COLLETOTRICHUM ACUTATUM: SIMMDS. F.SP. *PINEA* ASSOCIATED WITH "TERMINAL CROOK" DISEASE OF *PINUS* SPP.

JOAN M. DINGLEY
Plant Diseases Division, Department of Scientific and Industrial Research, Auckland,

and

J. W. GILMOUR
Forest Research Institute, New Zealand Forest Service, Rotorua

(Received for publication 6 September 1971)

ABSTRACT

Colletotrichum acutatum, Simmds. f.sp. **pinea** n.f. sp. has been shown to be the fungus associated with "terminal crook" disease of seedlings of **Pinus radiata** D. Don, **P. contorta** Dougl., **P. elliottii** Engelm., and **P. pinaster** Ait. in New Zealand forest nurseries in Northland, Central Auckland, South Auckland-Bay of Plenty, and East Coast. It is shown also to be a primary pathogen in **Lupinus arboreus** L, and **L. angustifolius** L. Laboratory inoculation tests have shown that it is capable of infecting seedlings of **Lathyrus odoratus** L. and **Vicia sativa L.** and is a wound pathogen of ripe tomato fruits (**Lycopersicon esculentum** Mill.). Isolates obtained from naturally infected plants of **Lathyrus odoratus** and **Lupinus arboreus** did not infect seedlings of **Pinus radiata.**

INTRODUCTION

In New Zealand, "terminal crook" disease is recorded on seedlings of *Pinus radiata* D. Don, *P. contorta* Dougl., P. *elliottii* Engelm., and *P. pinaster* Ait. The studies reported here were instigated primarily to clarify the identity of the fungus responsible for "terminal crook" disease of pine seedlings, and to find possible sources of infection in New Zealand.

Gilmour (1965) recorded a *Gloeosporium* sp. attacking tips of *P. radiata* seedlings in the New Zealand Forest Service nurseries at Woodhill in 1963, Kumeu in 1964, and Kaikohe in 1965. He noted that, unlike *Diplodia pinea* (Desm.) Kickx and *Botrytis cinerea Pers.* ex Fr., this fungus did not penetrate mature tissues through wounds but behaved as a primary pathogen attacking immature leaves and apical buds of young seedlings. The fungus penetrated immature stem tissue and, as a result, tips of young seedlings curved over and developed a characteristic terminal crook. Although apical growth was stopped the fungus infection appeared to stimulate diameter growth which resulted in stunted, thickened, malformed seedlings with hard stems. Subterminal buds which developed after some time produced healthy trees if there was no reinfection.

N.Z. Jl For. Sci. 2 (2): 192-201

Subsequently the disease has appeared in forest nurseries near Kawerau in 1966, Tokoroa in 1968, Gisborne and Kaingaroa in 1970, and Rotorua in 1971.

Simmonds (1965) recorded *Colletotrichum acutatum* Simmds. on *Pinus elliottii* in Queensland, Australia. It was associated with "terminal crook" disease of seedlings of *P. elliottii* in Queensland (Anon., 1967) and in 1968 the disease was recorded also on *P. caribaea* Morelot (Anon., 1968a). It was also found on *P. radiata* at Mt Stromlo nursery in Canberra (Anon., 1968b).

Gibson and Munga (1969) described "terminal crook" disease associated with *Colletotrichum acutatum* on *P. radiata* from nurseries in Kenya, East Africa (North Kinangop in 1956, Kimothon and Mount Elgon in 1962). Lim (1970) reported seedling blight of *P. caribaea* caused by *C. gloeosporioides* Penz. from nurseries in Kapong, West Malaysia, but symptoms of this disease differ from those of "terminal crook".

COLLETOTRICHUM ACUTATUM, ITS IDENTITY AND OCCURRENCE

The heterogenous nature of species classified under *Gloeosporium* Desm. & Mont., was noted by von Arx (1957a, 1957b, 1970). He pointed out that in the type species *G. castagnei* Desm. & Mont. conidia were typically two-celled whilst in most other species disposed in this genus by later authors, conidia were continuous. As *G. castagnei* is typical of species now included in *Marssonina* Magn., *Gloeosporium* was regarded by von Arx as a synonym of *Marssonina* and he disposed species of *Gloeosporium* with unicellular hyaline conidia under a number of different genera according to the manner in which conidia were formed. Von Arx (1957a, 1957b) included within *Colletotrichum* Cda. species where conidia were formed in basipetal succession on phialide-like cells borne on smooth or setose acervuli. In keying out species included in this genus he noted that conidia germinated to form dark coloured appressoria. He adopted a broad definition of many species and listed more than 600 names as synonyms of *C. gloeosporioides* Penz. the conidial form of *Glomerella cingulata* Stonem. & Schr. Already Mueller & von Arx (1954) had listed 100 synonyms for this ascigerous stage.

