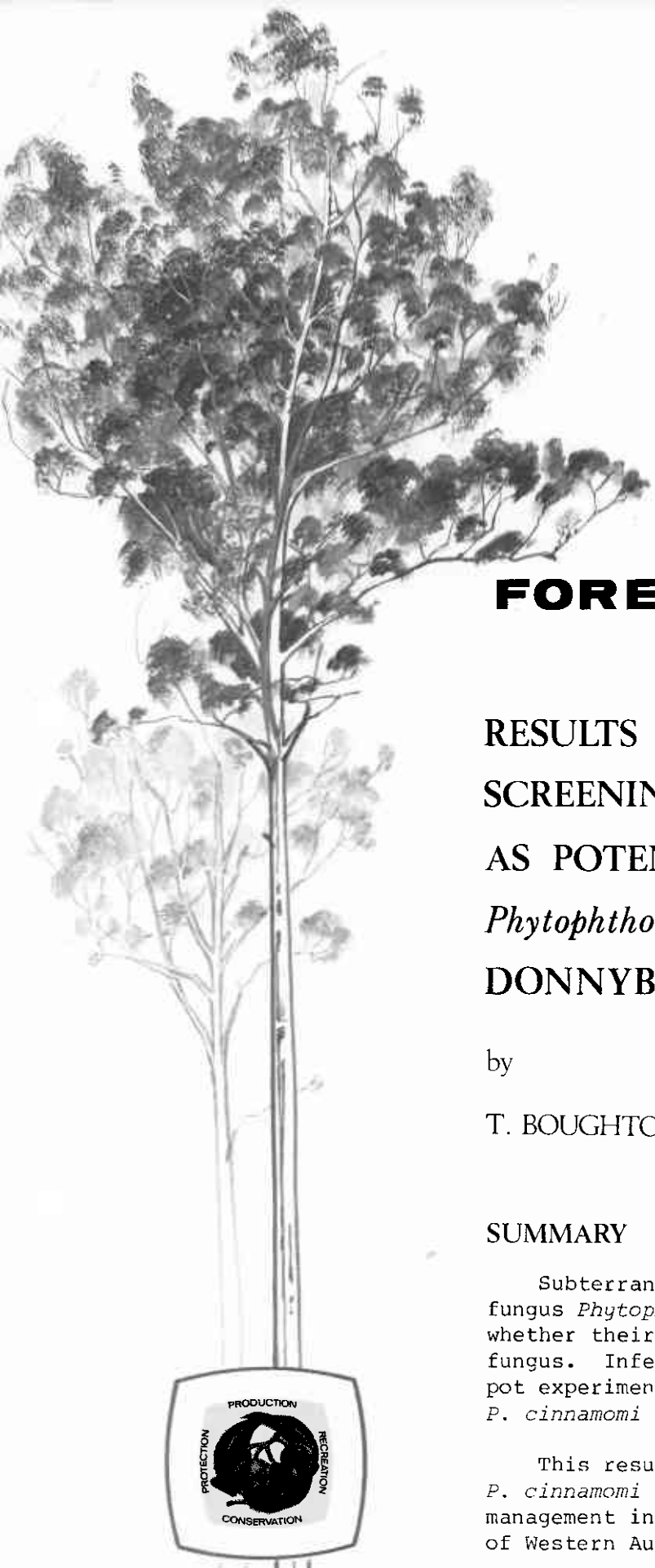

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RESULTS OF PRELIMINARY SCREENING OF CLOVER CULTIVARS AS POTENTIAL HOSTS FOR *Phytophthora cinnamomi* IN THE DONNYBROOK SUNKLAND

by

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SUMMARY

Subterranean clover plants were inoculated with the fungus *Phytophthora cinnamomi* (dieback disease) to test whether their roots could be infected or colonized by the fungus. Infection occurred in roots grown in a glasshouse pot experiment, and in roots and seed burrs adjacent to *P. cinnamomi* inoculum in the field.

This result is discussed in relation to raising *P. cinnamomi* susceptible trees under agro-forestry management in the Donnybrook Sunkland in the south-west of Western Australia.

More research will be required to show whether clover can increase *P. cinnamomi* inoculum potential in the Donnybrook Sunkland.

