identification. Unfortunately the separation of different species on the basis of SEM micrograps did not have good results.

The authors call the attention of specialists to the danger of separating the Gomphonema genus to new genera without comprehensive studies (to separate for example Gomphonema, Gomphoneis and small, sometimes unispecific genera). In the authors opinion besides the investigations of type materials the analyses of a large range of micrographs from the same form group, of different hydrobiological, ecological or geographical data would only make clear the taxonomical problems.

We cannot undertake the task of comparing all of the 3620 listed works with those cited in the four volumes. But certainly some of the works that were mentioned in the discussion of some genera and species (e.g. Cyclotella caspia Grunow — KISS et al. (1988), C. hakanssoniae WENDKER — WENDKER (1990), C. stelligera CLEVE et GRUNOW — HAWORTH (1986): Stephanodiscus agassizensis HAKANSSON et KLING — HAKANSSON et KLING (1989) etc.) are missing from the bibliography. There are also some studies in the bibliography that are not found in any of the volumes (e.g. GENKAL et HAKANSSON: 1990 — about Stephanodiscus minutulus (Kützing) CLEVE et MÖLLER, GENKAL et MAKAROVA: 1985 — about Cyclostephanos dubius (FRICKE), ROUND, BELCHER et SWALE: 1978 — about Skeletonema potamos (WEBER) HASLE and Cyclotella atomus HUSTEDT etc.).

Every volume of the now completed work is indispensible for every algologist, botanist and applied hydrobiologist. It is also an important book for university education.

K. T. KISS et A. SCHMIDT

KRAMMER, K.—LANGE-BERTALOT, H. (Unter Mitarbeit von HAKANSSON, H. und NÖRPEL, M.): Bacillariophyceae, 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. In: ETTL, H., GERLOFF, J., HEYNIG, H., MOLLENHAUER, D. (eds): Süsswasserflora von Mitteleuropa, Band 2/3, G. Fischer Verlag, Stuttgart, Jena, 1991. 576 pp.

The third volume of the series of books about diatoms is the most heterogeneous. One reason for this is that the book concerns not only the two Pennales families, but the 15 genera of the Centrales order, which are in many respects different from the Pennales. Another reason is that two colleagues assisted KRAMER and LANGE-BERTALOT, who co-authored the first two volumes, in writing some of the chapters in this volume:

Centrales:

— **Melosira, Orthoseira, Ellerbeckia, Aulacoseira, Skeletonema, Acanthoceras, Chaetoceros, Rhizosolenia, Pleurosira, Actinocyclus** — KRAMMER

— **Cyclotella, Cyclostephanos, Stephanodiscus, Thalassiosira, Stephanocostis** — HAKANSSON

Pennales:

— **Tetracyclus, Diatoma, Meridion, Asterionella** — KRAMMER

— **Tabellaria, Synedra, Fragilaria, Opephora, Hannaea, Centronella** — LANGE-BERTALOT

— **Eunotia, Actinella, Peronia** — LANGE-BERTALOT and NÖRPEL

The book is organized so that the editors' preface and the table of contents are followed by the authors' preface. The introduction deals primarily with terminology. It is practically an addition to the morphological glossary of volumes 2/1 and 2/2. Unfortunately, this volume includes only the German terminology, unlike volume 2/1 in which we can find the English, French and Latin equivalents.

The second chapter begins with the determination key of the genera of Centrales order. It is followed by a short general description of the first genus **(Melosira)**, the determination key of the species, and a morphological description of the species. This construction is typical for the remeinder of the book. The morphological description is primarily based on

marks seen in a light microscope, although for several species it mentions microstructure studied under electronmicroscope. The distribution of the species, habitat and life style, and occasionally their bioindicator (saprobity, trophity) characteristics are also given. All of these characteristics, with 166 picture tables containing 5-20 pictures each, help to determine each species. Most of the pictures are light microscopic (LM) micrographs; a few are electron-microscopic (EM) pictures. The book ends with a list of the species.

