SHOOT BLIGHT OF *EUCALYPTUS* SPP. CAUSED BY AN UNDESCRIBED SPECIES OF *RAMULARIA*

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(Plates xii, xiii)

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Synopsis

Ramularia pitereka n. sp. which causes a shoot blight and leaf spot of young plants of *Eucalyptus* spp. is described. It has been found only in eastern New South Wales on plants growing in nurseries and glasshouses. Inoculation tests and observations in nurseries indicate that only *Eucalyptus* spp. in the series Corymbosae-Peltatae of Blakely are susceptible. *Angophora costata* seedlings have been artificially infected. The possibility that this may have some taxonomic significance in the two host genera is mentioned. A possible relationship between *R. pitereka* and the canker fungus of *Eucalyptus ficifolia* in Western Australia is discussed.

Introduction

A shoot blight of *Eucalyptus* seedlings has been known in New South Wales for at least fifteen years. The only published information on it reports an outbreak in the N.S.W. Forestry Commission's nursery at West Pennant Hills in the autumn, winter and spring of 1960, and states : " An unidentified fungus was found causing severe blighting of the young shoot of seedlings of *E. maculata* and over 50% of some plantings of this species had been lost through the disease. The same fungus was found on a few seedlings of *E. eximia* " (N.S.W. Department of Agriculture, 1961). The disease was almost certainly described earlier than this by Mrs. J. de Bavay (personal communication) in notes she prepared in 1955. She observed a shoot blight on *Eucalyptus maculata* Hook. seedlings growing in a Sydney glasshouse and described it as follows : " Distortion was considerable in the young leaf and shoot tissue, in the infected zones. Scattered irregular necrotic areas appeared, over which a greyish white waxy bloom developed, due to the converging of masses of individual minute erumpent conidial pustules, each about $\frac{1}{3}$ to $\frac{1}{2}$ mm. in diameter." This is very similar to the disease described below.

Forestry officers have noticed the disease for several years on young plants in nurseries. It has been seen only on plants over three months old and, if not controlled by spraying, can kill or severely damage them. Although more common in spring and autumn, it has been collected throughout the year in nurseries, but has not been found on plants in the field.

Recent work has shown that the causal fungus is a previously undescribed species of *Ramularia*, which is described below. Standard abbreviations as given by Lanjouw and Stafleu (1964) are used in citing specimens in various herbaria.

Description

Ramularia pitereka sp. nov. (etym., *pitereka*, alba, in lingua aboriginum Australiae).

Pustulae in surculis juvenilibus tortis albae, confertae, usque ad 100 μm diametro, in caulibus erumpentes, in foliis praecipue hypophyllis et ex stomatibus orientes. Conidiophora in strato denso, cum stromate basali hyalino ex hyphis intertextis constata ; stromata 15–50 μm crassa. Conidiophora hyalina, usque

ad 50 μm longa, 2–2·5 μm lata, plerumque cicatricibus numerosis parvis praedita, saepe geniculata, in regione cicatricum non septata. Conidia acrogena, levia, non septata, formae valde variabilis, plerumque clavata vel elongato-clavata, saepe cylindrica vel anguste pyriformia, conidia minoria saepe obclavata vel ovalia, (5) 6–17 (20)×2·5–5 (6·5) μm, in catenis brevibus interdum portata, cicatrice vel basali vel in quoque extremo praedita, per hypham simplicem vel per

