## OOSPORE PRODUCTION IN PHYTOPHTHORA CINNAMOMI IN THE PRESENCE OF TRICHODERMA KONINGII\*

## By B. H. PRATT,<sup>†</sup> J. H. SEDGLEY,<sup>†</sup> W. A. HEATHER,<sup>†</sup> and C. J. SHEPHERD<sup>‡</sup>

The soil-borne fungus *Phytophthora cinnamomi* Rands is a particularly important pathogen in Australia because of its consistent association with root-rot disease of a wide variety of exotic and native plant species. It was thought originally to have been introduced from south-east Asia (Crandall and Gravatt 1967), but evidence recently obtained (Pratt, Heather, and Shepherd, unpublished data), suggests that it may be indigenous to eastern Australia and may have been partly instrumental in determining the distribution of certain susceptible species, particularly *Eucalyptus* spp.

The fungus occupies a wide range of climatic, geographic, and topographic sites and vegetational areas. Because of the possibility of continued adaptation by the fungus to new man-made habitats and the possibility of concomitant development of new pathogenic types, there has been considerable interest in the mechanism underlying variability and adaptation.

The fungus is claimed to be heterothallic with compatible mating strains  $A^1$ and  $A^2$  (Gallegly 1970). Both types occur in the United States of America, but until recently only type  $A^2$  was known to exist in Australia. Recently the authors (unpublished data) demonstrated oospore production in matings between isolates from different areas of Australia. Isolates compatible with Australian  $A^2$  isolates were compatible also with an  $A^2$  but not an  $A^1$  isolate from America, and were allocated to the  $A^1$  mating type. The identification of these latter isolates as  $A^1$  mating strains of *P. cinnamomi* was subsequently confirmed by the Commonwealth Mycological Institute, Kew, and will be reported separately.

Brasier (1971) demonstrated the ability of gaseous products of *Trichoderma* viride Pers. ex Fr. to stimulate American and British isolates of the  $A^2$  but not the  $A^1$  mating type of *P. cinnamomi* to form oospores in a seemingly homothallic manner within several days of bringing the two cultures together.

We have examined oospore production in cultures exposed to the volatile products of *Trichoderma* spp. in the following manner. Isolates of *P. cinnamomi* collected from different areas of Australia were grown in plastic Petri dishes on V-8 agar (20 g Oxoid agar, 20 g Campbell's V-8 juice, 1000 ml glass-distilled water) until the colonies were approximately 4 cm in diameter. *Trichoderma* spp. isolated from soils in different areas were grown on malt agar until about 6 cm in diameter. The lids

<sup>\*</sup> Manuscript received 29 May 1972.

<sup>†</sup> Department of Forestry, Australian National University, P.O. Box 4, Canberra, A.C.T. 2600.

<sup>&</sup>lt;sup>‡</sup> Division of Plant Industry, CSIRO, P.O. Box 1600, Canberra City, A.C.T. 2601.

SHORT COMMUNICATIONS

of the plastic dishes were discarded and the lower halves containing the developing cultures were brought together (i.e. "paired") and sealed with adhesive tape so that there was gaseous exchange but no immediate physical contact between the two cultures.

"Pairings" were made between P. cinnamomi and P. cinnamomi and between P. cinnamomi and Trichoderma spp. The plates were incubated in darkness at 23°C and examined for oospore production daily for 5 days.

Source of P. cinnamomi isolate	No. of P. cinnamomi isolates	Trichoderma spp.*							
		a	b	С	d	е	f	g	h
New South Wales	$10 A^2$	0	8	5	10	0	0	0	0
	$2 A^1$	0	0	0	0	0	0	0	0
Tasmania	$6 A^2$	0	<b>5</b>	4	6	0	0	0	0
Queensland	$5 A^2$	0	4	<b>4</b>	<b>5</b>	0	0	0	0
	1 A <sup>1</sup>	0	0	0	0	0	0	0	0
Western Australia	$5 A^2$	0	4	4	5	0	0	0	0
	$2 A^1$	0	0	0	0	0	0	0	0
South Australia	$3 A^2$	0	3	3	3	0	0	0	0
Victoria	$3 A^2$	0	3	3	3	0	0	0	0
Australian Capital Territory	$2 \ A^2$	0	<b>2</b>	<b>2</b>	<b>2</b>	0	0	0	0
United States of America	$1 A^2$	0	1	1	1	0	0	0	0
	1 A <sup>1</sup>	0	0	0	0	0	0	0	0
Total	$35 \ \mathrm{A^2}$	0	30	26	35	0	0	0	0
	$6 A^1$	0	0	0	0	0	0	0	0

