Aust. J. Biol. Sci., 1983, **36**, 191–203

Antifungal Activity of Western Australian Soil Actinomycetes against *Phytophthora* and *Pythium* Species and a Mycorrhizal Fungus, *Laccaria laccata*

D. Keast and C. Tonkin

Department of Microbiology, University of Western Australia, Nedlands, W.A. 6009.

Abstract

Soil pH, soil moisture content and soil organic matter content did not appear to influence significantly the total numbers of actinomycetes isolated from sample sites in Western Australia. However, seasonal influences exist with summer conditions leading to higher spore isolation. Substantial but non-specific antifungal activity against *Phytophthora cinnamomi*, *P. cryptogea*, *P. nicotiana*, *Pythium proliferum* and *L. laccata* was detected *in vitro* from many of the 2367 actinomycetes isolated. Antifungal activity may or may not occur in members of the same actinomycete group, suggesting segregation of antifungal capacity within all groups. A limited number of actinomycete groups was isolated from the rhizosphere of plants and these exhibited similar properties to their counterparts in soil or litter. Actinomycetes isolated from the rhizosphere of *Pinus radiata* produced a high degree of *in vitro* antifungal activity against the *Phytophthora* species but, in general, actinomycetes isolated from root surfaces exhibited antibiosis against all the fungi tested. More actinomycetes showed antifungal activity from soils where *P. cinnamomi* was causing dieback of jarrah and other understorey species.

Introduction

In Australia, and in particular Western Australia, one member of the genus *Phytophthora* de Bary, *P. cinnamomi* Rands, causes a very extensive disease of native flora—from jarrah trees (*Eucalyptus marginata* Donn ex Sm.) to understorey species such as *Banksia grandis* Willd. and shrub layer species such as blackboy (*Xanthorrhoea priessii* R. Br.) and *Macrozamia riedlei* Gaud. C. A. Gardn. (Podger *et al.* 1965).

Weste and Vithanage (1977) have shown that in eastern Australia there is an association between soil microbial population and *P. cinnamomi*-mediated dieback disease of forest flora. Weste and Vithanage (1977) concluded that the reduced microbial population might be linked to a reduction in the capacity of the soil to exert significant biological control on *P. cinnamomi*. The numbers of soil actinomycetes in particular were significantly reduced.

The role of soil microorganisms in the control of *P. cinnamomi* is being explored (Malajczuk 1982). The possibility of antibiotic production in the soil has been the subject of review for over 25 years (Brian 1957; Jackson 1965; Garrett 1970; Baker and Cook 1974*a*; Gottlieb 1976), but direct evidence that these agents are important

0004-9417/83/020191$02.00

in soil fungistasis is difficult to obtain (Lingappa and Lockwood 1961). Soil micro-organisms (bacteria, actinomycetes and fungi) produce antibiotic substances *in vitro* (Baker 1968; Baker and Cook 1974*b*; Arai 1976; Gottleib 1976; Rose *et al.* 1980). These antibiotic substances are not easily extracted from or detected in soil eluates because of their adsorptive or volatile natures (Brian 1957; Arai 1976; Pavlica *et al.* 1978).

Competition for space, nutrients and oxygen must form the basis for survival of organisms in the soil with carbon, nitrogen and vitamin sources being important in the survival of soil-borne plant pathogens (Baker 1968). In the absence of suitable host material, specific variations in growth cycles, formation of resting propagules and resistance to predation are important for survival (Garrett 1970). Antibiotic substances occurring in any of these systems should, therefore, give an additional survival potential to the 'resistant' organism on the one hand and control the 'antibiotic-sensitive' organism on the other. Widespread fungistasis in soil has been known for a considerable time (Lingappa and Lockwood 1961). As early as 1963, Tsao and Bickler showed that soils contained diffusible substances capable of inhibiting *Phytophthora* spp., and more recently the treatment of soil with carbon and nitrogen sources has been conducive to increasing the antagonistic effects of soils to members of the *Phytophthora* genus (Mehrota 1972; Malajczuk and Theodorou 1979). In all cases, increased bacterial predation of fungal mycelia occurred, which in some instances can be accompanied by spore formation (Tsao 1969). There is an increasing number of reports of soils suppressive to *Phytophthora* spp. (Broadbent and Baker 1974; Malajczuk 1979), in which the number and diversity of microbial population is being shown to be critical to their suppression of the fungal pathogens.

Mycorrhizal associations are often imperative to the survival and growth of plants (Littke *et al.* 1980; Schenck 1981) and their function as antagonists to pathogens including *P. cinnamomi*, which invades root systems including those of Australian eucalypts, is being investigated (Marx 1973; Pratt 1973; Malajczuk 1982). It has been reported that potentially pathogenic species of both *Pythium* and *Phytophthora* can be isolated from native vegetation in Australia (Pratt and Heather 1973; Gerrettson-Cornell *et al.* 1977). Therefore, although it is still unclear as to whether or not the production of antibiotic chemicals plays an important role within the soil, there are two important features of such antibiosis, if it occurs in Australian soils, that need investigation. The first concerns the extent to which antibiosis occurs through a group of organisms such as the actinomycetes and whether it is found to be associated with any particular isolate type. Secondly, it is important to know whether any antibiotic produced shows preferential activity against members of the *Phytophthora* genus or whether it will act universally on soil fungi such as *Pythium* and mycorrhizal fungi.