Corrigenda

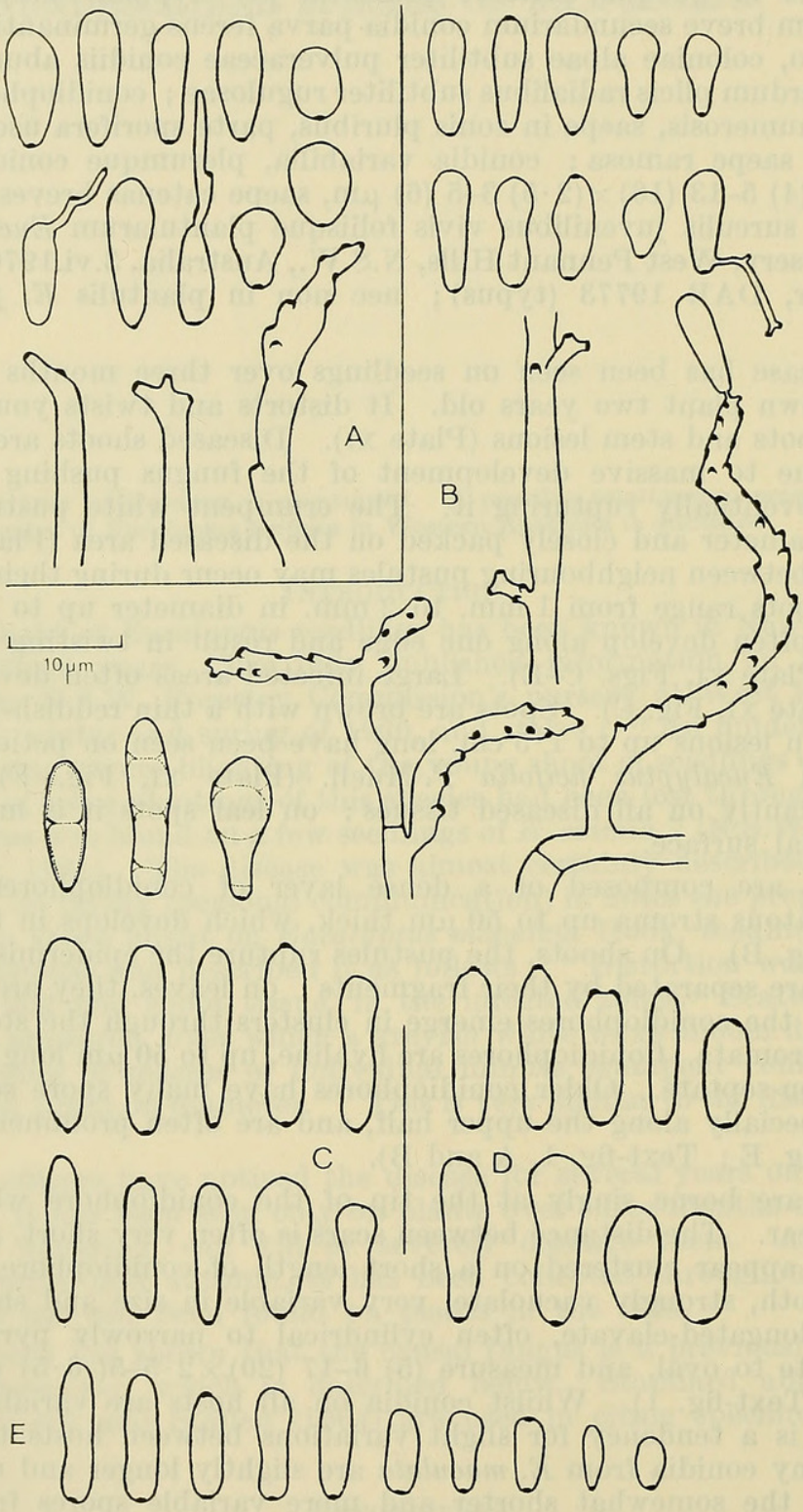
P. 109.—*For* Plate XI, *read* Plate XII.
For Plate XII, *read* Plate XIII.
P. 110.—*For* Plate XII, *read* Plate XIII.
P. 111.—*For* Plate XII, *read* Plate XIII.
P. 115.—*For* Plate XI, *read* Plate XII.
For Plate XII, *read* Plate XIII.