INTRODUCTION

Scattered deaths of *Pinus radiata* (D. Don) trees caused by *Phytophthora cinnamomi* (dieback disease) in the Donnybrook Sunkland, have led to investigations into pine plantation management techniques which could reduce the risk of serious disease spread occurring. An agro-forestry system of management using subterranean clover is now being developed in plantations in the Sunkland. Its benefits include improved tree nutrition, reduced fire hazards and increased financial return (McKinnell, 1979; McKinnell and Batini, 1978).

Species susceptible to *P. cinnamomi*, such as *Banksia grandis*, have a major role in the build-up of *P. cinnamomi* inoculum in the jarrah forest by providing a food base for the fungus and a protected site for its survival during summer (Schild *et al*, 1980). In avocado orchards, vigorous but susceptible plants such as *Lupinus angustifolius* are used as a green manure crop to promote soil microbial populations antagonistic to *P. cinnamomi* (Pegg, 1977). The danger with this technique is that if the lupins are cut at certain times of the year the resultant mulch may act as a substrate for *P. cinnamomi* rather than as a suppressant. (Broadbent, personal communication *). Little is known about the potential for the build-up of inoculum on the roots of non-susceptible agricultural plants.

United States reports have demonstrated that subterranean clover is susceptible to *Phytophthora megasperma* (Johnson and Keeling, 1969), and that clover species of the related genus *Pythium* are involved in the complex clover root rot disease which is prevalent in the south-west of Western Australia (Macnish *et al*. 1976).

The aim of the pot trials and field sampling was to discover whether clover, by becoming an alternative host of *P. cinnamomi*, had the potential to increase the risk of pine disease.

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METHOD

Experimental design

The experiment examined root infection of plants growing in pots of Sunkland soil that had been inoculated with *P. cinnamomi*. Nine clover cultivars were tested, using one inoculated (+ *P. cinnamomi*) pot and one uninoculated (- *P. cinnamomi*) pot for each cultivar. The inoculum used was artificially infected pine branch segments.

The clover cultivars tested were those which had already been planted in the Sunkland or those whose agricultural performance indicated they were suitable for the Sunkland: *Trifolium subterraneum* cultivars Clare, Dinninup Esperance, Mt Barker, Seaton Park, Larissa and Trikkala; *Trifolium fragiferum* (strawberry clover) and *Trifolium repens* (New Zealand white clover).

Pot experiment

Establishment and maintenance

Undrained plastic pots, 10 cm in diameter, were filled with 950 g air dry, type 4C, grey loamy sand (McCutcheon, 1978) collected from Compartment 2 at the Jarrahwood Plantation 30 km south of Busselton. The soil was saturated with distilled water, and the bases of the pots perforated, allowing the soil to drain. Eight pre-germinated clover seeds were placed in 0.5 cm depressions in the soil, 1 cm in from the edge of each pot. Each seed was inoculated with a suspension of *Rhizobium* strain WU 290 to ensure that nodulation and symbiotic nitrogen fixation occurred, as these are necessary conditions for clover growth in the nitrogen deficient soils of the Sunkland. Two control pots with 950 g air dry, type 4C, grey loamy sand were left unplanted. The seeds were covered with soil, and 165 mg of superphosphate copper zinc A (C.S.B.P. and Farmers, Kwinana, Western Australia), was broadcast over the surface of each pot. The pots were placed in a glasshouse with air temperatures ranging from 16° to 37° C, and were watered with distilled water twice weekly.

Inoculum was prepared from *P. radiata* branch segments (Shea *et al*., 1980). Three weeks after sowing, each + *P. cinnamomi* pot was inoculated by burying two infected

pine branch segments 1 cm below the surface of the soil in the centre of each pot. Autoclaved segments were buried in the uninoculated pots.

Harvest of experiment

The clover plants were harvested three months after sowing. Plant tops were removed and any visual effects of inoculation noted. The root ball was removed from each pot and a sample of approximately 50 g of soil and roots was taken from the base of the pot. This sample was lupin baited (Chee and Newhook, 1965) to test for movement of *P. cinnamomi* from the inoculum segment into the soil. Soil was washed from the root system of the clover plant and the inoculum segments were recovered.

