PHYTOPHTHORA CRYPTOGEA IN PINE FORESTS IN SOUTH AUSTRALIA

by

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STATEMENT

This dissertation has not previously been submitted for a degree at this or any other University and is the original work of the writer, except where due reference is made in the text.

Miervaldis Bumbieris

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SUMMARY

Decline and death of pine trees has frequently been observed in South Australian forest plantations. In the Adelaide hills forest reserves this disorder is often associated with sites which are subject to waterlogging in winter and drying out in summer. As the fungus *Phytophthora cryptogea* is associated with a number of decline sites its role in the decline of pines, mainly *Pinus radiata*, was investigated. In addition, factors likely to influence the susceptibility of *P. radiata* to *Ph. cryptogea* were also studied.

Field studies, mainly in the Kuitpo forest, showed that *Ph. cryptogea* was associated with healthy as well as with diseased *P. radiata*. The horizontal distribution of the fungus in a plantation, and in a cleared area was patchy, suggesting that in soil it may be associated with discrete niches probably in association with plant roots or as free-living chlamydospores. The population density of *Ph. cryptogea* was higher within the root zone of a *P. radiata* than in an area away from it.

In a field experiment with *P. radiata* and *P. pinaster* over 56% of the planted pines died during an abnormally wet winter. The experiment indicated that *P. radiata* is susceptible to *Ph. cryptogea* while *P. pinaster* is not. However, *P. pinaster* appeared to be more susceptible to waterlogging than *P. radiata*. When the experiment was repeated two years later, only about 9% of the planted young trees died, presumably because of much drier soil conditions.

In the glasshouse *Ph. cryptogea*, *Ph. cinnamomi*, *Pythium anandrum* and *P. irregulare* were pathogenic to *Pinus radiata* planted in sterilized

potting soil. Glasshouse tests also showed that waterlogging and transplanting render young *P. radiata* more susceptible to pythiaceous fungi. Transplanted young *P. radiata* were also more susceptible to waterlogging in the absence of pathogenic fungi. Deficiency of nitrogen and phosphorus in soil markedly affected growth of *P. radiata* in pot tests. *Ph. cryptogea* did not influence the growth of such deficient plants but did significantly retard the growth of young pines supplied with complete nutrient solution, and with a solution low in potassium. Although resistance of mycorrhizal pines to *Ph. cryptogea* was not demonstrated in this study, young *P. radiata* inoculated with the mycorrhizal fungus *Rhizopogon luteolus* appeared more healthy than non-mycorrhizal plants when grown in soil inoculated with *Ph. cryptogea*.

In laboratory tests *Ph. cryptogea* formed chlamydospores in roots of pines in soil; and, under the conditions used, the fungus colonized dead organic matter in soil. Production of sporangia by *Ph. cryptogea* was influenced by the temperature to which mycelium of the fungus had been pre-exposed, and the depth of water above the mycelium. Encysted zoospores of the fungus survived in Kuitpo forest soil for 14 days while germ tubes were lysed in 4 to 6 days. Zoospores of *Ph. cryptogea* were not strongly attracted to roots of *P. radiata*, and they did not accumulate in the area immediately behind the root tips. When young *P. radiata* were grown in sand inoculated with *Ph. cryptogea*, infection of roots was not confined to root tips.

It was concluded that *Phytophthora cryptogea* is a weak pathogen of *Pinus radiata* unless other factors detrimental to the trees are also present.

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I. INTRODUCTION

The total area of forest plantations in South Australia in 1973 was 72,574 ha (Thomas 1974). Of this area nearly 90 per cent has been planted with *Pinus radiata* D. Don. About 6,000 ha of land considered unsuitable for *P. radiata* have been planted with *P. pinaster* Ait., about 722 ha have been planted with other *Pinus* spp., and about 1,084 ha with various hardwoods and broadleaf species. Pine plantations in South Australia have been established in three main areas (Lewis and Harding 1963):

1. The podsolized sands and associated calcimorphic soils of the dune ranges of the South-East;
2. The Adelaide hills complexes of laterized relict, sedentary and alluvial soils;
3. The Northern dry country red-brown earths and alluvials in the lower Flinders Ranges.

Pine plantations in South Australia are classified into seven Site Qualities; the first 5 are healthy stands, SQ VI is of marginal health, and SQ VII ranges from marginal to failure. The following disorders have been observed in various forest plantations (Lewis and Harding 1963). Zinc deficiency has been common in most south-eastern soils, but has been eliminated by the standard practice of spraying zinc sulphate solution on trees at the age of 2-3 years. Summer deaths are common in the Adelaide hills reserves as a result of death of tops and decline of trees subjected to winter waterlogging; zones of high salinity also cause numerous tree deaths. 'Spindling' is largely

associated with low soil phosphorus, and is common on sites marginal for planting with *P. radiata*. 'Autumn brown top' is usually associated with summer droughts in the south-eastern calcimorphic soils. Also in the same area second rotation crops suffer from nitrogen deficiency because of suspected serious lowering of nitrogen status of these sites.

Although the above authors do not mention any pathogens, a number of disorders associated with plant pathogenic fungi have been observed in South Australian forest reserves. Occasional outbreaks of needle-cast caused by the fungus *Naemacyclus niveus* (Pers. ex Fr.) Sacc. have been observed in some areas (For. Res. Rpt. S.A. 1970-71). Various blue stain fungi are sometimes encountered, especially in trees suffering from damage by insects, as for example *Ips grandicollis* Eich. (Vaartaja; Pawsey unpubl.). The presence of root rot caused by *Armillariella* and timber rot have occasionally been observed (Pawsey and Bumbieris unpubl.). *Diplodia pinea* (Desm.) Kickx. has been found in association with tip die-back in the Adelaide hills plantations, and Kerr (pers. comm.) found that *D. pinea* could be pathogenic to young *Pinus radiata* after grafting.

Damping-off in forest nurseries may be severe when cold and wet conditions persist during the early seedling stage. Vaartaja (1967) reported that various fungi, mainly *Pythium* spp., *Fusarium* spp., *Rhizoctonia solani* Kühn and *Thielaviopsis basicola* (Berk. & Br.) Ferr. are associated with damping-off of pine seedlings in South Australia. Vaartaja and Bumbieris (1967) found that during hot and dry summer periods *Fusarium* spp. and *Macrophomina phaseolina* (Tassi) Goid. were mainly associated with root rot in pine nurseries. *M. phaseolina* was also found associated with death of 4-year old *Pinus radiata* in the Adelaide hills (Bumbieris unpubl.). Damping-off of pine seedlings has

now decreased in severity because of the standard practice of treating seed with the fungicides 'Captan' or 'Zineb' following a study by Vaartaja and Bumbieris (1965).

Death and decline of pines, both *Pinus radiata* and *P. pinaster*, occur in various forest plantations in South Australia. The symptoms associated with pine decline are frequently similar to those described by Campbell and Copeland (1954) for littleleaf disease of shortleaf and loblolly pines caused by *Phytophthora cinnamomi*. The affected trees have a sickly appearance, generally decline slowly, and die prematurely. Cases where death of trees occurs soon after the first appearance of symptoms, have also been observed. In some cases declining trees respond to applications of phosphorus fertilizers (Boardman, pers. comm.). This indicates that phosphorus deficiency is sometimes the cause of decline.

Davison and Bumbieris (1973) carried out a survey for pythiaceous fungi in forest reserves in South Australia. *Pythium irregulare* Buisman, *P. mamillatum* Meurs and *P. anandrum* Drechsler were frequently isolated from pine plantations. *Phytophthora cryptogea* Pethybridge and Lafferty was isolated in the Kuitpo forest reserve in the Adelaide hills where it was frequently associated with declining *Pinus radiata* on sites subject to waterlogging in winter. *Ph. megasperma* Drechsler var. *sojae* Hildebrand was isolated from one of the forest nurseries in the Adelaide hills, and the Penola forest reserve in the South-east. *Ph. cinnamomi* Rands was found in association with one *Pinus radiata* showing littleleaf symptoms in the Mount Bold seed orchard (Kuitpo forest reserve).

Various authors have reported the association of *Phytophthora* spp. with native vegetation in Australia. Podger, Doepel and Zentmyer (1965)

found *Ph. cinnamomi* in association with a widespread jarrah (*Eucalyptus marginata* Donn. ex Sm.) dieback in Western Australia. Weste and Taylor (1971) and Marks, Kassaby and Fagg (1972) studied the presence of *Ph. cinnamomi* in native vegetation in New South Wales, Tasmania and the Australian Capital Territory, and the presence of *Ph. drechsleri* Tucker in native vegetation in all Australian states except Western Australia. Bumbieris (1974) suggested that *Ph. drechsleri* and *Ph. cryptogea* are conspecific and that *Ph. cryptogea*, as the older name, has priority. This suggestion has since been supported to a certain extent by the studies of Halsall (1976) and Shepherd (1976). Davison (1972) found *Ph. cinnamomi* in association with native vegetation in South Australia.

Newhook (1959) reported that *Ph. cinnamomi* and other *Phytophthora* spp. were associated with death of *Pinus radiata* in shelterbelts and farm woodlots in New Zealand. Disease symptoms were confined almost entirely to trees over 20, and especially over 30 years of age. Isolations from soil yielded mainly *Ph. cinnamomi* and *Ph. cactorum* (Lebert & Cohn) Schroeter. *Ph. citricola* Sawada, *Ph. syringae* (Berk.) Kleb., and *Ph. cryptogea* were also isolated. He also found a highly significant correlation between the presence of *Phytophthora* spp. and disease symptoms, and a significant correlation between the severity of external symptoms and abundance of *Phytophthora* in soil. Some originally healthy-looking shelterbelts growing in soil with high populations of *Phytophthora* spp. later developed severe symptoms following an exceptionally wet autumn, winter and spring. Hepting and Newhook (1962) found *Ph. cinnamomi* in association with shortleaf, loblolly and longleaf pines showing littleleaf symptoms in various New Zealand plantations. With regard to the importance of *Phytophthora* in pine forests Newhook (1959) stated: '... it is obvious

that where *Phytophthora* spp. are present they must be regarded as pathogens, contributing to the disease complex of the forest'.

The association of *Phytophthora* with pine plantations has also been reported in Australia. Thus Oxenham and Winks (1963) found *Ph. cinnamomi* associated with disorders of *Pinus elliotii* Engelm., and *Ph. boehmeriae* Sawada with disorders of *Pinus patula* Schlecht in Queensland. Hartigan (1964) reported the isolation of *Ph. cinnamomi* from *Pinus radiata* showing dieback symptoms in New South Wales.

Various authors have reported the association of *Ph. cinnamomi* with littleleaf disease of pines in the United States of America (Campbell 1948; Kuhlman and Hendrix 1963; Roth ahd Kuhlman 1963; and others). Campbell and Copeland (1954) described the symptoms of littleleaf disease, pointing out that the early symptoms of the disease are difficult to distinguish from growth variations resulting from numerous other causes while in the final stages the scanty and chlorotic foliage is confined to the ends of the branches.

Numerous reports have shown that *Phytophthora* causes death and decline of fruit trees, as for example *Citrus* spp. (Klotz *et al.* 1958), avocados (Zentmyer *et al.* 1967), peaches (McIntosh 1964; Mircetich and Keil 1970), and the trunk rot of apples caused by *Ph. cactorum* has been known for many years (Welsh 1942). McIntosh (1964) reported that *Ph. cryptogea* was pathogenic to rootlets of pear, peach, apricot and cherry seedlings. Both *Ph. cryptogea* and *Ph. cinnamomi* were isolated from peaches suffering from collar and root rot in South Africa (Bumbieris unpubl.). Wicks and Volle (1976) reported the pathogenicity of *Ph. cryptogea* to chestnut trees in South Australia.

Robertson (1970) studied the susceptibility of exotic and

indigenous trees and shrubs to *Ph. cinnamomi* in New Zealand, and found that 23 species and cultivars not previously recorded as hosts of this fungus were susceptible under the experimental conditions.

During their survey Davison and Bumbieris (1973) found that *Ph. cryptogea* was present in 9 out of 14 sampled sites in the Kuitpo forest reserve which is situated in the Mount Lofty ranges about 50 km from Adelaide. Pines in this reserve are often planted on steeply sloping hillsides and valley floors with numerous creeks present in some areas. Sites where death of trees has occurred are often situated on valley floors on sandy soil overlying clay, liable to waterlogging in winter and drying out in summer. Death of pines associated with these sites in the Kuitpo forest is not widespread but is of importance because the affected trees generally are of better original quality than those growing on hilltops and sides of gullies. Poor growth of pines on hillsides and higher ridges may be associated with lack of water or with phosphorus deficiency in soil (Boardman pers. comm.), and generally becomes apparent early in the life of the trees. On the other hand, decline of trees growing on wet sites generally becomes obvious when the pines reach the age of 20 or 30 years.

The affected patches vary in size from a few square metres to about 1.5 ha. The age of these trees varies from 1 to 44 years although most are in the age group from 31 to 44 years. Apart from one case where one-year-old *Pinus pinaster* were showing decline symptoms, the rest of affected trees were *P. radiata* (Davison and Bumbieris 1973). It has also been noticed that in some cases the natural direction of drainage has been altered by compaction of soil along logging tracks.

Although Davison and Bumbieris (1973) found *Ph. cryptogea* associated with a number of sites where decline and death of pines had occurred, consistent association of the fungus with diseased trees was not established. Also, the authors did not isolate *Ph. cryptogea* directly from affected roots or investigate the pathogenicity of the fungus to *P. radiata*. As far as the author knows, the pathogenicity of *Ph. cryptogea* to pines has not previously been studied.

For the above reasons the first aim of this study was to investigate the association and pathogenicity of *Ph. cryptogea* to *P. radiata* as such information becomes important when replanting sites where decline and death of pines had occurred has to be considered. The second aim of the project was to investigate some environmental and biotic factors which might influence the relationship between *Ph. cryptogea* and *P. radiata*, and the process of decline. The investigations were carried out in the field, the glasshouse and in the laboratory.

II. MATERIALS AND METHODS

(1) Isolation of *Phytophthora* from soil and roots

In most cases *Phytophthora* was isolated from soil samples using the pear 'baiting' method (McIntosh 1964). Samples of soil and small roots were collected from one or two sides of trees at a distance of about 50 cm from their trunks and to a depth of about 15-20 cm, placed in plastic bags and taken to the laboratory. The same day about 200 g of each sample was placed in each of two plastic containers 12 cm in diameter and 6 cm deep. A semi-ripe pear of either the cultivar 'Packham' or 'Duchess', depending on availability, was placed in each container. Enough distilled water was added to the containers to cover the soil to a depth of about 2 cm. The containers were kept in the laboratory in diffused light until brown rot developed on the fruit at or near the water mark, but not longer than 7 days. Temperature in the room fluctuated between about 17°C and 25°C. In most cases rot developed within 3 to 5 days. To isolate *Phytophthora*, the pears were first washed in running tap-water, dried and passed briefly through a flame. Segments about 3x3 mm were cut from the edges of the rotting areas and plated on cornmeal agar containing Vancomycin at a concentration of 100 µg/ml to suppress growth of bacteria.

To determine the presence of *Ph. cryptogea* in soil from areas where the fungus had previously been isolated, and which had not yielded any other *Phytophthora* species, cotyledons of tomato seedlings were used as 'baits'. This method is similar to that used by Marks and Kassaby (1974) with cotyledons of *Eucalyptus sieberi* for the isolation of

Ph. cinnamomi. In this work cotyledons of tomatoes were preferred to those of *E. sieberi* because they appeared more susceptible to *Ph. cryptogea* when the two 'baits' were compared.

About 20 cc of a soil sample was placed in a small plastic cup and sufficient distilled water was added to cover the soil to a depth of about 1 cm. Four cotyledons of 2 to 3 week old tomato seedlings were floated on the water in each cup which was left in diffused light in the laboratory. The cotyledons became infected and sporangia developed around their edges in 3 to 4 days. To observe sporangia the cotyledons were transferred one by one to a small volume of water in the shallow lid of a small Petri dish and examined using a dissecting microscope. *Ph. cryptogea* could be recognised by the shape and size of sporangia, and the production of new sporangia by internal proliferation. In these cases isolation of the fungus was not deemed necessary.

To isolate *Ph. cryptogea* from individual pine roots, small root pieces were surface-sterilized in 1% sodium hypochlorite solution for 30 seconds and plated on Tsao and Ocana's (1969) $P_{10}VP$ medium (cornmeal agar with 10 µg/ml Pimaricin, 200 µg/ml Vancomycin and 100 µg/ml pentochloronitrobenzene).

Attempts were also made to isolate *Ph. cryptogea* from Kuitpo forest reserve soil using the following selective media: (1) Tsao and Ocana's $P_{10}VP$ medium, and (2) Lima bean agar with the same substances as in (1). The methods of isolation were: soil dilution plates (soil diluted 1:10); Warcup's (1950) soil plates with 0.1 cc of soil per plate; and 0.1 cc of soil distributed on the surface of solidified media. Ten replicate plates were prepared for each method with each of the two media.

The plates were incubated in the dark at 25°C for three days. Microscopic examination of the plates revealed no *Phytophthora*-like colonies on any of the plates, but numerous colonies of *Mortierella* and *Pythium* were seen. Only sporangia of *Pythium* developed when plates were flooded with water.

(2) The identification of *Phytophthora* species

To identify *Phytophthora* species, the fungi were grown on Difco Lima bean agar for 3 to 4 days, and then flooded with 1% non-sterile soil extract to stimulate production of sporangia. Stimulation of sporangial production of *Ph. cinnamomi* by non-sterile soil extract was first reported by Mehrlich (1935), and Zentmyer (1965) attributed this stimulation to certain soil bacteria. In the case of *Ph. cryptogea* the characteristic hyphal swellings developed when cultures of the fungus were flooded with soil extract of water. Although *Ph. cryptogea* grown on Lima bean agar produced sporangia when flooded with distilled water, non-sterile soil extract was often used to hasten production of these structures. However, use of non-sterile soil extract was not deemed necessary when examining roots of *Pinus radiata* removed from soil for the presence of the fungus, and distilled water was used in these cases to obtain production of sporangia. To induce release of zoospores, the dishes were chilled in a refrigerator for 20 minutes and then returned to room temperature.

The key of Waterhouse (1963) was used to identify *Phytophthora* species, and that of Middleton (1943) for *Pythium* species. Cultures of the fungi were maintained on cornmeal agar at room temperature in small McCartney bottles and sub-cultured twice a year.

III. THE PYTHIACEOUS FUNGI INVOLVED IN THE STUDY

The genus *Phytophthora* was described by de Bary in 1875 (Waterhouse 1970), the type species being *Phytophthora infestans* (Montagne) de Bary. *Phytophthora cryptogea* was described by Pethybridge and Lafferty (1919) from tomatoes and other plants in Ireland.

In her key to the species of *Phytophthora* Waterhouse (1963) segregates the genus into six groups based on characteristics such as the formation of sporangia by internal proliferation, width of the sporangial exit pore, production of oogonia in single strain culture, and the placement of antheridia (paragynous or amphigynous). *Ph. cryptogea* is placed in group 6 characterized by non-papillate sporangia with a thin apical thickening and a broad exit pore. The sporangia are rarely or never produced on solid substrates, and often develop by internal proliferation. Oospores are not always produced in single strain cultures but are produced when paired with the opposite strain of the same fungus or of *Ph. cinnamomi*. The antheridia are mostly amphigynous. The mycelium of *Ph. cryptogea* produces typical hyphal swellings when submerged in water. The cardinal growth temperatures as given by Waterhouse (1963) are: min. under 1^{o}C, opt. 22-25^{o}C, max. 31-33^{o}C.

Ph. cryptogea belongs to the same taxonomical group as *Ph. cinnamomi*, and morphologically is very similar to *Ph. drechsleri* as described by Waterhouse (1963). Bumbieris (1974) studied the morphological and some physiological characters of a number of isolates of *Ph. drechsleri* and *Ph. cryptogea*, and concluded that they should be

regarded as one species, and that *Ph. cryptogea*, as the older is the valid name. Appendix 1 shows the host list of *Ph. cryptogea* (including *Ph. drechsleri*) as far as it was possible to compile it from available literature.