In this paper, we report on *in vitro* studies of the antifungal activity of actinomycetes isolated from soil taken from 14 sample sites in Western Australia.

Materials and Methods

Field Sampling Sites

All sample sites are in the south-western forest and sand-plain area of Western Australia within 500 km of Perth, except for sample site 14, which is in the north-west of the State at Kununurra. Details of the same sites are given in Table 1.

Field Sampling Techniques

Soil sampling

Soil samples of weight approximately 5 g were collected by hand and processed in a mobile field laboratory. Duplicate samples were serially diluted (×10) in sterile distilled water and 0·1-ml samples were spread over the surface of selective agar plates. The field isolation medium was basically ISP medium 4 (Shirling and Gottlieb 1966) with further antibiotic supplementation (Pimafucin, 2 ml; Actidione, 2 ml of a stock solution containing 40 mg ml^{-1}; oxytetracycline, 0·25 ml (stock solution 10 mg ml^{-1}; polymyxin B, 0·24 ml). Each antibiotic was added to 500 ml of medium. After culturing at 27°C for 5–7 days, individual colonies were picked, without selection, onto yeast–malt agar slopes for identification. These slopes were of ISP medium 2 (Shirling and Gottlieb 1966). The total number of actinomycetes was recorded on all field isolation plates, and corrected to total number per gram of oven-dried soil.

Table 1. Description of field sample sites

Field Site No.	Day No. in 1980	Site description
1	87	Brackish lakeside, sand plain
2	101	Native scrub, sand plain
3	123	Farmland, granite/laterite soil
4	144	Farmland, granite/laterite soil
5	198	*Banksia* and jarrah forest, bauxite mine site
6	212	Coastal salt lake, sand plain
7	225	Coastal salt lake, high salinity sand
8	249	Native jarrah/*Banksia* forest, very poor laterite soil (very high fungal dieback)
9	254	Low desert scrub, desert sand plain
10	274	Long-established pine plantation, poor sand plain
11	293	Cultivated land, spearwood sand
12	300	Cultivated land, bassendean sand
13	315	Inner-city marsh lake, high organic sand
14	324	Tropical irrigation, flood plain

Rhizosphere sampling

Selected plants were carefully excavated to preserve as much of the fine root system as possible. All loose soil was shaken from the roots and the soil remaining was taken to represent that containing the rhizospheric microbial population (Alexander 1977). This soil was processed as described above.

Morphological Description of Actinomycetes

All isolates were classified to the International Streptomycetes Project colour criteria by soluble pigments produced, top colour of spores *en masse*, and reverse colony or substrate mycelial colour (Arai 1976). The presence or absence of aerial mycelia became an obligatory fourth classifying factor (Table 2). A number of isolates was assessed for melanin production and sporophore and spore morphology by electron microscopy.

Melanin production

In all, 170 isolates exhibiting minor morphological variations within each group were assessed for melanin production using ISP medium 6, peptone–yeast extract–iron medium (Shirling and Gottlieb 1966).

Sporophore and spore morphology

Sterile 10-mm diameter glass coverslips were embedded in yeast–malt extract–agar plates almost perpendicular to the surface. The 170 actinomycetes were inoculated along the coverslip–agar interface and the mycelia allowed to grow up the coverslip. Cultures were maintained for up to 6 days at 27°C. Coverslips containing mature mycelia and sporophores were aseptically removed from culture dishes and fixed in fresh 2·5% (v/v) glutaraldehyde for 18 h and after two washes in distilled water were dehydrated through a series of ethanol rinses (30, 50, 70, 80, 90, 100 superdry, percentage ethanol by volume), each for 15 min. Critical-point drying in a carbon dioxide bomb was then carried out and samples mounted in Philips scanning stubs with silver paste (Pelco No. 1603–2). Samples were finally vacuum-coated with 4·0% (w/v) Au–Pd. All samples were prepared in triplicate and scanned using a Philips stereoscan electron microscope (HT voltage 25 kV; spot size varied from 1250 to 320 nm because magnifications of 1700–700× were available). Sporophores were observed at 1250 nm (1700×) and spore detail at 640 nm (3500×).

Table 2. Simplified descriptive key for actinomycetes isolated mainly from the south-west of Western Australia

Isolate group No.	Aerial mycelia	Spore	Pigment	Reverse colour	Melanin production	Sporophore[A]	Spore[A]
0		Isolates with generally unique factor combinations				Very variable	
1	Present	White–pale lilac	None	Brown	Mainly − ve	R–F rarely, spiral	Smooth, rarely spiny
2	Present	Grey	Orange–dark brown	White–grey	Variable	R–F	Smooth, rarely spiny
3	Present	White and/or grey and/or yellow	Yellow–black	Brown–black	+ ve	R–F	Smooth
4	Present	White	Dark brown	Dark brown	− ve	R–F	Smooth
5	Present	White and/or orange and/or pink and/or red	Navy–brown	Red–brown	+ ve	Monoverti-cillium	Smooth
6	Present	Green	Olive–brown–yellow	Olive–yellow–brown	Variable	R–F	Smooth
7	Present	Violet and/or cinnamon or brown	Red–brown–none	Very variable: orange–red–brown–purple	Variable	Spiral rarely	Smooth
8	None	No spores	Variable	Yellow–black–brown	− ve	—	—
9	Present	White and/or grey–brown	Orange–brown	Orange beyond perimeter of growth	+ ve	R–F	Smooth
10	Present	White and pink	Red–red brown	Red	− ve	R–F	Smooth
11	Present	Orange	None–'oil slick'	Yellow–orange	+ ve	Spiral	Spiny
12	Present	White and orange	None	Yellow–brown	− ve	R–F	Smooth

[A] Morphological descriptions are based on those of Arai (1976): R–F, Rectus flexibilis.

