

RECOVERY OF POTENTIALLY PATHOGENIC *PHYTOPHTHORA* AND *PYTHIUM* SPP. FROM NATIVE VEGETATION IN AUSTRALIA

By B. H. PRATT* and W. A. HEATHER*

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Abstract

Soil and plant root samples from a variety of native forest habitats throughout Australia were examined for potentially pathogenic *Pythium* and *Phytophthora* spp. by lupin baiting.

Those recovered included *Pythium* (?)*acanthicum*, *P.* (?)*acanthophoron*, *P.* (?)*deliense*, *P. irregulare*, *P. middletonii*, *P.* (?)*oedochilum*, *P. splendens*, *P. ultimum* var. *sporangiferum*, *Phytophthora cinnamomi*, *Ph. citricola*, *Ph. drechsleri*, and *Ph. nico-tianae* var. *parasitica*.

It is suggested that disease of native plant species normally attributed to *Ph. cinnamomi* could be caused by other *Pythium* and *Phytophthora* species, acting singly or alone, with or without *Ph. cinnamomi*.

I. INTRODUCTION

Phytophthora cinnamomi Rands has been frequently recovered by the lupin-baiting technique of Chee and Newhook (1965) from samples of soil and plant roots obtained from native forest areas of Australia (Pratt and Heather 1972). The presence of the fungus is sometimes but not always associated with a root rot and dieback disease of native plant species (Podger *et al.* 1965; Pratt and Heather 1973). While there is general acceptance of *Ph. cinnamomi* as the causal agent of disease in Western Australia, the evidence supporting such a viewpoint for other areas is largely circumstantial and there is no published information on the presence or absence of other potential pathogens in disease sites from which *Ph. cinnamomi* has been isolated.

In order to examine the possible involvement of other organisms, soil and plant root samples were obtained from within and adjacent to a wide range of diseased native forest areas throughout Australia. Following lupin baiting, a number of potentially pathogenic *Phytophthora* and *Pythium* species, in addition to *Ph. cinnamomi*, were isolated from these samples.

II. MATERIALS AND METHODS

(a) Sample Collection

Between 1969 and 1972 approximately 12,000 samples of soil and plant roots were obtained in eastern Australia from a zone approximately 2000 miles long and 100 miles wide, extending from near Cairns in north Queensland to the Catamaran River in southern Tasmania. Further samples were taken from other sites in all Australian States and the Northern Territory, using techniques

* Department of Forestry, Australian National University, P.O. Box 475, Canberra, A.C.T. 2601.

described by Pratt and Heather (1972). Usually samples were taken from the root zones of native plants within natural forest communities, or more rarely from native and exotic species in plantations, farm windbreaks and Botanic Gardens. Samples were obtained from both diseased and apparently non-diseased areas.

(b) Recovery and Identification of Fungi

Samples were assayed by lupin baiting using the technique of Chee and Newhook (1965) modified by Pratt and Heather (1972). Although *Ph. cinnamomi* could be readily identified by direct microscopic examination of sporangia formed in lesions developing on infected lupin roots, this technique was unreliable for other *Phytophthora* spp. or for *Pythium* spp. Consequently, following baiting lupin roots were surface-sterilized with 70% ethanol and plated on water agar to facilitate recovery of colonizing organisms.

All *Pythium* and *Phytophthora* isolates recovered from lupin roots were cultured on V-8 agar at 23°C and examined to determine mycelial form, and the morphology and variability of hyphal swellings, chlamydospores, sporangia, and sexual structures.

Some isolates did not produce fruiting structures readily on solid media. Hence 6-mm disks of 3-day-old cultures of each isolate on V-8 agar were removed to sterile glass-distilled water or to 20% soil extract prepared by incubating 20 g garden soil with 100 ml glass-distilled water for 48 hr then filtering through Whatman No. 1 filter paper. Chilling was used to stimulate zoospore formation and release where necessary.

Phytophthora spp. which did not readily produce sexual structures in single culture were stimulated to do so by mating them with A1 and A2 strains of *Ph. cinnamomi* and A2 strains of *Ph. drechsleri* Tucker, and also by exposing them to gaseous products of *Trichoderma koningii* Oud. agg., using the techniques of Brasier (1971), Pratt *et al.* (1972a, 1972c). Known mating strains of other *Phytophthora* spp. were not available for study.