The isolate of *Phytophthora cryptogea* (P 30) used in most experiments was isolated from Kuitpo forest soil, and has been deposited in the American Type Culture Collection (Accession Number 28194). The isolate formed colonies with little aerial growth when grown on cornmeal agar but colonies on Lima bean agar were quite fluffy. Regular hyphal swellings, mainly in groups, were formed when mycelium of the isolate was submerged in distilled water. The net-like appearance of the mycelium in water described by Waterhouse (1963) was also observed.

Abundant sporangia were formed within 24 hours when cultures were submerged in 1% non-sterile soil extract, but sporangial production in distilled water was less prolific. Sporangia were ovoid to obpyriform, non-papillate with an average size of 47.6 x 32.9 µm (35-65 x 25-38 µm). Mature sporangia often possessed a conspicuous central vacuole. No sporangia were seen in non-irrigated cultures. Oogonia were not produced in single strain cultures but were formed within 11 days when growth together with the A-1 strain of the fungus on Campbell's V-8 juice agar (Davison pers. comm.). The isolate did not grow at 1°C but grew 0.2 mm/day on cornmeal agar at 5°C; on the same agar it grew 0.2 mm/day at 35°C but did not grow at 37°C. All the observed characteristics agreed with those given by Waterhouse (1963) for the species within the limits of experimental error.

Isolate P 50 of *Ph. cryptogea* isolated from roots of *Pinus radiata*, and used in some later tests was similar to isolate P 30 with regard to its morphological characteristics, and its effect to *Pinus radiata*.

Ph. cinnamomi, isolate P 26, used in some tests, was obtained from the root zone of a *P. radiata* at the Mount Bold seed orchard in the Adelaide hills. When cultured on cornmeal agar the isolate showed the typical coralloid growth pattern, and the characteristic spherical swellings developed within two to four days. The swellings were more numerous and more chlamydospore-like when the fungus was grown on Lima bean agar.

The internally proliferating sporangia of the fungus which developed when Lima bean agar cultures were submerged in 1% non-sterile soil extract, were larger than those of *Ph. cryptogea* (average 54.7 x 35.2 µm). They were non-papillate with a fairly wide exit pore (8-10 µm), and ellipsoid or ovoid, sometimes also obpyriform in shape. It was interesting to notice that often zoospores were also released from the spherical hyphal swellings. Oogonia were not formed in single strain cultures but were formed within 11 days when growth together with the A-1 strain of the same fungus, and within 35 days when grown together with A-1 strain of *Ph. cryptogea* on V-8 juice agar (Davison pers. comm.). All the characteristics agreed with those of *Ph. cinnamomi* (Waterhouse 1963).

Although this work deals mainly with *Ph. cryptogea*, *Pythium irregulare* was included in one test because of its common occurrence in pine forests, and because it had been shown to be involved in a decline

of peach trees (Hendrix *et al.* 1966). The isolate used (Py 15) was obtained from the Mount Bold seed orchard, and has been deposited in the American Type Culture Collection (Accession Number 28196). On cornmeal agar the isolate had spherical to ellipsoid sporangia borne both terminally and intercalarily, with a mean diameter of 20.9 µm. The aplerotic oogonia, with a mean diameter of 19.3 µm, had the irregular appearance characteristic of *P. irregulare*. Oogonia often possessed one or two spines. Antheridia were typically monoclinous, and in most cases one per oogonium.

Pythium anandrum was included in one test because of its frequent occurrence in pine forests in South Australia. Its identity was confirmed at the Commonwealth Mycological Institute (Davison and Bumbieris 1973). In cornmeal agar cultures the fungus produced the very characteristic echinulate oogonia with acutely tipped spines. The oogonial stalk widened near the oogonium, and in many cases was limited from the oogonium by a dome-shaped septum as seen in Figure 16 of Middleton's (1943) description of the species. No antheridia were seen in this isolate. Sporangia did not develop in agar cultures but were produced when grass stems infected by the fungus were placed in water and left in the open air outside the building (at about 15^{o}C).

When cotyledons of tomato seedlings were used as 'baits' to isolate *Phytophthora cryptogea* from soil, they often became infected by *Pythium anandrum*, and numerous sporangia of *P. anandrum* were observed around the edges of the cotyledons. They were elongated, ellipsoidal and papillate, and were often formed by internal proliferation.

IV. DESCRIPTION OF THE FOREST AREAS

The field work, collection of soil samples and roots for isolation of *Phytophthora* were done in the Kuitpo and Mount Crawford forests.

The area of Kuitpo forest used for most of the work is situated about 0.5 km east of the Prospect Hill - Willunga road, and is part of compartment 1936/162 of the Kuitpo forest reserve. Field experiments were carried out on a site where trees suffering from severe decline had been salvaged seven years earlier. The size of the site, which will be referred to as site A, is about 1.5 ha, and it has not been replanted. Root material and soil samples were also collected from the plantation adjoining site A. Although some of the pines at the edge of site A showed symptoms of decline, trees in the plantation generally appeared to be healthy.

Rix and Hutton (1953) describe the landscape of the Hundred of Kuitpo as a blockfaulted, uplifted and dissected peneplain. These authors group the various soil types of this area under four main headings: laterized and relict soils, occurring on tops of hills and spurs; sedentary soils, occurring on a few isolated hills and sides of gullies; transported soils, occurring on floors of valleys; and alluvial soils which occur along watercourses. The soils of site A, and adjoining plantations belong to the following types: Meadows fine sand (Mfs), Euchunga sand (Es) and alluvial complexes (A-C). In the Mfs and Es types the A horizon consists of white to grey to yellow sand while the B horizon is characterized by various types of clay. The A-C complexes consist of an intricate mosaic of the various valley

soil types together with a number of different alluvial deposits.

Soil samples for the isolation of *Phytophthora* were also collected from compartment 1941/227 of Mount Crawford forest which is situated about 45 km north-east of Adelaide in the Mount Lofty ranges. A number of *Pinus radiata* in this compartment had died about 15 years previously, their death at the time being attributed to high salinity of the soil (Harding pers. comm.). There is the possibility, however, that *Phytophthora* may have been involved.

V. FIELD EXPERIMENTS

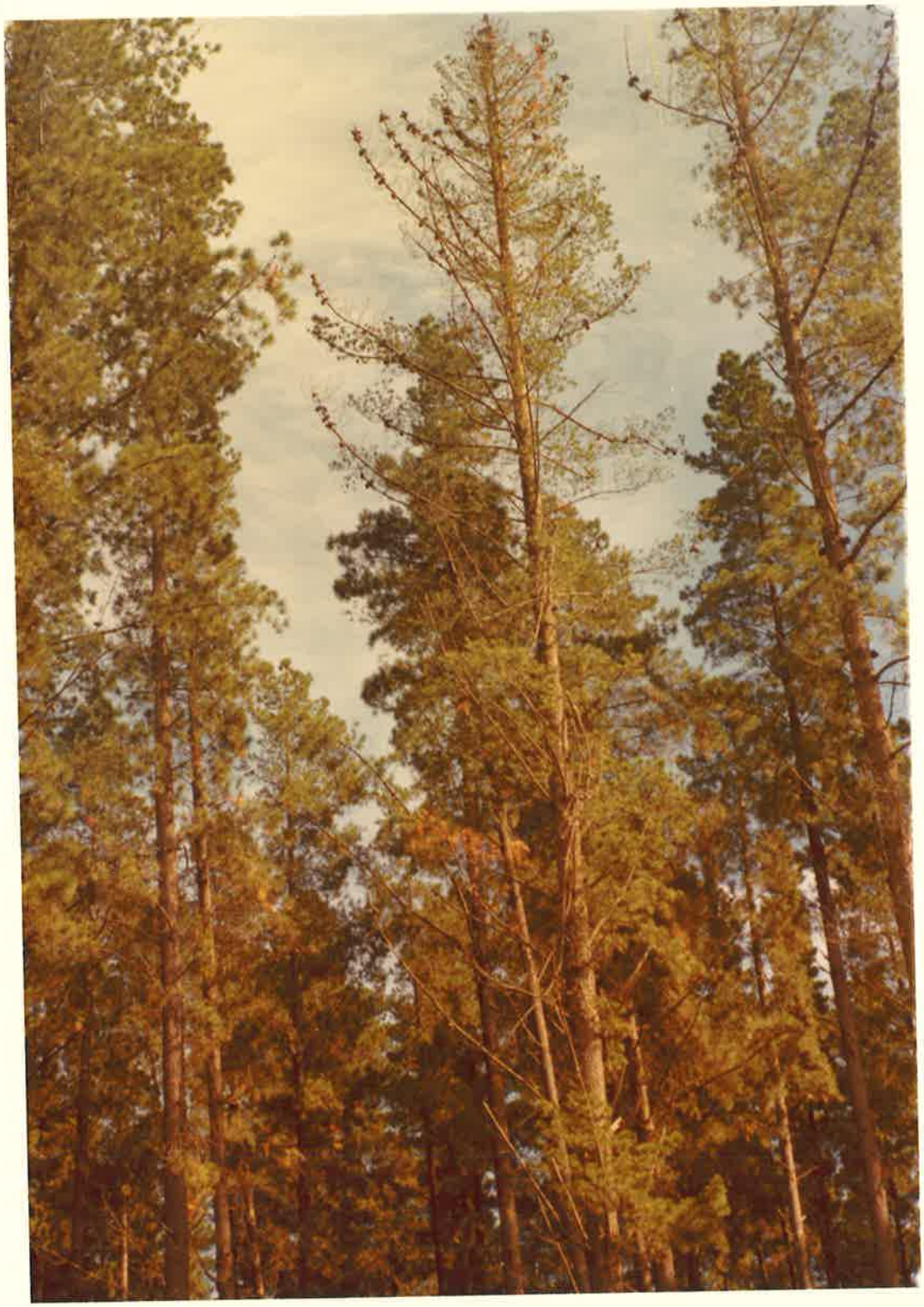
To show that a certain organism is the causal agent of a disease, the rules known as Koch's postulates are followed where possible. These rules require that: the organism should be consistently associated with the particular disease; that it should be isolated in pure culture; that when inoculated into a healthy plant under favourable disease conditions, the organism should reproduce the symptoms; and that it should be reisolated from the inoculated plant, compared with the original, and found to be the same.

Davison and Bumbieris (1973) isolated *Phytophthora cryptogea* from the root zones of 21 of the sampled 101 *Pinus radiata* showing decline symptoms in the Kuitpo forest reserve. In this respect the fungus does not appear to satisfy the first of Koch's postulates as its consistent association with declining trees was not demonstrated. However, negative results obtained in samplings with the 'baiting' method such as used for *Phytophthora* cannot always be regarded as conclusive. There are various factors such as soil temperature, soil moisture, etc., which influence the results so that negative results are not necessarily reliable. On the other hand, the isolations showed that *Ph. cryptogea* is present in sites where decline of *P. radiata* occurs.

(1) Isolation of *Phytophthora cryptogea* from healthy trees

As a preliminary step in the investigation of the role of *Ph. cryptogea* in pine decline, it was decided to examine apparently healthy trees for the presence of the fungus in their root zones. This examination was carried out in the Kuitpo and Mount Crawford forests.

Figure 1. Two *Pinus radiata* showing symptoms of pine decline.



Soil samples were collected from near the trunks of trees of healthy appearance situated near the decline sites but on higher ground, and the pear 'baiting' method used to isolate the fungus.

Table 1 shows that 21 of the 42 trees sampled yielded *Ph. cryptogea*. Thus the fungus was present in the root zones of at least 50 per cent of trees which appeared healthy.

Thus this and the previous work established the presence of *Ph. cryptogea* in the root zones of both declining and apparently healthy *Pinus radiata* in Kuitpo and Mount Crawford forests. Although isolation of *Phytophthora* from soil around diseased plants is often used to show the association of these fungi with their hosts, it does not distinguish between fungi present in soil, and fungi present in host roots. Thus *Ph. cryptogea* isolated from the root zones of apparently healthy trees may have originated from soil rather than the roots of the trees. Therefore it was decided to examine the roots of an apparently healthy tree for the presence of the fungus.

As a preliminary step, a group of 10 trees situated near site A were sampled for the presence of *Ph. cryptogea* in their root zones using the pear 'baiting' method. The trees, all *P. radiata*, were numbered for further reference. *Ph. cryptogea* was isolated from tree No. 5 which was then used for further work.

To obtain roots from the tree, a block of soil about 25 x 25 x 20 cm was removed from the root zone with a spade, placed in a plastic bag and transported to the laboratory. The roots were then washed out under a tap. Root pieces about 1 cm long were plated on the selective $P_{10}VP$ medium. The sampling was repeated six times during the

Table 1.

Results of isolations from apparently healthy trees

Locality	Age of Trees (years)	No. of trees sampled	No. of trees yielding *Ph. cryptogea*
Mount Crawford	32	10	3
Kuitpo	various	10	7
Kuitpo	37	22	11
Total		42	21

period from November 1974 to April 1975. Table 2 shows that only 17 root pieces of the total number of 1345 yielded *Ph. cryptogea*. Although the number of root pieces yielding *Ph. cryptogea* was very small (about 1.3%), the examination showed that the fungus is associated with roots of an apparently healthy *P. radiata*.

(2) Presence of *Phytophthora cryptogea* in roots of two trees of different vigour

To obtain information about the presence of *Ph. cryptogea* in trees differing in vigour, two *Pinus radiata* situated at the eastern end of site A were chosen for comparison. Tree (1) appeared more healthy but with a fairly narrow crown while tree (2) had a wider crown but showed more definite decline and littleleaf symptoms. Undoubtedly it would have been better to choose two more contrasting trees but the trees chosen were growing next to each other, and both yielded *Ph. cryptogea* from their root zones at a preliminary sampling.

Roots were obtained as previously described from blocks of soil from the root zones of both trees. Sampling was repeated 5 times during the months of May and June, 1975. One hundred root pieces from each tree at each sampling were plated as described previously except that at the last sampling only 80 pieces could be obtained from the soil blocks. Thus a total of 480 root pieces were plated from each tree. It is seen from Table 3 that there was no difference in the number of root pieces yielding *Ph. cryptogea*.

(3) Horizontal distribution of *Phytophthora cryptogea* in soil

To determine the horizontal distribution of *Ph. cryptogea* in soil, soil and root samples were collected from three rectangular areas

Table 2.

Isolation of *Phytophthora cryptogea* from roots of an apparently healthy *Pinus radiata*

Sampling dates	No. of root pieces plated	No. of root pieces yielding *Ph. cryptogea*
15.11.74	230	7
8.1.75	230	1
20.1.75	215	6
13.2.75	215	2
19.3.75	230	0
3.4.75	225	1
Total	1345	17

Table 3.

Isolation of *Phytophthora cryptogea* from roots of trees of high vigour (1) and low vigour (2)

No. of root pieces yielding *Ph. cryptogea*	
Tree (1)	Tree (2)
10	18
13	20
14	12
12	20
27	6
76	76

at site A, and from three similar areas in the adjacent plantation. The latter areas were situated in the root zone of tree No. 5 used in the work previously described. The size of the areas was 2.5 x 1.5 m, and samples were taken from points 50 cm apart with a soil sampler 2.7 cm in diameter inserted to a depth of 15 cm. A total of 24 samples were collected from each area. In the laboratory 20 cc of soil from each sample were placed in each of two 25 cc graduated plastic cups. The presence of *Ph. cryptogea* in soil samples was determined by 'baiting' with cotyledons of tomato seedlings as previously described.

Figure 2 shows the horizontal distribution of *Ph. cryptogea* in the sampled areas. It is seen that there is no correlation between sampling points yielding the fungus, and location of trees, stumps or furrows where soil could be expected to be wetter.

(4) Population density of *Phytophthora cryptogea* in two areas

As *Ph. cryptogea* was found to be present in both the cleared site A, and in the root zone of a *Pinus radiata* in the adjacent plantation, it was decided to compare the population density of the fungus in the two locations. Soil and root samples were collected along two transects: one marked on site A, and the other in the adjacent plantation. Each of the transects was 40 m long with sampling sites 10 m apart. Thus samples were collected from 5 sites along each transect. Transect (1) passed through the first three sampling areas used for the determination of horizontal distribution of *Ph. cryptogea* described in the previous section. Site (e) of transect (2) was situated at tree No. 5 sampled previously. Figure 3 shows the location and sampling sites of the transects.

Figure 2. Horizontal distribution of *Ph. cryptogea* on a cleared site and in adjoining plantation.

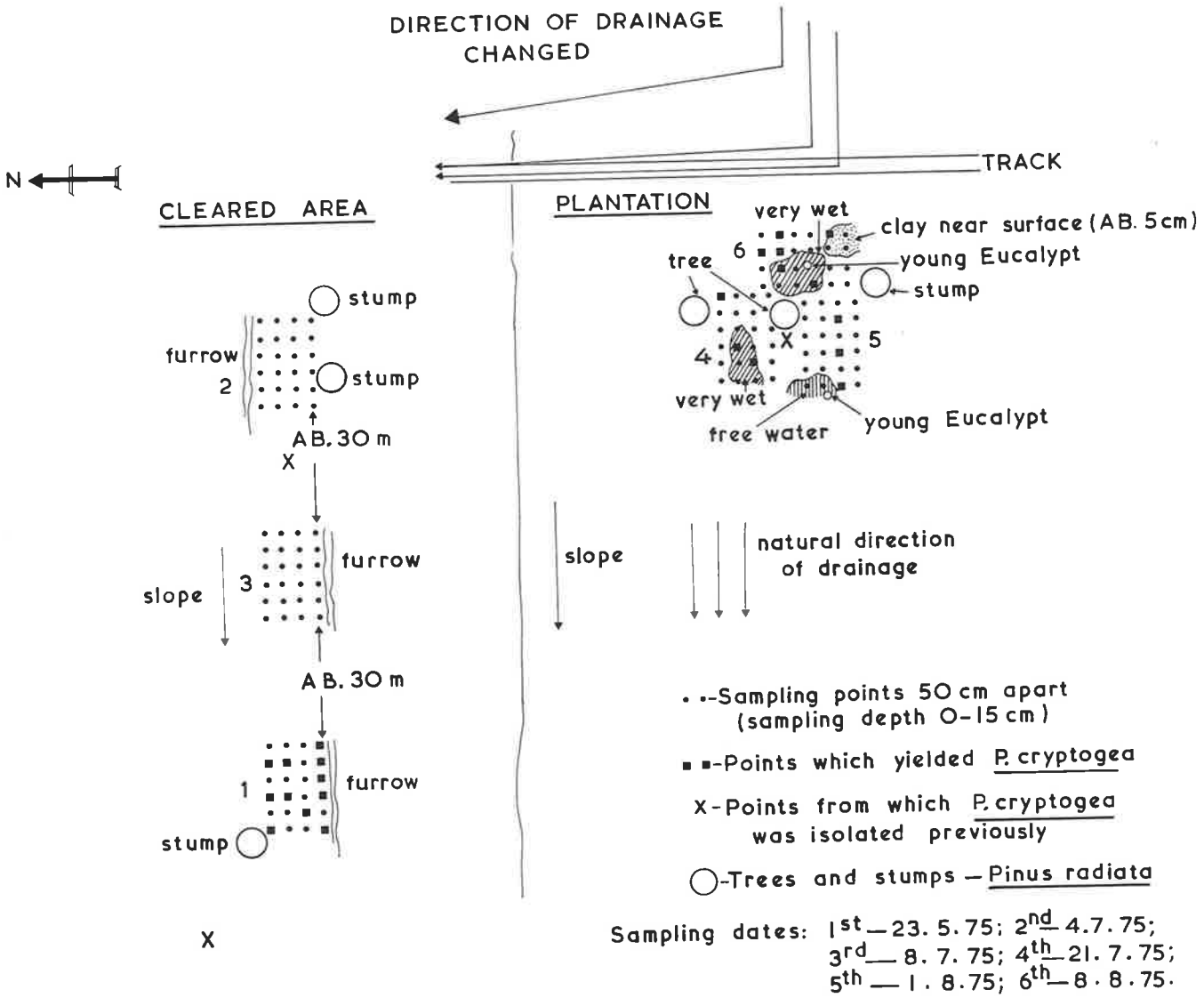
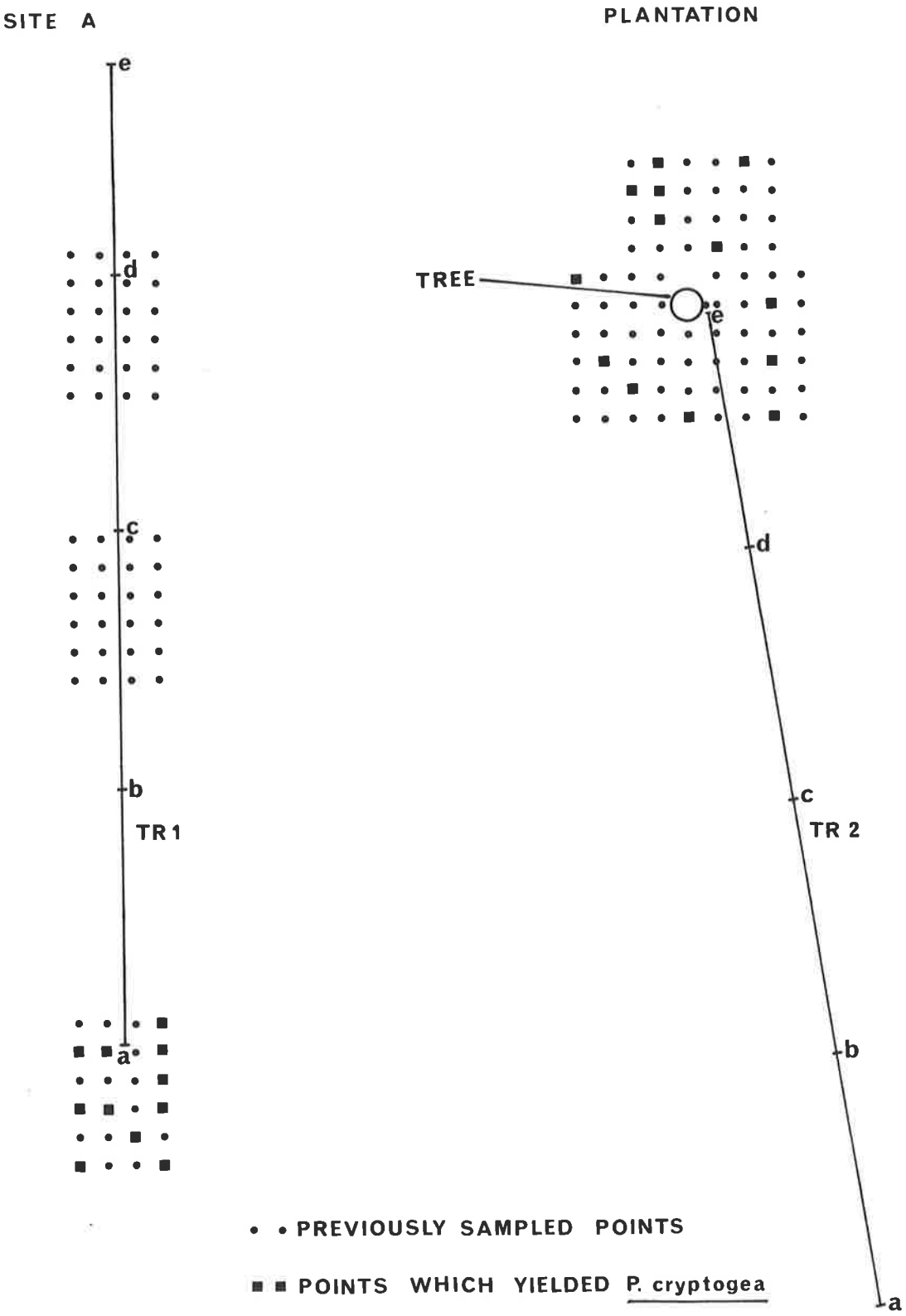

