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Since commencement at C.S.I.R.O., Kelmscott, in December, 1980, the writer has been mainly involved with research into certain aspects of the possible effects on jarrah dieback of replacing the proteaceous understorey (primarily *Banksia grandis*) with an understorey of the leguminous fireweed, *Acacia pulchella*. In addition, a pilot study was undertaken to assess effects of low intensity controlled burning of leaf litter on sporangium production in soil by the dieback pathogen, *Phytophthora cinnamomi*. During March, 1982, preliminary work on a third project was initiated and this is concerned with basic research into the mechanism of sporangium induction in *P. cinnamomi*.

PROJECT NO. 1 : The biological basis for alleviation of jarrah dieback by replacement of the proteaceous understorey with *Acacia pulchella*.

INTRODUCTION

It has been suggested (Shea, S.R. & Malajczuk, 1977) that replacement of the proteaceous understorey (notably *B. grandis*) with *A. pulchella* might protect *Eucalyptus marginata* from infection by *P. cinnamomi* in freely drained, upland sites in the jarrah forest and that such protection might have, in part, a biological basis.

Firstly, it is considered that while *P. cinnamomi* probably cannot persist in soil in freely drained sites, during dry periods, it can survive in previously invaded roots of highly susceptible hosts until conditions again become conducive to emergence of the fungus and rapid production of the infective zoospore stage. Therefore, replacement of susceptible *B. grandis* with *A. pulchella*, in effect, represents a potential reduction in the quantity of *Phytophthora* inoculum available for infection of jarrah. This of course, assumes that the legume does not provide a significant food base for pathogen survival. Secondly, there are indications (Shea, S.R. & Malajczuk, N., 1977) that the soil microbiological environment associated with *A. pulchella* is less favourable to activity by *P. cinnamomi*, compared to that associated with *B. grandis*.

OBJECTIVES

(a) To show conclusively whether *Phytophthora* sporangium production and zoospore release are reduced in soils with *A. pulchella* compared to soils supporting *B. grandis* or the moderately susceptible *E. marginata*.

(b) To compare the relative survivability of *P. cinnamomi* in *Acacia*, *Banksia* and jarrah roots, and in soils supporting (or not) these hosts.

(c) To compare microbial populations found in connection with *Acacia*, *Banksia* and jarrah, with particular attention directed towards organisms which significantly affect different stages of the pathogen life cycle.

RESULTS

(a) *Preliminary studies including development of methods.*

The early work on microbial populations was complicated by the lack of suitable methods for detection or quantification of soil microbes affecting sporangium production by *P. cinnamomi*. However, several workers have indicated that sporangium production is usually accompanied by reduced mycelial growth. Based on this idea, that there might be an inverse correlation between the two processes, it was decided to investigate whether populations of organisms stimulatory to sporangium formation could be detected through their antagonistic effects on mycelial growth. Accordingly, the triple-agar-layer technique, for isolation of antagonistic bacteria and actinomycetes (Herr, L.J., 1969), was adapted for use with *P. cinnamomi*. This involved a series of trials using different agar media. Essentially, the triple layer comprises: (i) a basal layer of agar, providing an even surface for overlying layers and enhancing the clarity of inhibition zones in the uppermost layer; (ii) a second layer including a dilute suspension of test soil; and (iii) a top layer containing *P. cinnamomi* propagules. Where antagonistic colonies form in the middle layer, clear circular zones of inhibition become apparent (after incubation) in an otherwise increasingly opaque lawn of *Phytophthora*. The method has now been perfected and allows a statistical comparison to be made between population densities of antagonists in different soil samples. Evidence has also been obtained to support the hypothesis that sporangial stimulatory microbes can be quantified using this technique.

Fungal populations in different soils are examined using dilution plates and here, attention is mainly directed towards fungi which appear, for example, in soil from *Acacia* or *Banksia*, but not both, or, whole population density is obviously much greater in association with either plant species compared to the other. Organisms meeting these criteria are isolated and tested individually for effects on mycelial growth and sporangium production by *P. cinnamomi*. Also, total populations of fungi and bacteria are compared using dilution plates.