The data were checked for constant classification of isolates into one of 12 characteristic groups by a computer program devised previously (Keast *et al.*, unpublished data; program available by negotiation from Prof. T. Speed, Department of Mathematical Statistics, University of Western Australia, Crawley, W.A.)

The statistical package for the Social Sciences (SPSS) Version 8 from the Vogelback Computing Centre, North Western University, Illinois, U.S.A., was used to sort and analyse all data.

Field Data

Soil was sampled by hand in approximately 5-g amounts from the sides of a sample pit. Each sampling depth was measured in centimetres from the surface. Plants were excavated at each site, classified and their roots sampled as described.

The percentage moisture content of soil samples was obtained by oven-drying and the organic matter content by the flash furnace burning technique of an over-dried sample. The pH of root samples was determined using a 1 : 5 (w/v) soil–distilled water mixture in the field. Samples were shaken for 1–3 min before the pH was recorded using pH papers (Merck Spezialindikator, No. 9542 narrow range). The pH was recorded again on return to the laboratory using a Tacussel glass-electrode pH-meter. The mean pH was taken as that of the soil.

Fungi

Isolates of *Phytophthora cinnamomi*, *P. cryptogea* Peth. & Laff., *P. nicotiana* Van Breda de Haan var. *parasitica* (Dastur) Waterhouse, *Pythium proliferum* de Bary and *L. laccata* (Scop. ex Fr.) Berk. & Br. were routinely maintained on potato dextrose agar.

Antifungal Activity Testing

Once actinomycete isolates were established on slopes of ISP medium 2 as pure cultures, they were subcultured onto 9-cm potato dextrose agar plates, four isolates per plate. When colonies were approximately 1 cm in diameter, 5-mm central plugs of agar, containing 7–10-day-old cultures of one of the four fungi under test were placed onto the plates. Five to seven days later, the diameter of any zones of inhibition of fungal growth around the actinomycete isolates was recorded in centimetres.

Isolates were first screened for activity against *Phytophthora cinnamomi* and then retested for activity against the other four fungi. For tabulation, zones of fungal inhibition of less than 3 mm diameter were coded as 0, 4–12 mm were coded as 1, and zones greater than 13 mm were coded as 2.

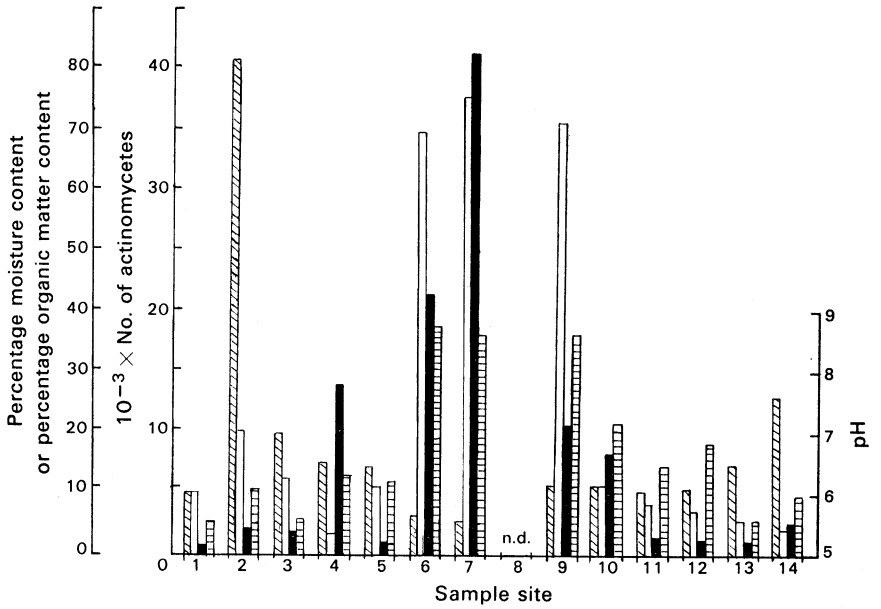

Fig. 1. Histograms of the total numbers of actinomycetes (diagonal hatching) isolated per gram dry weight of soil, and of the organic matter content (no hatching), the moisture content (solid hatching) and the pH (horizontal hatching) of the soil at all sample sites. n.d., not done.

Results

Fig. 1 is a summary of the associations between the total number of actinomycetes recovered per gram dry weight of soil and the soil characteristics—water content,

organic matter content and pH. Although visually there appears to be some general relationships between the total numbers of isolations made and the various soil parameters at each sampling site, no significant direct relationships could be substantiated statistically (Fig. 2). It is, however, clear that there is a large variation in the total number of isolates from the various sampling sites.