This volume does not achieve the quality of volumes 2/1 and 2/2, which can possibly be attributed to the four authors. Before starting our critical comments, we would like to mention that we are well aware of the great difficulty that is most noticeable in the Centrales order. New genera, many new species and sub-species taxa have been described in the Centrales order in the last 10-15 years as a consequence of the ever extending use of the electronmicroscope. Many of these descriptions are not thorough or cautious enough. Therefore unifying them in a common system is a hard task. Unfortunately, the authors were not able to fulfil this task entirely.

— Those who do not know the Centrales order thoroughly can be misguided even at the level of genera. E.g., according to 10a (determination key of Centrales — see page 6) — areoles are arranged in sectors — we cannot get to **Thalassiosira**, as this feature is characteristic only in the **Actinocyclus** and **Coscinodiscus** genera. Possible misguidance as a result of imprecise or inaccurate specification might also occur in the **Cyclotella, Cyclostephanos, Stephanodiscus, Thalassiosira** species. It is even more disturbing since the key should be the base to distinguishing species.

— It would be very important to have the main characteristics of EM structure in addition to light microscopic structure especially for the tiny **Cyclotella, Cyclostephanos, Stephanodiscus, Thalassiosira** species. They are often missing or incomplete. In these cases descriptions should have been supplemented with TEM or SEM photos. As a consequence, the separation of very closely related species is almost impossible. (E.g. **Cyclotella caspia** GRUN, — **C. hakanssoniae** WENDKER, or **Cyclostephanos costatilimbus** (KOBAYASHI et KOBAYASHI) STOERMER, HAKANSSON et THERIOT, **C. tholiformis** STOERMER, HAKANSSON et THERIOT the list can be continued.) The situation is similar with more separable species in which cell sizes overlap (e.g. the **Cyclotella hakanssoniae** WENDKER in Fig. 3 in Table 46 can be confused with the **Cyclotella michigenia** SKVORTZOW in Fig. 12, other examples can be found).

— It is an old tradition of the series — and a favored one — that, since many of the species are cosmopolitan, more than just Central-European species are included in the book. Therefore, it is acceptable that **Cyclotella stylorum** BRIGHTWELL from tropical seas; **C. baikaliensis** SKVORTZOW known only from Lake Baikal; **Cyclostephanos tholiformis** STOERMER, HAKANSSON et THERIOT and **Stephanodiscus agassizensis** HAKANSSON et KLING from North America; **S. aegyptiacus** EHRENBERG etc. got a place in the book. It is not understandable why other species also found in Central Europe were left out or not described in detail (e.g. **Cyclotella cryptica** REIN, LEWIN et GUILL., **C. costei** DRUART et STRAUB, **C. meduanae** GERMAIN, **Stephanodiscus delicatus** GENKAL, **S. makarovae** GENKAL, **Thalassiosira faurii** (GASSE) HASLE, **Th. guillardii** (HASLE). The reason for omission can in some cases be the species' uncertain taxonomical position. Although these species should nevertheless have been mentioned and discussed. The fact that they were not mentioned is especially disturbing because there are possibly synonymous species like **Cyclotella hakanssoniae** WENDKER or Stephanodiscus parvus STROERMER et HAKANSSON, which is considered a synonymous or invalid, taxon by many authors. On the other hand some uncertain Stephanodiscus species can be rightly found in a separate chapter.

— It might be reasonable to exclude Cyclotella woterecki HUSTEDT, but why is there a picture of it? or, if there is a picture of C. stelligeroides HUSTEDT, why is it not mentioned? All the ambiguous "stelligeroid" Cyclotella species should have been discussed together.

— The quotations are inconsistent, too. E.g. the main works are found at the end of the general description of Stephanodiscus. HAKANSSON is the author or co-author of 10 of the 25 papers, but other important names like GENKAL, KOBAYASHI or KLEE et STEINBERG are missing. It is probably only a mistake that the papers HAKANSSON et KLING, 1989, 1990 listed in the important works are missing from the references.

— The following can be seen just as minor mistakes. Next to the two super SEM micrographs of Skeletonema potamos (WEBER) by HASLE in Table 85, there are two LM pictures of a blue-green alga species.

In spite of these critical comments, I recommend this book not even to those who already possess the first two volumes. It will be very useful for algologists in their daily work, botanists dealing with general taxonomy, and applied hydrobiologists. It is an essential, basic systematic work for university education.