When studying *Colletotrichum* associated with ripe fruit rots in Queensland, Australia, Simmonds (1965, 1968) recognised in part von Arx's broad concept of *C. gloeosporioides* but described the closely related *C. acutatum* as a new species. Simmonds (1965) noted that conidia of *C. acutatum* were fusiform rather than oval or oblong, 2.5-4.5 μm broad, and variable in length. Sub-cultures of *C. acutatum* from Simmonds, have been compared with New Zealand material of *Colletotrichum* sp. with fusiform rather than oval conidia and were found to be identical. Von Arx (1970) noted that in *C. acutatum* conidia were cylindrical with attenuated or pointed ends 8-15 $\times$ 3-4 μm.

In New Zealand, conidia from diseased pine needles and from cultures from seedlings infected with "terminal crook" disease are typically cylindrical to fusiform, 9.5-15 $\times$ 3-4 μm. They are phialospores borne on simple conidiophores, the conidiophores usually united to form a sporodochium or acervulus; brown setae 25-64 $\times$ 2.5 μm may or may not be present on the acervuli (Fig. 1). In culture, especially on potato dextrose agar, sectoring is common in both *C. gloeosporioides* and *C. acutatum;* so only fresh cultures should be studied. On this medium *C. acutatum* differs from *C. gloeosporioides*

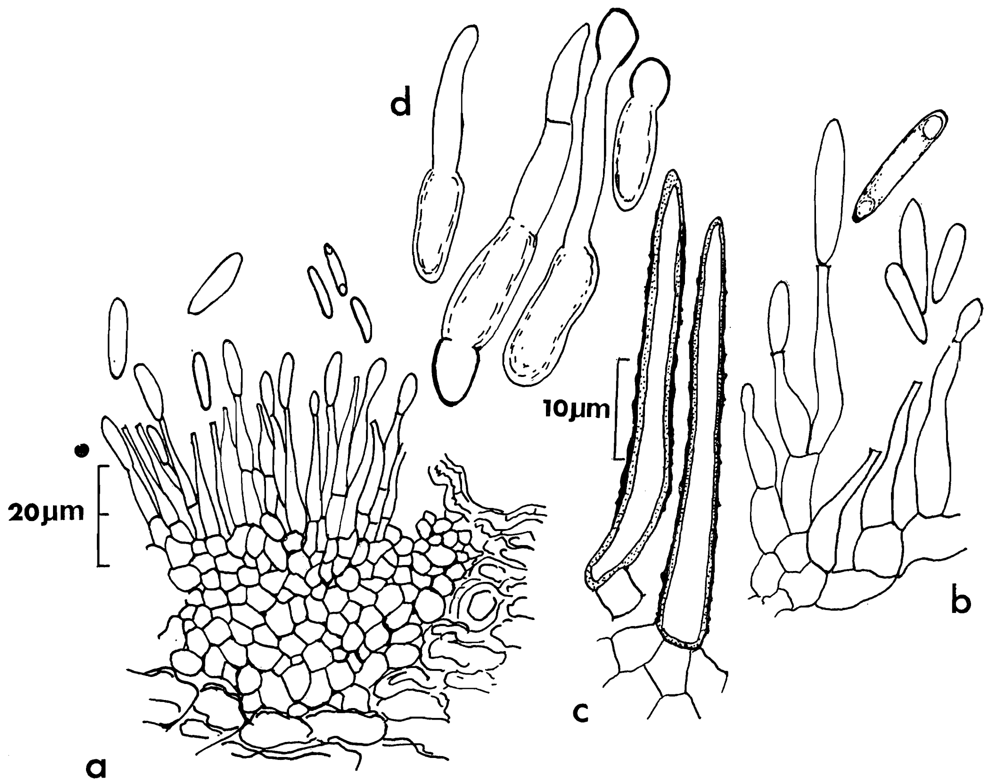


FIG. 1—**Colletotrichum acutatum** f.sp. **pinea** n.f. sp. (a) Acervulus ((b) Conidia produced in basipetal succession on phialide-like conidiophores (c) Germinating conidia (d) Setae.