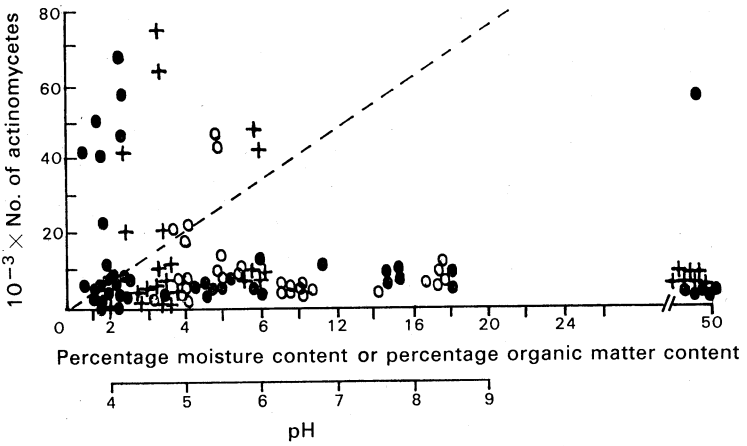

Fig. 2. Relationship between the total number of actinomycetes isolated per gram dry weight of soil content and percentage moisture (●), organic matter content (+) and pH (○) of the soils sampled. – – – Line of best fit of all data.

The occurrence of various groups of actinomycetes over soil depths to 23 cm is summarized in Table 3. Overall groups 0, 1, 2 and 3 were most commonly isolated. The percentage distribution of isolates at each level sampled suggests that, in general,

Table 3. Total numbers of actinomycetes isolated over all samples sites and their percentage group distribution in the soil profile

— No isolates detected

Soil depth (cm)	10^{-3} × total No. in group	Actinomycete-group distribution (%)													
		0	1	2	3	4	5	6	7	8	9	10	11	12	Others[A]
0	41·2	8	43	16	12	2	9	—	7	1	—	0·1	0·5	—	1
2–4	23·8	3	35	40	2	0·4	1	8	6	2	1	0·2	2	—	—
5–8	41·8	16	33	11	12	0·4	0·1	16	15	3	0·4	0·5	1	—	2
9–23	10·6	17	45	16	3	1	12	1	3	1	—	1	0	0	—
Total	1183·6	10	38	19	9	1	5	8	6	2	0·2	0·3	1	0	1

[A] Bacteria and/or fungi.

an increasing number of unclassifiable isolates (group 0) is found in the deeper samples from the soil. Groups 2, 6, 9 and 11 appear to be more associated with the rhizosphere soil, whereas the remaining groups are more evenly distributed through all levels sampled.

Table 4. Antifungal activity directed against *Phytophthora cinnamomi*, *P. cryptogea*, *P. nicotiana*, *Pythium proliferum* and *Laccaria laccata* for all groups of actinomycetes sampled from all sites, grouped according to the depth from which the soil sample was taken

P. cinn., Phytophthora cinnamomi; P. cry., P. cryptogea; P. nic., P. nicotiana; Py. pro., Pythium proliferum; L. lacc., L. laccata

Actinomycete group	Soil depth (cm)	$10^{-3}\times$ total No. of samples	% of total No. of samples	No. showing antifungal activity (as % of total) against: *P. cinn.*	*P. cry.*	*P. nic.*	*Py. pro.*	*L. lacc.*
0	0	3·8	8	65	44	40	19	42
	2–4	0·6	3	92	75	83	58	75
	5–8	6·8	16	24	17	19	7	10
	>9	1·9	17	87	70	78	43	32
1	0	17·9	43	41	26	25	7	25
	2–4	8·2	35	79	56	57	28	41
	5–8	14·3	33	50	33	41	19	27
	>9	4·8	45	77	44	47	29	24
2	0	6·6	16	55	55	56	13	46
	2–4	9·5	40	75	52	52	33	62
	5–8	4·8	11	46	26	36	15	19
	>9	1·7	16	44	24	29	15	15
3	0	4·9	12	60	60	54	32	37
	2–4	0·5	2	44	33	22	11	11
	5–8	5·2	12	41	25	25	20	14
	>9	0·3	3	33	33	33	33	33
4	0	1·0	2	42	42	42	21	26
	2–4	0·1	0·4	0	0	0	0	0
	5–8	0·2	0·4	67	0	33	0	33
	>9	0·1	1	100	100	100	100	100
5	0	3·8	9	58	48	60	8	39
	2–4	0·2	1	75	50	75	25	0
	5–8	0·1	0·1	100	0	0	0	0
	>9	1·3	12	92	73	73	69	27
6	0	0·0	0	0	0	0	0	0
	2–4	2·0	8	23	22	18	13	8
	5–8	6·9	16	20	26	26	16	13
	>9	0·1	1	100	100	100	50	50
7	0	3·0	7	40	40	43	32	52
	2–4	1·5	6	52	35	24	21	24
	5–8	2·0	5	31	30	31	10	13
	>9	0·3	3	83	68	83	67	50
8	0	0·5	1	20	25	10	0	10
	2–4	0·6	2	91	55	64	56	82
	5–8	1·2	3	54	17	17	17	13
	>9	0·1	1	50	50	100	50	50
9	0	0	0	0	0	0	0	0
	2–4	0·2	1	50	33	50	0	25
	5–8	0·2	0·4	67	50	67	33	67
	>9	0·1	1	0	0	0	0	0

