MARASMIELLIUS COLLAR ROT OF JAPANESE MINT

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ABSTRACT

A description is given of a collar rot of Japanese mint from which Marasmiellus epochnous *was isolated. Cultural characters of the two isolates are described. The inoculation of two mint cultivars, reproduction of symptoms and re-isolation of the pathogen are reported.*

INTRODUCTION

Japanese mint (*Mentha arvensis* var. *piperascens* Malinvaud is a recent introduction to Papua New Guinea. Experimental plantings of the two Japanese cultivars Okako and Ryokubi were first made in 1968 at a number of stations. In 1972 wilted patches were noted by Mr P. D. L. Ranson (Agronomist) in plantings at Popondetta on the lowlands of north-east Papua. Specimens were collected in September by Ranson (PNG 8277, cultivar unspecified) and by Dr D. E. Shaw (Chief Plant Pathologist) and Mrs E. G. Cartledge (Plant Pathologist) (PNG 8307, cultivar Okako).

Both collections had a collar rot, and the diseased tissue yielded cultures and fruiting bodies of a basidiomycete. This report describes the disease and inoculation experiments with the isolates.

DISEASE SYMPTOMS

The collar rot is followed by wilting and death of shoots. Rotted tissue is brown, with buff to dull red mycelium over the collar surface. The hyphae of the surface mycelium are thin-walled, with clamp connections.

ISOLATIONS FROM SPECIMENS

Isolates were obtained from the three following kinds of tissue of both accessions: (i) surface mycelium (PNG 8277 only); (ii) collar tissue which had been surface-sterilized with 0.1 per cent mercuric chloride; and (iii) the stipes of fruit bodies which developed both in culture and also on diseased stems incubated in a humid atmosphere. Cultures from all these sources have the same colony and hyphal characters.

Colonies incubated in darkness on 1.25 per cent malt extract agar produce moderate growth of pale-pinkish buff, cottony mycelium which grows up dish sides and over lids. Both on glass and agar, some hyphae aggregate into thin strands, but there is negligible penetration of the medium.

On agar slopes, fruit bodies develop after four weeks. Radial growth of fresh isolates was 8 cm in seven days. Extracellular oxidase, estimated by the guaiacum test (Nobles 1958) is produced at low to moderate levels. Hyphae are thin-walled, 0.5 to 6 *mu* m diameter, with both simple septa and clamp connections; hyphal branches are moderately frequent, often arising opposite clamp connections.

IDENTITY OF THE FUNGUS

Collar tissue from both accessions was maintained in a humid atmosphere and sporophores developed after six weeks. The fructifications are mushroom-like, with a surface diameter of 4 to 10 mm, whitish to buffish-brown, on a stalk (stipe) up to 1 mm wide, with a length 1 to 2.5 times the diameter of the cap (*Plate* I). The full description of the fruit bodies is given below.

Macroscopic Characters

Pileus round to deeply bayed, concave to somewhat undulate, depressed over stipe, margin slightly wavy, downcurved, variably crenate, uniform whitish to whitish-and-buffish-brown in a broad irregular pattern to uniform red-brown with faint brown radial striae corresponding with the gills, diameter 4 to 10 mm, surface dull, smooth to slightly hairy, flesh translucent whitish to buffish-brown; tough when dry, expanding when wet.

Gills of 3 lengths, translucent whitish to buffish when fresh, drying to buff-brown, free collarium