Isolation of *P. cinnamomi*

Phytophthora cinnamomi was isolated from the clover roots in each pot using the following procedure. The root system was divided into two sections. One section was surface sterilized in 70 per cent ethanol for 30 seconds and then rinsed in running distilled water for another 30 seconds. The other section was left untreated. Four sub-samples of roots were cut from each section, then bulked and floated in sterile distilled water. Root segments approximately 1 cm long and free of attached organic matter were taken from the bulked sample and plated onto P10 VP (H) medium selective for *Phytophthora* (Masago *et al.*, 1977). The remainder of each root system with attached organic matter was placed in a litre beaker, covered with distilled water and lupin baited to test for the presence of *P. cinnamomi*. Samples showing no recovery of the fungus at the first baiting were rebaited.

The plates were incubated at 25° C for 36 hours, after which *P. cinnamomi* hyphae were seen growing from the clover roots. Each *P. cinnamomi* outgrowth was classed as one infection. The total length of roots on each plate was measured and the results expressed as the number of infections per metre of root.

Statistical analysis of pot experiment results

Owing to the unreplicated experimental design and the skewed distribution of the root infection results, statistical analysis was limited to a comparison of the infection levels of untreated roots with those of surface sterilized roots, using a Sign test (Siegel, 1956).

Sampling of clover roots inoculated in the field

An inoculated *P. radiata* plot at the Vasse Plantation 20 km south-west of Busselton was used to test the potential of subterranean clover as an alternative host for *P. cinnamomi* in the field.

The plot was located adjacent to a creek on type 4D organic, grey loamy sand (McCutcheon, 1978). In autumn 1980 the site was mounded and seeded with a mixture of Trikkala and Esperance clover at 12 kg. ha^{-1}, and topdressed with 500 $kg.ha^{-1}$ superphosphate Cu, Zu, Mo No. 2 (C.S.B.P. and Farmers, Kwinana, Western Australia). Inoculum segments were buried 5 cm below the soil surface in the centre of the mounds on 25 June 1980.

On 20 November 1980, four samples of clover roots were taken. Two of these samples were blocks of soil and roots surrounding the inoculum plugs. The other samples were blocks of soil and roots taken from between 100 and 200 mm from the inoculum.

The samples were gently washed to remove soil from the roots. Roots in the following classes were plated without surface sterilization:

(1) roots less than 5 mm from the inoculum segment;

(2) roots 5 to 10 mm from the inoculum segment;

(3) roots 100 to 200 mm from the inoculum segment.

Where present, seed burrs and nodules were plated separately. Recovery of *P. cinnamomi* from the samples was measured in the same way as in the pot experiment.

RESULTS

Pot Experiment

Phytophthora cinnamomi killed one Seaton Park plant in this trial. The fungus was recovered from the plant's root system in this pot and from a petiole that was in

contact with the soil surface. Extensive root necrosis was only observed in the plants in the inoculated Seaton Park and strawberry clover pots. Inoculation had no effect on the top growth or on the appearance of root systems on the remaining 61 inoculated plants.

Plating recovered *P. cinnamomi* from the untreated roots of all cultivars and from eight of the nine surface sterilized root samples (Table 1). Seaton Park, Esperance, Mt. Barker, Dinninup and Clare were the only cultivars which yielded *P. cinnamomi* from both direct plated and lupin baited roots.

Surface sterilization of roots significantly reduced ($P<0.5$) the number of infections per metre of roots for almost all cultivars. The extent of this reduction ranged from 12 per cent to 100 per cent. This suggests that a proportion of the infections detected in untreated roots was on the surface of the root, and hence more readily affected by sterilants than established infections in the root.

Phytophthora cinnamomi was not recovered from any of the samples in the uninoculated pots. All inoculum segments remained viable at the end of the experiment.