DIRECTION OF DRAINAGE CHANGED
N
TRACK
CLEARED AREA
PLANTATION
stump
furrow
2
stump
AB. 30 m
X
slope
3
furrow
AB. 30 m
1
furrow
stump
X
very wet
clay near surface (AB. 5cm)
tree
6
young Eucalypt
stump
4
X
5
very wet
free water
young Eucalypt
slope
natural direction of drainage
• •-Sampling points 50 cm apart (sampling depth 0-15 cm)
■ ■-Points which yielded P. cryptogea
X-Points from which P. cryptogea was isolated previously
◯-Trees and stumps — Pinus radiata
Sampling dates: 1st — 23. 5. 75; 2nd — 4. 7. 75;
3rd — 8. 7. 75; 4th — 21. 7. 75;
5th — 1. 8. 75; 6th — 8. 8. 75.

Figure 3. Location of two transects.


SITE A
PLANTATION
e
d
c
b
TR 1
a
TREE
e
d
c
TR 2
b
a
• • PREVIOUSLY SAMPLED POINTS
■ ■ POINTS WHICH YIELDED P. cryptogea

Six subsamples of soil were collected from each of the sampling sites at a distance of 15 cm from a central point, bulked, placed in a plastic bag and taken to the laboratory. The method of Tsao (1960) was used to determine the population density of *Ph. cryptogea* in the collected samples. A known quantity of field soil was thoroughly mixed with an equal quantity of sterile sand, and half of the mixture was divided into three small plastic cups (20 cc of mixture per cup). The other half of the mixture was again mixed with an equal volume of sterile sand and treated as before. Thus a series of dilutions were obtained ranging from 1 : 0 to 1 : 64. Reciprocals of the dilution ratios are known as population density indices. The method was modified by Marks and Mitchell (1970) when they studied the presence of *Ph. megasperma* in lucerne fields. Weste and Ruppin (1975) and Marks, Kassaby and Fagg (1973) used this method to study population densities of *Ph. cinnamomi* in native forests in Victoria. The presence of *Ph. cryptogea* was determined by the tomato cotyledon method.

Table 4 shows that none of the sampled sites of transect (1) yielded *Ph. cryptogea* although sampling site (a) was situated in the area where several points had yielded the fungus before. However, the fungus was obtained from two sites of transect (2): site (c) where only undiluted soil yielded *Ph. cryptogea*, and site (e) where the population density index was 8. Sampling from transect (1) was repeated a month later but the result was negative again.

(5) Attempted isolation of *Phytophthora cryptogea* from various soil fractions

An attempt was made to isolate *Ph. cryptogea* from different soil fractions to learn whether the fungus would be found in association with

Table 4.

Population density of *Phytophthora cryptogea* along two transects

Transects and sampling sites	Reciprocals of dilutions							
	0	1	2	4	8	16	32	64
1 (a)	-	-	-	-	-	-	-	-
1 (b)	-	-	-	-	-	-	-	-
1 (c)	-	-	-	-	-	-	-	-
1 (d)	-	-	-	-	-	-	-	-
1 (e)	-	-	-	-	-	-	-	-
2 (a)	-	-	-	-	-	-	-	-
2 (b)	-	-	-	-	-	-	-	-
2 (c)	+	-	-	-	-	-	-	-
2 (d)	-	-	-	-	-	-	-	-
2 (e)	+	-	+	+	+	-	-	-

— = negative result

\+ = positive result

organic matter in the soil, or as free chlamydospores in which case, because of the size of these propagules, the fungus could be expected to be associated with the fine soil fraction.

Soil was collected from all sites of transect (2), bulked together and thoroughly mixed. The following steps were then carried out:

(1) About 2 kg of soil was sieved through a 2 mm sieve which retained most of the roots and larger organic debris;

(2) The residue was stirred in a blender at low speed for 2 minutes with 5 volumes of water;

(3) The suspension was passed through a series of graded sieves: 259 µm, 104 µm, and 53 µm mesh;

(4) The residue retained on the first sieve (250 µm) which appeared to consist mainly of organic matter, was stirred again with 300 ml of water and passed through the sieves twice to remove fine soil particles;

(5) The coarse fraction obtained by passing soil through the 2 mm sieve was stirred in a blender as before, and passed through the same series of graded sieves.

The following fractions were thus obtained: fraction No. 1 consisting of organic matter; fraction 2 (> 250 µm) consisting mainly of coarse sand and some organic matter; fraction 3 (104-250 µm) consisting mostly of sand; fraction 4 (53-104 µm) consisting of sand and possibly some silt; and fraction 5 (< 53 µm), collected in a tray, consisting mostly of clay. Five small plastic cups were used for each

of the fractions to determine the presence of *Ph. cryptogea* by 'baiting' with tomato cotyledons but no *Phytophthora* was obtained from any of the fractions. The procedure was repeated once more with the same result.

(6) Isolation of *Phytophthora cryptogea* from other plant species

Various other plants are frequently found among pines in thinned stands. Some of the species found among *Pinus radiata* in the general area studied are: *Acacia armata* R.Br. ex Ait. (kangaroo thorn), *Cirsium vulgare* (Savi) Ten. (thistle), *Eucalyptus camaldulensis* Dehnh. (red gum), *Leptospermum juniperinum* Sm. (tea-tree), *Rosa canina* L. (dog rose), *Rubus fructicosus* L. (blackberry) and *Senecio pterophorus* De. (African daisy).

To see if these plants were hosts of *Ph. cryptogea*, roots were collected and taken to the laboratory although all plants appeared healthy except the *Leptospermum juniperinum* which occasionally showed some dying branches. In the laboratory root pieces, about 1 cm long, of each species were plated on the selective $P_{10}VP$ medium.

Table 5 shows that *Ph. cryptogea* was found in association with the roots of *Cirsium vulgare* and *Eucalyptus camaldulensis*. This shows that other plant species, although appearing healthy, may harbour *Ph. cryptogea* in or on their roots, and thus be of importance in the survival of the fungus.

(7) Discussion

Often, when an organism is suspected of causing a disease, healthy plants in the vicinity are also examined for the presence of the organism. To see if *Phytophthora cryptogea* is associated with apparently healthy *Pinus radiata*, a total of 42 such trees were sampled in Kuitpo

Table 5.

Isolation of *Phytophthora cryptogea* from other plant species.

Plants	No. of root pieces plated	No. of root pieces yielding *Ph. cryptogea*
Acacia armata	60	-
Cirsium vulgare	50	5
Eucalyptus camaldulensis	60	4
Leptospermum juniperinum	50	-
Rosa canina	50	-
Rubus fructicosus	50	-
Senecio pterophorus	50	-

and Mount Crawford forest reserves; of these 21 (50%) yielded the fungus. The sampled trees were growing next to decline sites but were situated on higher ground because of the sloping nature of the site in Kuitpo forest, and because they had been planted on mounds in Mount Crawford forest. The presence of *Ph. cryptogea* in the root zones of apparently healthy trees tends to discount any claim that the fungus is the cause of the observed decline. On the other hand, it is difficult or even impossible to say that these trees were growing as well as they would be if the fungus were not present.

A more direct method of establishing the association of an organism with a host plant is its isolation from affected parts of the host, in this case pine roots. When direct isolation of *Ph. cryptogea* was attempted from roots of an apparently healthy *P. radiata*, 17 root pieces of a total of 1345 pieces plated on a selective medium yielded the fungus (Table 2). Although *Ph. cryptogea* had been isolated from the root zone of this tree before, only a small number of root pieces yielded the fungus when direct isolation from roots was attempted. Failure to isolate the fungus from more roots of this tree is difficult to explain. When roots of two adjoining trees differing in vigour were examined, 76 root pieces of 480 pieces plated from each tree yielded *Ph. cryptogea* (Table 3). Thus examination of trees using the two isolation methods showed that *Ph. cryptogea* was associated with declining as well as apparently healthy trees.

As already mentioned, the apparently healthy trees were growing on higher ground where soil was probably drier than at the decline sites. It is now generally accepted that various factors present in soil, because of their interactions with the host or the pathogen, play a part

in soil-borne diseases. Kassaby, Fagg and Marks (1977) studied the colonization of mountain forest soil by *Ph. cinnamomi*, and concluded that the establishment of the fungus and expression of disease are not encouraged by low soil temperature. The interactions of various biological factors may also influence soil-borne plant diseases. Marx (1970) studied the effect of mycorrhizas on root rot of pines caused by *Ph. cinnamomi*. The significance of various microbial interactions in soil has been discussed by Griffin (1972). Sitepu and Wallace (1974) investigated correlations between soil moisture, soil pH, soil texture, and the presence of *Ph. cryptogea* and *Pythium vexans* de Bary in relation to retarded growth of apple trees. The effect of soil moisture on *P. radiata* in the presence and absence of *Ph. cryptogea* is examined later.

In Kuitpo forest *P. radiata* suffering from severe decline had been salvaged from site A seven years prior to this study. It was of interest to know whether the fungus was still present, and what would be the distribution of the fungus at the salvaged site and in the adjoining plantation. In this study only the horizontal distribution was investigated. The vertical distribution of the fungus was not studied as it was considered that the vertical distribution of *Ph. cryptogea* would be similar to that of *Ph. cinnamomi*.

Weste, Cooke and Taylor (1973) studied the vertical distribution of *Ph. cinnamomi* in a eucalypt forest in Victoria, and isolated the fungus from the depth of 56 cm. Campbell (1951) examined the vertical distribution of *Ph. cinnamomi* in soil under littleleaf-diseased shortleaf pines and found that the fungus was most abundant at the depth of 5 to 7.5 cm, falling rapidly to a low point at 15 and 17.5 cm, after which it

was again more abundant at 20 and 22.5 cm. No doubt, the depth at which the fungus is found varies depending on such interacting factors as soil type, aeration and available nutrients. Information about the horizontal distribution of *Ph. cryptogea* is of interest as it would affect results of sampling for the fungus at a given site.

Examination of the horizontal distribution of *Ph. cryptogea* at site A and in the adjoining plantation showed that in the salvaged area the fungus was present at 11 of the 24 sampled points in grid 1. Although 5 of the 6 points which yielded *Ph. cryptogea* in grid 1 were situated next to a furrow where soil could be expected to be wetter, such a correlation was not seen in grids 2 and 3. Figure 2 shows that grid 1 was situated on lower ground due to the sloping nature of the site which may explain the presence of the fungus in grid 1 but not in grids 2 and 3. The test showed that the horizontal distribution of *Ph. cryptogea* at the cleared site is patchy. Because of such distribution, results from samplings from such sites can only be reliable if a sufficiently large number of points are sampled.

Grids 4, 5 and 6 which were situated around a tree, all yielded *Ph. cryptogea* from a number of points (Figure 2). As the fungus had been isolated from the roots of the same tree before (Table 2), its presence in all three grids was to be expected. The horizontal distribution of the fungus in these grids was also patchy.

The test also showed that *Ph. cryptogea* had survived in soil for 7 years in the absence of living *P. radiata*. The fungus may survive in soil for long periods either by means of resistant structures, or by infecting alternative hosts or organic matter present at the site. The

first possibility was not investigated during this study as such an investigation would need to extend over several years. However, isolation from soil only slightly moist collected at the site and kept in a plastic bag in the laboratory still yielded *Ph. cryptogea* after two years (unpubl. data). The possibility of the fungus surviving in alternative hosts is discussed later.

When attempts were made to examine the population density of *Ph. cryptogea* at site A and in the adjoining plantation as shown in Figure 3, the fungus was not isolated from any of the sampling points along transect 1. Two sampling points along transect 2 yielded the fungus: point 2(c) and point 2(e) the population density indices being 1 and 8 respectively. As point 2(e) was situated in the root zone of a tree which had yielded *Ph. cryptogea* before, a higher population density index was to be expected at this point.

It is difficult to understand why *Ph. cryptogea* was not isolated from point (a) of transect 1 as it was placed in grid 1 which had yielded the fungus previously. As the method used for determination of the fungus in soil was the same in both investigations, failure to isolate it in the second examination would not be a reflection of the method used. It is possible that a larger number of subsamples would have given a positive result as it appears that in soil *Ph. cryptogea* is present only in discrete niches where it may be associated with a plant root or some organic particle, or as a resistant structure. On the other hand, there may have been changes in some factor which influenced isolation of the fungus either at the isolation site or in the laboratory.

An attempt was also made to isolate *Ph. cryptogea* from various soil fractions. The test was expected to show whether the fungus occurred

in association with organic matter in the soil, or as free living chlamydospores. However, although the soil used in the test was collected from a site which had yielded *Ph. cryptogea* before, the fungus was not isolated from any of the fractions. Isolation of *Ph. cryptogea* from soil using a 'baiting' method depends on the fungus producing sporangia and releasing zoospores which infect the 'bait'. The finest fraction consisted mainly of clay suspended in a relatively large quantity of water. After the clay had settled on the bottom of the cups used for 'baiting', the depth of water above the clay was about 25 mm, and production of sporangia may have been inhibited by lack of aeration at this depth (see p. 100). However, production of sporangia would not have been inhibited in the coarsest fractions as depth of water above these fractions did not exceed 7 to 8 mm.

The possible association of *Ph. cryptogea* with roots of alternative hosts was also examined. Cother and Griffin (1973b) studied the role of alternative hosts in the survival of *Ph. drechsleri* (considered to be conspecific with *Ph. cryptogea*) in relation to safflower in New South Wales. They found that various weeds could be artificially infected with the fungus, and concluded that rotations on agricultural land with 'non-susceptible' crops would be of little value. Of the various plant species present in cleared forest areas and thinned plantations the fungus was obtained from the roots of red gum (*Eucalyptus camaldulensis*) and a thistle (*Cirsium vulgare*). As thistles are present in most of cleared forest areas, the ability of the fungus to infect these plants would ensure its survival for long periods.

An attempt was also made to determine the susceptibility of the two plant species to *Ph. cryptogea*. Attempts to germinate seeds of *C. vulgare* in the glasshouse failed. Seeds of *E. camaldulensis* were sown in sterilized John Innes potting soil and grown for 6 months. The young plants were then inoculated with hyphal-sporangial suspension of *Ph. cryptogea* at the rate of 50 ml per pot (see p. 59). The test was replicated 8 times, and the height of each plant was measured at the time of inoculation, and again three months later. Average increase in height of uninoculated plants was 95.0 mm, and of plants inoculated with *Ph. cryptogea*, 111.2 mm. Thus *Ph. cryptogea* was not pathogenic to *E. camaldulensis* under the experimental conditions, and it appears that the fungus may be able to exist in association with alternative hosts without affecting their growth.

VI. REPLANTING A DECLINE SITE IN THE FOREST

(1) Experimental

The third of Koch's postulates require that the pathogen be inoculated in a healthy host where it should reproduce the symptoms observed before. This requirement is difficult to satisfy with older trees growing in a forest. *Phytophthora cryptogea* in the forest attacks mainly the smaller feeder roots of trees, the destruction of which results in impeded uptake of water and nutrients. Therefore in inoculation tests the fungus would need to be introduced in the root zone of a tree where there is already an established microbial population present both in the soil and in the rhizosphere. Difficulties of introducing an 'alien' organism in soil and achieving infection of plants have been reported (Park 1955). Alternative approaches are pathogenicity tests with young trees planted in areas known to contain the pathogen, or in soil inoculated with the pathogen in a glasshouse. However, the degree to which such tests can be related to older trees in the forest is questionable. But quite apart from this, tests with young trees are necessary if affected areas of forests are to be replanted.