in that *C. acutatum* always produces a carmine pigment, sporodochia are more brightly coloured, and not usually associated with dark sclerotial-like bodies. Sometimes conidia are borne singly among aerial mycelium on terminal phialide-like cells. At the optimum temperature (24°-26°C) growth is slower than that of *C. gloeosporioides* but, after continuous sub-culturing, the cultures tend to become grey-white, floccose and sterile. In some respects *C. acutatum* may be confused with *C. coccodes* (Wallr.) Hughes as well as with *C. gloeosporioides.* A comparison of morphology and cultural characters of New Zealand isolates of these three species is given in Table 1.

Analysis of measurements of conidia from a number of cultures selected at random, all of which could be included within the broad concept of *C. gloeosporioides* as defined by von Arx (1957a), showed that length alone was of no value for discriminating between different isolates. The function of width over length (W/L) gave highly significant differences as did L/W (although this has a smaller F value). It was finally found that the best linear function for discriminating species was length × 6 width. Analysis of measurements of conidia using this method showed that conidia of *C. acutatum* were significantly distinct from *C. gloeosporioides.* No significant differences were found in conidia of cultures of *C. acutatum* obtained from rots of ripe fruits of tomato (*Lycopersicon esculentum*), tree tomato (*Cyphomandra betaceae* (Cav.) Sendt.),

TABLE 1—Comparison of **Colletotrichum coccodes, C. acutatum** and **C. gloeosporioides** grown in culture on potato dextrose agar

Species	Macro-characters	Conidia (μm)	Setae (μm)	Appressoria (%)	Optimum Temp. (°C)	Growth 17 days (cm)
C. coccodes	No superficial mycelium. Sclerotia large and abundant. Media pigmented, sometimes pale salmon, often brown.	On sclerotia. Cylindrical-oval. 8.5-26.5 × 2.5-3.5	Present. 65-135 × 2.5-3	Approx 75 Irregularly shaped.	24-28	8-5
C. acutatum	Grey superficial mycelium. Sclerotia poorly developed and rare. Media pigmented, carmine.	On sporodochia or singly.	Present. 25-64 × 2.5-3	Under 50 Globose	24-26	6
C. gloeosporioides	White to grey superficial mycelium. Sclerotia large and abundant. Media not pigmented.	On sporodochia. Oblong or cylindrical. 10-16 × 4-7.	Present. 45-108 × 3.5-4	50-75. Irregularly shaped.	24-28	8.5

feijoa (*Acca sellowiana* (O. Berg) Burret); flowers and tip dieback of *Jasminum mesnyi* Hance, lesions on pods of sweet peas (*Lathyrus odoratus* L.) and from lesions on seedlings of tree lupin (*Lupinus arboreus* L.), blue lupin (*L. angustifolius* L.) and *Pinus radiata.* Conidia from naturally infected plant material were identical to those grown on potato dextrose agar. Other morphological characters, both in culture and on plant materials, such as the formation and position of fruiting bodies, occurrence of setae, and the formation of appressoria on germination of conidia, were rather too erratic in their occurrence to be used for the separation of species.

PATHOGENICITY OF CULTURES OF *C. ACUTATUM*

Glasshouse-grown seedlings were inoculated with a suspension of conidia *(ca.* 10^6 conidia/ml) in sterile water. The conidia were obtained from cultures grown on potato dextrose agar. Sufficient inoculum was applied to each plant to thoroughly wet the foliage. Inoculated plants were placed for three days in a cabinet at $16° \pm 2°C$ in a saturated atmosphere. Plants were maintained in this cabinet for another three days at this temperature but at a humidity of 90%-95% before being returned to the glasshouse bench. Results were recorded 10 days after inoculation (Table 2). Whenever pathogenicity was recorded the presence of the fungus was confirmed by re-isolation on potato dextrose agar.