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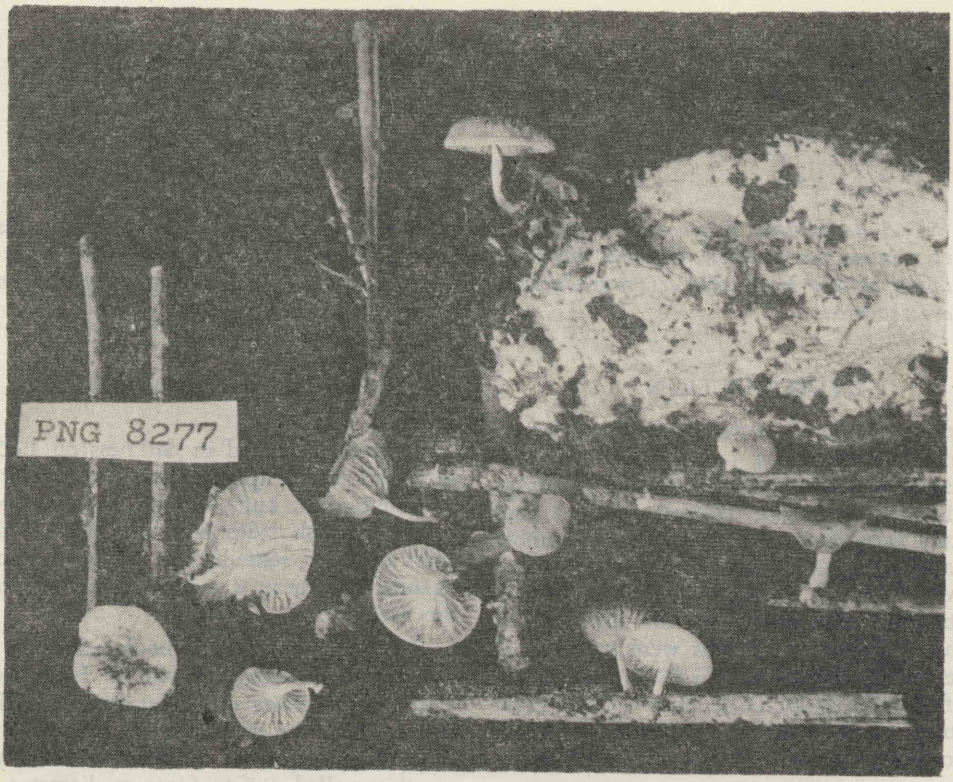


Plate I—Fructifications showing normal habit as well as some inverted to show gills on the under-surface of the pileus (Approx. x 1) (Photo by D. Shaw)

lacking, rather crowded, rather thick, the longer gills occasionally forked but not interveined.

Stipe central (but in one, adjoins deep bay in the pileus), diam. 0.3 to 1.0 mm, not relatively thick or thin, stuffed with paler hyphae, not hollow, concolorous with pileus, surface dull and smooth to slightly hairy, of equal diam. throughout length or wider below, length 1 to 2.5 times diam. of pileus.

Microscopic Characters

Pilear surface hyphae lightly encrusted, pale yellowish, thin-walled, the majority at least of septa being clamp-connections (septa which appeared to be simple may have had clamps below, obscured from view), diam. (3.5-) 5 to 10 *mu* m (*Figure* 1a). Infrequent, emergent, prostrate to erect broom cells (? pilocystidia) with short, simple or branched projections, diam. including projections 7.5 to 13 *mu* m. Projections often on one side only (*Figure* 1b), giving a comb-like appearance. No amyloid reactions in these or any other structures of the fruit body.

Trama of gills a loose weft of branching, thin-walled, broad hyphae, the majority if not all with clamps, staining moderately in lacto-phenol cotton blue (LPCB), becoming more tightly woven in upper gills and pileus.

Gill edge sterile, consisting of clavate, hyaline cheilocystidia (*Figure* 1c) with irregular, rather short, blunt projections, diam. including projections 5.0 to 12.5 *mu* m.

Hymenium of densely packed hyaline, clavate basidia (*Figure* 1d) all with 4 sterigmata, diam. 5.5 to 10.0 *mu*. m Cystidia lacking. Basidial-subhymenial layer 55 to 90 *mu* m deep not forming distinct sublayers, densely staining in LPCB.