To gain some information about the role of *Ph. cryptogea* in the decline and/or death of young pines in the forest, a field experiment was set up in the Kuitpo forest. Site A where pines had died or shown decline symptoms, was chosen as the test site, and the experiment was designed as follows:

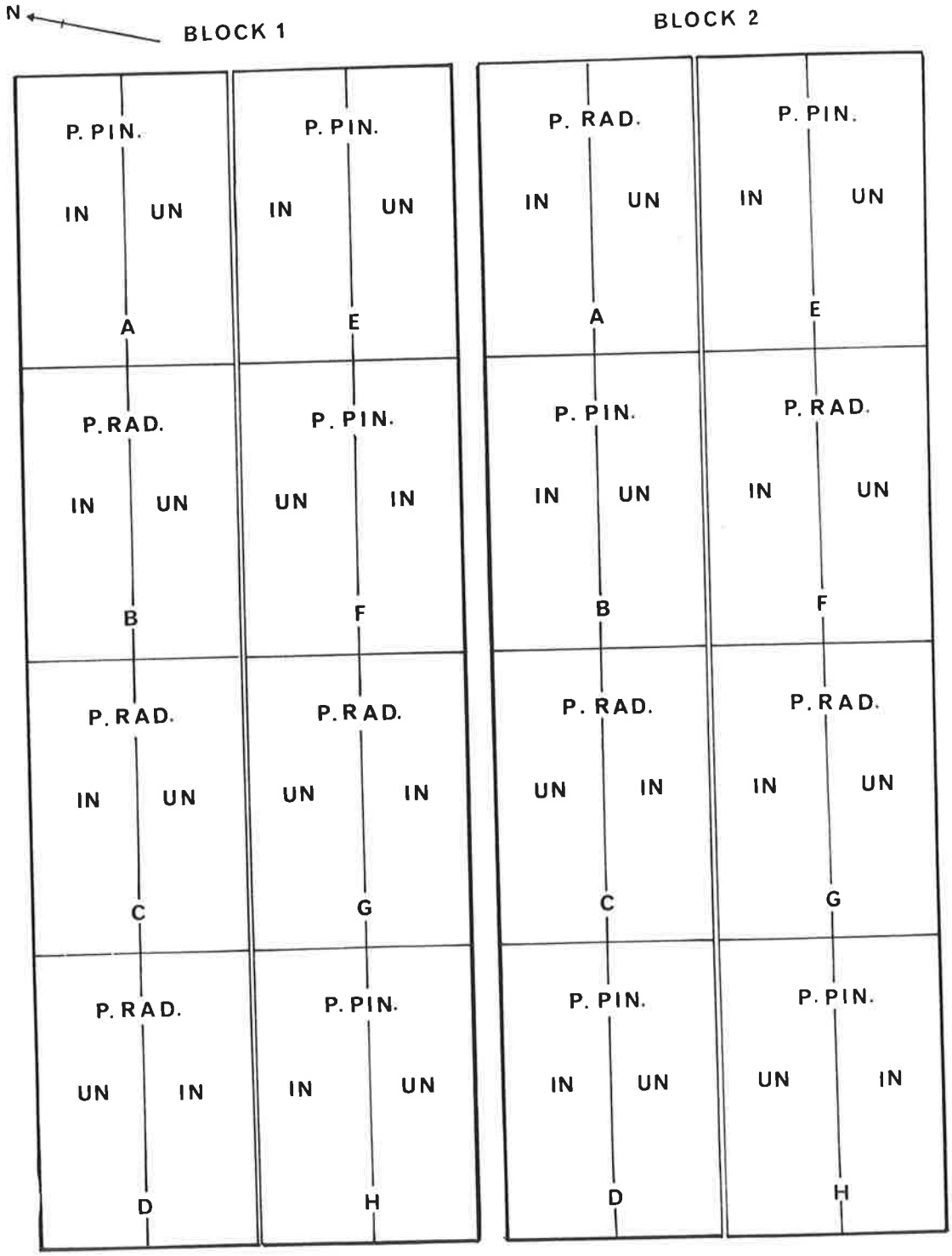
(1) The experimental area was divided into two blocks, each 39 x 17 m. Block (1) was ripped to provide drainage for surface water, the rips being 2.14 m apart. Block (2) was ploughed and ripped as block (1).

Figure 4. Design of field experiment.

Legend:

P.PIN. - *Pinus pinaster* IN - inoculated

P.RAD. - *P. radiata* UN - uninoculated


N
BLOCK 1
BLOCK 2
P. PIN.
IN UN
A
P. PIN.
IN UN
E
P. RAD.
IN UN
A
P. PIN.
IN UN
E
P. RAD.
IN UN
B
P. PIN.
UN IN
F
P. PIN.
IN UN
B
P. RAD.
IN UN
F
P. RAD.
IN UN
C
P. RAD.
UN IN
G
P. RAD.
UN IN
C
P. RAD.
IN UN
G
P. RAD.
UN IN
D
P. PIN.
IN UN
H
P. PIN.
IN UN
D
P. PIN.
UN IN
H

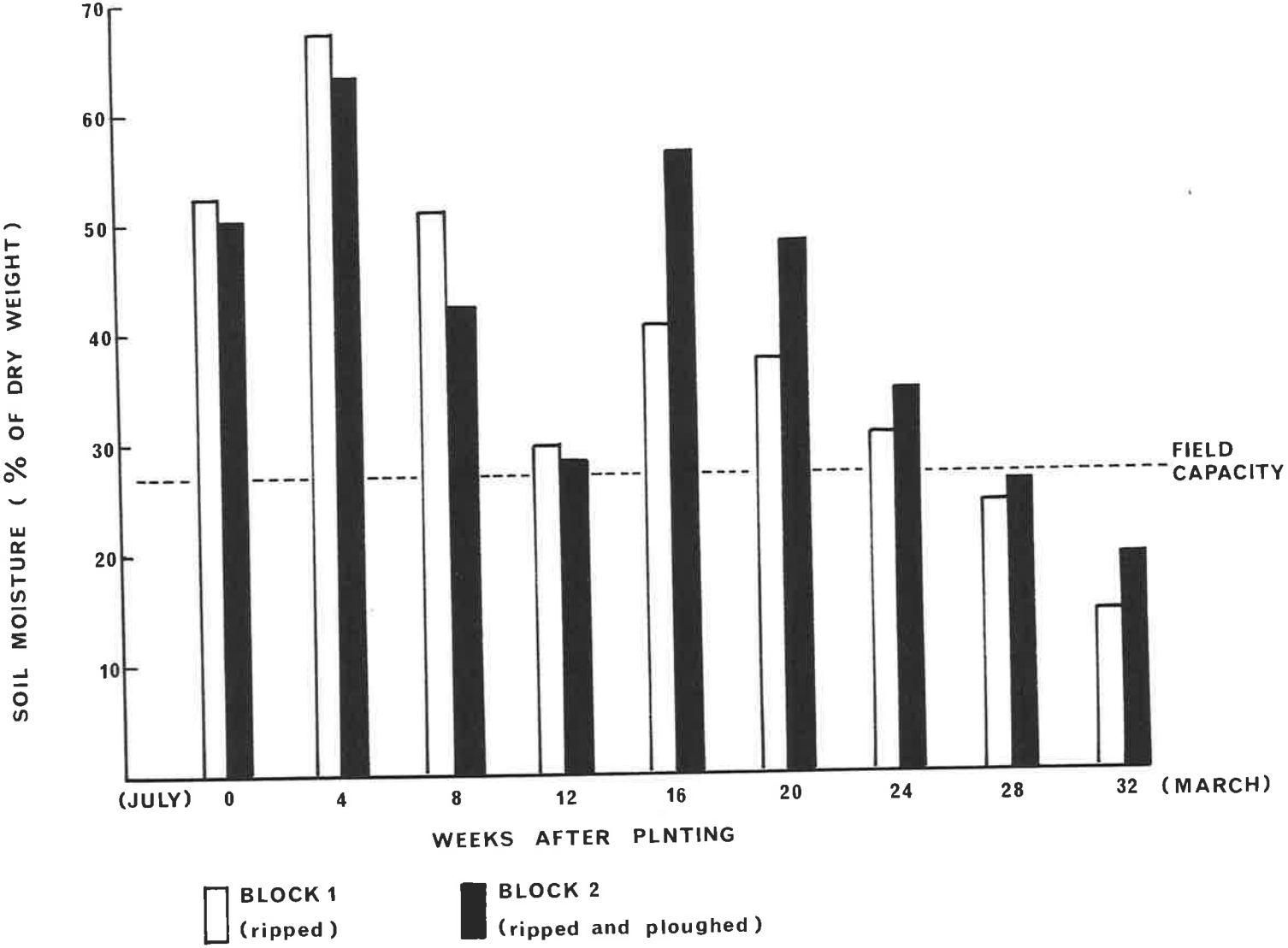
(2) Each block was of randomized split plot design with 4 replications of each of the two pine species, *Pinus radiata* and *P. pinaster*. Seventy-two trees were planted in each plot arranged in 8 rows of 9 trees. Spacing between plants and between rows was 1.06 m.

(3) Each plot was split in two sections, and the trees in one of the sections received 20 g of a six-week-old sand-cornmeal culture of *Ph. cryptogea* in the planting hole to supplement the natural population of the fungus in the soil. The same quantity of pure sand-cornmeal was added to uninoculated plants.

(4) The trees with wrenched roots, and obtained from a forest nursery, were planted in the first week of July 1973, and within two weeks after planting each tree was supplied with phosphorus fertilizer in the form of one Foslump placed 7.5 cm from a tree just below ground level. About 6 weeks after planting, the weedicide Vorok AA was applied to the experimental area at standard rates (fertilizer application and weedicide treatment as well as planting of trees were done by the staff of the Woods and Forests Department). Figure 4 shows the design of the experiment.

During the period from July 1973 to March 1974 soil samples were collected at weekly intervals from all plots for determination of soil moisture. Soil samples were taken from a central point of each plot to a depth of about 15 cm, and samples at each sampling were collected as near as practically possible from the same points. Each sample was placed in an aluminium weighing dish and transported to the laboratory for immediate processing. Soil moisture was determined for each sample individually by first weighing the soil, and then drying it for 24 hours

Figure 5. Average soil moisture in ripped and ploughed plots in 1973/1974.


SOIL MOISTURE (% OF DRY WEIGHT)
70
60
50
40
30
20
10
FIELD CAPACITY
(JULY)
0
4
8
12
16
20
24
28
32
(MARCH)
WEEKS AFTER PLNTING
BLOCK 1
(ripped)
BLOCK 2
(ripped and ploughed)

in a drying oven adjusted to 90°C. The dried soil was weighed again, and soil moisture was calculated on dry weight basis. Figure 5 shows the average soil moisture for the two blocks and all plots at four-weekly intervals.

The number of dead trees in the experimental plots was ascertained at various intervals. Table 6 shows that, of the two pine species, significantly more *P. pinaster* died than *P. radiata*. When the number of trees which died in the split plots inoculated with *Ph. cryptogea* is compared to the number of trees which died in uninoculated plots, it is seen that significantly more *P. radiata* died in the inoculated plots. Such a difference was not seen with regard to *P. pinaster*. It is also seen that significantly more *P. radiata* died in inoculated plots in Block 1 than in Block 2. The numbers shown in Table 6 were obtained 9 months after planting.

A number of dead plants were collected 4 months after planting, and root pieces of these plants were plated on the selective $P_{10}VP$ medium. Table 7 shows that *Ph. cryptogea* was isolated from the roots of *P. radiata* collected from only two plots. The Table also shows that two *Pythium* species, not previously mentioned, were also isolated. One of them was *P. paroecandrum* Drechsler, and the other *P. acanthicum* Drechsler, both were identified using the key of Middleton (1943).

On observing the plots it soom became apparent that trees died mostly in deeper furrows which, in some cases, were about 20 cm deep, and in which water accumulated and persisted for long periods. There were also noticeable differences in tree vigour between those planted on higher ground, and those on lower ground. About 15 months after planting the growth of randomly chosen *P. radiata* collected from

Table 6.

Number of dead trees in 1973/1974 trial

Plot No.	Block 1				Block 2			
	P. radiata		*P. pinaster*		*P. radiata*		*P. pinaster*	
	In.	Un.	In.	Un.	In.	Un.	In.	Un.
1	20	11	26	29	15	9	27	25
2	18	17	26	28	12	12	27	27
3	20	18	30	24	18	14	21	29
4	15	9	21	28	14	12	25	21
Mean	18.2	13.8	25.7	27.3	14.7	11.7	25.0	25.5

In. - Inoculated

Un. - Uninoculated

L.S.D. (P = 0.05) - 2.6

(P = 0.01) - 3.6

N.B. Total number of trees lost - 648 or 56.4%.

Table 7.

Fungi isolated from dead pines

Block and Plot No.	Inoculum	Host	No. of root pieces plated	Organisms isolated
1 A	+	*P. pinaster*	12*	-
1 A	-	"	8	-
1 B	+	*P. radiata*	12	-
1 C	+	"	8	-
1 D	+	"	8	-
1 D	-	"	6	-
1 E	+	*P. pinaster*	8	-
1 E	-	"	12	*Pythium irregulare*
1 F	+	"	12	*P. irregulare*
1 F	-	"	12	*P. irregulare*, *P. pareocandrum*
1 G	+	*P. radiata*	6	-
1 H	+	*P. pinaster*	12	-
1 H	-	"	12	*P. pareocandrum*, *P. acanthicum*
2 A	+	*P. radiata*	12	*P. irregulare*
2 B	+	*P. pinaster*	6	-
2 C	+	*P. radiata*	8	*Phytophthora cryptogea*
2 C	-	"	6	-
2 D	+	*P. pinaster*	6	*Pythium irregulare*
2 D	-	"	12	-
2 E	+	"	8	*P. irregulare*
2 E	-	"	6	-
2 F	+	*P. radiata*	8	-
2 F	-	"	12	*Phytophthora cryptogea*
2 G	+	"	8	-
2 G	-	"	12	-
2 H	+	*P. pinaster*	12	-
2 H	-	"	4	*Pythium irregulare*

* Number of root pieces plated varied according to availability as some plants had practically no roots.

higher ground and lower ground, and the appearance of their roots were compared. Table 8 shows that trees from higher ground were significantly taller than those from lower ground as measured from soil level.

There were also obvious differences between the root systems of trees from higher ground and those from lower ground. Table 9 compares the roots of these trees. It is seen that trees from higher ground generally had more mycorrhizal roots than those from lower ground, and roots of trees from lower ground were generally darker brown with a large proportion of completely black roots. Table 9 also shows that development of mycorrhizal roots, although better in trees from higher ground, was generally poor. Figure 6 shows two plants from higher ground, and two from lower ground.

Because of the abnormally wet winter during the first trial, a second trial was started in July 1975 to observe the effect of possible differences in soil moisture on the growth of young pines in the field.

It consisted of two treatments designed to facilitate drainage of surface water, and to minimize waterlogging of soil around the trees: (a) ground ripped with trees planted in the rips, and (b) ground mounded with trees planted on tops of mounds. Again the two species, *P. radiata* and *P. pinaster*, were planted. No additional inoculum of *Ph. cryptogea* was added to the soil. There were 8 plots in the experiment: 4 ripped and 4 mounded plots. Half of each of the 4 plots were planted with *P. radiata*, the other half with *P. pinaster*. The number of trees planted in each plot was 24. Soil moisture was determined as before at 16 points of the experimental site at

Table 8.

Height of trees on higher and lower ground

Height of trees in cm	
higher ground	lower ground
73	39
69	42
77	31
71	32
73	43
74	48
58	42
69	42
63	49
Mean 69.7(1)	40.9(1)

(1) Difference significant at < 1%.

Table 9.

Presence of mycorrhizas, and the amount of dark roots in trees from higher and lower ground

Higher Ground		Lower Ground	
Mycorrhizas (1)	Degree of blackening (ii)	Mycorrhizas	Degree of blackening
+	2	-	4
+	1	-	5
-	4	-	5
+	1	+	1
++	2	-	5
++	2	+	5
-	2	+	5
++	4	+	5
++	3	-	5

(1) Mycorrhizas: + trace only

++ small amount of mycorrhizal roots present

(ii) Root blackening rated from 1 to 5 where 1 signifies up to 20% of very dark roots, and 5 where the whole root system appeared almost completely black.

Figure 6. Trees from higher ground (left), and lower ground (right).

fortnightly intervals. Of the points, 8 were situated in the ripped plots and 8 in the mounded plots. Figure 7 shows the average soil moisture for ripped and mounded plots at 4-weekly intervals. It is seen that soil was moister in the ripped than in the mounded plots.

Table 10 shows the mean height of trees measured from ground level, and the number of trees dead or missing 6 months after planting. The trees in ripped plots were somewhat larger than those in mounded plots, presumably because of the higher soil moisture in the ripped plots which would be beneficial to the trees during the warm weather, from about November to January. However, the difference in height of plants was not statistically significant. The number of trees dead or missing was: ripped plots *P. radiata* 1, *P. pinaster* 3; mounded plots *P. radiata* 6, *P. pinaster* 8. Of this number at least 7 trees appeared to be broken off probably by animals. Thus the total number of trees dead or missing was 18 or 9.37% of the total number planted.

(2) Discussion

Table 6 shows that 648 pines, both *Pinus radiata* and *P. pinaster*, or 56.4% of the total planted died during the 1973/1974 trial. When the various treatments are compared, it is seen that significantly more inoculated *P. radiata* died than uninoculated ones. This suggests that the added inoculum had an effect on the trees. There were also significantly more deaths of inoculated *P. radiata* in ripped plots (Block 1) than in ripped and ploughed plots (Block 2) where soil moisture was lower during the first 12 weeks of the experiment (Figure 5).

Figure 7. Average soil moisture in ripped and mounded plots in 1975/1976.

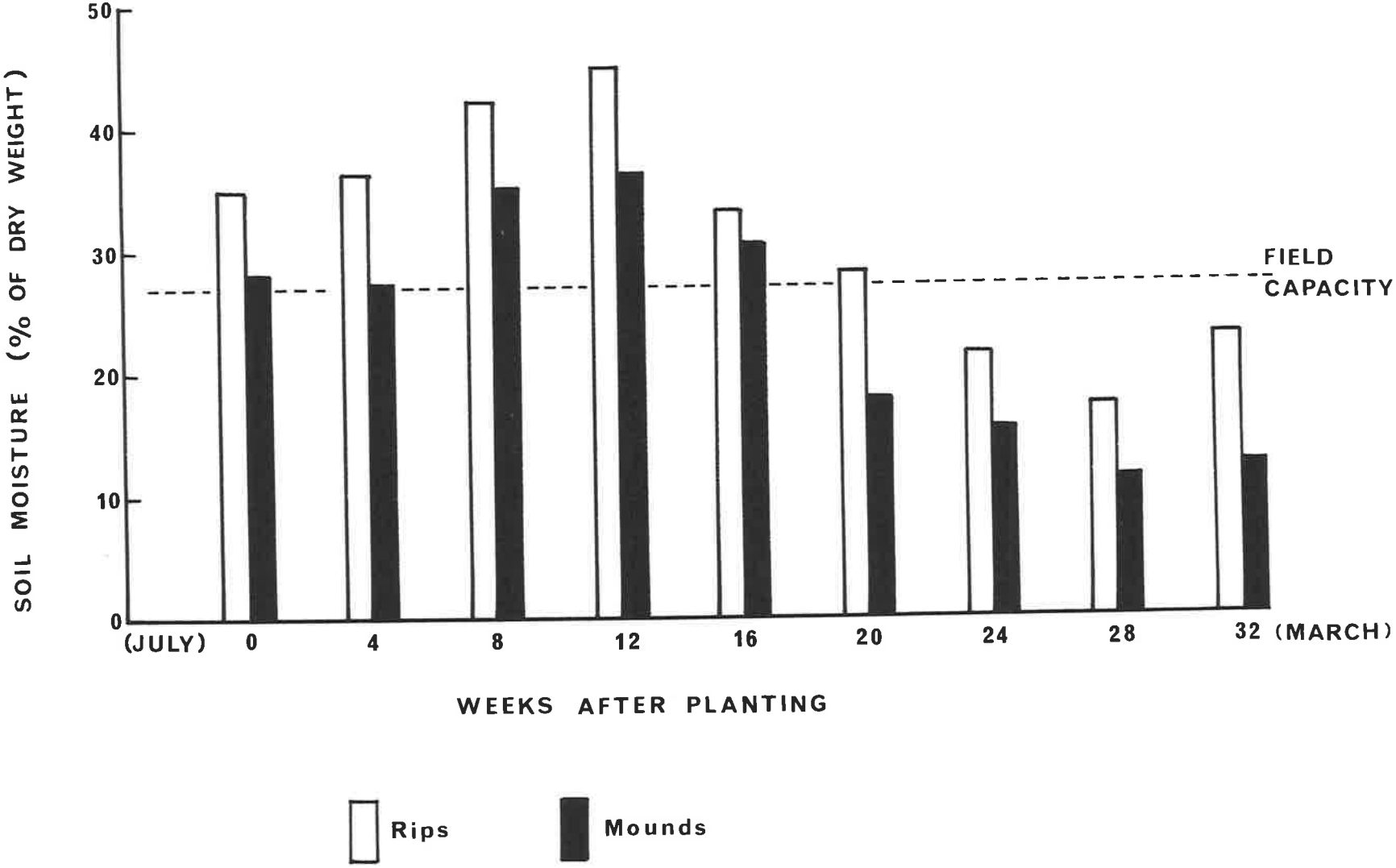

SOIL MOISTURE (% OF DRY WEIGHT)
50
40
30
20
10
0
FIELD CAPACITY
(JULY) 0 4 8 12 16 20 24 28 32 (MARCH)
WEEKS AFTER PLANTING
Rips
Mounds

Table 10.