Table 4 (*continued*)

Actinomycete group	Soil depth (cm)	$10^{-3}\times$ total No. of samples	% of total No. of samples	No. showing antifungal activity (as % of total) against: *P. cinn.*	*P. cry.*	*P. nic.*	*Py. pro.*	*L. lacc.*
10	0	0·1	0·1	0	0	0	0	0
	2–4	0·1	0·2	0	0	0	0	0
	5–8	0·2	0·5	50	0	24	0	0
	>9	0·1	1	50	0	100	0	50
11	0	0·2	0·5	100	100	75	75	25
	2–4	0·4	2	86	43	43	57	14
	5–8	0·6	1	82	82	64	82	46
	>9	0	0	0	0	0	0	0

The antifungal activity of all groups of actinomycetes isolated from all sample sites was compared *in vitro* for the three *Phytophthora* species, *Pythium proliferum* and *L. laccata* at four depths in the soil (Table 4). Where the numbers of individual group isolates are significant, members of that group may or may not produce anti-*Phytophthora* antibiotic activity (Table 4). The percentage of isolates within any one group exhibiting an ability to produce anti-*Phytophthora* activity does not vary significantly through the depths of soil sampled. Furthermore, there does not appear to be any high degree of species specificity of the antibiotic activity recorded against the three members of the *Phytophthora* genus tested (Table 4). The results are similar when *L. laccata* is the indicator organism for antibiotic activity. However, there is a substantial loss of activity away from the zones of inhibition of diameter 13–30 mm to zones of less than 12 mm diameter. When *Pythium proliferum* is the indicator organism, there is a substantial reduction in the percentage of actinomycetes exhibiting antibiotic activity (Table 4).

Table 5 records the association of the groups of actinomycetes isolated from the soil rhizospheres of plants from 12 plant genera. These can be compared with the pooled data on the distribution of the actinomycetes isolated from litter samples from the sample sites. When the antifungal activities of isolates in the rhizosphere of each plant genus are compared, there is a similar distribution of activity to that shown in Table 4 with one exception (Table 6). The antifungal activity against *Phytophthora cinnamomi* and other members of the genus is significant in all the groups of actinomycetes isolated from the rhizospheres of all plant genera. However, in the case of the rhizosphere of *Pinus radiata*, the number of groups of actinomycetes isolated is greatly reduced in comparison to that expected from litter (Table 6). In addition, antifungal activity against *Pythium proliferum* and the mycorrhizal fungus *L. laccata* is low (Table 6).

The general distribution of antifungal activity of actinomycete isolates against *P. cinnamomi* varies considerably from site to site and is generally related to the dieback disease status of flora at the site (Table 7). Four sites of significant dieback activity yielded 70–83% of the isolates with significant antifungal activity. There are two noticeable exceptions—site 10, a pine plantation, and site 13, the shores of an inner-city lake. Significant antifungal activity is also detected when *Phytophthora cryptogea*, *P. nicotiana*, *Pythium proliferum* and *L. laccata* are the target fungi (Table 7).

When the anti-*P. cinnamomi* activity produced an inhibition zone of greater than 12 mm diameter, the percentage of isolates producing antifungal activity of this intensity is greatly reduced overall. The exceptions are areas of high dieback activity, the pine plantation and the city-lake areas (Table 7).

Table 5. Numbers of actinomycetes in each morphological group isolated from 1 g of rhizosphere soil of each plant genus

Plant genus	10^{-2} × No. of actinomycetes in group:													Total (%)
	0	1	2	3	4	5	6	7	8	9	10	11	12	
Litter[A]	85	360	186	67	12	52	32	52	11	2	1	3	0	863 (71)
Acacia	3	17	2	3	0	1	3	5	3	2	1	2	0	48 (4)
Banksia	1	19	1	1	0	0	1	0	0	0	0	0	0	23 (2)
Dryandra	0	8	1	2	1	0	0	0	0	0	0	0	0	12 (1)
Melaleuca	0	6	2	27	1	0	8	1	9	0	2	0	0	56 (5)
Anthoceris	4	5	4	5	0	0	0	1	2	0	0	2	0	23 (2)
Juncus	0	2	0	1	0	0	13	2	0	0	1	1	0	20 (2)
Lemna	1	0	2	0	0	0	1	1	0	0	0	3	0	8 (0·7)
Templetonia	1	1	2	2	0	1	1	0	0	0	0	0	0	8 (0·7)
Tetragonia	8	2	0	1	0	0	13	2	0	0	0	2	0	28 (2)
Spinifex	6	2	16	1	0	0	19	4	0	0	2	2	0	54 (4)
Arctotheca	1	0	11	0	0	0	0	0	0	0	0	0	0	12 (1)
Pinus	16	32	4	0	0	0	1	2	0	0	0	0	0	55 (5)

[A] Represents the pooled litter samples from all sample sites from which the plants were taken.