Multiple isolates of each species of *Pythium* and *Phytophthora* were studied where possible, to determine the homogeneity of populations of each species, and thus cross-check the initial identifications. *Pythium* spp. were identified according to the key of Middleton (1943), and with the assistance of the Commonwealth Mycological Institute, Kew, *Phytophthora* spp. were identified according to Waterhouse (1963, 1970) and by comparison of morphological and reproductive characters with those of *Phytophthora* spp. obtained from the following sources: *Ph. cinnamomi* (A1 and A2 strains, G. A. Zentmyer), *Ph. cactorum* (Leb. & Cohn) Schroet., *Ph. cambivora* Petri, *Ph. citricola* Sawada, *Ph. citrophthora* (Smith & Smith) Leon., *Ph. cryptogea* Pethybr. & Laff., *Ph. megasperma* Drechs. var. *sojae* Hildebrand, *Ph. nicotianae* Breda de Haan var. *parasitica* (Dastur) Waterhouse, *Ph. syringae* (Kleb.) (all from Forest Research Institute, Western Australia), *Ph. nicotianae* Breda de Haan var. *nicotianae* Tucker (Division of Plant Industry, CSIRO, Canberra), and *Ph. drechsleri* (A2 strain, New South Wales Department of Agriculture; Western Australian Department of Agriculture).

No attempt was made to quantify the populations of the fungi in field sites, nor were all isolates of *Pythium* and *Phytophthora* from all sites identified.

III. RESULTS

Fourteen morphologically different types of *Pythium* were recovered from plated lupin roots. Eight of these were identified by the Commonwealth Mycological Institute as follows: *Pythium* (?)*acanthium* Drechsler, *P.* (?)*acanthophoron* Sideris, *P.* (?)*deliense* Meurs, *P. irregulare* Buisman, *P. middletonii* Sparrow, *P.* (?)*oedochilum* Drechsler, *P. splendens* Braun, and *P. ultimum* var. *sporangiferum* Drechsler. The areas from which they were recovered are shown in Table 1.

Seven *Phytophthora* spp. were recovered, of which four were formally identified. *Ph. cinnamomi* Rands was most frequently recovered, and it occurred in all States and the Australian Capital Territory, in diseased and apparently non-diseased areas.

TABLE 1

DISTRIBUTION AND DISEASE ASSOCIATION OF *PYTHIUM* AND *PHYTOPHTHORA* SPECIES IN AND ADJACENT TO NATIVE VEGETATION IN AUSTRALIA

+, disease symptoms obvious; —, disease symptoms absent

Area	Species recorded*	Disease occurrence	Major <i>Eucalyptus</i> spp. affected by disease
Queensland			
Koomboooloomba	9	+	<i>E. microcorys</i> F. Muell.
Cooroy	9	+	<i>Eucalyptus</i> sp.
Petrie	9	—	—
New South Wales			
Bega	8, 10	+	} <i>E. sieberi</i> L. Johnson
Eden	2, 9, 10	+	
Batemans Bay	9, 10	+	
Termeil	4, 9, 10	+	
Tallaganda	9	+	<i>E. viminalis</i> Labill, <i>E. sieberi</i>
Bermagui	9, 12	—	—
Ourimbah	10, 11	+	<i>E. saligna</i> Sm.
Coffs Harbour	4, 9, 10, 12	+	<i>E. pilularis</i> Sm.
Jindabyne	9	+	} <i>E. stellulata</i> Sieb. ex DC
Tom Groggin	9	+	
Australian Capital Territory			
Black Mountain	9, 10	+	<i>E. macrorrhyncha</i> F. Muell. ex Benth., <i>E. rossi</i> R. T. Bak. & H. G. Sm.
Black Mountain	4, 9, 10	+	†
Condor Creek	9, 10		<i>E. viminalis</i>
Victoria			
Silver Creek	9, 10	+	<i>E. obliqua</i> L'Herit.
Carrajung	9, 10	+	<i>E. radiata</i> Sieb. ex DC
Brisbane Ranges	3, 10	+	<i>E. obliqua</i>
Tasmania			
Launceston	1, 3, 4, 5, 10	+	<i>E. amygdalina</i> Labill.
King Island	2	+	‡
Wesley Vale	5, 8, 9	+	§
South-eastern region	2, 3, 6, 7, 9, 10	+	<i>E. regnans</i> F. Muell., <i>E. obliqua</i>
South Australia			
Mt. Lofty	4, 10	+	Understorey species
Mt. Lofty	1, 10		†
Mt. Bold	4, 9, 10		‡
Western Australia			
Dwellingup	3, 6, 10	+	<i>E. marginata</i> Sm.
Marrinup	4, 10	+	<i>E. marginata</i>

* 1, *Pythium* (?)*acanthicum*; 2, *P.* (?)*acanthophoron*; 3, *P.* (?)*deliense*; 4, *P. irregulare*; 5, *P. middletonii*; 6, *P. ultimum* var. *sporangiferum*; 7, *P. splendens*; 8, *P.* (?)*oedochilum*; 9, *Phytophthora drechsleri*; 10, *Ph. cinnamomi*; 11, *Ph. citricola*; 12, *Ph. nicotianae* var. *parasitica*.