Height of trees in the 1975/1976 trials[1]
(in mm)

	P. radiata		*P. pinaster*	
	Ripped plots	Mounded plots	Ripped plots	Mounded plots
	514	289	254	216
	307	339	289	234
	449	313	324	196
	496	501	270	291
Mean	441	360	284	234

(1) Values are means for number of trees alive in each plot.

It appears that the moister soil in Block 1, especially in the presence of *Phytophthora cryptogea*, may have affected the trees during the early part of their growth when they were still subject to transplanting stress.

Table 6 also shows that the number of dead *P. pinaster* was higher than the number of dead *P. radiata* in the corresponding treatments. The only factor which could be responsible for such a difference is soil moisture as there were no differences in the numbers of dead *P. pinaster* between inoculated and uninoculated plots. Thus the field experiment of 1973/1974 suggested that *Ph. cryptogea* is pathogenic to young *P. radiata* but not to *P. pinaster*, while *P. pinaster* are more susceptible to waterlogging than *P. radiata*.

Table 8 shows that *P. radiata* grew better on higher ground than on lower ground, and Table 9 shows that trees from higher ground had more mycorrhizal roots and the roots were generally lighter in colour than those of plants from lower ground. These results indicate that high soil moisture is detrimental to formation of mycorrhizas and to plant growth. In a review about factors influencing formation of mycorrhizas, Slankis (1974) mentioned soil moisture as one such factor. Bowen and Theodorou (1972) reported that colonization of *P. radiata* roots by *Rhizopogon luteolus* declined when soil moisture was both above field capacity and below 50% of field capacity.

Comparison of Figures 5 and 7 shows that soil moisture was generally considerably higher in the first experiment than in the second experiment. When the results of the two experiments are compared, it is seen that 56.4% of the planted pines were lost during the first experiment but only 9.37% were lost during the second experiment. This difference can only be explained by the different

soil moisture levels during the two experiments.

VII. SOME FACTORS AFFECTING GROWTH OF PINES IN GLASSHOUSE EXPERIMENTS

Although the field experiments suggested that *Phytophthora cryptogea* is pathogenic to young *Pinus radiata*, the results were not clearcut, and it was necessary to verify the field results by glasshouse experiments where experimental conditions may be more standardized. The field experiments also indicated that soil moisture may be an important factor which affects the growth of young pines in soil infested with *Ph. cryptogea*. To examine the effect of soil moisture on the growth of young *P. radiata*, experiments were carried out in the glasshouse with young pines growing in the presence and absence of *Ph. cryptogea* at different soil moistures. Other factors such as nutrient deficiency, and the presence of mycorrhizas were also examined.

(1) Pathogenicity of some pythiaceous fungi to *Pinus radiata*

A pathogenicity test was done to determine the susceptibility of young *P. radiata* seedlings to *Phytophthora cryptogea*, *Ph. cinnamomi* and *Pythium anandrum*. The latter was included because of its frequent occurrence in pine forests in South Australia.

Seedlings of *P. radiata* were grown for 5 weeks in sterilized John Innes potting soil (5 parts of loam, 5 parts of coarse river sand, 4 parts of peatmoss; with 1.19 kg blood manure, 1.13 kg superphosphate, 0.57 kg potassium sulphate and 0.37 kg ground limestone per cubic yard of mixture). Seedlings were then replanted to further John Innes potting soil in 10 cm pots. The soil for each pot was inoculated individually with a test fungus using a one month old sand-cornmeal

culture at the rate of 3% w/w. Four seedlings were transplanted in each pot, and 10 replicate pots used for each treatment. Uninoculated sand-cornmeal medium was added to the control pots. The pots were watered once a day up to field capacity.

Results were assessed after a further 5 weeks by counting the number of dead plants in each pot (Table 11). It is seen that all the test fungi were pathogenic to 5-week-old *P. radiata* under the experimental conditions, although *Pythium anandrum* was considerably less pathogenic than the two *Phytophthora* species.

Thus the test showed that the three fungi could be regarded as potential pathogens of *P. radiata*, at least in pine nurseries. The association of various *Pythium* species with damping-off and root rots of *P. radiata* in South Australian forest nurseries has been reported by Vaartaja (1967) and Vaartaja and Bumbieris (1967). However, the above pythiaceous fungi were not isolated from nurseries in South Australia. *Ph. cinnamomi* has been reported as a serious pathogen in pine nurseries in Queensland (Oxenham and Winks 1963).

(2) Effect of waterlogging on growth of young *Pinus radiata*

The previously discussed field experiments suggested that waterlogging is an important factor involved in the decline and death of young pines. The importance of waterlogging in the presence and absence of *Phytophthora cryptogea* was examined in a series of glasshouse tests.

The first of these tests was done with *Pinus radiata* seedlings grown in Kuitpo forest soil known to contain *Ph. cryptogea* and various

Table 11.

Pathogenicity of *Pythium anandrum*, *Phytophthora cinnamomi* and *Ph. cryptogea* to *Pinus radiata*

Replicate No.	No. of dead plants per pot			
	Uninoculated	*Phythium anandrum*	*Ph. cinnamomi*	*Ph. cryptogea*
1	2	4	3	4
2	0	1	3	3
3	0	2	3	4
4	1	3	3	4
5	0	2	4	4
6	0	2	4	4
7	0	2	2	4
8	1	3	4	4
9	0	0	4	4
10	0	1	4	4
Mean	0.4	2.0	3.4	3.9

L.S.D. (P = 0.05) - 1.1

(P = 0.01) - 1.4

Pythium species. Seeds of *P. radiata* were planted at the rate of 10 seeds per 10 cm pot, and half of the pots were watered once a day up to field capacity while the other half were kept saturated with water by immersing the pots in water-filled plastic containers 6 cm deep. Complete waterlogging of soil was ensured by maintaining the water level in the plastic containers to within 1-2 cm from the tops of the pots.

Table 12 shows that the average number of seedlings which emerged in moist soil was 6.8 per pot, the number in waterlogged soil was 5.6; the difference between the two values was not significant. However, while only 2.2% of the emerged seedlings died in the moist soil, the percentage post-emergence death in waterlogged soil was 72%. Thus the difference in post-emergence deaths between the two soil moisture levels was highly significant.

In the next experiment the pathogenicity of three pythiaceous fungi to *P. radiata* was determined at two soil moisture regimes. One-year-old pines were replanted singly in 15 cm pots using John Innes potting soil inoculated as previously with *Ph. cryptogea, Ph. cinnamomi* or *Pythium irregulare*. (*P. irregulare* was included in the test because of its frequent isolation from forest soils.) Four pots of each treatment were watered once a day up to field capacity, and 4 pots were kept waterlogged for 5 weeks. After an initial 5 week period all pots were then watered once a day for a further period of 8 weeks, after which time the experiment was terminated. The height of all plants was measured from soil level to the tips of the top needles at the time of planting, and at the end of the experiment.

Table 12.

Effect of two soil moistures on damping-off of pine seedlings in non-sterilized field soil

(Data are means of 5 replicates)

Treatment	No. of emerged seedlings per pot (max. 10)	Percentage post-emergence deaths
Moist soil	6.8	2.2
Waterlogged soil	5.6	72.0 (1)

(1) Significantly different from number of seedlings in moist soil (P = 0.01).

Table 13 gives the mean increase of height of plants during the 13 weeks of the experiment, and the number of dead plants for each treatment. All the test fungi were pathogenic to one-year-old *Pinus radiata* in moist soil, although *Pythium irregulare* was significantly less pathogenic than either *Phytophthora cryptogea* or *Ph. cinnamomi*. In waterlogged soil all plants were severely stunted, even in uninoculated soil, and eventually died. Thus in this experiment one-year-old *P. radiata* planted in potting soil died under waterlogged soil conditions in the absence of pythiaceous fungi. In moist soil *Pythium irregulare* only caused some stunting of the transplanted pines while the two *Phytophthora* spp. killed most of the plants.

In previous experiments soil was inoculated with the various test fungi using sand-cornmeal cultures of the fungi. With this method uniform distribution of the inoculum depends on inoculating soil for each pot individually, and thorough mixing of the inoculum with the soil. The method is time-consuming, especially for larger experiments.

For the above reasons the following method was adopted for later glasshouse experiments. The test fungi were first grown for one week in 100 ml of sterile liquid medium containing 10% of Campbell's V-8 juice and 90% distilled water in 300 ml flasks. The medium in each flask was then replaced with 100 ml of distilled water, and the flasks were kept for two days under a fluorescent light at 25°C to induce production of sporangia. Each culture was then homogenized in a blender at low speed for 30 seconds. Fifty ml of the hyphal-sporangial suspension were added to each pot in holes about 5 cm deep made at two sides of a test plant. The pots were immediately watered

Table 13.

Pathogenicity of *Pythium irregulare*, *Phytophthora cryptogea* and *Ph. cinnamomi* to young pines at two soil moistures

Organism	Moist soil		Waterlogged soil	
	Mean increase in height (mm)	No. of dead plants (out of 4)	Mean increase in height (mm)	Number of dead plants (out of 4)
Pythium irregulare	94.75	0	9.00	4
Ph. cryptogea	20.25	3	4.00	4
Ph. cinnamomi	36.00	3	11.50	4
Controls	145.50	0	6.25	4

L.S.D. (P = 0.05) - 21.13

(P = 0.01) - 28.76

to wash the inoculum into soil as much as possible. The method was pre-tested as follows:

Ten-week-old *Pinus radiata* were transplanted to John Innes potting soil in 10 cm pots and inoculated with *Phytophthora cryptogea* as described above. Inoculum was added at the rates of 30, 40 and 50 ml per pot. The treatments were replicated 4 times, and the pots were watered as required up to field capacity. Table 14 shows that all plants were killed by the fungus within 10 weeks when applied at the rates of 40 and 50 ml of inoculum per pot.

The method was not tested with other fungi as inoculation methods using either suspensions of hyphal fragments or zoospores are widely used. In the modification used in this work both hyphae and sporangia are present which may result in further production of sporangia in soil as well as release of zoospores, thus increasing the inoculum potential of the fungus.

The following experiment was designed to examine the effect of various periods of waterlogging on *P. radiata* growing in soil in the presence and absence of *Ph. cryptogea*.

P. radiata seedlings were grown in sterilized John Innes potting soil in 10 cm pots for three months, and the following treatments were then applied: (1) uninoculated and watered as required up to field capacity, (2) uninoculated and kept waterlogged for 10 days, (3) uninoculated - kept waterlogged for 20 days, and (4) uninoculated - kept waterlogged for 30 days. Treatments (5), (6), (7) and (8) were kept under the same soil moisture conditions as the first 4 treatments but were inoculated with hyphal-sporangial

Table 14.

Inoculation of *Pinus radiata* with different levels of hyphal-sporangia suspension of *Phytophthora cryptogea*

Treatments	No. of dead plants after 10 weeks		
	30 ml of inoculum	40 ml of inoculum	50 ml of inoculum
Uninoculated	0	0	0
Inoculated	2	4	4

suspension of *Ph. cryptogea* at the rate of 50 ml per pot as previously described. The treatments were replicated 6 times. The height of each tree was measured at the beginning of the test, and three months later when the experiment was terminated.

The data in Table 15 show that growth of trees was reduced with increasing periods of waterlogging, but the only statistically significant difference in growth between uninoculated and inoculated trees was after 20 days of waterlogging. In all cases differences in growth between plants watered up to field capacity and those kept waterlogged for various periods were statistically significant.

After the experiment was finished, all trees were washed from pots and 5 root pieces from each tree were plated on the selective $P_{10}VP$ medium. Table 16 shows that *Ph. cryptogea* was isolated from roots of inoculated plants except those kept waterlogged for 30 days. No *Phytophthora* or *Pythium* species were seen on any of the plates from plants from the uninoculated treatments. Mycorrhizal roots were not seen in any of the pots. Figure 9 shows the effect of various periods of waterlogging on roots of uninoculated plants.

(3) Effect of two temperatures and four soil moisture levels on young pines in field soil

To determine possible differences in the effects waterlogging may have on young pines at different temperatures the following experiment was carried out using natural field soil.

Seeds of *Pinus radiata* were sown in Kuitpo forest soil collected at site A and adjusted to 7.6% soil moisture content in 10 cm pots and plants grown for 4 months. The soil at this moisture

Table 15.

Growth of young *Pinus radiata* with various periods of waterlogging in the presence and absence of *Phytophthora cryptogea*

Period of waterlogging (days)	Mean increase in height in mm	
	Uninoculated	*Ph. cryptogea*
0	107.0	105.5
10	88.8	77.8
20	81.3	45.7
30	53.5	54.5

L.S.D. (P = 0.05) - 15.4
(P = 0.01) - 20.2

Table 16.

Isolation of *Phytophthora cryptogea* from *Pinus radiata* kept waterlogged for various periods

Treatment	No. of trees yielding *Ph. cryptogea*
Uninoculated:	
waterlogged for 0 days	0
" " 10 "	0
" " 20 "	0
" " 30 "	0
Inoculated:	
waterlogged for 0 days	4
" " 10 "	5
" " 20 "	1
" " 30 "	0

Figure 8. Effect of various periods of waterlogging on roots of uninoculated *Pinus radiata*.

Soil waterlogged for:
A - 0 days
B - 10 days
C - 20 days
D - 30 days


A
B
C
D

was quite dry but still contained enough moisture for plant growth. Soil moisture was maintained at 7.6% in all pots by weighing the pots at each watering. After 4 months 64 pots with as uniform seedlings as possible were chosen for the experiment which consisted of the following treatments: (1) soil moisture maintained at 7.6%, (2) soil moisture maintained at 14.0%, (3) soil moisture maintained at 22.0%, and (4) soil kept waterlogged for two months; during the third month these pots were watered once a day up to field capacity. Half of the pots were kept in a growth cabinet adjusted to 25°C day and 20°C night temperature, the other half were kept in a cabinet adjusted to 15°C day and 10°C night temperature. Length of daylight in both cabinets was 16 hours. The treatments were replicated 8 times. The respective soil moistures were maintained by weighing the pots at each watering. Height of each plant was measured at the beginning and the end of the experiment.

The matric potentials for soils maintained at 7.6%, 14% and 22% were determined using the filter paper method of Fawcet and Collis-George (1967). Soil with 7.6% water content corresponded with about -4 bar matric potential, 14% soil moisture corresponded with about -2.6 bar, and 22% soil moisture with about -0.1 bar (= field capacity). The waterlogged soil contained 38.8% water.

The results given in Table 17 show that plants grew better at the two intermediate soil moistures than in the drier soil but their growth was retarded when soil was kept waterlogged for two months at either temperature. The young pines also grew better at the higher temperature except in waterlogged soil. Thus in this experiment increasing soil moisture up to about field capacity was

Table 17.

Growth of young *Pinus radiata* at two different temperatures and four soil moistures

Temperature	Mean increase in height in mm			
	7.6% soil moisture	14% soil moisture	22% soil moisture	Waterlogged soil
25/20°C	8.1	26.7	37.6	9.0
15/10°C	5.0	8.1	12.6	9.6

L.S.D. (P = 0.05) - 6.8

(P = 0.01) - 9.0

beneficial to growth of young *P. radiata*. As the pines were growing in soil naturally containing *Phytophthora cryptogea*, and there were no uninoculated controls, the test did not show whether the lower temperature had any effect on infection of the plants by the fungus.

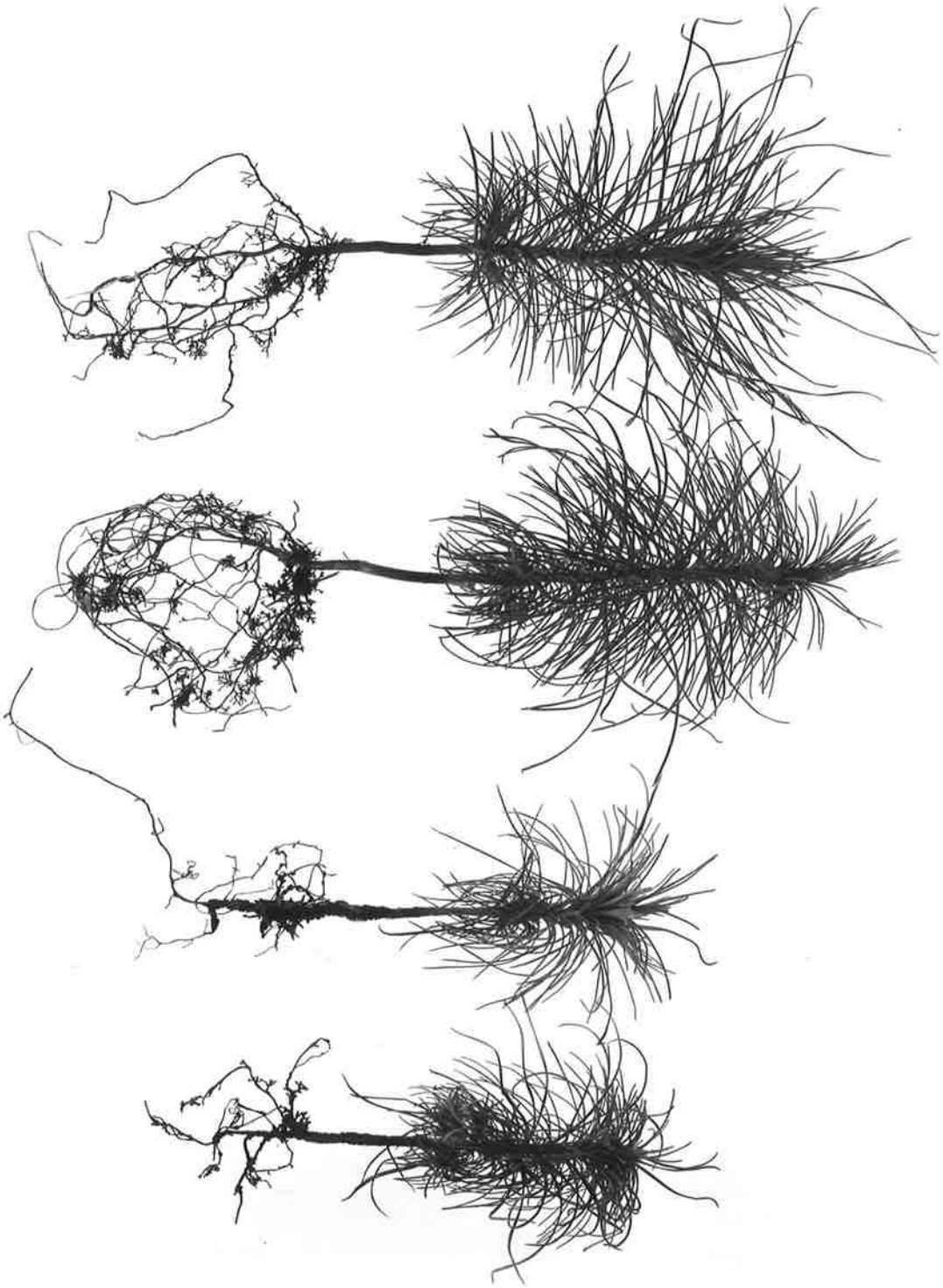
At the end of the experiment 5 root pieces from each plant were plated on the selective $P_{10}VP$ medium but no *Phytophthora* was isolated from any of the roots. Abundant mycorrhizal roots were present in the plants kept at intermediate moistures but only a few mycorrhizas were seen in plants grown in waterlogged soil, and in the drier soil. Plants from waterlogged soil were not only severely stunted but also showed extensive yellowing and browning of needles. Figure 9 shows two plants grown in soil with 22% water content, and two grown in waterlogged soil from the 25/20°C series.

(4) The effect of transplanting on the susceptibility of *Pinus radiata* to *Phytophthora cryptogea*

Results of the previously discussed experiments suggested that *Pinus radiata* may be more susceptible to *Phytophthora cryptogea* and waterlogging, if they are transplanted shortly before being subjected to either of these factors.