Table 6. Antifungal activity of the morphological groups of actinomycetes found in the rhizosphere of *Pinus radiata*

P. cinn., Phytophthora cinnamomi; P. cry., P. cryptogea; P. nic., P. nicotiana; Py. pro., Pythium proliferum; L. lacc., Laccaria laccata

Actinomycete group No.	10^{-2} × Total No.	Percentage expressing antifungal activity to: *P. cinn.*	*P. cry.*	*P. nic.*	*Py. pro.*	*L. lacc.*[A]
0	16·5	91	72	82	44	34
1	31·5	80	33	43	14	7
2	4·0	63	38	50	50	25
6	3·5	17	100	100	0	0
7	1·5	100	100	100	100	33

[A] There was no zone of antifungal activity with a diameter greater than 12 mm in this group.

Discussion

Physical features of soils, such as moisture content, pH and organic matter content, must influence the total numbers of microorganisms present even though they may not relate strictly to any antimicrobial activity within the soils. Soil fungistatic activity, which undergoes seasonal changes (Dutta and Isaac 1979) often appears to be associated with the microbial flora of the soil. As a group, actinomycetes may be influenced by such factors as soil pH and moisture content to a greater degree than other soil microorganisms (Williams *et al.* 1971; Williams and Mayfield 1971) and their distribution, in particular at the rhizosphere, may be even more influenced by

local environmental factors (Bowen and Rovira 1976). In the present study, all sampling sites were combined in an attempt to relate an overall significance, if any, of soil pH, moisture content and organic matter content of soil to the total number of

Table 7. Comparison of the percentage antifungal activity at each sample site of actinomycetes isolated from soil with the dieback status of the area

The percentage of actinomycetes exhibiting the two inhibitory zone sizes of 4–12 mm and >13 mm against *P. cinnamomi* are compared for their activity against the other test fungi. *P. cinn.*, *Phytophthora cinnamomi; P. cry.*, *P. cryptogea; P. nic.*, *P. nicotiana; Py. pro.*, *Pythium proliferum; L. lacc.*, *Laccaria laccata*

Site No.	Dieback status[A]	No. of isolates showing antifungal activity (as % of total) against:				
		P. cinn.	*P. cry.*	*P. nic.*	*Py. pro.*	*L. lacc.*
		Inhibition zone size of 4–12 mm				
1	L	37	60	50	10	60
2	L	48	11	39	15	14
3	L	56	69	82	36	51
4	L	16	100	100	100	0
5	M	70	96	90	88	84
6	L	15	90	90	85	70
7	L	20	43	43	36	7
8	VH	58	60	63	16	59
9	VL	18	75	77	38	30
10	L	61	53	67	18	19
11	M*	64	67	67	43	30
12	M*	60	48	48	13	51
13	L	33	55	54	18	75
14	L	34	62	67	37	44
		Inhibition zone size of >13 mm				
1	L	0	0	0	0	0
2	L	0	0	0	0	0
3	L	4	67	33	67	67
4	L	17	100	100	0	0
5	M	0	0	0	0	0
6	L	6	86	100	57	43
7	L	11	88	75	50	38
8	VH	23	84	80	42	56
9	VL	6	100	94	53	35
10	L	14	74	82	49	33
11	M*	19	89	89	89	56
12	M*	12	44	67	50	39
13	L	53	86	86	68	93
14	L	9	82	78	70	57

[A] VH, forest exhibiting the 'graveyard effect' with peripherally dying forests; M, over- and understorey vegetation dying at the time of sampling; M*, city environs, moderate localized dieback activity; L, evidence of dieback activity in understorey species; VL, evidence of few infected plants

actinomycetes isolated from Western Australian soils (Fig. 1). Some degree of association between the total numbers of actinomycetes isolated and the physical parameters measured exists (Fig. 1), but no statistically significant relationships were

found (Fig. 2). It has been argued that in studies on the actinomycetes found in soil, isolation procedures preferentially detect spores rather than vegetative propagules (Mayfield *et al.* 1972). There was a tendency for more isolations to be made over the drier summer periods of the year, suggesting that the detection of spores was forming the basis of our isolation techniques (Mayfield *et al.* 1972).

Although not all sites were sampled at more than one depth, the combination of results from all sites indicated that the actinomycetes were found in highest numbers in soil depths of less than 8 cm (Table 3). Four groups—0, 1, 2 and 3—were isolated frequently from Western Australian soils with group 0 (made up of isolates that exhibited morphological features not included in the classification scheme) being more frequent at the lower depths of the soil profile. There was also a tendency for isolates from groups 2, 6, 9 and 11 to be located in the rhizospheres of plants (Table 3).

Opinions differ on the significance of the production of antibiotics within the soil and their effects on soil microbial ecology (Brian 1957; Gottlieb 1976; Malajczuk 1982). However, in this study, where a considerable range of plant dieback disease associated with *Phytophthora cinnamomi* was found over the sites sampled, a substantial proportion of actinomycete isolates from all groups exhibited the ability to exert antifungal activity to three members of the *Phytophthora* genus *in vitro* (Table 4). Isolates from all groups at various soil depths exhibited a similar distribution of activity against *Phytophthora* spp. The characteristics of the antifungal activity of the actinomycetes ranged from suspension of mycelial development, distortion and excessive branching with hyphal protuberances, to about 1% of the actinomycetes inducing an apparent cessation of hyphal growth with concomitant sporulation.