cuticle and eventually rupturing it. The erumpent white pustules are up to 100 μm in diameter and closely packed on the diseased area (Plate XI, Fig. B). Some fusion between neighbouring pustules may occur during their development. On leaves, spots range from 1 mm. to 2 mm. in diameter up to large irregular areas which often develop along one edge and result in twisting and distortion of the leaf (Plate XI, Figs. C–E). Large infected areas often develop along the mid-vein (Plate XI, Fig. C). Spots are brown with a thin reddish-purple margin. Sunken brown lesions up to 1·5 cm. long have been seen on petioles and stems, especially on *Eucalyptus ficifolia* F. Muell. (Plate XI, Fig. F). Sporulation occurs abundantly on all diseased tissues ; on leaf spots it is more prominent on the abaxial surface.

Pustules are composed of a dense layer of conidiophores borne on a plectenchymatous stroma up to 50 μm thick, which develops in the host tissue (Plate XII, Fig. B). On shoots, the pustules rupture the epidermis and the thick cuticle, and are separated by their fragments ; on leaves, they are mainly hypophyllous and the conidiophores emerge in clusters through the stomates from a sub-stomal stromata. Conidiophores are hyaline, up to 50 μm long and 2–2·5 μm wide, and non-septate. Older conidiophores have many spore scars (up to 14 or more), especially along the upper half, and are often prominently geniculate (Plate XII, Fig. E ; Text-fig. 1, A and B).

Conidia are borne singly at the tip of the conidiophore which grows on around the scar. The distance between scars is often very short, so that several conidia may appear clustered on a short length of conidiophore. Conidia are hyaline, smooth, strongly vacuolate, very variable in size and shape, generally clavate to elongated-clavate, often cylindrical to narrowly pyriform, smaller ones obclavate to oval, and measure (5) 6–17 (20)×2·5–5(6·5) μm (Plate XII, Figs. C–E ; Text-fig. 1). Whilst conidia on all hosts are variable in size and shape, there is a tendency for slight variations between hosts to occur. For example, many conidia from *E. maculata* are slightly longer and more regularly clavate than the somewhat shorter and more variable spores from *E. eximia* Schau. They often form short chains and have either a basal spore scar or scars at each end. Conidia sometimes germinate in the pustule, and they often form short secondary conidiophores bearing small secondary conidia (Text-fig. 1, A). Germination usually occurs by one to two germ tubes anywhere on the spore, except that germination through the scar has not been seen.

On agar media, colonies are white, raised in the centre and when older, often wrinkled by fine radial folds (Plate XII, Fig. A). They are covered with an abundant dry growth of conidiophores and conidia. Conidiophores are of indeterminate length. Spore-bearing areas up to 120 μm long have been seen



Text-fig. 1. A–D, *Ramularia pitereka* conidia and conidiophores. A, from *Eucalyptus eximia* (DAR 19773), two conidia showing secondary conidiophores. B, from culture ex DAR 19773, showing types of conidiophore development, and three spores with vacuolate contents. C, from *E. maculata* (DAR 19769), natural infection. D, from *E. maculata* (DAR 19770), artificial inoculation. E, " *Sporotrichum destructor* " spores from IMI 7368.

and often several zones of scars occur along a conidiophore. Because of these zones of sporulation, conidia appear to be borne in clusters of varying size along the conidiophore. The arrangement of scars on the conidiophore is very similar to that described and illustrated by Hughes (1951, Fig. 2, E) for *Acrotheca acuta* Grove in culture. Often, conidiophores are branched and, apart from the spore scars, are very similar to vegetative hyphae. Conidia from all hosts are similar in culture and show a similar variability in shape to those from infected plants, but are generally slightly smaller, measuring (4) 5–13 (16) × (2·5) 3–5 (6) μm. Short chains are often formed (Plate XII, Fig. C ; Text-fig. 1).

