ISSN 0156-0972

AUSTRALASIAN PLANT PATHOLOGY

VOL. 11 NUMBER 1 MARCH 1982

RESEARCH NOTES

Stimulation of Oospore Production in *Phytophthora Cinnamomi* by *Trichoderma* Species Isolated from Eucalypt Roots

G.C. Johnson
CSIRO Division of Chemical Technology
Highett, Vic. 3190
and
W.A. Heather,
Department of Forestry,
The Australian National University,
Canberra, A.C.T. 2600

Volatile products from cultures of certain *Trichoderma* species and from *Gliocladium roseum* Bainier can stimulate the A2 mating strain of *Phytophthora cinnamomi* Rands to produce oospores (2,4). Such induction could have considerable survival value to a root pathogen which has short-lived mycelium and which depends on oospores and chlamydospores to survive in the soil (3). At certain times and places the A1 mating type may be comparatively rare (1,8,9), and the likelihood of interaction between an A2 strain and a stimulatory fungus might be greater than that between an A1 and an A2 strain (3). The oospores of *P. cinnamomi* cannot be germinated regularly and reliably. Hence the significance of these structures, whether normally or *Trichoderma* induced, in increasing variability of the organism is unknown.

In view of the possible ecological significance of this '*Trichoderma* effect', certain Fungi Imperfecti recovered from eucalypt roots were examined for their ability to stimulate oospore formation. These fungi were isolated from the washed (7) roots of *Eucalyptus viminalis* Labill. and *E. fastigata* Deane et Maiden. These species, shown previously in greenhouse trials to be, respectively, resistant and susceptible to *Phytophthora cinnamomi* (W.A. Heather, pers. comm.), had been grown in their respective forest soils in a greenhouse for up to 47 weeks. Representatives of all identified *Trichoderma* species, plus an isolate similar to *T. aureoviride* Rifai, were tested. An isolate of *Clonostachys* sp. was included because of its taxonomic similarity to *Gliocladium roseum* (5). The *Oidiodendron* species and *Penicillium frequentans* Westling, commonly isolated from eucalypt roots, were tested also. Brasier's method (1), utilizing culture-derived volatile compounds as stimulants, was used to test the reaction of an A2 isolate of *P. cinnomomi* (ANU Department of Forestry Culture PC 176) to the root fungi.

Table 1. Fungi isolated from eucalypt roots and tested for stimulation of oospore formation in an A2 culture of *P. cinnamomi.*

Fungi tested	Oospore formation
Clonostachys sp.	No
Oidiodendron tenuissimum (Peck) Hughes	No
O. truncatum Barron	No
Penicillium frequentans Westling	No
Trichoderma hamatum (Bon.) Bain. (3 isolates)	No
T. koningii Oudemans	Yes
T. longibrachiatum Rifai	No
Trichoderma sp. (? *aureoviride* Rifai)	No
T. viride Pers. ex S.F. Gray (2 isolates)	Yes

In this study, only *Trichoderma koningii* Oudemans and *T. viride* Pers. ex S.F. Gray induced oospore formation (Table1). Although *T. koningii* and one of the *T. viride* isolates each stimulated *P. cinnamomi* to produce a few oospores a second *T. viride* culture stimulated the production of many oospores. Although some fully developed

Table 2. Occurrence of each *Trichoderma* species as a percentage of total *Trichoderma* isolates on roots of *Pinus* (6) and *Eucalyptus.*

	Source of isolates	
Species isolated	*Pinus*[1]	*Eucalyptus*[2]
T. hamatum	1	70
T. harzianum	9	0
T. koningii[3]	1	7
T. longibrachiatum	0	5
T. piluliferum Rifai & Webster[3]	30	0
T. polysporum (Link ex Pers.) Rifai[3]	1	0
Trichoderma sp. (? *aureoviride)*	0	13
T. viride[3]	58	5
Total species likely to demonstrate the '*Trichoderma* effect'	90	12

[1] Data from Gibbs (1976); acidic soil study.
[2] Data from present study; figures represent total for both eucalypts.
[3] Species able to stimulate oospore production.

Registered by Australia Post — Publication No. VBG256

oospores were observed many appeared to have an incomplete wall.

Table 3. Number of isolates of *Trichoderma* species recovered from live and dead eucalypt roots.

Trichoderma species	E. fastigata		E. viminalis	
	live root	dead root	live root	dead root
T. koningii	0	0	1	3
T. viride	2	0	1	0

Brasier (3), in searching for information on the distribution of *Trichoderma* species on roots, cited a study of the relative frequency of each species to the total *Trichoderma* population of *Pinus* roots (6). For comparison, these results were included in Table 2 along with equivalent data collected in the present study from eucalypt roots.

Whereas 90% of the *Trichoderma* isolates from *Pinus* roots were species likely to elicit the '*Trichoderma* effect', only 12% of those from eucalypt roots were likely to be stimulatory. Although comparative information is not available for the pine, some 3200 washed and plated segments (2 mm long) of eucalypt root yielded only seven isolates of species known to cause the '*Trichoderma* effect' *in vitro.* In the study some 3400 fungal isolates were collected from 130 plants over a 330-day period. The source of the *Trichoderma* isolates is shown in Table 3.