K. T. KISS

ROUND, F. E. (ed.): Algae and the Aquatic Environment. Contributions in honour of J. W. G. LUND, C.B.E., F.R.S. — Biopress Ltd., Bristol, 1988. 460 pp.

ROUND wrote the following in the preface "This volume is dedicated to John LUND in his 75th year. The contributors are all phychologists or limnologists who have in some way or other been associated with John either personally or by the nature of their work. The result is 24 papers covering a wide range of topics and as editing proceeded, it was realized that every contributor without making a special effort had written a paper which reflected the interests of John LUND." He also wrote a long appreciation of LUND, the phychologist polymath listing his publications (143 from 1935 to 1988) in the preface as well. The topics of the articles are certain species, species groups, their biology and taxonomy; the seasonal variation of a species or the phytoplankton; the phytoplankton of the Lake District; the geological and chemical regulation of processes in lakes, nutrient cycles, the application of the LUND tube technics; research on rivers. This division is arbitrary in many respects, as the papers often cover more than one of the above mentioned topics i.e. TALLING and HEANEY's article (Long-term Changes in Some English (Cumbrian) Lake Subjected to Increased Nutrient Inputs) includes not only the seasonal changes of SiO_2, NO_3-N and PO_4-P between 1945 and 1986 but also describes the hypolimnetic oxygen depletion and the phytoplankton writing about the abundance of sevaral predominant species as well. ATKINSON, EVANS, BAILEY-WATTS and FRIFEROVA also deal with the seasonal changes of a phytoplankton species. FOGG and BAJPAI investigated the seasonal changes in the phytoplankton as a function of the temperature. The ecological or taxonomical investigation of a species or genus is the topic of GIBSON and FOY's (Melosira italica ssp. subarctica), MANTON's (Stelexomonas-Aulomonas), BOURRELLY's (Micrasterias lundii nov. sp.), and HAWORTH's (Melosira, mainly Aulacoseira spp.) work. HAWORTH reports on a few considerable taxonomical results helping the cognition of Melosira genus consisting of hardly determinable species. The same can be stated CRAWFORD (Melosira arenaria, M. teres), and ROUND (Pinnularia cardinaliculus) articles summarizing the results of EM diatom investigations. A special interest should be given to the papers of HAPPY-WOOD, KENWAY, ONG, CHITTENDEN and EDWARDS investigating the role of nano and picoalgae in the phytoplankton production. The first article on picoplankton and its often very important role in the primary production of freshwaters was published only a couple of years ago. These 0.5-2 μm long planktonic organism were often listed as a part of the bacterioplankton. Fluorescence labelling made clear that many of them photosynthesize. The very important picoplankton role in the production of freshwaters has also only recently been investigated.

From among the papers on running waters REYNOLD's work must be mentioned, that summarizes and reevaluates the key problems of potamoplankton while it also gives an outline of the probable future research (Potamoplankton: paradigms, paradoxes and prognoses).

The Algae and the Aquatic Environment volume provides a large-scale overview of algology by discussing different brand-new results. The presswork, the diagrams and micrographs are excellent making this book even more valuable. This book edited by ROUND can highly be recommended to all algologists, hydrobiologists and ecologists working on freshwaters.

K. T. KISS

TZARENKO, P. M.: Kratkij opredelitel chlorococcovih vodoroslei Ukrainskoi SSR (A Guide for the Determination of Chlorococcales species Occurring in the Ukraine). — Naukova Dumka, Kiew, 1990. 207 pp.

The young Ukrainian author touches on a great tradition marked by such basic algological works as that of KORSIKOFF (1953), which are still in use today. In the introduction TZARENKO tell us that of the 1200 species belonging to the Chlorococcales order 450 are known in the Ukraine. These algae are important components of several ecosystems. They play an important role in both primary production, as producers of organic material and oxygen, and directly and indirectly in secondary production, as fish food. They are actively involved in the self purification of waters and in establishing water quality. Many species are indicators of saprobity and trophity. Moreover, mass cultivation of these algae can surely provide a basis for both human and animal food. These are all reasons why the author finds it important to broaden our knowledge of this order. He gives valuable data on 239 species and 286 subspecies taxa.