Isolation and Growth in Culture

Ramularia pitereka is isolated easily from spores. It grows slowly on potato dextrose and malt agars and has an optimum temperature for radial growth between 20° and 25° C. After 24 days, colonies on malt agar are 12 mm. in diameter at 25° C. and 6 mm. in diameter at 30° C. No growth occurs at 35° C. and growth is very slight at 5° C., colonies being only 2 mm. in diameter after 45 days. Colonies are white above and pale cream below. In cultures three to four months old, colonies often develop a faint cream to pink tint, and the medium is slightly darkened.

Infection and Host Range

Artificial inoculations were carried out on a range of species of *Eucalyptus* and on *Angophora costata* (Gaertn.) Druce. Conidia from cultures were suspended in sterile water and applied with a fine brush to young shoots of seedlings in the glasshouse. Plants were kept in a humid atmosphere for 24 days after inoculation and then placed on the glasshouse bench. The characteristic symptoms of the disease and sporulation of the fungus were seen usually within three to four weeks of inoculation. The range of species tested and the results obtained are shown in Table 1.

Table 1

Reaction of Seedlings of Eucalyptus *and* Angophora *to Inoculation with* Ramularia pitereka

Section*	Series	Species	Number Inoculated	Number Infected
Macrantherae	Corymbosae-Peltatae	*E. eximia* Schau.	10	9
		E. ficifolia F. Muell.	10	10
		E. maculata Hook.	10	10
	Transversae	*E. longifolia* Link and Otto	10	0
		E. punctata DC.	10	0
	Exsertae	*E. camaldulensis* Dehn.	10	0
Macrantherae (Normales)	Globulares	*E. bridgesiana* R. T. Bak.	10	0
		E. pulverulenta Sims	10	0
Renantherae (Normales)	Pseudo-stringybarks	*E. pilularis* Sm.	10	0
	Pachyphloiae	*E. caliginosa* Blakely and McKie	10	0
Porantheroideae (Normales)	Buxeales	*E. albens* Benth.	10	0
Terminales	——	*E. sideroxylon* A. Cunn. ex Woolls	10	0
Platyantherae	Subulatae	*E. flocktoniae* Maiden	10	0
——	——	*Angophora costata* (Gaertn.) Druce	20	15

* The *Eucalyptus* spp. are arranged according to the sections and series given by Blakely (1965) and their nomenclature follows Johnston and Marryatt (1965).

In these tests the only *Eucalyptus* spp. attacked were in Blakely's (1965) series Corymbosae-Peltatae. Several plants of *Angophora costata* were also infected. The significance of these results is discussed below.

Possible Relationship to Canker Fungus of *Eucalyptus ficifolia*

Possibly closely related to *R. pitereka* is the fungus that causes canker disease of adult trees of red-flowering gum (*E. ficifolia* F. Muell.) in Western Australia. Little has been published about this organism. In 1936 Mr. H. A. Pittman, then Government Plant Pathologist, reported on a canker disease of *E. ficifolia* in Western Australia (Anon., 1936). It had apparently been known for some years before this (Beard, 1963 ; Cass Smith, 1970), but Pittman was the first to isolate the causal fungus and suggest control measures. At the time he also sent a culture to the Commonwealth Mycological Institute. Subsequently, the fungus was referred to in various publications as " *Sporotrichum destructor* Pittman ". MacNish (1963) lists it as " *Sporotrichum destructor* Pittman n. sp." and in a footnote states " pathogen named 1936 ". Later authors dealing with canker have used this name (Beard, 1963 ; Cass Smith, 1970) or have referred to the disease as *Sporotrichum* canker (Pryor and Willing, 1963).