There was a substantial reduction in the number of isolates active against the *Phytophthora* spp. that were also active against *Pythium proliferum* and the fungus *L. laccata* (Table 4). There is no evidence for either a soil zonal distribution or a specificity of antifungal activity for these two genera of fungi.

Bowen and Rovira (1976) have discussed in detail factors that may affect microbial colonization of plant roots. As predicted by these workers, the number and distribution of actinomycete groups are greatly restricted in all cases except at the roots of members of the *Acacia* genus. Tippett and Malajczuk (1979) have shown members of *Acacia* to be 'field resistant' to *Phytophthora cinnamomi* and have shown a diverse microbial population at these roots. In all cases except one, the distribution of antifungal activity of isolates was similar to that of the same groups found in the soil. Thus, the presence of the root had not influenced either the total capacity or the distribution of antifungal activity against the test organisms (Table 4). However, notable exceptions were the groups of actinomycetes associated with the rhizosphere of *Pinus radiata* (Table 6). There were only five actinomycete groups detected out of the possible 12 and of the five only two (groups 0 and 1) were present in high numbers. Both of these groups produced very high levels of anti-*Phytophthora* activity *in vitro*. However, this antifungal activity did not appear to cross-react to a high degree to either *Pythium proliferum* or *L. laccata* (Table 6). This was the only indication of any specificity in the antifungal activity recorded *in vitro*.

When the degree of plant dieback in the area of the sampling site could be assessed, antifungal activity *in vitro* of the actinomycetes isolated from that area was high (Table 7). There were two exceptions to this: activity of isolates from the pine plantation and from an inner-city lake. However, the antifungal activity at the city lake was general in nature, being active against all fungi tested.

The actinomycete populations of the Western Australian soils sampled exhibit widespread but non-specific antifungal activity *in vitro*. There were some indications of increased antifungal activity by isolates from areas where plant dieback disease is most active. This may reflect a direct response of the actinomycetes to the presence of *Phytophthora cinnamomi* or it may result from metabolic stress on the actinomycetes as a result of nutritional deficiency. This deficiency could be induced by direct competition with *P. cinnamomi* or as a result of a decrease in root metabolite leakage due to the fungal infection of the flora. Spore formation might result in association with secondary antifungal conservative and chemical pathways (Rose 1979). However, in two plants considered tolerant of *P. cinnamomi* infection, there are two different colonization patterns of their roots. *Acacia* spp. support a large and varied population of actinomycetes, all members of which exert varying degrees of *in vitro* activity to the range of fungi tested, and *Pinus* genus supported a restricted root population of actinomycetes with high antifungal activity. Whether these contribute to the tolerance of these plants to invasion by *P. cinnamomi* or not remains to be established.

Acknowledgements

We thank Mr J. Shannahan and Ms B. Bowra for collecting the original isolates and Mrs C. Woods for typing the manuscript. We thank the Merck Co., Rahway, New Jersey, U.S.A. for making this study possible.

References

Alexander, M. (1977). 'Introduction to Soil Microbiology.' 2nd Edn. p. 423. (Wiley and Sons: New York.)

Arai, T. (1976). 'Actinomycetes: the Boundary Microorganisms.' (University Park Press: Baltimore).

Baker, R. (1968). Mechanisms of biological control of soil-borne pathogens. *Annu. Rev. Phytopath.* **6**, 263–94.

Baker, K. F., and Cook, R. J. (1974*a*). Biological control of soil-borne pathogens. *Annu. Rev. Phytopathol.* **6**, 283–94.

Baker, K. F., and Cook, R. J. (1974*b*). 'Biological Control of Plant Pathogens.' (Freeman: San Francisco.)

Bowen, G. D., and Rovira, A. D. (1976). Microbial colonization of plant roots. *Annu. Rev. Phytopathol.* **14**, 121–44.

Brian, P. W. (1957). The ecological significance of antibiotic production. In 'Microbiological Ecology.' (Eds R. E. O. Williams and C. C. Spencer.) pp. 168–87. (Cambridge University Press: Cambridge.)

Broadbent, P., and Baker, K. F. (1974). Behaviour of *Phytophthora cinnamomi* in soils suppressive and conductive to root rot. *Aust. J. Agric. Res.* **25**, 21–37.

Dutta, B. K., and Isaac, I. (1979). Seasonal variation of fungistasis in some soils. *Trans. Br. Mycol. Soc.* **73**, 157–9.

Garrett, S. D. (1970). 'Pathogenic Root-infecting Fungi.' (Cambridge University Press: Cambridge.)

Gerrettson-Cornell, L., Dowden, H. G. M., Bridges, R. G., and Townsend, S. R. (1979). The incidence of *Phytophthora cinnamomi* and *Pythium* species in thinned eucalyptus. *Plant Dis. Rep.* **63**, 490–3.

Gottlieb, D. (1976). The production and role of antibiotics in soil. *J. Antibiot.* **29**, 987–1000.