† Botanic Garden, indigenous plant species.

‡ *Pinus radiata* D. Don planting.

§ *Melaleuca* sp. windbreak.

Ph. citricola Sawada was recovered from a single diseased *Eucalyptus saligna* Sm. site in New South Wales and *Ph. nicotianae* B. de Haan var. *parasitica* (Dastur) Waterh. from diseased *E. pilularis* Sm. and apparently non-diseased *E. sieberi* L. Johnson sites in New South Wales.

Ph. drechsleri occurred frequently in diseased, and occasionally in non-diseased areas in the Australian Capital Territory and all States except Western Australia (Table 1). Fifty-five isolates of the organism, which appeared similar on superficial examination, were separated into two distinct types following more detailed examination, as follows.

Thirty-five isolates from the Australian Capital Territory, central and southern New South Wales, Victoria, Tasmania, and South Australia appeared to be of the same type, and were designated "southern" type. The remaining 20 from other sites in the Australian Capital Territory, central and northern New South Wales, and Queensland appeared to be of another type and were designated "northern" type.

The hyphae produced by both southern and northern isolates were of similar width, morphological appearance, and degree of variability, and developed identical branching and mycelial patterns. Radial growth rates of isolates from each group were similar. Sporangia were rare or absent on solid media but formed within 12–48 hr on mycelial disks immersed in water or soil extract. The sporangia were of similar size, shape, and variability in northern and southern isolates but were usually produced more abundantly by the latter. Hyphal swellings were common in disks of southern isolates immersed in sterile water but rare or absent in northern isolates under similar conditions.

Some isolates from both groups produced a few apparently homothallic oospores in inoculum plugs of subcultures. The oogonia, amphigynous antheridia, and oospores were of the same size, shape, colour, and degree of variability in each group.

The two groups differed markedly in their response to mating with *Ph. cinnamomi* and exposure to *Trichoderma koningii*. All southern isolates produced abundant oospores when mated with the A1 strain of *Ph. cinnamomi* but not when mated with the A2 strain of *Ph. cinnamomi* or the A2 strain of *Ph. drechsleri*. In addition some southern isolates produced oospores when exposed to *T. koningii* cultures for 3–5 days.

By contrast, northern isolates did not produce an oospore when mated with *Ph. cinnamomi* or *Ph. drechsleri* or when exposed to *T. koningii*.

The morphology of the southern-type isolates agreed with the description of *Ph. drechsleri* in the Waterhouse (1963) key, and mating reactions indicated all were of the A2 strain. This identification was supported by comparison with the *Ph. drechsleri* isolate PC11 (IMI 129907) recovered from citrus soil in Western Australia by R. Doepel, and K166 (IMI 164185) and K184 isolated from diseased safflower in New South Wales by E. Cother and G. Stovold respectively.

Identification of the northern type was more difficult. Morphologically it could not be separated from the southern type isolates or from *Ph. drechsleri* isolate PC11, K166, and K184, and differed from these only in the lack of oospore production when mated with *Ph. cinnamomi* or exposed to *T. koningii*. In all characters it appeared identical with isolates PC12 (IMI 133597) recovered from diseased safflower in New

South Wales by E. Cother, and with K165 and K186 isolated from diseased safflower and gooseberry respectively in New South Wales by G. Stovold, all of which had been tentatively identified as *Ph. drechsleri* on morphological characters.

Southern and northern isolates, and the horticultural isolates of Doepel, Cother, and Stovold were mated in all possible combinations of pairs, but none produced oospores.

On the basis of morphological characters, the northern isolates were tentatively identified as *Ph. drechsleri*, but of an undetermined mating type.