TABLE 1

oospore production in *Phytophthora Cinnamomi* isolates "paired" with isolates of TRICHODERMA spp. for 5 days at  $23^{\circ}C$ 

\* a = T. koningii Oud agg., Western Australia;

b = T. koningii Oud agg., South Australia;

c = T. koningii Oud agg., New South Wales;

d = T. koningii Oud agg., New South Wales;

e = T. hamatum (Bonord.) Bain. agg., United States of America;

f = T. hamatum (Bonord.) Bain. agg., South Australia;

g = T. harzianum Rifai agg., South Australia;

h = T. viride (Pers.) Gray, Australian Capital Territory.

a, b, f, and g from Dr. J. Warcup, University of Adelaide, South Australia; c and h from Mr. R. Rickards and Mr. G. Chilvers, Australian National University, Canberra.

Oospores of *P. cinnamomi*, complete with amphigynous antheridia, were formed within 2–5 days, in some but not all of the *P. cinnamomi–Trichoderma* spp. "pairings" (Table 1). Oospores were not formed in the *P. cinnamomi–P. cinnamomi* "pairings".

Oospores were produced only in the presence of T. koningii, and only in isolates of the  $A^2$  strain. The pattern of oospore development was similar to that ascribed to gaseous stimulation of P. cinnamomi by T. viride, as described by Brasier (1971). Isolates of T. koningii differed, however, in their stimulatory ability, varying from non-stimulation by a Western Australian isolate to stimulation of all  $A^2$  isolates tested by a New South Wales isolate. In addition to its broader range, isolated

862

(Table 1) stimulated production of a higher concentration of oospores per unit area than did other stimulatory isolates.

T. koningii appears to be common in Australian soils and has been found by the authors in sites in eastern Australia where P. cinnamomi seems indigenous. If T. koningii stimulates oospore production in the field as it does in culture, it may contribute to the survival of P. cinnamomi by inducing production of what is presumed to be a resistant spore by a pathway alternative to that of  $A^1$  and  $A^2$  mating. The significance of this apparently homothallic type of oospore production on the genetic variability of P. cinnamomi cannot be assessed until the mechanism underlying the process has been determined.

This study illustrates the complex interrelationships between soil fungi and the necessity for understanding these in relation to disease. *P. cinnamomi* is associated with serious diseases which have not responded to chemical or other more routine control measures. The possibility of achieving control of the diseases by manipulation of the soil environment to suppress organisms stimulatory to the fungus and promote populations of antagonists will require a detailed knowledge of the interactions between *P. cinnamomi* and other members of the soil microflora.

## Acknowledgments

We are indebted to our colleagues Dr. J. Warcup, Mr. R. Rickards, and Mr. G. Chilvers for supply of *Trichoderma* spp., to Professor G. A. Zentmyer for supply of  $A^1$  and  $A^2$  isolates of *P. cinnamomi*, and to the Commonwealth Mycological Institute for identification of *Trichoderma* spp. isolates and  $A^1$  mating types of *P. cinnamomi*.

This work was carried out while Dr. Pratt was a recipient of grants from the Forests Department of Western Australia, the Forestry Commission of Tasmania, the Forestry Commission of New South Wales, the Forests Commission of Victoria, the Department of Forestry, Queensland, the Woods and Forests Department, South Australia, the Associated Pulp and Paper Mills Ltd., the Australian Newsprint Mills Ltd., and A.P.M. Forests Pty. Ltd. Dr. Pratt and Dr. Heather were joint holders of a grant from the Australian Research Grants Committee.

## References

BRASIER, C. M. (1971).—Induction of sexual reproduction in single A<sup>2</sup> isolates of *Phytophthora* species by *Trichoderma viride*. Nature New Biology 251, 283.

CRANDALL, B. S., and GRAVATT, G. F. (1967).—The distribution of *Phytophthora cinnamomi*. Ceiba 13, 43-55, 57-8.

GALLEGLY, M. E. (1970).-Genetics of Phytophthora. Phytopathology 60, 1135-41.