The name " *Sporotrichum destructor* " has never been validly published, and no description of the fungus has been found in the literature. Mr. Pittman (personal communication) wrote that, whilst he proved its pathogenicity and tentatively referred to it as *Sporotrichum*, he did not call it " *S. destructor* ". However, a description under this name has been obtained from Mr. H. L. Harvey, of the Department of Agriculture, Perth, Western Australia. This description, dated 20.x.1936, came from files of correspondence with the Commonwealth Mycological Institute, and was prepared there by Mr. E. W. Mason (F. C. Deighton, personal communication). It describes the fungus in culture. Colonies are described as " floccose, then powdery, finally showing radiating striations ", at first white and later pinkish-buff in colour. The fertile hyphae are described as " usually long (up to 150 μ), more rarely lateral and shorter, unbranched, cylindrical, typically straight and bearing in a stellate manner a single whorl, or occasionally a spiral of a few (up to 10) conidia, sometimes gently bent and provided with two or even more verticills or spirals. Conidia hyaline, cylindrical or oblong, rounded at both ends, 0-septate, sessile (that is—without sterigmata), 6–8 μ long, 2 to 2·5 μ broad."

The only specimen filed under the name " *Sporotrichum destructor* " at the Commonwealth Mycological Institute has been examined. It is a dried malt agar culture, light buff (Ridgeway, 1912) in colour, now showing as a fairly compact felted mat of hyphae on the dried agar. Abundant hyaline, unicellular conidia are present. They are variable in shape, cylindrical to clavate, with smaller conidia oval to obovate, measuring (2·5) 3–10 (12·5) × (2) 2·5–3·5 (4) μm, and have an indistinct spore scar at one end. Evidence of chain formation was present as some spores had a scar at each end. Conidiophores could not be clearly distinguished, but some fragments with probable indistinct spore scars were seen. In some cases, clusters of conidia were seen around probable conidiophores.

The spores from this specimen are smaller than those of *R. pitereka* in culture. From Mr. Mason's description, its cultural characters and some aspects of its conidiophore and conidium morphology are similar to those of *R. pitereka*, but until it can be studied from fresh specimens, its identity remains uncertain.

Specimens Examined

Ramularia pitereka J. Walker and A. L. Bertus—New South Wales : on *Eucalyptus eximia* Schau., in nursery, Forestry Commission of N.S.W., West Pennant Hills, A. L. Bertus and J. Walker, 3.vi.1970, DAR 19773

(type) (portion filed as IMI 151369) ; same locality, J. Walker, 18.xi.1960, DAR 6469 ; same locality, A. L. Bertus, 5.v.1970, DAR 20366 ; artificial inoculation, glasshouse, Department of Agriculture, Rydalmere, A. L. Bertus, 15.xi.1967, DAR 19771. On *Eucalyptus ficifolia* F. Muell., in nursery, Forestry Commission of N.S.W., West Pennant Hills, A. L. Bertus and J. Walker, 3.vi.1970, DAR 20361. On *Eucalyptus maculata* Hook., in nursery, Forestry Commission of N.S.W., West Pennant Hills, J. Walker, 30.vi.1960, DAR 5867a ; in commercial nursery, Beverley Hills, J. Stronach, 24.ii.1967, DAR 19768 ; same locality, J. Stronach, 28.ii.1967, DAR 19769 (portion filed as IMI 151368) ; artificial inoculation, glasshouse, Department of Agriculture, Rydalmere, A. L. Bertus, 15.xi.1967, DAR 19770 ; in commercial nursery, Glenhaven, A. L. Bertus, 5.xi.1970, DAR 20468. On *Angophora costata* (Gaertn.) Druce, artificial inoculation, glasshouse, Department of Agriculture, Rydalmere, J. Walker, 16.xi.1970, DAR 20469.

" *Sporotrichum destructor* "—Western Australia : in dried malt agar culture, H. A. Pittman, 1936, IMI 7368 (slides filed as DAR 20410). On *Eucalyptus ficifolia* F. Muell., King's Park, Perth, L. Harvey, Sept., 1970, DAR 20409 (cankers only, no fungus seen).

Discussion

No previous description of a species of *Ramularia* on hosts in the family Myrtaceae has been found. Most species of *Ramularia* occur on herbaceous hosts and generally cause comparatively minor damage such as leaf spots and blotches. *R. pitereka* occurs on young growth of woody hosts and causes more severe damage than is usually seen with many other *Ramularia* spp. Whilst comparatively few species of *Ramularia* have been studied in culture, those that have generally shown sparse sporulation on artificial media ; however, *R. pitereka* produces abundant spores in culture.