Spores (*Plate* IIa and b; *Figure* 1e) hyaline, ellipsoid with one side flattened, apiculate, one-to-several-guttulate, 6 to 10x (3.5-) 4 to 5 *mu*.

Cultures from sporophore tissue were the same as those from diseased plant tissue.

After three years in culture, neither isolate fruited under laboratory conditions, either on agar media (malt extract agar or potato dextrose agar) or on autoclaved grass stems maintained in a humid atmosphere. However, when cultures on autoclaved grass stems were put on moist soil in pots out of doors, fruit bodies developed on the stems after four weeks.

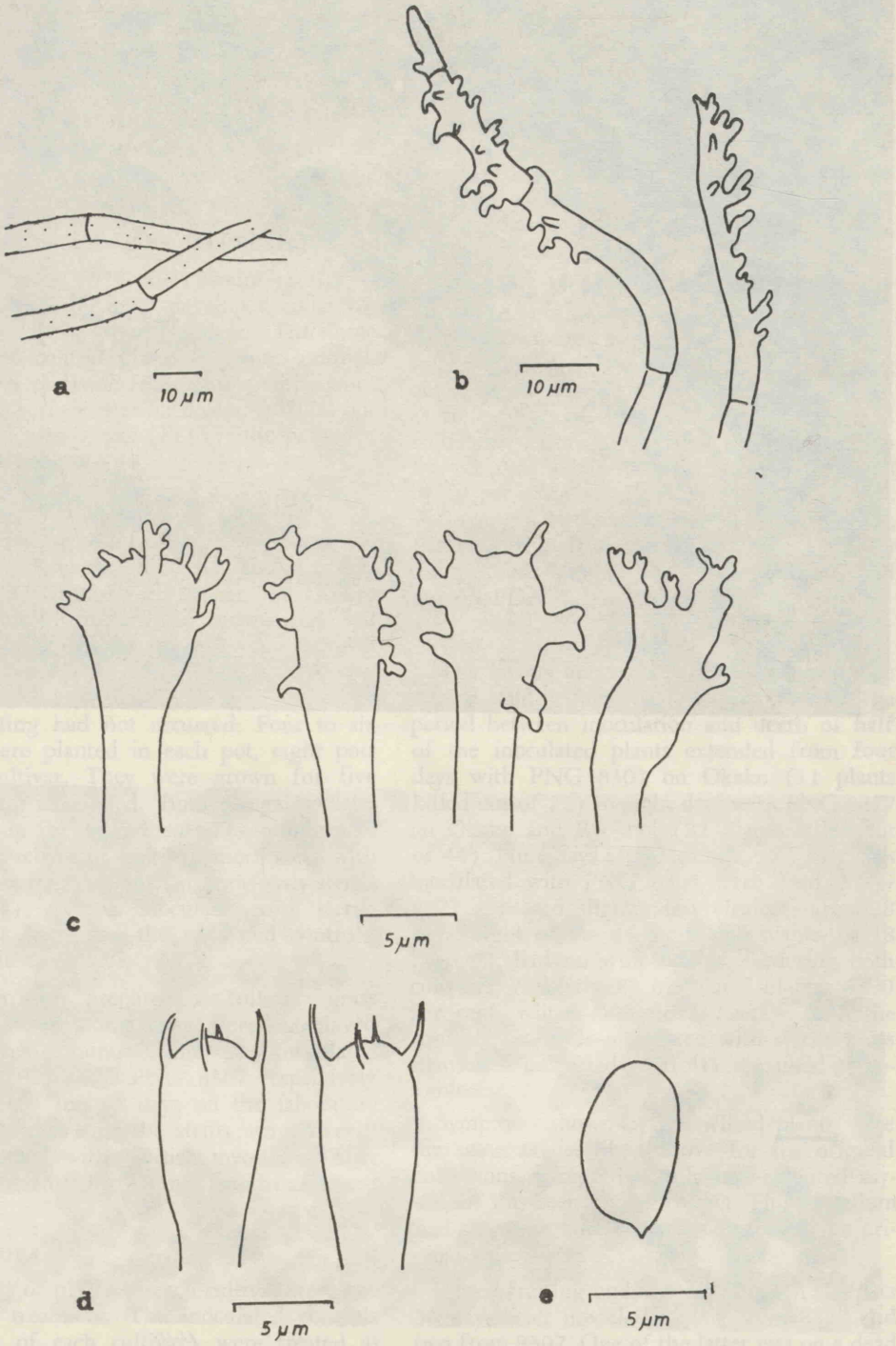
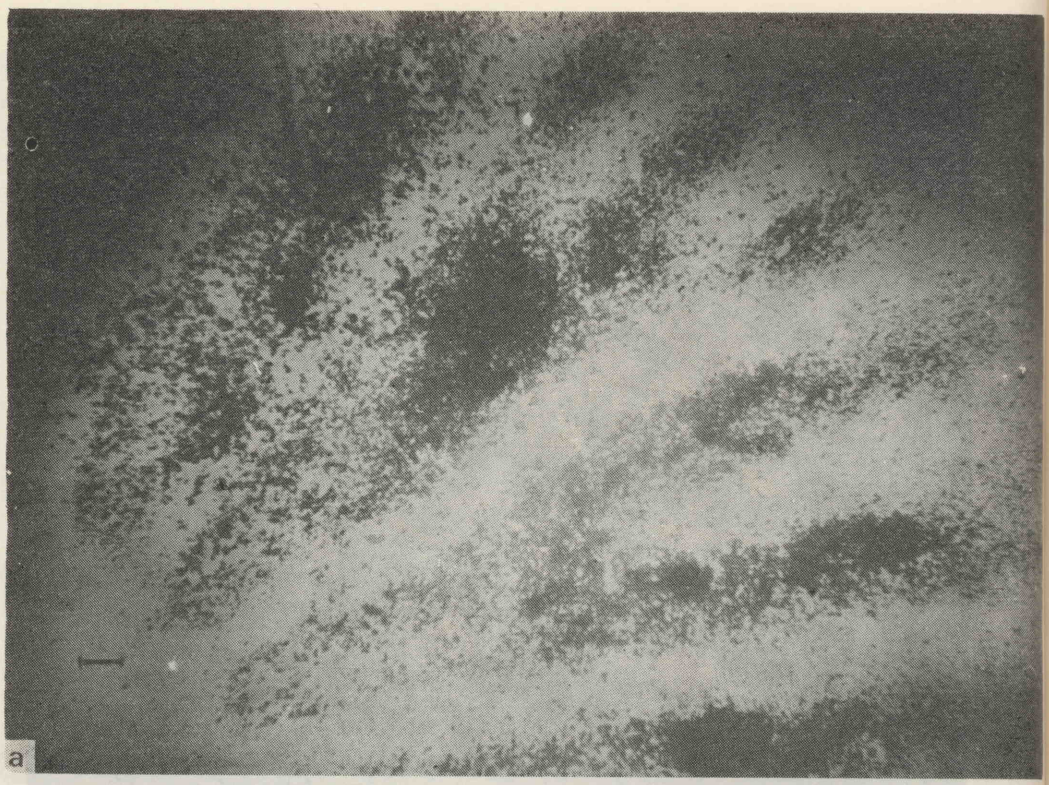