This study was restricted to certain fungi isolated from the roots of only two eucalypt species. Furthermore, the eucalypts were grown under greenhouse, not field, conditions and the ability of root isolates to elicit the '*Trichoderma* effect' was assayed *in vitro.* Within the limits of this study, it would appear that the roots of both a *P. cinnamomi* resistant eucalypt and a *P. cinnamomi*-susceptible eucalypt harbour few fungi known to stimulate oospore production in *P. cinnamomi.*

REFERENCES

(1) Arentz, F., Pratt, B.H. and Heather, W.A.(1972) - Occurrence of the A1 mating type of *Phytophthora cinnamomi* Rands on the South Coast of New South Wales. *Aust. Plant Pathol. Soc. Newsletter* **1** (4): 27.

(2) Brasier, C.M. (1975)- Stimulation of sex organ formation in *Phytophthora* by antangonistic species of *Trichoderma.* 1. The effect *in vitro. New Phytol.* **74**: 183-94.

(3) Brasier, C.M. (1975)- Stimulation of sex organ formation in *Phytophthora* by antagonistic species of *Trichoderma.* 11. Ecological implications. *New Phytol.* **74**: 195-8.

(4) Chang, S.T., Shepherd, C.J. and Pratt, B.H. (1974)- Control of sexuality in *Phytophthora cinnamomi. Aust. J. Bot.* **22**: 669-79.

(5) Gams, W. (1971)- '*Cephalosporium* - artige Schimmelpilze'. (G. Fisher: Stuttgart.) p. 262.

(6) Gibbs, J.N. (1967)- A study of the epiphytic growth habit of *Fomes annosus. Ann. Bot. N.S.* **31**: 755-74.

(7) Harley, J.L. and Waid, J.S. (1955)- A method of studying active mycelia on living roots and other surfaces in the soil. *Trans. Br. Mycol. Soc.* **38** (2): 104-18.

(8) Pratt, B.H., Heather, W.A. and Shepherd, C.J. (1972)- Transcontinental occurrence of A1 and A2 strains of *Phytophthora cinnamomi* in Australia. Aust. J. Biol. Sci. **25**: 1099-1100.

(9) Shepherd, C.J., Pratt, B.H. and Taylor, P.A. (1974)- Comparative morphology and behaviour of A1 and A2 isolates of *Phytophthora cinnamomi. Aust. J. Bot.* **22**: 461-70.

Observations of the Infection Process of *Phytophthora cinnamomi* on *Eucalyptus sieberi.*

I. W. Smith and G. C. Marks
Forests Commission of Victoria
Box 4018 GPO Melbourne
3001

Until recently *Phytophthora cinnamomi* has been considered to be only a root rot organism of the fine roots of *Eucalyptus* spp. (8). Reports of stem lesion development in *E. regnans, E. obliqua* and *E. sieberi* (3,4,5) and in *E. marginata* (6) however indicate that stem infections may be much more frequent than suspected and that, as is the case with many other genera of plants, sudden death of eucalypts may be due to collar girdling rather than only rotting of the fine roots. This report details some observations regarding stem lesion development in artificially inoculated *E. sieberi* seedlings.

Eucalyptus sieberi seedlings were grown in 20 cm pots containing a soil conductive to *P. cinnamomi* root rot. At 6 months of age the pots were inoculated with *P. cinnamomi* according to the method of Marks and Kassaby (1). After approximately 30 days 60% of the seedlings died and in all cases death was associated with a dark brown lesion at the collar. Hand sections taken from the collar revealed that tyloses had formed in the xylem vessels similar to those reported by Marks and Tippett (2) for seedling roots. *P. cinnamomi* was isolated from the main roots and collar.

In the other 40% of seedlings, collar infection did not immediately result in wilting and seedling death. The fungus was channelled into the stem along only one root and instead of girdling, the collar produced a continuous lesion up the stem and into the veins of the leaves (Fig. 1) from which *P. cinnamomi* was isolated. The leaves wilted progressively, commencing at the bottom, as each was cut off from the stem by the lesion. Hand sections taken from the stem revealed mycelium in the vessels (Fig. 2) which upon plating on a selective medium (7) produced pure cultures of *P. cinnamomi* (Fig 3). The fungus could not be isolated from sections taken from healthy tissue above the lesion, however tyloses were seen in the vessels in this region. After 60 days, infection had resulted in the death of the remaining seedlings.

The reason for the differential reaction to infection within this species, may be due to variation in both the physiological resistance due to genetic factors, and to the number of infected major roots channelling the fungus into the collar. A similar pattern of disease development, after initial infection, may also occur in larger seedlings under field conditions. Disease progression in this situation however may also depend on factors such as soil temperature, drought stress within the tree and the amount and activity of soil antagonistic microflora influencing fungal activity with the root. Future studies on *P. cinnamomi* disease in eucalypt species should integrate these aspects of the problem that up to now have been examined separately.

ACKNOWLEDGEMENTS

We gratefully acknowledge the technical assistance of Mr P Clements.