The following experiment was designed to examine this question. *P. radiata* were sown in sterilized John Innes potting soil in 10 cm pots and grown for three months. Half of the seedlings were then transplanted to new pots of the same soil. Half the number of both transplanted and non-transplanted seedlings were inoculated with *Ph. cryptogea* by adding 50 ml of hyphal-sporangial suspension of the

Figure 9. Young *Pinus radiata* grown in natural forest soil at 22% soil moisture (left), and in waterlogged soil (right).



fungus to each pot. The treatments were replicated 6 times. For the first two months of the experiment the pots were kept in trays filled with water to a depth of about 2 to 3 cm, for the third month the plants were watered once a day up to field capacity. Height of all plants was measured at the beginning and the end of the experiment.

Table 18 shows that after three months those plants transplanted and inoculated with *Ph. cryptogea* had grown much less than the non-transplanted and inoculated plants. Two of the transplanted and inoculated plants died after about two months, and the rest showed severe browning of needles. The difference in growth between non-transplanted and transplanted uninoculated plants was also significant.

Thus the experiment confirmed that transplanted young *P. radiata* are more susceptible to *Ph. cryptogea* and waterlogging than non-transplanted ones. Schoeneweiss (1975) discussed the effect of transplanting in a review article, and stated that "... the most severe economic losses caused by disease organisms often occur following transplanting". It appears that injury and loss of roots which occur at transplanting are the most likely factors to be responsible for transplanting stress, but plants growing under good conditions may be able to recover.

(5) Nutrient deficiency and susceptibility of *Pinus radiata* to *Phytophthora cryptogea*

Copeland (1960) reported that slow decline and dieback of southern pines associated with *Phytophthora cinnamomi* were partially arrested by heavy applications of nitrogenous fertilizers. Newhook (1970) achieved improvement in the growth of *Pinus radiata* in soils heavily infested with *Ph. cinnamomi* after applying phosphate fertilizer.

Table 18.

Effect on transplanted and non-transplanted *Pinus radiata* of waterlogging for 2 months with and without inoculation with *Phytophthora cryptogea*

Treatment	Mean increase in height in mm	
	Uninoculated	Inoculated
Non-transplanted	104.3	49.0
Transplanted	20.7	13.0

L.S.D. (P = 0.05) - 28.1
(P = 0.01) - 38.9

These reports suggest that nutrient deficiency may be another factor to be considered in relation to pine decline associated with *Ph. cryptogea*.

The effects of nitrogen, phosphorus and potassium deficiency on the susceptibility of *P. radiata* to *Ph. cryptogea* were examined. *P. radiata* were sown in 10 cm pots in washed and crushed quartz (16-30 mesh) and grown for two months. During this time the pots were irrigated with a complete nutrient solution for 5 days, and water for two days a week. The composition of the complete nutrient solution as well as the composition of the solutions with low N, low P and low K is shown in Table 19. The solutions were made up following Walker, Gessel and Haddock (1955).

After two months the following treatments were applied: (1) complete nutrient, (2) low N, (3) low P and (4) low K. Half of the pots in each treatment were inoculated with hyphal-sporangial suspension of *Ph. cryptogea* at the rate of 50 ml per pot. Thus there were 8 treatments which were replicated 6 times. The height of each plant was measured at the beginning of the experiment, and three months later at the termination of the experiment. During the first 8 weeks of the experiment all plants received the respective nutrient solutions for 5 days a week and water for the remaining two. During the last 4 weeks they were irrigated with water. The pots were always irrigated up to field capacity.

The results recorded in Table 20 show that low N and low P had a significant effect on plant growth but not low K; and that *Ph. cryptogea* had the greatest effect on plants receiving complete

Table 19.

Composition of nutrient solutions

Compounds	Concentration of compounds (mols/litre)			
	Complete solution	With low N	With low P	With low K
$NH_4H_2PO_4$	0.0005	0.000005	0.000005	0.0005
KNO_3	0.003	-	0.003	0.00002
$Ca(NO_3)_2$	0.002	0.000035	0.002	0.002
$MgSO_4$	0.001	0.001	0.001	0.001
NaH_2PO_4	-	0.000495	-	-
K_2SO_4	-	0.0015	-	-
$CaCl_2$	-	0.001965	-	-
NH_4Cl	-	-	0.000495	-
$NaNO_3$	-	-	-	0.00298

Micronutrients were added to all solutions as follows:

Fe (as $FeCl_2$)	-	0.5 ppm	Cu (as $CuCl_2$)	-	0.005 ppm
B (as H_3BO_3)	-	0.125 ppm	Zn (as $ZnCl_2$)	-	0.0125 ppm
Mn (as $MnCl_4$)	-	0.125 ppm	Mo (as Na_2MoO_4)	-	0.0125 ppm

Table 20.

Growth of young *Pinus radiata* with various nutrient deficiencies with and without inoculation with *Phytophthora cryptogea*

Treatments	Mean increase in height in mm	
	Uninoculated	Inoculated
Complete nutrient	112.7	33.3
Low K	98.7	65.0
Low N	81.5	53.2
Low P	17.0	21.3

L.S.D. (P = 0.05) - 29.6
(P = 0.01) - 38.9

nutrient solution, and a lesser effect on plants receiving the low K. The difference in growth between uninoculated plants and inoculated plants receiving a solution with low N failed to be significant at the 5% level only by 1.3 mm. *Ph. cryptogea* had no effect on the plants supplied with a solution low in P. The number of inoculated plants which died during the experiment was: 3 in pots supplied with complete nutrient, 3 with low K, 1 with low N, and none with low P. Table 20 also shows that the difference in growth between inoculated and uninoculated plants becomes smaller as the effect of nutrient deficiency increases.

(6) Mycorrhizas and susceptibility of *Pinus radiata* to *Phytophthora cryptogea*

Marx (1970) studied the effect of the fungi *Thelephora terrestris* (Ehrh.) Fr. and *Pisolithus tinctorius* (Pers.) Coker & Couch on the susceptibility of shortleaf pine seedlings to *Phytophthora cinnamomi*, and found that mature ectomycorrhizas formed by either fungal symbiont were resistant to infection from zoospores and vegetative mycelium of the pathogen. He stated that the resistance of ectomycorrhizas to infections by *Ph. cinnamomi* strengthens the concept that ectomycorrhizas function as biological deterrents to infection of feeder roots by pathogenic fungi. Marx (1973) also suggested that propagules of *Ph. cinnamomi* may have been killed directly on the root surface by volatile antibiotics, terpenes and sequiterpenes produced by ectomycorrhizas.

To investigate the effect of mycorrhizas on the growth of young *Pinus radiata* in the presence and absence of *Ph. cryptogea*, seeds of *P. radiata* were first surface sterilized with 0.5% $HgCl_2$ for one

minute and washed in distilled water. They were then germinated on malt agar at 25°C and planted in John Innes potting soil in 10 cm pots. The mycorrhizal fungus *Rhizopogon luteolus* Fr. et Nordh. was introduced into a number of pots by adding small discs (7 mm in diameter) of 18 days old Melin-Norkrans (Melin 1959) agar cultures of the fungus to planting holes at the time of planting.

After three months a number of plants were inoculated with *Ph. cryptogea* by adding 50 ml of hyphal-sporangial suspension of the fungus to each pot. Thus the experiment consisted of the following treatments which were replicated 10 times: (1) uninoculated plants, (2) plants inoculated with *Ph. cryptogea*, (3) plants inoculated with *R. luteolus*, (4) plants inoculated with *R. luteolus* and *Ph. cryptogea*, and (5) plants inoculated with both fungi, and kept waterlogged for one month. The height of each plant was measured at the beginning and end of the experiment three months later.

Results of the experiment are summarized in Table 21. It is seen that under the experimental conditions the mycorrhizal fungus did not significantly influence the growth of young *P. radiata*, and there was no difference in growth between plants inoculated with both fungi and those inoculated with *Ph. cryptogea* alone. However, the pines inoculated with both fungi appeared healthier than those inoculated with *Ph. cryptogea* (Figure 10); three of the latter plants died during the experiment and two others showed browning of needles. In the treatment where the plants were inoculated with both fungi, one plant was wilting at the end of the experiment. Plants inoculated with both fungi and kept waterlogged were severely stunted; three of them died, and two were wilting at the end of the experiment.

Figure 10. Growth of *Pinus radiata* in soil inoculated with *Phytophthora cryptogea* in the presence (right), and in the absence of *Rhizopogon luteolus* (left).

Table 21.

Growth of plants inoculated with *Phytophthora cryptogea* in the presence and absence of *Rhizopogon luteolus*

Treatments	Mean increase in height in mm	Presence of mycorrhizas
Uninoculated	83.8	1.4
Inoculated with *Ph. cryptogea*	39.4	1.5
Inoculated with *R. luteolus*	92.7	3.0
Inoculated with both fungi	39.1	2.1
Inoculated with both fungi and kept waterlogged	17.7	0.7

L.S.D. (for increase in height) (P = 0.05) - 23.1

(P = 0.01) - 30.4

Examination of plant roots showed that plants not inoculated with *R. luteolus* also had mycorrhizas, probably because of air-borne contamination. The presence of mycorrhizas in these plants may explain the small difference in growth rates of plants inoculated with *R. luteolus* and uninoculated plants. The presence of mycorrhizal roots was rated on a scale from 0 to 5 where 0 indicates complete absence of mycorrhizas, and 5 abundant mycorrhizas present. The mean ratings for mycorrhizas in each treatment are shown in Table 21.

The experiment showed that although there was no significant difference in the growth of plants inoculated both with *Ph. cryptogea* and *R. luteolus*, and *Ph. cryptogea* alone, the appearance of these plants suggested that the presence of mycorrhizas may have had some beneficial effect on the young pines inoculated with *Ph. cryptogea*. The severe stunting of plants kept waterlogged for one month, and the nearly complete absence of mycorrhizas on their roots, showed that waterlogging had affected the establishment of mycorrhizas, and that the plants were affected by waterlogging and the presence of *Ph. cryptogea*.

(7) Discussion

The glasshouse experiments showed that waterlogging is an important factor contributing to decline and death of young *Pinus radiata* both in the presence and absence of *Phytophthora cryptogea*. Although experiments with young pines in a glasshouse cannot be directly related to older trees in the forest, it is likely that older trees also would not be severely affected by the fungus unless other detrimental factors, such as waterlogging, were present. The fact that *Ph. cryptogea* was isolated from roots of apparently healthy trees in the forest, supports this assumption.

The effect of waterlogging on pines has been discussed by Remezov and Pogrebnyak (1965), who suggest that oxygen deficiency and/or presence of toxic substances in waterlogged soil affect the growth of pines. However, pine species differ in their tolerance to waterlogging. Hunt (1951) studied the effect of flooded soil on growth of pine seedlings and concluded that seedlings of *P. echinata*, *P. taeda* and *P. rigida* in general were unusually resistant to injury by flooding. In his tests seedlings grew better in soil moistened to near field capacity than in soil flooded with standing water for 12 weeks, but the original differences disappeared within 7 months when flooding of soil was discontinued. Differences in susceptibility to waterlogging between *P. radiata* and *P. pinaster* were also indicated by the field work done during this study.

Heather and Pratt (1975) reported the association of *Ph. drechsleri* (= *Ph. cryptogea*) with death of *P. radiata* in wind-break plantings. They observed that sites showing disease symptoms were considerably wetter than the surrounding areas. However, these authors did not obtain disease symptoms when they kept the test pots in trays filled with water to a depth of 1.0 cm. Under such conditions complete waterlogging as used in the tests of this study would not occur, and the effect would be much less drastic.

Jehne (1971) studied soil conditions and occurrence of *Ph. cinnamomi* in relation to deaths in young plantations of *P. radiata* in New South Wales. He concluded that *Ph. cinnamomi* appears to be ubiquitous throughout the region, but the evidence indicated that it is not the primary factor responsible for deaths in the plantations.

The immediate cause of the tree deaths appeared to have been drought stress arising from the inability of trees with restricted root systems to absorb sufficient water to meet transpiration demand. The inadequate root systems in the high mortality areas are attributed directly to the effects of an adverse soil environment, as the soil in high mortality areas was shallow, less permeable and of higher bulk density than in healthy plantations. These factors led to waterlogged soil conditions after heavy rainfall in the previous summer before death of trees appeared.

The soil conditions described by Jehne (1971) resemble those existing in some areas in Kuitpo forest where a shallow sand covers a hard clay. Under these conditions vertical drainage is restricted, waterlogging occurs during winter and spring and the soils become very dry in summer. Sutherland, Newhook and Levy (1959) examined the influence of soil drainage on decline of *P. radiata* associated with *Ph. cinnamomi* in New Zealand, and concluded that susceptibility of *P. radiata* increased and likewise the recovery rate of trees which had developed symptoms decreased with increasingly poor soil drainage.

When the effect of nitrogen, potassium or phosphorus deficiencies on the susceptibility of *P. radiata* to *Ph. cryptogea* was examined, the behaviour of *Ph. cryptogea* appeared to be similar to that of some obligate parasites. Bainbridge (1974) examined the effect of nitrogen nutrition of the host on barley powdery mildew, and found that the number of mildew infections, pustule growth and conidia production were all increased by giving more nitrogen to barley plants in pots.

The results obtained in this study agree with those of Marks,

Kassaby and Fagg (1973); they found that eucalypts and conifers which had been shown to be susceptible to *Ph. cinnamomi* in pathogenicity tests became more sensitive to the pathogen by improved nutrition.

The described experiment does not explain the nature of the phenomenon but it is possible that deficiency of certain nutrients alters the chemical nature of root exudates rendering them less attractive to the fungus. Hickman (1970) reported that various amino acids, sugars and some organic acids contained in root exudates attract *Phytophthora* zoospores, but *Phytophthora* species vary with regard to the substances they prefer. All amino acids contain N, and deficiency of this element may alter the constitution of root exudates. In these tests *Ph. cryptogea* did not have a significant effect on young pines suffering from N deficiency. The possibility also exists that the various nutrient solutions had a direct effect on the fungus. Thus Chen and Zentmyer (1970) found that Ca and Fe stimulated production of sporangia by *Ph. cinnamomi* in axenic cultures; and Mircetich and Zentmyer (1969) reported that low quantities of nutrients in natural soil appeared to be the primary factor limiting germination of chlamydospores of *Ph. cinnamomi*. On the other hand, the possibility also exists that plants suffering from nutrient deficiency did not produce new roots which could be more attractive to *Ph. cryptogea*.

In the test which examined the effect of mycorrhiza on the susceptibility of young *P. radiata* to *Ph. cryptogea*, there was no difference in growth between plants inoculated with the pathogen in the presence, and in the absence of *Rhizopogon luteolus*, although the plants inoculated with the mycorrhizal fungus appeared more healthy. Thus the

test failed to provide evidence that mycorrhiza protects young pines from attack by *Ph. cryptogea*. This failure may be attributed to the experimental conditions. It is possible that repeating the test under more controlled conditions, and exclusion of possible air-borne contamination might give different results. However, the healthier appearance of the young pines inoculated with both the pathogen and the mycorrhizal fungus suggests that *R. luteolus* may have had some beneficial effect in the test.

VIII. LABORATORY EXPERIMENTS

Understanding the behaviour of an organism which causes a disease in plants, and the study of the various factors which influence this behaviour, are important components of plant pathological investigations. The study of disease organisms becomes difficult when one deals with soil-borne fungi. Warcup (1967) stated: "Compared with some other fungal habitats, soil has proved difficult to study. This is a consequence of the multitude of organisms which occur in soil, of the complexities of fungal life cycles, together with the difficulties inherent to investigating soil because of its opacity, its heterogenous nature and its complex structure". Various methods have been devised for the study of soil fungi, but because of the mentioned difficulties, they are more or less indirect, and the conditions under which soil fungi are studied, are to a certain degree artificial. Nevertheless, these methods, providing we realize their shortcomings, used either in the field or in the laboratory, provide useful information about soil fungi and their ecology. Obviously, experiments have to be done under conditions as closely as possible corresponding to the natural situation, and care must be taken in interpretation of obtained results. Important information about the behaviour of soil fungi has in the past been obtained by laboratory work.

The life cycle of *Phytophthora cryptogea* consists of various stages such as hyphal growth in either dead or living substrates, production of sexual and asexual fruiting structures, germination of fruiting structures either by germ tubes or release of zoospores, and

infection of fresh substrates. All these stages are affected by environmental factors, both biological and physical, and study of these factors is necessary when investigating the fungus. Griffin (1972) stressed that "... an ecological approach is a necessity when considering soil-borne diseases of plants".

Some aspects of the behaviour of *Ph. cryptogea* in soil and roots were investigated in laboratory tests during this study.

(1) Behaviour of *Phytophthora cryptogea* in colonized roots

To obtain information about the behaviour of *Ph. cryptogea* in colonized pine roots, small root pieces, about 1.5-2.0 cm long and about 1 mm in diameter, of seedlings of *Pinus radiata* were first placed on an agar culture of *Ph. cryptogea* and incubated for 6 days at 25°C to allow them to become colonized by the fungus. The root pieces were then transferred to levelled surfaces of Kuitpo forest soil in 5 cm Petri dishes ensuring good contact between the root pieces and soil. The soil had previously been adjusted to three moisture levels: dry (5% moist content), moist (14% soil moisture) and wet (22% soil moisture). The plates were incubated at 20°C in a moist chamber.

After various intervals the root pieces were examined for structures of *Ph. cryptogea*. Three root pieces were removed from each dish at each examination and cut in halves. One half of each piece was placed in distilled water for 24 hours for production of sporangia. The other half was first boiled in 50% lactic acid, and then dissected by means of dissecting needles on a microscope slide. The material was stained with 0.1% aqueous acid fuchsin and examined under a microscope. The root pieces which had been kept in water for 24 hours

were stained and examined in the same way.

After 20 days a few large hyphal swellings were seen in root tissues from moist and wet soils. Although it was not possible to say with certainty that these had originated from hyphae of *Ph. cryptogea*, their frequent occurrence, and their association with aseptate hyphae suggested that they were enlarged swellings of this fungus. Sporangia developed on the other halves of these root pieces kept in water for 24 hours. No structures other than hyphae, which appeared to have lost some of its contents, were seen in the tissues of root pieces from dry soil when they were examined immediately after removal from soil, and sporangia did not develop when these roots were kept in water for 24 hours, presumably because hyphae of the fungus had become at least partly desiccated under the dry conditions.

After 27 days the hyphal swellings in the tissues of root pieces kept on moist and wet soils had become more rounded, and had the appearance of chlamydospores, although their walls were not very thick at this stage. When root pieces were kept in water for 24 hours, sporangia developed only on those pieces which had been kept on moist soil.

After 30 days sporangia again developed only on the root pieces which had been kept on moist soil. Definite chlamydospore-like structures with a mean diameter of 9.7 µm (8-13 µm), and with 1-2 µm thick walls were seen in the tissues of these roots. A number of them had germinated during the 24 hours in water, and a few of them had sporangia at the tips of the germ tubes which, because of their shape, were recognizable as sporangia of *Ph. cryptogea*. More

sporangia were seen which apparently had become dislodged from germinated chlamydospores during preparation of the slide. On the basis of the above observations it was concluded that *Ph. cryptogea* produces chlamydospores in pine roots in soil. Figure 11 shows one germinated and one ungerminated chlamydospore.

Although some swellings were seen in the roots kept on wet soil, these did not develop into definite chlamydospores. The root pieces kept on dry soil were quite dry at this stage, and no structures were observed except some collapsed hyphae which could not be identified.

It is possible that chlamydospores would have been formed in the root pieces kept on wet soil after a longer period, but the results suggest that formation of chlamydospores may be inhibited in very wet soils.

Cother and Griffin (1973a) observed formation of chlamydospores by *Ph. drechsleri* in lupin roots in soil within three weeks. The average size of chlamydospores observed by these authors was 7.9 µm. Chlamydospores developed in pine roots in this study within 30 days. The slight difference with regard to size of chlamydospores, and the length of time during which they were produced, could be explained by different experimental conditions, and by different hosts. There is also a possibility that the fungi used in the two studies belonged to different ecotypes. The existence of two ecotypes of this fungus was reported by Shepherd and Pratt (1973). Thus the observations of Cother and Griffin (1973a), and the observations in these tests show that *Ph. cryptogea* forms chlamydospores in root tissues in soil.

Figure 11. One germinated and one ungerminated chlamydospore of *Ph. cryptogea* from tissues of pine roots.