Figure 1—a. Hyphae of pilear surface showing clamps and fine incrustations. b. Broom cells. c. Cheilocystidia. d. Basidia with sterigmata. e. Basidiospore



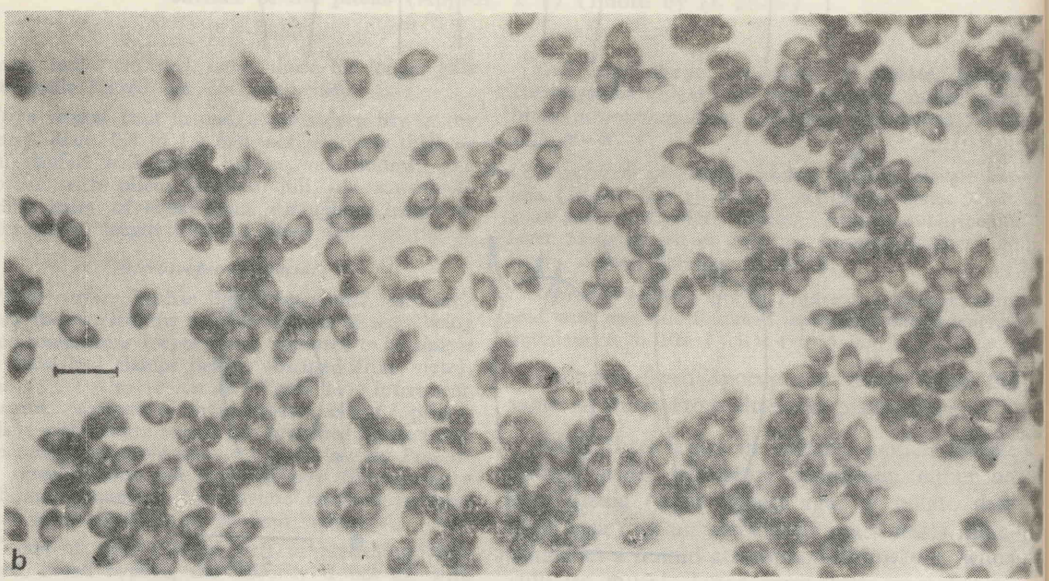


Plate II—a. Portion of spore print from mature pileus, bar = 100 μm. b. Basidiospores in spore print, bar = 10 μm. (Both a and b stained with cotton blue lactophenol) (Photos by D. Shaw)

The fruit bodies were examined by Dr D. N. Pegler of the Royal Botanic Gardens, Kew, who identified them as *Marasmiellus epochnous* (Berk. & Br.) Singer. He stated that they are identical with the original collection of *M. epochnous* from Sri Lanka, and further agree with the subsequent descriptions given by Singer for Central American collections.

FIRST INOCULATION EXPERIMENT

In November, 1972, all 11 mint shoots inoculated with PNG 8307 developed collar rot and severe wilt within nine days. The three uninoculated control plants remained normal. Thirty pieces of tissue from shoots with symptoms were surface sterilized and plated out on potato dextrose agar (PDA); the pathogen was re-isolated from 18.

SECOND INOCULATION EXPERIMENT

Two Japanese mint cultivars were used, Okako and Ryokubi. Sixteen pots, diameter 15 cm, were sterilized with 95 per cent alcohol and filled with unsterilized brown clay soil from the Brown River area. Rooted cuttings of both cultivars showing no symptoms of disease were obtained from areas at Popondetta where wilting had not occurred. Four to six cuttings were planted in each pot, eight pots of each cultivar. They were grown for five weeks, then inoculated. Both fungal isolates were used in the experiment. The numbers of plants of each mint cultivar inoculated with cultures of both accessions (on previously sterile grass stems), controls inoculated with sterile grass stems only, and the untreated controls, are given in the *Table*.

Inoculum was prepared as follows: grass stems on moist cotton wool were autoclaved at 121° C for 15 minutes. They were inoculated with PNG 8277 and PNG 8307 respectively and incubated for 13 days on the laboratory bench, by which time the stems were covered and permeated with whitish mycelium. They were then cut into 5 to 10 mm lengths and used as inocula.