TABLE 1

Recovery of *P. cinnamomi* from direct plated clover roots and lupin baited soil and root samples

	Total number of infections per metre of direct plated roots		Recovery of *P. cinnamomi* from lupin baited samples[3]		
Cultivar	U[1]	SS[2]	Soil and root sample from base of pot	U[1]	SS[2]
Seaton Park	25.5	9.0	+	+	+
Esperance	7.6	4.2	+	+	+
Mt Barker	4.9	0.5	-	+	+
Strawberry	1.9	0.5	-	+	-
Trikkala	1.9	0	-	-	+
Dinninup	1.7	0.8	-	+	+
Clare	1.5	0.4	+	+	+
New Zealand White	1.2	3.2	-	-	+
Larissa	0.8	0.7	+	-	+
Unplanted control			+		

1 roots untreated

2 roots surface sterilized in 70 per cent ethanol for 30 seconds

3 + indicates *P. cinnamomi* recovered

\- indicates *P. cinnamomi* not recovered

Field sampling

In the field, *P. cinnamomi* was isolated from clover roots within 5 mm of the inoculum segments (Table 2). The level of intensity of infection and/or colonization (8.3 infections per metre of root) equalled the levels observed in the pot experiment. Seed burrs were also able to harbour the fungus. *Phytophthora cinnamomi* was not recovered from nodules or from roots further than 5 mm from the inoculum.

DISCUSSION

The results showed that *P. cinnamomi* is able to colonize and infect clover plants growing in Sunkland soil, usually without the clover plants showing any symptoms of the disease. This result was not surprising, as more detailed observations by Hinch and Weste (1979) have shown that *P. cinnamomi* zoospores are attracted to, and are able to infect, the roots of the non-susceptible grass *Themeda australis*. The results of this experiment indicate that the response of subterranean clover to *P. cinnamomi* lies between the hypersensitive reaction to infection shown by the resistant species *Acacia pulchella* (Tippett and Malajczuk, 1979), and that of highly susceptible species where infection usually results in the death of the plant.

This range in reactions to *P. cinnamomi* infection occurs as a result of differences in the plants' genotypes and the effects of different growing environments. Irwin *et al.*, (1979) showed that different lucerne cultivars vary in their reaction to *P. megasperma* inoculation. Although the results of the present experiment suggest that a similar range of reactions may be present in clover cultivars, replicated experiments will be necessary to support this finding.

Many of the papers on clover root rot reviewed by Burgess *et al.*, (1973) suggested that the fungi causing the disease were weak parasites which only become important when plants have been predisposed to infection by the loss of food reserves, through age, grazing or during the winter. Environmental stresses such as water logging, shading, infestation by red legged earth mites (*Halogydeus destructor*), infections by leaf pathogens such as clover scorch (*Kabatiella caulivora*) and pepper spot (*Leptosphaerulina trifolii*), and grazing by agricultural and native animals occur in Sunkland areas managed for agroforestry. It is possible that these stressed may predispose clover to *P. cinnamomi* infection.

In the pot experiment and field sampling, non-pathogenic infection occurred when clover

TABLE 2

Recovery of *P. cinnamomi* from inoculated clover in the field.

Sample type	Distance of sample from inoculum segment (mm)		
	5	5-10	100-200
root[1]	6/723	0/1850	0/2330
seedburr[2]	n.d.[4]	2/20	n.d.[4]
nodule[3]	n.d.[4]	0/4	0.35

[1] Total number of *P. cinnamomi* infections/total root length (mm)

[2] Total number of infected burrs/total number plated

[3] Total number of infected nodules/total number plated

[4] n.d. represents no sample taken

roots grew around a localized source of inoculum. *Phytophthora cinnamomi* was recovered from roots 10 cm from the inoculum plug in the pot experiment, but only from roots 1 cm from the inoculum in the field. Unless large-scale dispersal of *P. cinnamomi* zoospores occurs as a result of sporulation and overland runoff, it is likely that infection levels comparable with those obtained in this experiment would only occur around fragments of existing infected plant material, such as banksia roots. The distribution of these fragments in the field is unknown. However, as a result of the dense nature of the clover sward and the great length of clover roots, the probability of some clover roots coming in contact with old infected roots is high. Planting of clover on Sunkland sites causes an increase in the biomass of live roots in the soil. The existence of this extensive root system would also increase the probability of introduced infected soil or water-borne zoospores establishing an infection in live tissue.

The experiment does not provide a clear answer as to whether the use of clover in the Sunkland is deleterious or not. Further investigations are desirable.

If it appears that subterranean clover will increase the disease risk in plantations, it may be necessary to select agricultural or native legumes whose roots are less likely to be infected by *P. cinnamomi*.

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