It was also found that cultures obtained from seedlings of *Pinus contorta, P. elliottii, P. pinaster,* and *P. radiata* were all pathogenic to *P. radiata* seedlings. Seedling pines not more than 6 months old were more susceptible to the fungus than older seedlings, and only immature primary leaves surrounding the apical bud were attacked. Small tan coloured lesions were formed, often growing down the leaves into the stem. Fruiting bodies were freely produced on the infected leaves (Fig. 2). Lesions retarded growth of the stem but healthy tissue continued to grow and an apical crook was formed (Fig. 3).

Isolates of *Colletotrichum acutatum* from pine seedlings also infected seedlings of lupins (both *Lupinus arboreus* and *L. angustifolius*), sweet peas and vetches. Isolates from diseased lupins grown among pine seedlings, infected pine seedlings. None of the cultures which were isolated from tree lupin seedlings growing on sand dunes about Auckland were pathogenic to pine seedlings, but most were pathogenic to other legumes. Similarly, isolates from sweet peas were found to be pathogenic to legumes only. No isolates from ripe fruit rots that were pathogenic to pines or legumes could be found. All the isolates used in this study caused a fruit rot when wound-inoculated into ripe tomato fruits.

DISCUSSION

The species *Colletotrichum acutatum* has been shown to be distinct from *C. gloeosporioides* both in morphology and in cultural characters. In the field *C. acutatum* occurred as a primary pathogen, as an agent causing a ripe fruit rot, or as a saprophyte on moribund tissue. Some isolates studied were primary pathogens, but were more or less host specific. Only isolates from pine or lupin seedlings obtained from forest nurseries were shown to be pathogenic on immature leaves of pine seedlings and to be responsible for "terminal crook" disease.

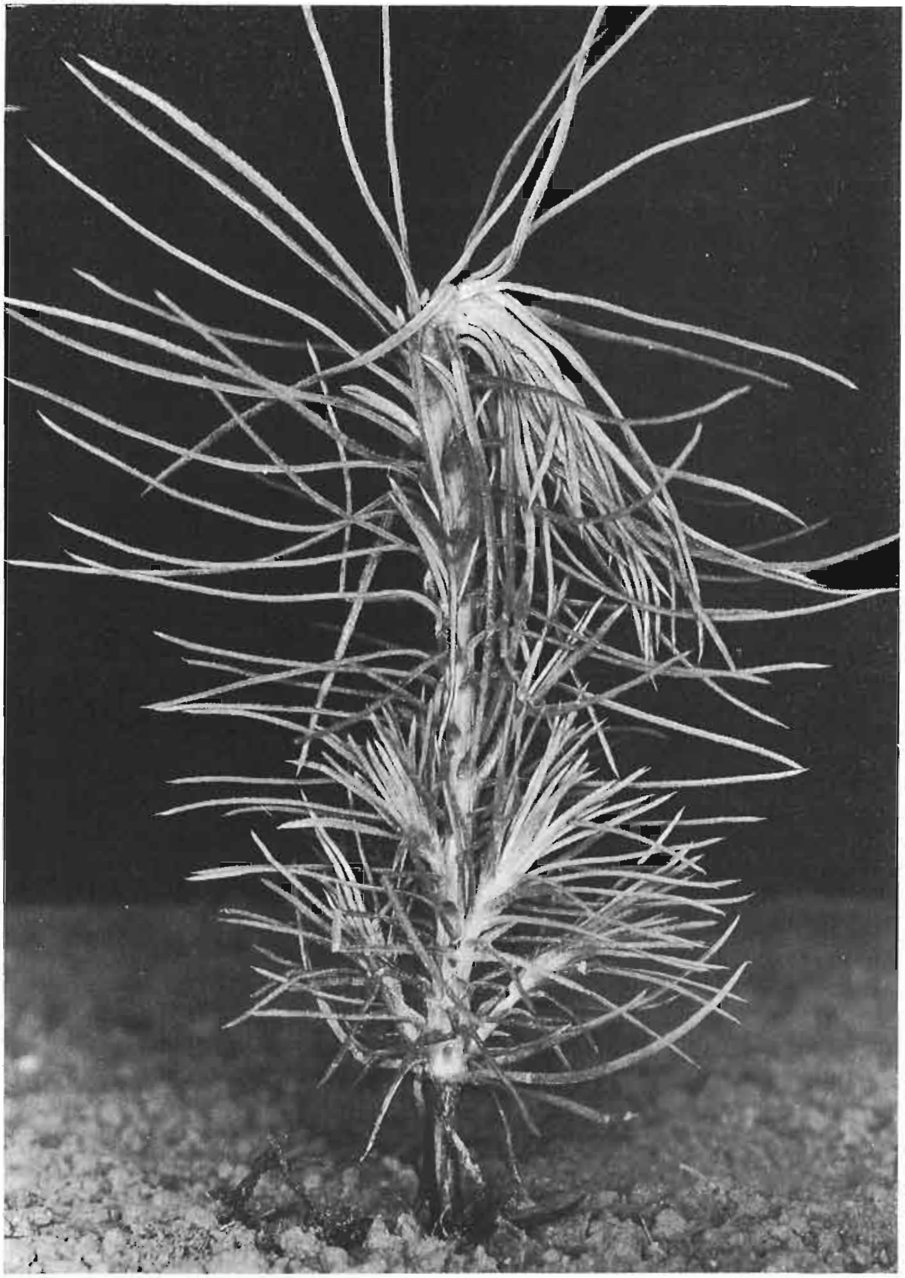
Sheldon (1905) when recording "anthracnose" of sweet peas caused by a