(2) Competitive saprophytic colonization of dead pine roots by *Phytophthora cryptogea*

Garrett (1970) listed five different ways in which root-disease fungi survive in soil, the first of them being survival as competitive saprophytes on dead organic substrates. He stated that for any particular fungus the share of potential substrate that it can obtain will be determined: (1) directly by its competitive saprophytic ability, which is an intrinsic characteristic of the fungal species; (2) directly by its inoculum potential on the surface of the substrate; (3) inversely by the inoculum potential of its competitors.

Phytophthora cryptogea is a soil-borne fungus which mainly attacks smaller feeder roots of its host plants. After these small roots become rotten and disintegrate, structures of the fungus, such as chlamydospores or possibly oospores, remain in the soil. Survival of the fungus in soil for longer periods in such cases would be enhanced if it were able to colonize dead organic matter in soil when living host roots are not present. The ability to colonize dead organic matter would also be important when zoospores of the fungus are deposited at sites where living host roots are absent. As the fungus in such cases would have to compete with other soil organisms, the competitive saprophytic colonization of dead pine roots by *Ph. cryptogea* was examined in the following tests.

The Cambridge method of Garrett (1970) was used in the first. Two to three cm long root pieces (ab.2 mm in diameter) of *Pinus radiata* killed by autoclaving at 121^{o}C for 15 minutes were used as the substrate to be colonized. *Ph. cryptogea* was grown on sand-cornmeal medium for

three weeks, and the culture was then mixed with Kuitpo forest soil to obtain mixtures containing 100, 98, 90, 50, 10, 2 and 0 percent of the fungal culture. Fifty root pieces were buried in each mixture in containers with tightly fitting lids which were also sealed with a plastic tape to prevent the soil from drying out. The containers were incubated for two weeks at 25°C.

After two weeks the root pieces were removed from the mixtures and placed in distilled water for production of sporangia. After one day in water they were examined using a dissecting microscope. Root pieces not showing sporangia were examined again after two days in water but no more sporangia were seen. Table 22 shows that a large number of the root pieces had become colonized by *Ph. cryptogea* in mixtures with very high inoculum potentials, but only 9 root pieces produced sporangia when the mixture contained 50% of the fungal culture. While some colonization may have occurred from natural inoculum present in the soil this seems unlikely as no sporangia occurred on root pieces incubated without added inoculum of *Ph. cryptogea*.

Because zoospores are likely to be the main means of spread for *Phytophthora* spp. (Kuhlman 1964), the experiment was repeated as follows: about 10 g of autoclaved roots of *Pinus radiata* were first placed on a culture of *Ph. cryptogea* and incubated at 25°C for one week, during which time they became colonized by the fungus. About 5 g of the roots were then placed on a 2 cm layer of Kuitpo forest soil in each of two glass dishes 4.5 cm deep and 7.5 cm in diameter, and covered by a 1 cm thick layer of the same soil. Twenty pieces of autoclaved pine roots about 1.5 cm long, were then placed on the soil surface in each dish (Figure 12). The soil in one dish was saturated with water while in the other it was

Table 22.

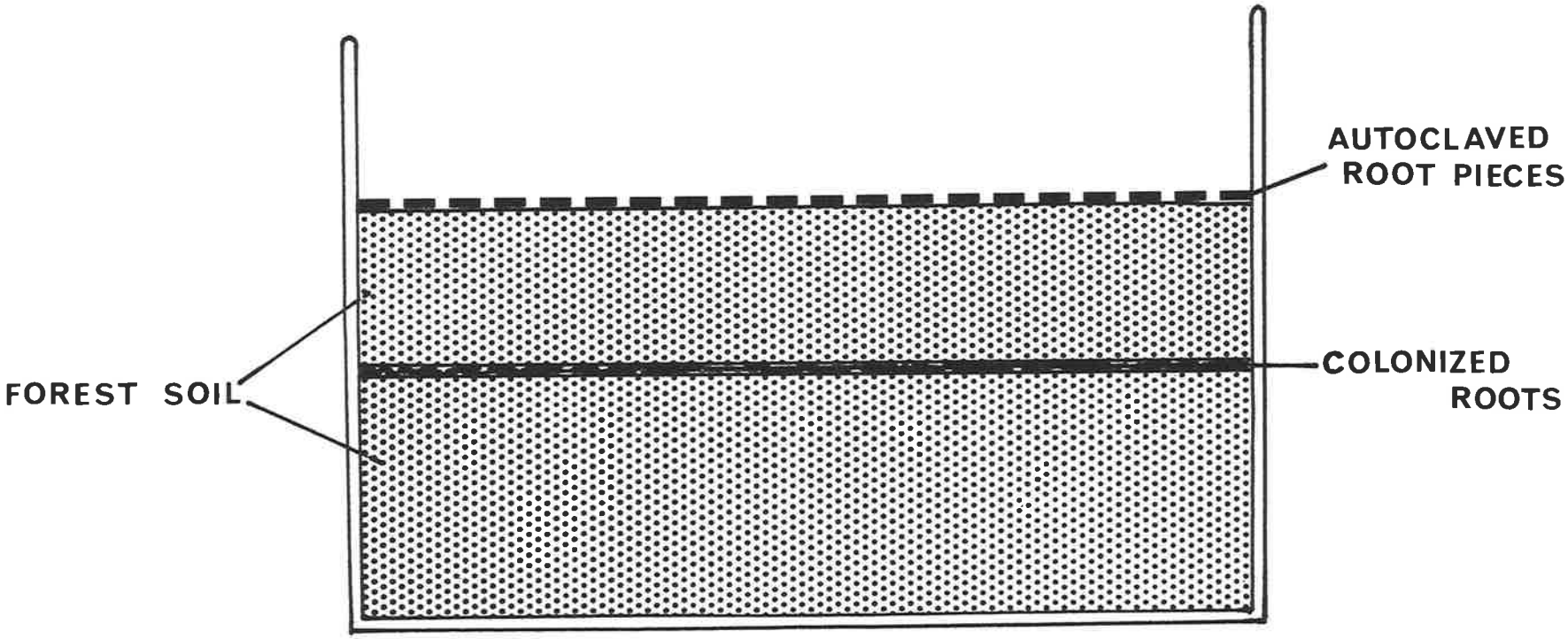
Competitive saprophytic colonization of dead roots of *Pinus radiata* by *Phytophthora cryptogea*

(Number of root pieces colonized by the fungus)

% inoculum	100	98	90	50	10	2	0 (1)
Ph. cryptogea	46	45	41	9	0	1	0

(1) Natural populations of the fungus may be present in test soil.

Figure 12. Glass dish containing forest soil, autoclaved roots, and roots of *Pinus radiata* colonized by *Phytophthora cryptogea*.


AUTOCLAVED
ROOT PIECES
COLONIZED
ROOTS
FOREST SOIL

moistened to about 12% water content. Both dishes were incubated in a moist chamber at 25°C. After one week colonization of the surface root pieces were examined by placing them in water for 24 hours and then checking for the presence of sporangia.

It was seen that 11 root pieces (55%) from the soil kept waterlogged had produced sporangia typical of *Ph. cryptogea*. No sporangia were seen on the root pieces kept on moist soil. It is assumed that infection of root pieces on wet soil occurred from zoospores because no infection of root pieces occurred on moist soil. Bumbieris and Llyod (1967) showed that the rate of lysis of fungal hyphae in soil increases considerably in wet soil, and therefore hyphae of *Ph. cryptogea* could be expected to grow better in moist soil than in waterlogged soil.

The results show that *Ph. cryptogea* is able to colonize dead organic matter in natural forest soil in competition with other microorganisms present in the soil. However, further work is needed to show whether the fungus is able to colonize organic matter in soil pre-colonized by other organisms.

(3) Effect of temperature on subsequent production of sporangia by *Phytophthora cryptogea* under sterile and non-sterile conditions

Kuhlman (1964) discussed survival and pathogenicity of *Phytophthora cinnamomi* in several soils, and stated that zoospores are the natural means of spread for this pathogen. Undoubtedly this is also true for *Ph. cryptogea*. The quantity of zoospores present at a given site is obviously dependent on the production of sporangia by the fungus. Similar to other stages in the life cycle of the fungus, production of sporangia may be affected by various environmental factors. This is indicated by the fact that isolation of *Phytophthora* from soil

using a "baiting" method, which is dependent on production of sporangia and zoospores, is not always successful even with soils known to contain the fungus. It is generally believed that isolation of these fungi from dry soils during hot summer periods is seldom possible.

The effect of exposing mycelium of *Ph. cryptogea* to various temperatures on subsequent production of sporangia was examined in the next experiment. Lima bean agar plates, each containing 20 ml of the medium, were inoculated with cornmeal agar culture discs of *Ph. cryptogea* 5 mm in diameter. The Lima bean agar cultures were grown for three days at 25°C, and then kept for 4 days at 5, 15 and 25°C. Squares of 1x1 cm were cut from the cultures and transferred to small Petri dishes containing 10 ml of non-sterile soil extract each. All plates were then kept under a fluorescent light at 25°C for two days for production of sporangia. After the two days sporangia were counted in 8 randomly chosen microscope fields of the dissecting microscope, 8x objective, around the edges of each square. Table 23 shows that the highest number of sporangia were produced by the culture pre-exposed to 15°C.

As the above test was done under sterile conditions, the experiment was repeated to examine the effect of various temperatures on the production of sporangia by *Ph. cryptogea* on colonized roots of *Pinus radiata* under sterile and non-sterile conditions. One cm long root pieces of *Pinus radiata* were first autoclaved, and then kept for one week on agar cultures of *Ph. cryptogea* to allow colonization to occur. The root pieces were transferred to 6 small Petri dishes containing unsterilized Kuitpo forest soil, to 2 dishes containing sterilized moist filter paper, and to 2 dishes containing

Table 23.

Numbers of sporangia produced on Lima bean agar by cultures of *Phytophthora cryptogea* pre-exposed to temperatures of 5, 15 and 25°C

	Temperature °C		
	5	15	25
Mean number of sporangia	3.1	56.9	34.7

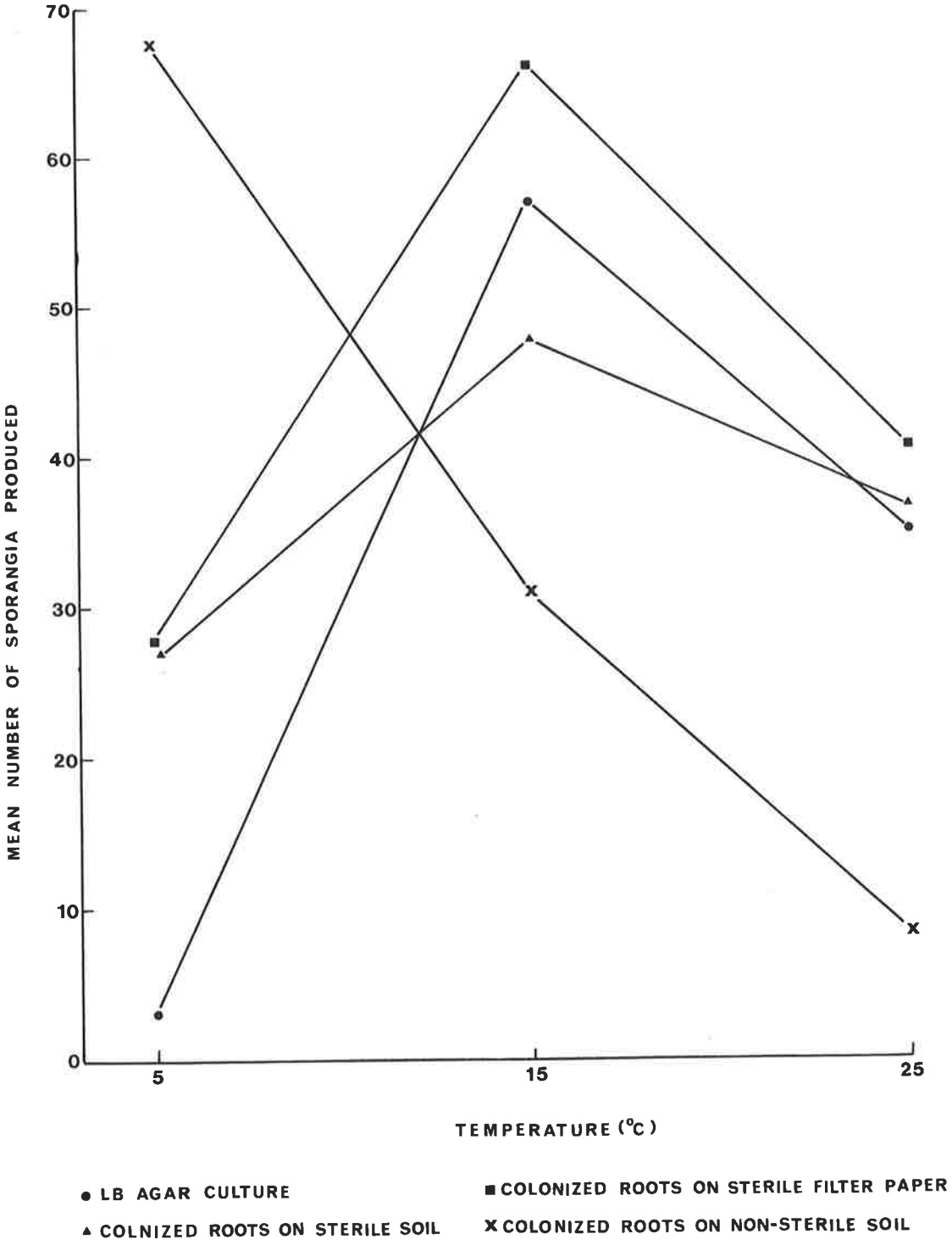
sterilized Kuitpo forest soil. Five root pieces were placed in each dish, and the dishes were incubated in moist chambers at 5, 15 and 25°C for one week. Then production of sporangia was induced as before except that distilled water was used instead of non-sterile soil extract. The number of sporangia produced on each root piece was counted using a dissecting microscope.

Figure 13 shows that the highest number of sporangia on root pieces kept on non-sterile soil were produced when the pieces were kept at 5°C. The number of sporangia decreased on root pieces kept at 15°C, and even more on those kept at 25°C. The same trend was observed with root pieces from both dishes containing unsterilized soil, therefore data for only one of the dishes are shown in Figure 13. On the other hand, when the root pieces were kept on sterile soil or filter paper, the optimal pre-exposure temperature for subsequent production of sporangia was again 15°C.

The results suggest that although the optimal pre-exposure temperature for production of sporangia by *Ph. cryptogea* may be about 15°C, in non-sterile soil production of sporangia may be influenced by other soil microorganisms. Thus the situation may be similar to that discussed by Griffin (1972) with regard to increased saprophytic colonization of wheat straw by *Gibberella zeae*, *Fusarium culmorum* and *Cochliobolus sativum* as temperatures decreased within the range of 10-30°C. He pointed out that in all cases the experiments suggested that the level of antagonism was the major factor that determined successful saprophytism.

Root pieces colonized by *Ph. cryptogea*, and placed on moist natural soil in a Petri dish in an atmosphere of nearly 100% relative

Figure 13. Mean numbers of sporangia produced by *Phytophthora cryptogea* after exposure to various temperatures under non-sterile and sterile conditions.


70
60
50
40
30
20
10
0
MEAN NUMBER OF SPORANGIA PRODUCED
5
15
25
TEMPERATURE (°C)
● LB AGAR CULTURE
■ COLONIZED ROOTS ON STERILE FILTER PAPER
▲ COLNIZED ROOTS ON STERILE SOIL
X COLONIZED ROOTS ON NON-STERILE SOIL

humidity, and at temperatures suitable for the growth of microorganisms, would soon become colonized by large numbers of bacteria which could affect hyphae of the fungus. Although certain soil bacteria stimulate production of sporangia by *Phytophthora* (Zentmyer 1965) such stimulation seems to play a part only during the actual production of sporangia, and would not cancel any previous antagonistic effect on the fungus by other organisms. It is difficult to relate these results to a field situation, but it seems likely that production of sporangia in the field is also influenced by the soil microflora.

(4) Effect of depth of soil extract on production of sporangia by *Phytophthora cryptogea*

In some of the previous tests it appeared that the number of sporangia produced by *Phytophthora cryptogea* decreased as the depth at which agar pieces with fungal mycelium had been submerged, increased. It was decided to examine the effect of this factor on the production of sporangia by the fungus.

Ph. cryptogea was grown on Lima bean agar for 5 days. Discs 10 mm in diameter were cut from the culture and placed in glass vials 75 mm in height and 23 mm in diameter. Three discs were placed in each vial to which 1% non-sterile soil extract was then added to obtain depths of about 6, 13, 20, 25 and 32 mm above the culture discs. The vials were placed under a fluorsecent light at 25^{o}C for two days. The numbers of sporangia produced were counted under a microscope (10x objective and 10x eyepieces) in 8 randomly chosen microscope fields around the edges of the discs.

Table 24 shows that the number of sporangia produced decreased sharply when the depth of liquid increased from 6 mm to 13 mm, then decreased gradually with the increasing depth of liquid until no sporangia were seen on the agar discs kept at the depth of 32 mm. The experiment suggests that decreasing concentration of O_2 at greater depth of liquid might be responsible for the reduction of numbers of sporangia produced.

The results of this test also shows that the depth of water above soil when isolating *Phytophthora* from soil using a "baiting" method, must not be too great. In this study depth of liquid above soil when using pears as "baits" was about 2 cm, and with tomato cotyledons as "baits" about 1 cm. Because of the above results, care is now taken in this laboratory to use a minimal quantity of water when isolating *Phytophthora* from soil.

Duniway (1975) studied the formation of sporangia by *Ph. drechsleri* (= *Ph. cryptogea*) in soil at high matric potentials, and found that aeration at a depth of 5 mm in saturated soil was inadequate for sporangia to form. Thus the observations of this author support the results of these tests, and confirm that aeration is a factor which influences the formation of sporangia by *Ph. cryptogea*. It is of interest that in Duniway's (1975) tests with waterlogged soil, formation of sporangia was already inhibited at a depth of 5 mm. It appears that in waterlogged soil some factor in addition to lack of aeration, as for example toxic substances, may affect the formation of sporangia.

Table 24.

Production of sporangia by *Phytophthora cryptogea* at different depths in liquid

Replicate No.	Depth of liquid in mm, and numbers of sporangia produced at each depth				
	6	13	20	25	32
1	73	5	1	2	0
2	71	18	2	0	0
3	92	0	7	3	0
4	9	3	11	1	0
5	4	2	0	3	0
6	64	4	4	1	0
7	25	3	2	1	0
8	64	2	1	0	0
Mean	50.25	4.63	3.50	1.38	0

L.S.D. (P = 0.05) - 19.35

(P = 0.01) - 26.11

(5) Survival of zoospores and germ tubes of *Phytophthora cryptogea* in soil

As mentioned before, zoospores are the natural means of spread of *Phytophthora* species, and therefore the period they are able to survive in soil is an important factor in the life cycle of these fungi. Hickman and Ho (1966) reviewed the behaviour of zoospores of plant pathogenic phycomycetes, and indicated that their zoospores rapidly loose infectivity in the absence of a host plant. But they also pointed out exceptions to the general rule, and gave examples where zoospores (or structures produced by them) have been reported to be still viable in moist soil after 6 months. There is no doubt that the period fungal structures are able to survive varies from soil to soil. Bumbieris and Lloyd (1967) showed that germ tubes of various fungi were lysed faster in a garden soil than in a wheat-field soil.

To examine the survival of encysted but ungerminated zoospores of *Ph. cryptogea* in Kuitpo forest soil, zoospores of the fungus were obtained as described in p.10. Two to three drops of the zoospore suspension were added to the smoothed surfaces of Kuitpo forest soil in small Petri dishes and incubated in a moist chamber at 25°C. The zoospore suspension was added to the soil before the zoospores had a chance to germinate.

Viability of the encysted zoospores was examined after intervals of 2-3 days by adding a few drops (2-3) of 1% glucose-peptone solution to the soil surface to induce their germination. Germinated and ungerminated zoospores were then recovered from the soil several hours later as described by Bumbieris and Lloyd (1967). The test shows that encysted zoospores of *Ph. cryptogea* were still able to germinate after 14 days on Kuitpo forest soil under the experimental conditions. After

17 days one doubtful germling was recovered from the soil.