The introduction, which deals with general questions, begins with a description of the main morphological features of the Chorococcales species. Unfortunately, pictures are shown only when there is an explanation of cell and chloroplasts shapes. Summarizing other morphological characteristics in pictures would have been helpful. The author gives a short overview of ecological problems, the role of Chlorococcales species in the life of nature and man, the methods of investigation and sample collection, and the taxonomy of Chlorococcales. This chapter ends with a list of the families and genera of the Chlorococcales species.

The following chapter, in which the author introduces all the Chlorococcales families found in the Ukraine, is very detailed. This includes the determination key of the families and then a separate section on each of the families. Within the family description we find the keys of the subfamilies and genera. There are families from which only one species is mentioned, and there some form which none is mentioned (Chlorochytriaceae (G.S.WEST)SETCHELL et GARD.), Dicranochaetaceae BOURR.).

Each section begins with the morphological and taxonomical characteristics of the family, subfamily and genus. We can find data on distribution and habitat in the description of species. In addition to a detailed morphological description, high quality drawings (usually 2-4) help in the determination of species. The author's original drawings of many species are included. The taxonomical position of the species is mentioned with correct references in many cases. The book ends with the bibliography, a list of the discussed taxa and the table of contents.

Some further critical comments need to be made. The first and most important criticism is that, although the author uses many references in his book and knows the latest works, these are not mentioned in the bibliography, which includes only 32 studies, a small fraction of those mentioned in the text. The proper references are missing from the newly described genera and species, or combinations (e.g. Sphaerocystidaceae FOTT ex TZARENKO (1991) family, Topaczevskiella MASSJUK (1985) genus, Topaczevskiella nautococcoides MASSJUK (1985), Ankyra viridis (MASSJUK) TZARENKO (1990), Coenochloris fottii (HIND:) TZARENKO (1990), Characium sieboldii var. simplex (KORSCH) TZARENKO (1990), Scenedesmus polessicus TZARENKO (1984), S. serrato-pectinatus (CHOD.)TZARENKO (1990)). It would have been useful to present a greater number of international literature, especially for the Russian speaking algologists working in the former Soviet Union. Likewise, there are many studies in Russian and Ukrainian that are unknown to foreign scientists because of the difficulties in obtaining Soviet periodicals and journals.

The author follows the approach of the latest taxonomical works in some of the species, but he is more conservative in others. In several cases he changes sub-species taxa (writing "varietas" instead of "form") without justification (e.g. Scenedesmus acuminatus var. tortuosus SKUJA (1936), which SKUJA originally described this taxon as a form; Scenedesmus apiculatus var. indicus (HORTOB.)HORTOB. (1969), although it was originally S. apiculatus (W. et G.S.WEST) R. CHOD. var. indicus HORTOB., and later this taxon became the basionym of Steinedesmus indicus (HORTOB.)COMAS et KOM.). There are other inconsistencies in TZARENKO's work, but this sort of inconsistency appears in other books on Chlorococcales.

There are also mistakes in the references to illustrations. On page 71 one can read that Fig. 15/7 is a drawing of FILIPOSE; in fact, this is the iconotype of Micractinium crassisetum HORTOB.

The book's greatest disadvantage is that it was published in Russian and is scarcely available outside the Ukraine, and even there it is not easy to find. We should not forget, though, that the book was written mainly for algologists working in the Soviet Union. In spite of this "geographic isolation", TZARENKO's book can be of use to every algologist. It is, consequently, recommended for everyone interested.

A. SCHMIDT and K. T. KISS

WHITTON, B. A.—ROTT, E.—FRIEDRICH, G. (eds): Use of algae for monitoring rivers. — Proceedings International Symposium held at the Landesamt für Abfall Nordrhein-Westfalen Düsseldorf, Germany, 26—28 May 1991. ISBN 3-9500090-0-0, 193 pp.

Since the study of rivers began almost a 100 years ago, a large amount of data has been gathered, and the relationships and principles concerning biological processes in rivers have been established. Our knowledge in rheobiology is still poorer than our limnobiological knowledge. One, though not the dominant, reason for this is that limnological studies are older. A more important reason seems to be that studying rivers in methodologically much more difficult.