TABLE 2—Results of inoculation of seedlings and fruits with different isolates of **Colletotrichum acutatum**

Inoculum source	Species inoculated					
	Pinus radiata seedlings	**Lathyrus odoratus** seedlings (sweet pea)	**Lupinus arboreus** seedlings (tree lupin)	**L. angustifolius** seedlings (blue lupin)	**Vicia sativa** seedlings (vetch)	**Lycopersicon esculentum** fruits (tomato)
Pinus radiata seedlings (Pine nursery, Kumeu)	+	+	+	+	+	+
P. radiata seedlings (Pine nursery, Woodhill)	+	+	+	+	+	+
P. radiata seedlings (Pine nursery, Tokoroa)	+	+	+	+	+	+
Lathyrus odoratus seedlings (Auckland)	—	+	+	+	+	+
Lupinus arboreus seedlings (Pine nursery, Kumeu)	+	+	+	+	+	+
L. arboreus seedlings (Sand dunes, Bethels Beach)	—	—	+	+	+	+
L. angustifolius (Pine nursery, Kumeu)	+	+	+	+	+	+
Lycopersicon esculentum (Tomato fruit, Auckland)	—	—	—	—	—	+
Acca sellowiana (Feijoa fruit, Auckland)	—	—	—	—	—	+
Jasminum mesnyi (Die-back, Auckland)	—	—	—	—	—	+

N.Z. Forest Service 421 (S. Downward)

FIG. 2—Acervuli (arrowed) of **Colletotrichum acutatum** f.sp. **pinea** on infected primary leaves of the terminal shoot of a **P. radiata** seedling.



N.Z. Forest Service 20 157 (H. G. Hemming)

FIG. 3—**Pinus radiata** seedlings, 6 months old, showing the typical "terminal crook" symptom, approximately 2 months after attack by **Colletotrichum acutatum** f. sp. **pinea n.f. sp.** Infection occurred in the terminal bud and only those needles within one or two millimetres of the terminal bud have been killed. Note the recovery buds developing near the apex of the "crook".

Gloeosporium sp. in West Virginia, USA, noted that inoculation tests suggested that the organism responsible for the disease also caused bitter rot of ripe fruits of apples and grapes. Peterson (1955) noted that in South Carolina, USA *Glomerella cingulata* causing "anthracnose" of peach fruits did not overwinter on peach trees but on blue lupins which, when grown adjacent to a peach orchard, could cause serious disease problems in the following season's peach crop. He showed that isolates from peaches could also infect a large number of leguminous plants. From these findings it is feasible to conclude that as in *Fusarium* Link ex Fr. as illustrated and discussed by Snyder and Hansen (1940), Gordon (1965) and Armstrong and Armstrong (1968), there are in some species of *Colletotrichum* a number of biological strains which may occur as primary pathogens on specific host plants. This accounts for the large numbers of synonyms listed for *C. gloeosporioides* by von Arx (1957a) and for the ascigerous stage *Glomerella cingulata* (Mueller and von Arx, 1954). In the case of *Colletotrichum acutatum* it would appear that even strains that are host specific primary pathogens will cause rots of ripe fruit and occur on moribund tissues. Yearly cropping in an area with one or two plant species will select from a field population, a biological strain specifically suited to them. As these strains do not differ morphologically they are not worthy of specific status and therefore, as in the case of *Fusarium,* the concept of "formae speciales" is adopted. It is proposed that the biological strain of *Colletotrichum acutatum* associated with "terminal crook" disease of pine seedlings in Queensland, Kenya, and New Zealand should be defined as *C. acutatum* Simmonds f.sp. *pinea* n.f.sp. It must be noted that this form is also pathogenic to some legumes.