Inoculations

Two pots of plants of each cultivar were not given any treatment. The inoculated controls (two pots of each cultivar) were treated as follows: two to three 10 mm lengths of sterilized grass stem were placed in contact with each mint stem, and covered by about 10 mm of soil. No part of the plant, such as roots or stem, was disturbed or damaged in the process. Inoculated plants (four pots of each cultivar) were treated in the same way as the inoculated controls except that inoculum was substituted for sterilized grass. Pots in saucers were placed on level ground in part shade. Cultivars and treatments were distributed at random over the site.

Inoculum pieces had become attached by hyphae to the majority of inoculated stems by the time symptoms appeared. All such remnants were removed and discarded prior to plating out, and the diseased mint stems then treated as follows. Pieces from the margins of rotted collars were surface-sterilized by immersing in 50 per cent alcohol for 15 seconds followed by 0.1 per cent mercuric chloride for 35 seconds. Following a two-minute rinse in sterile water, tissue from each of the four inoculum-cultivar combinations was cut into 15 pieces and plated out on PDA.

RESULTS

The plants inoculated with *M. epochnous* started wilting three days after treatment. The period between inoculation and death of half of the inoculated plants extended from four days with PNG 8307 on Okako (11 plants killed out of 22) to eight days with PNG 8277 on Okako and Ryokubi (22 plants killed out of 44). Nine days after treatment, all 39 plants inoculated with PNG 8307 were dead. PNG 8277 appeared slightly less virulent; after 28 days, eight of the 44 inoculated plants, or 18 per cent, had no symptoms. Considering both cultivars, 75 of the 83 inoculated plants, or 90 per cent, wilted and died (*Table*). Both the controls (controls inoculated with sterile grass stems and untreated controls) remained symptomless.

Symptoms shown by the wilted plants were the same as described above for the original collections, except that only buff-coloured mycelium was seen on the collars. This mycelium had the same microscopic characters as the original collections.

Three fruiting bodies were noted in the pots 39 days after inoculation, one from 8277 and two from 8307. One of the latter was on a dead mint stem, the other two were growing from pieces of inoculum. The fruiting bodies were

Table—Summary of results of inoculating two mint cultivars with *Marasmiellus epochnous* (isolates PNG 8277 and PNG 8307)

Treatment	Mint cultivar	Plants		Period between inoculation and death of half the number of plants (days)		Plants killed after 28 days		
		No.	Total	No.	Mean	No.	Total	%
Control, untreated	Okako	13		—		0		
	Ryokubi	10		—		0		
	Both cultivars		23	—			0	0
Control, inoculated with sterile grass pieces	Okako	16		—		0		
	Ryokubi	19		—		0		
	Both cultivars		35	—			0	0
Inoculated, isolate PNG 8277	Okako	23		8		18		
	Ryokubi	21		8		18		
	Both cultivars		44		8		36	82
Inoculated, isolate PNG 8307	Okako	22		4		22		
	Ryokubi	17		8		17		
	Both cultivars		39		6		39	100
Both isolates and both cultivars			83		7		75	95

the same as those which developed on the original collections.

Re-isolation of the pathogen

Sixty pieces of collar tissue from wilted plants were surface-sterilized and plated out into nutrient agar. Thirty-six pieces, or 60 per cent, yielded colonies with the same cultural characters as the original isolates.

DISCUSSION

The results show that the fungus isolated from PNG 8277 and PNG 8307 is involved in a wilt disease of at least two cultivars of Japanese mint. *M. epochnous* does not appear to have been recorded previously on this host, nor were records found of Agaricaceae on any *Mentha* species. No records were found of *M. epochnous* on any host.

Singer, Lucas and Warren (1973) described a blight of American beachgrass (*Ammophila breviligulata* Fern.) caused by *M. mesosporus* in which the symptoms are similar to the present disease. The PNG sporophores differ from those authors' description of *M. mesosporus* in having smaller and more numerous projections from the cheilocystidia. The PNG fungus also resembles the description by Sabet, Ashour, Samra and Abdel-Azim (1970) of *M. inoderma* on maize, differing in having hyphae of the epicutis coarsely rather than finely encrusted.

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