Studying large rivers that run through several countries requires considerable coordination of data collection and investigation. Coordination is complicated even for rivers such as the Danube, for which an international organization (IAD = International Arbeitsgemeinschaft der Donauforschung) has been in existence for more than 25 years.

River algology (phytoplankton, phytobenthon), a very important aspect of rheobiology, was the topic of an international symposium held between 26—28 May 1991 in Düsseldorf, Germany. Papers in this book provide answers to the many questions that arose and show that algological studies are pursued differently in different European countries.

Because the topics of the 28 papers are so varied, the table of contents would be particularly informative.

As each paper refers to many other works, their bibliographies give wide-ranging information concerning algological studies in rivers.

In addition to the papers there are two short chapters in the book. One is the list of the authors' current addressess; the other is the introduction.

The book can be recommended primarily to hydrobiologists in both theoretical and applied areas, algologists, and specialists in water management and environmental protection. This volume is especially pertinent in Hungary today because some hydrobiological methods are about to be standardized and the system of water and environmental classification is soon to be reorganized.

A. SCHMIDT

WORBES, M.: Lebensbedingungen und Holzwachstum in zentralamazonischen Überschwemmungswäldern. — E. Goltze GmbH et Co. KG, Göttingen, 1986. 122 pp.

The book is divided into the following chapters: 1. Introduction, 2. The studied area, 3. Width expansion of trees, 4. Discussion: Strategic coordination of the life conditions of trees, 5. Summary, 6. English summary, 7. Portuguese summary, 8. Bibliography (144 references).

The author studied the width expansion of tree species in the vegetation of two areas (Taruma Mirim-Igapó, Ilha de Marchantaria-Várzea) in the tropical forest of the catchment area of the middle Amazon. The areas are situated 20 kms northwest and 20 kms southeast of the town

of Manaus, around the Rio Negro and Rio Solimoes. Annual precipitation is over 2000 mm. The mean annual temperature is 27.2 °C. The vegetation of the catchment area is flooded in May, June and July under up to 4 m of water. The author studied the area's nutrient and mineral supply and the materials with which the floods enrich or deplete the soil. He also studied the manner in which floods change the physical state of the soil and the influence of these changes on plant development. He found that the two areas have different species composition. The Igapó area has 54 species from 20 families. The Várzea area has 33 species from 22 families. These species are woody, semi-woody. They seem to be unusually low in number.

He measured the width of the tree rings in the Moraceae family **Pseudoxandra polyphleba** (Annonaceae), Ilex sp. (Aquifoliaceae), **Rourea** sp. (Connaraceae), **Nectandra amazonum** (Lauraceae), **Swartzia** sp. (Fabaceae), **Parkia auriculata** (Mimosaceae), **Sorocea duckei** (Moraceae), and carried out anatomical investigations of 13 tree species.

WORBES' anatomical and developmental studies on the trees of the Igapó and Várzea rain forest are pioneering. We can recommend this book, which is illustrated with 72 figures and photos, to vegetation-researchers, ecologists and those interested in hystology.

K. BABOS

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3. References
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Examples:

Jakucs, P. 1961: Die Phytozönologischen Verhältnisse der Flaumeichen-Buschwälder Südostmitteleuropas. Akadémiai Kiadó, Budapest.

Fekete, G., Précsényi, I. 1981: Niche structure of a perennial sandy grassland. In: Stefanovits, P., Berczik, Á., Fekete, G., Seidl, M. (eds): Man and the Biosphere Programme. Survey of 10 Years Activity in Hungary. Budapest, 68–102.

Borhidi, A., Muñiz, O., Del Risco, E. 1979*a*: Clasificación fitocenologica de la vegetación de Cuba. Acta Bot. Hung. **25:** 263–301.

Jakucs, P. 1973: „Síkfőkút Projekt". Egy tölgyes ökoszisztéma környezetbiológiai kutatása a bioszféra program keretein belül (Síkfőkút-Project. Environmental-biological research of an oakwood ecosystem within the framework of the Biosphere program). MTA Biol. Oszt. Közl. **16:** 11–25.

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