From the pattern of pathogenicity tests obtained in the laboratory it is suggested that the epidemics of "terminal crook" in pine seedlings in forest nurseries at Woodhill and Tokoroa were brought about by the continuous rotation of pine seedlings with a cover crop of blue lupins. However, in the absence of repeated lupin cover crops this disease has become epidemic in several newly-established nurseries in Central Auckland, South Auckland, Bay of Plenty, and East Coast provinces*. The results of research by Petersen (1955) would suggest that many pasture legumes can carry the infection in a manner similar to lupins.

* For provincial boundaries see New Zealand Official Year Book, 1969; map NZMS 84A (5th ed.), June 1966.

ACKNOWLEDGMENTS

The authors thank Mr J. H. Simmonds and Dr I. A. S. Gibson for cultures, Mrs M. Hollies for the statistical method, and Mrs S. Davidson and Mr B. R. Young for technical assistance.

REFERENCES

ANON. 1967: Queensland Annual Report, Department of Forestry, year 1966-67, p. 11.

ANON. 1968a: Queensland Annual Report, Department of Forestry, year 1967-68, p. 11.

ANON. 1968b: Commonwealth of Australia, Department of National Development. Forestry and Timber Bureau. Annual Report Year 1967, p. 41.

ARMSTRONG, G. M. and ARMSTRONG, Joanne K. 1968: Formae speciales and races of **Fusarium oxysporum** causing a tracheomycosis in the syndrome of disease. **Phytopathology 58:** 1242-6.

GIBSON, I. A. S., and MUNGA, F. M. 1969: A note on "terminal crook" disease of pines in Kenya. **East African Agriculture and Forestry Journal 35:** 135-40.

GILMOUR, J. W. 1965: **Gloeosporium** "terminal crook" a new disease of **Pinus radiata** seedlings. **New Zealand Forest Service, Forest Research Institute, Rotorua, Research Leaflet** 10. 4pp.

GORDON, W. L. 1965: Pathogenic strains of **Fusarium oxysporum. Canadian Journal of Botany 43:** 1309-18.

LIM, T. M. 1970: Seedling blight of pine. **Food and Agriculture Organisation of the United Nations, Rome, Plant Protection Bulletin 17:** 119-20.

MUELLER, E. and von ARX, J. A. 1954. Die Gattungen der amerosporen Pyrenomyceten. **Beitrage zur Kryptogamen flora der Schweiz, 11 (1).** 434pp.

PETERSEN, D. H. 1955: Additional hosts of **Glomerella cingulata** in the families of Gramineae and Leguminosae. **Plant Disease Reporter 39:** 576-7.

SHELDON, J. L. 1905: Concerning the identity of the fungi causing an anthracnose of the sweet pea and the bitter rot of the apple. **Science, n.s. 22** (550): 51-2.

SIMMONDS, J. H. 1965: A study of the species of **Colletotrichum** causing ripe fruit rots in Queensland. **Queensland Journal of Agricultural and Animal Science 22:** 437-59.

——— 1968. Type specimens of **Colletotrichum gloeosporioides** var. **minor** and **C. acutatum. Queensland Journal of Agricultural and Animal Science 25:** p. 177a.

SNYDER, W. C. and HANSEN, H. M. 1940: The species concept in **Fusarium. American Journal of Botany 27:** 64-7.

von ARX, J. A. 1957a: Die arten der gattung **Colletotrichum** Cda. **Phytopathologische Zeitschrift 29:** 413-68.

——— 1957b: Revision der zu Gloeosporium gestellten Pilze. **Verhandelingen der K. Nederlandse Akademie van Wetenschappen Afdeeling Natuurkunde II, 51 (3).** 153pp.

——— 1970: A revision of the fungi classified as **Gloeosporium. Bibliotheca Mycologia 24.** 203pp.