In the limited inoculation tests carried out so far only *Eucalyptus* spp. in the series Corymbosae-Peltatae of Blakely (1965) have been susceptible. The species used have also been arranged (Table 2) according to a new classification and coding of the genus (Pryor and Johnson, 1971) and in this system all the susceptible species fall into the subgenus C *Corymbia*. The one species of *Angophora* tested, *A. costata*, was experimentally infected. It is possible that this may have some taxonomic significance in the two host genera, and tests on a wider range of species will be carried out. The affinity in essential oil and certain morphological characters between species of *Eucalyptus* in the above and related series and the genus *Angophora* has been pointed out previously (Baker and Smith, 1920).

Finally, *R. pitereka* may be the same as the undescribed fungus causing canker of red-flowering gums in Western Australia, and referred to in the literature as " *Sporotrichum destructor* Pittman ". If so, it may have been introduced into eastern Australia from the west. Cass Smith (1970) indicates that in Western Australia the disease occurs naturally on *Eucalyptus calophylla* R. Br. ex Lindl., and that infections on cultivated *E. ficifolia* probably originated from naturally infected *E. calophylla*. So far, no plants infected with *R. pitereka* have been found in nature in N.S.W. Cass Smith (1970) also lists *E. haematoxylon* Maiden as another host of the canker fungus. All the known hosts of " *Sporotrichum destructor* " thus occur in Blakely's series Corymbosae-Peltatae (or subgenus C *Corymbia* in Pryor and Johnson's (1971) classification), and this provides a further indication of a possible relationship between the canker fungus and *R. pitereka*.

TABLE 2

Species of Eucalyptus *and* Angophora *Arranged According to the Classification and Coding of Pryor and Johnson (1971)*

Subgenus		Section		Series		Subseries		Species Code	Species
C	Corymbia	CA	Rufaria	CAF	Gummiferae	CAFO	Ficifolinae	CAFOA	*E. ficifolia* F. Muell.
		CA	Rufaria	CAF	Gummiferae	CAFU	Gummiferinae	CAFUA	*E. calophylla* R. Br. ex Lindl.
		CC	Ochraria	CCA	Eximiae	——		CCA:E	*E. eximia* Schau.
		CC	Ochraria	CCC	Maculatae	——		CCC:B	*E. maculata* Hook.
S	Symphyomyrtus	SE	Transversaria	SEC	Salignae	SECG	Longifolinae	SECGA	*E. longifolia* Link and Otto
						SECE	Punctatinae	SECED	*E. punctata* DC.
		SI	Bisectaria	SIT	Oleosae	——		SIT:T	*E. flocktoniae* Maiden
		SN	Exsertaria	SNE	Tereticornes	SNEE	Tereticorninae	SNEEP	*E. camaldulensis* Dehn.
		SP	Maidenaria	SPI	Viminales	SPID	Bridgesianinae	SPIDC	*E. bridgesiana* R. T. Bak.
						SPIN	Cordatinae	SPINQ	*E. pulverulenta* Sims
		SU	Adnataria	SUL	Moluccanae	——		SUL:G	*E. albens* Benth.
				SUX	Melliodorae	——		SUX:I	*E. sideroxylon* A. Cunn. ex Woolls
M	Monocalyptus	MA	Renantheria	MAI	Pilulares	MAIA	Pilularinae	MAIAA	*E. pilularis* Sm.
				MAH	Capitellatae	MAHE	Eugenioideinae	MAHED	*E. caliginosa* Blakely and McKie
A	*Angophora* (genus)	AA	Lemuria	AAA	Costatae	AAAD	Costatinae	AAADA	*Angophora costata* (Gaertn.) Druce


A
C
B
D
E
F