During development of the methods outlined above, antagonistic actinomycetes and fungi were isolated from jarrah forest soils in stands of *B. grandis* and *A. pulchella*. A few bacteria were also obtained, but these lost their antagonistic ability in pure culture, whereas fungi or actinomycetes remained strongly antagonistic after several months in culture.

Results of an experiment in which eleven "mycelial" antagonists were tested against *P. cinnamomi* for effects on sporangium production are given in Table 1. All of the test organisms were strongly inhibitory to mycelial growth of the pathogen on precolonised substrate units suspended in non-sterile soil extract together with antagonist inoculum. Three actinomycetes also inhibited formation of sporangia. The other seven actinomycetes and one fungus appeared to stimulate sporangium formation to a remarkable extent. More recently, the fungus (a species of *Aspergillus*) has been retested against *P. cinnamomi* under sterile conditions and results confirm an effect on sporogenesis, and also on sporangium size. This fungus was recovered from soil in stands of *B. grandis* at a high soil inoculum density, but could not be isolated in connection with *A. pulchella*. In contrast, the actinomycetes were associated with stands of both host species. Further studies on sporangial stimulatory abilities of actinomycetes and fungi will be repeated using organisms isolated in pot trials and field studies mentioned below.

(b) *Pot trials investigating effects of different hosts on soil microbial populations and on sporangium formation and zoospore release by* P. cinnamomi.

Two extensive pot trials have been harvested recently and results are presently being analysed. Currently available data shows conclusively that, under the experimental conditions observed, the total soil population of sporing fungi

TABLE 1. *Effect of actinomycetes and a fungus on production of sporangia by* P. cinnamomi *on substrates suspended in non-sterile soil extracts for 3 or 4 days*

Treatment*	No. of sporangia mm^{-2}	
	3 days	4 days
Control	0·2**	17·6
A9	0·0	0·0
A10	95·6	278·4
A15	130·6	249·4
A20	97·8	201·4
A22	73·4	191·7
A25	98·6	296·7
A26	30·9	231·5
A32	47·8	215·8
A36	0·0	0·0
A41	0·1	1·8
D6	76·3	246·1

* Actinomycetes (A) or a fungus (D) isolated from different soils/sites in the jarrah forest.

**Each value is the mean of four replicates.

associated with *B. grandis* was consistently and significantly (P = 0·001) greater than in soils in which *A. pulchella* was grown. Populations in connection with *E. marginata* were either not significantly (P = 0·05) different from those in soils with *Acacia*, or, in one case, were mid-way between population sizes in treatments with the other two hosts. Soils which were not planted with any host species contained populations similar to those in *Acacia* treatments. The results suggest that *B. grandis* is stimulatory to sporulation of soil fungi, particularly *Penicillium* and *Aspergillus* spp., whereas *A. pulchella* appears to have no effect in this regard. This may be important since many species of *Penicillium* and *Aspergillus*, which inhibit mycelial growth of *P. cinnamomi*, may also stimulate sporangium production.

There was no significant (P = 0·05) difference between the population densities of actinomycetes (antagonistic to *P. cinnamomi*) in different treatments. Total populations of bacteria and actinomycetes varied considerably between replicates within an experiment and between experiments. At this stage, no conclusions can be drawn with respect to the possible importance of these organisms in relation to the intensity of sporulation of *P. cinnamomi* under different host plants.

Qualitative differences in the species composition of antagonistic actinomycetes associated with the three host plants were not detected, but a number of organisms were isolated. Several fungi, occurring disproportionately in connection with a particular host, were also recovered from dilution plates. All isolated species will subsequently be tested for effects on mycelial growth, sporangium production, zoospore release and zoospore germination.