To examine the survival of germ tubes of *Ph. cryptogea* in soil, zoospores were obtained as before but were left in the soil extract for 3-4 hours during which time most of them encysted and germinated. Two to three drops of the suspension containing germinated zoospores were then added to the smoothed surfaces of Kuitpo forest soil in small Petri dishes and incubated as before. After 2, 4 and 6 days the germlings were recovered from the soil as above. Some lysis of germ tubes was seen after two days on soil. Lysis had progressed considerably after 4 days, and after 6 days lysis was estimated as well over 90%. When assessing the degree of lysis of fungal hyphae in soil, a rating of over 90% lysis indicates that all of the hyphae have been completely or partly lysed (Bumbieris and Lloyd 1967). Thus germ tubes of *Ph. cryptogea* were able to survive in Kuitpo forest soil for about 4 to 6 days at 25°C.

In these tests encysted zoospores of *Ph. cryptogea* were still able to germinate after 14 days, and germ tubes survived for at least 4 days in Kuitpo forest soil. A period of 14, or even 4 days, is ample time for germinated or ungerminated encysted zoospores to be spread from one area in the forest to another, or to distant plantations by water or soil adhering to implements or vehicles.

(6) Attraction of zoospores of *Phytophthora cryptogea* to roots of *Pinus radiata*

The attraction of zoospores by roots of susceptible host plants (chemotaxis) has often been described. It has been shown in such cases that zoospores accumulate in clusters around the root, especially near

the root tip. To examine attraction of *Phytophthora cryptogea* zoospores to roots of *Pinus radiata*, zoospores of the fungus were obtained as in previous tests. About 5 cm long roots of *P. radiata*, grown in sand, were excised from young plants, taking care not to damage the root tips. These were then immediately placed in a zoospore suspension in a small Petri dish with the cut ends resting on the edge of the dish, and observed using a microscope.

Zoospores were soon seen swimming around the roots. The density of zoospores around a root, as far as could be judged, was not much greater than at some distance away. A number of zoospores landed on the root itself where they encysted. But some zoospores were also seen swimming away from the root after initial contact. Furthermore, the zoospores did not appear to show any preference for the area immediately behind the root tip. Thus it appeared that there was not a strong attraction of zoospores of *Ph. cryptogea* to roots of *P. radiata* when examined under the condition described above. Hickman and Ho (1966) stated that accumulation of zoospores on plant tissues is very widespread. Root models have been used to demonstrate attraction of zoospores of pythiaceous fungi by various chemical substances which are constituents of root exudates. Royle and Hickman (1964), for example, analyzed the behaviour of zoospores of *Pythium aphanidermatum* towards a wide range of single compounds and mixtures, and observed trapping and encystment of zoospores in response to 1% casein hydrolysate plus fructose, glucose and sucrose, all at concentrations of 1%.

In some cases zoospores may be attracted to sites of wounds but not to intact root surfaces as shown by Kraft and Endo (1966) for

Pythium aphanidermatum on bentgrass. In this study zoospores of *Ph. cryptogea* were not strongly attracted to intact roots of young *P. radiata* grown in sand, and zoospores did not appear to show any preference for the area immediately behind the root tip. However, zoospore attraction to roots may be affected by differences in the physiological conditions of roots grown under different conditions (Hickman and Ho 1966).

(7) Infection of *Pinus radiata* by *Phytophthora cryptogea*

To gain information about the process of infection of roots of *Pinus radiata* by *Phytophthora cryptogea*, tests were done to find out whether the fungus has any preferences with regard to infection sites on a root, and what kinds of roots are infected. For this purpose roots were classified as young-white roots, long and suberized roots, short roots - mycorrhizal and short roots - non-mycorrhizal. The following tests were done:

A. Three month old *P. radiata* grown in washed sand with complete nutrient solution (Table 19) were inoculated with *Ph. cryptogea* using 50 ml of hyphal-sporangial suspension of the fungus per pot. Beginning one week after inoculation, plants were removed from different pots at weekly intervals for examination of their roots. The roots were first washed under a tap and then spread out on a laboratory bench on a white background. Each root system was copied on paper and all roots were numbered. The roots were then excised and placed in distilled water in individual numbered Petri dishes. After 24 hours at 25°C the roots were examined for the presence or sporangia of *Ph. cryptogea*.

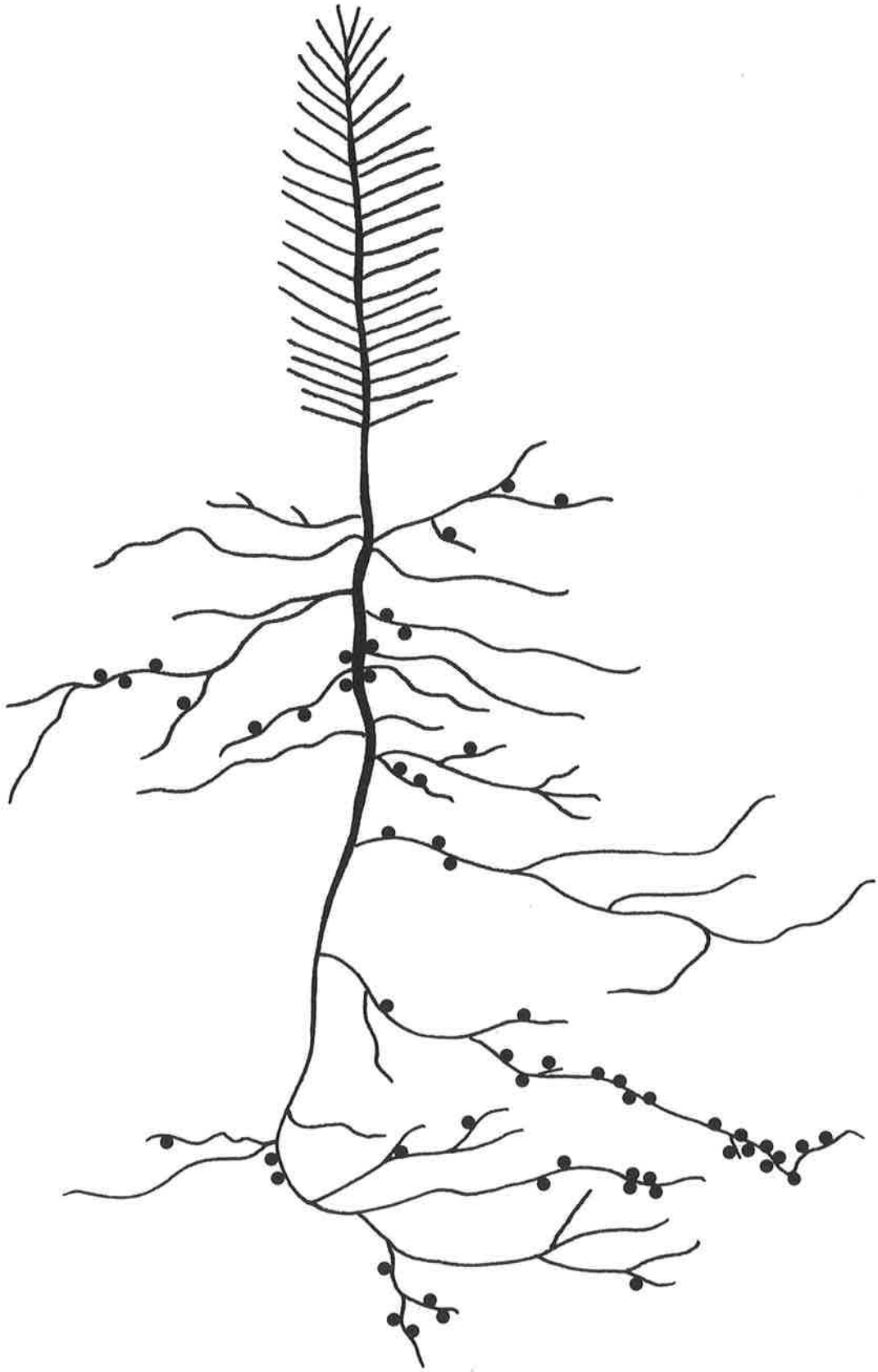
Recognition did not present a problem for there were no other *Phytophthora* spp. present in the pots, and also internal proliferation

of sporangia was often observed. At first, roots on which no sporangia were seen after 24 hours, were incubated for further 24 hours but longer periods of incubation did not result in the production of sporangia. Therefore incubation for 24 hours was considered sufficient in later observations. In addition, longer periods of incubation may cause secondary infections by zoospores released by the first crop of sporangia.

Examination of roots showed that only two plants had been infected under the experimental conditions. These were plants removed from pots 1 and 6 weeks after inoculation. In the first case only three sites had been infected; one about 5 cm from the tip of a suberized long root, the second at the base of a short root, and the third at the tip of a short root. The short roots did not appear to be mycorrhizal. Figure 14 shows the infection sites on the roots of the plant removed from a pot 6 weeks after inoculation. In most cases the infection sites, where large numbers of sporangia were present, were found at various distances from root tips. Infections near root tips had occurred only occasionally. It is seen that not only branch roots but also the tap root had been infected in this case. As few young white roots were seen in this plant, most of the infected roots were already suberized.

Abundance of sporangia at most of the infection sites indicated that they had originated from infections and not from occasional chlamydospores present on the roots. Infection near root tips had occurred only rarely. This pattern of infection corresponds to the rather weak attraction of zoospores by roots of young *P. radiata* noted previously.

Figure 14. Infection of *Pinus radiata* roots by *Phytophthora cryptogea* in inoculated sand.


• Infected sites

B. In a second test the roots of a 4 month old *P. radiata* grown in washed sand were suspended in a zoospore suspension of *Ph. cryptogea*. After two days infection of the roots was examined as in the previous test. A number of the short roots of this plant appeared to be mycorrhizal, they were light creamy in colour and swollen. Microscopic examination of these roots showed abundant fungal mycelium, consisting of septate hyphae forming a sheath around the roots, and also extending into the cortex. These roots thus appeared mycorrhizal although no mycorrhizal fungi had been introduced in the pots; infection with the fungus must have occurred by air-borne spores.

When the roots were examined for the presence of sporangia, a total of 7 infected sites were seen: three of them were on suberized long roots, one at the base of a short root, and three at tips of short roots. The infected short roots did not appear to be mycorrhizal as they were not swollen and were dark brown in colour.

No valid comparison can be made between the infection of mycorrhizal and non-mycorrhizal roots by *Ph. cryptogea* in this case as the number of infection sites was too small.

(8) Discussion

Results obtained show that *Phytophthora cryptogea* forms chlamydospores in decaying roots of *Pinus radiata* in soil. No doubt, these structures play an important role in the survival of the fungus in soil in the absence of host roots, and could be transported with soil to new locations. The survival of the fungus in slightly moist soil stored in a plastic bag for two years may be entirely due to these structures. Their ability to germinate and produce sporangia within 24 hours when roots were placed in water, shows that new crops of

zoospores would soon be produced when soil becomes wet, for example after a heavy rainfall.

As far as the author knows, no previous work has been done on the ability of *Ph. cryptogea* to colonize dead organic matter in soil. In these tests *Ph. cryptogea* was able to colonize roots of *P. radiata* killed by autoclaving when separated from the roots by 1 cm of soil. However, there is a possibility that infection of the roots also occurred from propagules present in the soil.

Growth of *Phytophthora* hyphae in soil has not been extensively studied but it appears that their growth in natural soil may be limited. Kuhlman (1964) could not recover *Ph. cinnamomi* from soil at a distance of 2.5 cm from an inoculum disc placed on non-sterile forest soil. Bumbieris (1973) observed that growth of hyphae of *Ph. cinnamomi* was influenced by the balance between available nutrients and inhibitory factors. Thus it appears that zoospores may be the main means of spread of these fungi as suggested by Kuhlman (1964).

The inoculum potential of *Ph. cryptogea* at a given infection site depends partly on the number of infective propagules present, which may be mainly zoospores. Thus the production of sporangia and zoospores becomes an important phase in the life cycle of *Ph. cryptogea*. In these tests production of sporangia was influenced firstly by the temperature at which mycelium of the fungus had been pre-exposed. It appears that temperature may affect production of sporangia directly, or indirectly by stimulating antagonistic soil microorganisms. Secondly, production of sporangia was also influenced by the degree of aeration present. It is likely that the mentioned factors also affect production of sporangia by *Ph. cryptogea* in the field. But in the complex soil

environment other factors may also be important, and these should be further investigated.

In these tests exogenous nutrients were used to stimulate germination of encysted zoospores of *Ph. cryptogea* on natural forest soil to ascertain their viability. As encysted zoospores of the fungus germinate in water but not on soil in an atmosphere of nearly 100% relative humidity when a free film of water would be present on the smoothed soil surface, it appears that these spores, similar to many other spores of soil-borne fungi, are subject to soil fungistasis.

In a further test where young *P. radiata* were grown in sand supplied with a complete nutrient solution and then inoculated with *Ph. cryptogea*, the roots of only 2 out of 7 young pines became infected by the fungus. This further supports the assumption that *Ph. cryptogea* is only a weak pathogen of *P. radiata*.

IX. GENERAL DISCUSSION

The study showed that *Phytophthora cryptogea* is associated with declining as well as with healthy *Pinus radiata* in forest plantations in the Adelaide hills. However, in glasshouse tests the effect of the fungus was insignificant on non-transplanted young *P. radiata* growing in moist soil. On the other hand, waterlogging affected the growth of young pines both in the absence and presence of the fungus. Thus waterlogging is an important factor in the decline of *P. radiata*, and drainage should be improved when replanting wet sites with these trees. Although *P. pinaster* appeared to be resistant to *Ph. cryptogea* the value of this species for replanting wet sites is doubtful as it appeared to be more susceptible to waterlogging than *P. radiata*.

Although various workers have shown that improved soil nutrition (Copeland 1960; Newhook 1970), and the presence of mycorrhizas (Marx 1970 and 1973) improve the growth of pines in soils infested with *Ph. cinnamomi*, the beneficial effects of these factors was not demonstrated in short term experiments under the experimental conditions. Further studies under different experimental conditions, and using different techniques may yield different results.

Although *Ph. cryptogea* was found in association with some other plants in the forest, the elimination of such hosts would be of little value in controlling the fungus as it could be easily reintroduced to such sites by movement of water or soil. Laboratory studies showed that encysted zoospores of *Ph. cryptogea* were able to germinate in unsterilized soil after 14 days. Zoospores released during periods of high soil moisture could be carried by drainage or irrigation water

for considerable distances, and would still be able to germinate and infect fresh host roots. The presence of *Ph. cryptogea* in irrigation water was recently demonstrated by Taylor (1977).

Laboratory tests showed that *Ph. cryptogea* forms chlamydospores in decaying pine roots in soil by means of which the fungus may be able to survive in soil for long periods. However, the survival of the fungus in soil should be further investigated, especially under very dry conditions such as often prevail in the forests during summer periods. Laboratory tests also showed that *Ph. cryptogea* colonized dead organic matter in soil under the experimental conditions. This property would further enhance the survival of the fungus in forest soil in the absence of host plants. The described properties of *Ph. cryptogea* indicate that the fungus is very well suited to its soil environment both with regard to its survival and spread, and elimination of the fungus from a given area would be very difficult.

Although the described study cannot be directly related to older *Pinus radiata* in forest plantations, it is likely that *Phytophthora cryptogea* is only a weak pathogen of trees growing under conditions favourable for tree growth. Under unfavourable conditions there is no evidence to suggest that trees are less tolerant to infection by the fungus. In fact any effect of the fungus on pines in the forest is probably overshadowed by other factors detrimental to the trees, especially waterlogging.

X. APPENDICES

Appendix 1

Host List of *Phytophthora cryptogea*
(Includes references for *Ph. drechsleri* as both species are considered to be conspecific by the author.)

1. *Achimenes cardinalis* A.Dietr.	(Middleton, Tucker, and Tompkins, 1944)
2. *A. grandiflora* DC	(")
3. *A. longiflora* DC	(")
4. *Aeschynantus lobbiana* Hook	(")
5. *A. speciosus* Hook	(")
6. *Alloplectus schlimii* Planch. and Lind.	(")
7. *Arctostaphylos* sp.*	(Middleton and Baxter 1955)
8. *Calceolaris crenatiflora* Cav.	(Middleton, Tucker, and Tompkins, 1944)
9. *Callistephus chinensis* Nees.	(Tompkins and Tucker, 1937)
10. *Carthamus lanatus* L.	(Cother and Griffin, 1973 b)
11. *C. tinctorius* L.	(Erwin, 1952; Stovold, 1973)
12. *Castanea* sp.	(Wicks and Volle, 1976)
13. *Celosia argentea* var. *cristata* (L) O. Ktze.	(Middleton, Tucker, and Tompkins, 1944)
14. *Chondrilla juncea* L.	(Cother and Griffin, 1973 b)
15. *Citrullus lanatus* (Thernb.) Matsumura & Nakai	(")
16. *Darlingtonia* sp.*	(Middleton and Baxter, 1955)
17. *Echium lycopsis* (*E. plantagineum* L.)	(Cother and Griffin, 1973 b)
18. *Episcia cupreata* Hanst.	(Middleton, Tucker, and Tompkins, 1944)
19. *Erodium botrys* (Cav.) Bertol.	(Cother and Griffin, 1973 b)
20. *Eucalyptus* sp.*	(Pratt and Heather, 1973)
21. *E. microcorys* F. Muell.*	(")
22. *E. sieberi* L. Johnson*	(")
23. *E. viminalis* Labill.*	(")
24. *E. pilularis* Sm.*	(")
25. *E. stellulata* Sieb. ex D.C.*	(")
26. *E. macrorryncha* F. Muell. ex Benth.*	(")
27. *E. obliqua* L'Herit.	(")
28. *E. radiata* Sieb. ex DC*	(")
29. *E. regnans* F.Muell.*	(")
30. *Gerbera jamesonii* Hook. var. *transvaalensis* Hort.	(Tompkins and Tucker, 1937)

31. *Gesneria cardinalis* Lehm.	(Middleton, Tucker, and Tompkins, 1944)
32. *Godatia grandiflora* (Lindl.)	(")
33. *Isolama amabile* Mott.	(")
34. *I. hirsutum* Regel.	(")
35. *Lycopersicum esculentum* Mill.	(Pethyridge and Lafferty, 1919)
36. *Malus domestica* Borkh.	(Sitepu and Bumbieris, 1972)
37. *Mathiola incana* R.Br. var. *annua* Voss.	(Tompkins and Tucker, 1937)
38. *Mimulus* sp.*	(Middleton and Baxter, 1955)
39. *Naegelia cinnabarina* Lind.	(Middleton, Tucker, and Tompkins, 1944)
40. *N. multiflora* Hook.	(")
41. *N. zebrina* Regel.	(")
42. *Pinus* sp.*	(Middleton and Baxter, 1955)
43. *P. radiata* D.Don	(Bumbieris unpubl.)
44. *P. pinaster**	(Davison and Bumbieris, 1973)
45. *Prunus armeniaca* L.	(McIntosh, 1964)
46. *P. cerasus* L. or *P. avium* L.	(")
47. *P. persica* (L) Batsch.	(")
48. *Pyrus communis* L.	(")
49. *Saintpaulia ionantha* Wandl.	(Middleton, Tucker, and Tompkins, 1944)
50. *Senecio cruentus* DC	(")
51. *Sequoia* sp.*	(Middleton and Baxter, 1955)
52. *Sinningia speciosa* Benth. & Hokk.	(Middleton, Tucker, and Tompkins, 1944)
53. *Solanum tuberosum* L.	(Drechsler, 1929)
54. *S. melongena* L.	(Tucker, 1931)
55. *Streptocarpus kewensis* Hort.	(Middleton, Tucker, and Tompkins, 1944)
56. *Taxus* sp.*	(Middleton and Baxter, 1955)
57. *Xanthium spinosum* L.	(Cother and Griffin, 1973b)

* Plant species to which pathogenicity of *Phytophthora cryptogea* has not been demonstrated.

Note: Erwin (1954) reported that *Ph. cryptogea* causes root rot of lucerne (*Medicago sativa* L.) but it is doubtful that the fungus was correctly identified as it produced oogonia abundantly in single strain cultures, and *Ph. cryptogea* isolates obtained from other sources were not pathogenic to lucerne.

Appendix 2

Previous Publications on *Phytophthora*.

Davison, E.M. and Bumbieris, M. (1973). Phytophthora and Pythium SPP. from pine plantations in South Australia. *Australian Journal of Biological Sciences, 26*(1), 163-169.

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