IV. DISCUSSION

Species of *Pythium* and *Phytophthora* have been recognized for many years as pathogens of a wide variety of plant species, most commonly affecting seedling and fine root tissues. Some are thought to be generally distributed in soils throughout the world while others appear to be limited to an association with specific plant hosts. Of the 12 *Pythium* and *Phytophthora* species identified during this study, only the following seven species have been associated previously with disease in Australia: *Ph. cinnamomi*, *Ph. citricola*, *Ph. drechsleri*, *Ph. nicotianae* var. *parasitica*, *P. acanthicum*, *P. irregulare*, and *P. splendens* (Anon. 1953; Anon. 1964; Anon. 1968; Teakle 1960; Vaartaja and Bumbieris 1964; Vaartaja 1967). Only *Ph. cinnamomi* had been recovered from native vegetation in natural surroundings (Pratt *et al.* 1972b). In other parts of the world, however, all of the species recovered during this study, with the possible exception of *P. ultimum* var. *sporangiferum*, have been found associated with disease of horticultural and forest crops and are considered likely causal agents of disease, acting singly or in combination with other organisms (Wardlaw 1927; Meurs 1934; Drechsler 1941; Campbell and Hendrix 1967; Anon. 1968; Ragunathan 1968; Hendrix and Campbell 1970).

Ph. drechsleri has been found affecting a wide range of plant species in many countries [Commonwealth Mycological Institute (CMI) Map 281] and is best known as the causal agent of a serious root rot disease of safflower (*Carthamus tinctorius* L.) in the United States of America (Erwin 1950). It was found in Australia associated with disease of velvet bean [*Mucuna deeringiana* (Bort.) Merr.] in Queensland (Anon. 1963), and safflower in central and northern New South Wales (Anon. 1970; G. Stovold, personal communication).

Ph. cinnamomi has a world-wide distribution (CMI Map 302), and has been found associated with diseases of an exceedingly wide range of plant species (Zentmyer and Thorn 1967; Titze and Palzer 1969). In Australia it has been found associated with disease of native plant communities in all States and the Australian Capital Territory and was found throughout extensive areas of both diseased and apparently non-diseased native forest in eastern and southern Australia (Pratt and Heather 1973). Consistent recovery of the organism from remote areas of eastern Australia suggests that the fungus could be indigenous to this area (Pratt *et al.* 1972b).

Ph. nicotianae var. *parasitica* and *Ph. citricola* have a world-wide distribution and are pathogenic to numerous plant species including citrus in Australia (Doepel 1966; Anon. 1968; Oxenham and Stone 1969).

Although various workers have shown an association between the presence of *Ph. cinnamomi* and the occurrence of disease in native plant communities, it has not

been demonstrated conclusively that this fungus is the causal agent of disease in all of these communities. The present work emphasizes two relevant points. Firstly, disease similar to that normally associated with the presence of *Ph. cinnamomi* can occur in sites where *Ph. cinnamomi* cannot be recovered by lupin baiting—for example, in stands of *E. obliqua* L'Herit in Tasmania and *E. viminalis* Labill. in New South Wales. Secondly, in these sites, and in other sites where *Ph. cinnamomi* was associated with disease, there were also populations of organisms which are known to be pathogenic in other parts of the world.

Root rot and dieback disease in native plant communities may be induced by organisms other than *Ph. cinnamomi*. One possibility is that the organisms act as a complex, with or without *Ph. cinnamomi*, to induce disease. Such combined action appears to be common in *Pythium* and *Phytophthora*-induced diseases in horticulture and effectively increases the pathogenic ability of the organisms (Robertson 1959; Kerr 1963; Pratt 1965; Hendrix *et al.* 1966).

The effect of *Pythium*- and *Phytophthora*-induced disease is often more pronounced in wet rather than dry situations, and in other work Pratt and Heather (1973) have reported that disease of native plant communities is initiated usually where disturbance has resulted in an increase in soil moisture levels. A similar conclusion was reached in the study reported herein. Disease may develop as a result of increased soil moisture levels in those communities favouring development of abnormally high populations of *Pythium* and *Phytophthora*, or abnormal activity of these organisms.

The relative pathogenicity of *Pythium* and *Phytophthora* spp. from native plant communities is being determined under controlled environmental conditions. This study, involving a range of *Eucalyptus* spp., also includes cultivars of safflower because of the known disease association between *Ph. drechsleri* and this species.

The origin of the *Pythium* and *Phytophthora* spp. recovered during this Australia-wide survey has been difficult to determine and probably can never be fully ascertained. Some sample sites were relatively close to areas where fungal introductions could have occurred with plantings of conifers and agricultural and horticultural crops. However, others were found in areas remote from cultivation and other activities of European man and this may indicate that the organisms are indigenous to eastern Australia or at least have been present in the area for an exceedingly long period. This would be in accord with a prior suggestion we have made to explain the widespread distribution of *Ph. cinnamomi* in Australian native plant communities (Pratt *et al.* 1972b).

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