To investigate effects of the three hosts on sporangium production and zoospore release, substrate units colonised by *P. cinnamomi* were buried in pots of soil supporting *Acacia*, *Banksia* or jarrah. After a period of incubation, the units were recovered and preserved for examination. The numbers of released and intact sporangia are presently being estimated. Early indications suggest no effect of host on sporangium production, but a possible inhibitory effect of *Acacia* on zoospore release. However, it should be stressed that the results are incomplete and no conclusions can be drawn before statistical analyses are finalised. It is also intended to analyse the data for interactions between various microbial population densities and the level of *Phytophthora* sporangium formation. This should provide an insight into the relative importance of certain microorganisms in relation to *Phytophthora* sporulation.

(d) *Field trials investigating effects of different hosts on soil microbial populations and on sporangium formation and zoospore release by* P. cinnamomi.

These trials, which will follow the same general pattern as the pot experiments mentioned above, are due to commence in May, 1982, and should be completed in December, 1982.

(e) *Pot trials investigating the relative survivability of* P. cinnamomi *in roots of different hosts and in soil.*

Results from this work will become available between October, 1982 - March, 1983. Here, it is intended to demonstrate whether *A. pulchella* roots are capable of providing a niche which enhances survival of *P. cinnamomi* during periods when soil conditions are not conducive to growth and sporulation of the fungus.

PROJECT NO. 2 : Effect of removing surface leaf litter on sporangial formation by *P. cinnamomi* in jarrah forest soils.

INTRODUCTION

Dr. S.R. Shea of the W.A. State Forest Dept. has suggested the possibility that sporangium production by *P. cinnamomi* may be influenced by the practice of low-intensity burning used to remove the leaf litter fire hazard in parts of the jarrah forest.

OBJECTIVE

To test under controlled conditions whether sporangium production by *P. cinnamomi* is affected by removal of leaf litter through low-intensity burns.

RESULTS

No difference in sporangium production was found in a preliminary experiment where substrates colonised by *P. cinnamomi* were incubated in twenty undisturbed, burnt or non-burnt soil samples in the glasshouse. In this test, burning of leaf litter was effected under artificial conditions and it has been decided to repeat the trial using soil samples from naturally burnt (or non-burnt) areas in the forest. Depending on the outcome, field studies may be initiated and it is envisaged that this would be conducted jointly with the State Forests Dept.

<u>PROJECT NO. 3</u> : Induction of zoosporangium formation in *P. cinnamomi*.

As indicated on Page 1, work on this project commenced very recently and results will not become available until late 1982 onwards.

Based on a preliminary literature survey, some early observations and previous experience of the writer in work on sporogenesis with other fungi, sporangium induction is likely to be triggered by changes in the availability of certain growth factors, or other metabolites, produced by soil-borne microbes. Nutrient depletion in the face of intense microbial competition and damage to mycelium caused by a strongly fluctuating soil moisture/temperature regime may also be important interacting factors.

OBJECTIVES

(a) The major objective is to elucidate the basic mechanism of sporangium induction in *P. cinnamomi*.

(b) To critically compare and improve techniques for assessment of sporangial stimulatory properties of jarrah forest soils, soil leachates and sterile salt solutions.

(c) To identify the environmental conditions conducive or suppressive to sporogenesis and ultimately, to determine the optimum sequence of conditions for sporangium production in the field situation.

COMMENTS

1. Elucidation of the basic mechanism of sporangial induction would be a research finding of major importance. Not only would this promote a better understanding of the epidemiology of jarrah dieback, but much of the information obtained might be relevant to studies of other soil-borne fungal phytopathogens, particularly pythiaceous fungi.
2. It is anticipated that Project No. 1 should be completed during 1983 and that this work should yield at least three publications.

REFERENCES

HERR, L.J. (1969). A method of assaying soils for numbers of actinomycetes antagonistic to fungal pathogens. *Phytopathology* 49, 270-273.

SHEA, S.R. (1977). Potential for control of eucalypt dieback in Western Australia. *Forests Department of Western Australia*, Reprint No. 3.

NOTE