Jackson, R. M. (1965). Antibiosis and fungistasis of soil microorganisms. In 'Ecology of Soil Borne Plant Pathogens.' (Eds K. F. Baker and W. C. Snyder.) pp. 363–73. (University of California Press.)

Lingappa, B. T., and Lockwood, J. L. (1961). The nature of the widespread soil fungistasis. *J. Gen. Microbiol.* **26**, 473–85.

Littke, W. R., Bledsoe, C. S., Nadkarni, N. M., and Edmonds, R. L. (1980). Technique for rapid mycorrhizal colonization of container-grown Douglas-fir by *Hebeloma crustuliniforme*. *Soil Biol. Biochem.* **12**, 575–8.

Malajczuk, N. (1979). Biological suppression of *Phytophthora cinnamomi* in eucalypts and avocados in Australia. In 'Soil Borne Plant Pathogens.' (Eds B. Schippers and W. Gams.) pp. 635–52. (Academic Press: London, New York.)

Malajczuk, N. (1982). Microbial antagonism to *Phytophthora*. In 'Biology, Ecology and Pathology of *Phytophthora*.' (Eds D. C. Erwin, P. M. Tsao and S. Bartnicki-Garcia.) Ch. 16. (Phytopathology Society.)

Malajczuk N., and Theodorou, C. (1979). Influence of water potential on growth and cultural characteristics of *Phytophthora cinnamomi*. *Trans. Br. Mycol. Soc.* **72**, 15–18.

Marx, D. H. (1972). Ectomycorrhizae as biological deterrents to pathogenic root infections. *Annu. Rev. Phytopathol.* **10**, 429–54.

Marx, D. H. (1973). Mycorrhizae and feeder root disease. In 'Physiological Ecology.' (Eds G. G. Marks, and T. T. Kozlowski.) Ch. 9. (Academic Press: London.)

Mayfield, C. I., Williams, S. T., Ruddick, S. M., and Hatfield, H. L. (1972). Studies on the ecology of actinomycetes in soil. Observations on the form and growth of streptomycetes in soil. *Soil Biol. Biochem.* **4**, 79–91.

Mehrotra, R. S. (1972). Behaviour of zoospores of *Phytophthora megasperma* var. *sojae* and *P. drechsleri* in soil. *Can. J. Bot.* **50**, 2125–30.

Pavlica, D. A., Hora, T. S., Bradshaw, J. T., Skogerboe, R. K., and Baker, R. (1978). Volatiles from soil influencing activities of soil fungi. *Phytopathology* **68**, 758–65.

Podger, F. D., Doepel, R. F., and Zentmyer, G. A. (1965). Association of *Phytophthora cinnamomi* with a disease of *Eucalyptus marginata* forest in W.A. *Plant Dis. Rep.* **49**, 943–7.

Pratt, B. H. (1973). Isolation of basidiomycetes from Australian eucalypt forest and assessment of their antagonism to *Phytophthora cinnamomi*. Seed and Nursery Trader, November issue, pp. 31–7. (Surrey Beatty & Sons: Melbourne.)

Pratt, B. H., and Heather, W. A. (1973). Recovery of potentially pathogenic *Phytophthora* and *Pythium* spp. from native vegetation in Australia. *Aust. J. Biol. Sci.* **26**, 575–82.

Rose, A. M. (1979). Secondary products of metabolism. In 'Economic Microbiology'. Vol. 3. (Academic Press: London, New York.)

Rose, S. L., Li, C. Y., and Hutchins, A. S. (1980). A streptomycete antagonistic to *Phollinus weirri*, *Fomes annosus* and *Phytophthora cinnamomi*. *Can. J. Microbiol.* **26**, 583–7.

Schenck, N. C. (1981). Can mycorrhizae control root disease? *Plant Dis. Rep.* **65**, 231–4.

Shirling, E. B., and Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.* **16**, 313–40.

Tippett, J., and Malajczuk, N. (1979). Interaction of *Phytothphora cinnamomi* and a resistant host *Acacia pulchella*. *Phytopathology* **69**, 764–76.

Tsao, P. H. (1969). Studies on the saphophytic behaviour of *Phytophthora parasitica* in soil. Proc. 1st Int. Cirtus Symp. (Ed. H. D. Chapman.) Vol. 3. pp. 1221–30. (University of California: Riverside, California.)

Tsao, P. H., and Bickler, J. L. (1963). Growth inhibition of *Phytophthora cinnamomi* by soil diffusates on agar medium. *Phytopathology* **53**, 892.

Weste, G., and Vithanage, K. (1977). Microbial populations in three forest soils. Seasonal variations and changes associated with *Phytophthora cinnamomi*. *Aust. J. Bot.* **25**, 377–83.

Williams, S. T., Davies, F. L., Mayfield, C. I., and Khan, M. R. (1971). Studies on the ecology of actinomycetes in soil. II. The pH requirements of streptomycetes from two acid soils. *Soil Biol. Biochem.* **3**, 187–95.

Williams, S. T., and Mayfield, C. I. (1971). Studies on the ecology of actinomycetes in soil. III. The behaviour of neutrophilic streptomycetes in acid soil. *Soil Biol. Biochem.* **3**, 197–208.

Manuscript received 16 August 1982, accepted 3 February 1983