Heterothallic *Phytophthora*: Evidence for Hormonal Regulation of Sexual Reproduction

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Both A¹ and A² mating types of *Phytophthora cinnamomi*, *Phytophthora parasitica* and *Phytophthora palmivora* formed oospores by selfing when they were paired with different mating types on opposite sides of polycarbonate membranes. The selfing of one mating type in the presence of the other mating type demonstrates the production of diffusible substances like plant hormones as found in related fungi. Young cultures and A² isolates were better hormone producers, whereas old cultures and A¹ isolates were more responsive to hormones in both intra- and interspecific pairings. The polycarbonate membrane method should facilitate identification and genetic studies of heterothallic species of *Phytophthora*.

INTRODUCTION

Heterothallic species of *Phytophthora* readily produce oospores when two compatible types of the same species or of different species are paired in cultures (Smoot *et al.*, 1958; Savage *et al.*, 1968; Haasis & Nelson, 1963). Two early reports (Galloway, 1936; Kouyeas, 1953) provided data supporting the mechanism of chemical stimulation of oospore formation originally proposed by Ashby (1929). However, subsequent efforts to confirm these results using similar and different techniques have not been successful (Stamps, 1953; Apple, 1959; Haasis & Nelson, 1963; Marx *et al.*, 1965; Brasier, 1972; Chang, Shepherd & Pratt, 1974). Indirect evidence suggesting the involvement of stimulatory substances in sexual reproduction of heterothallic species of *Phytophthora* includes: (i) induction of oospore formation in A² isolates of *Phytophthora* species by *Trichoderma viride* (Brasier, 1971) and *T. koningii* (Pratt *et al.*, 1972); (ii) stimulation of sexual reproduction of *Phytophthora parasitica* by a soil bacterium (Mukerjee & Roy, 1962); and (iii) stimulation of oospore formation in an A² isolate of *Phytophthora cinnamomi* by an extract of avocado roots (Zentmyer, 1952; Ho & Zentmyer, 1977). I report here direct evidence for hormonal regulation of oospore formation by compatible isolates of three heterothallic species of *Phytophthora*.

METHODS

Organisms. Species of *Phytophthora* used were *P. cinnamomi* (UCR97, A¹ and 64F, A²), *P. parasitica* (p991, A¹ and p731, A²), and *P. palmivora* (p611, A¹ and p255, A²). All isolates were supplied by G. A. Zentmyer with the exception of 64F which was isolated from a macadamia root in Hawaii. The opposite mating types of heterothallic *Pythium splendens* (no. 117 and no. 461) were obtained from M. Aragaki.

Mating. Both A¹ and A² isolates of *P. cinnamomi*, *P. parasitica* and *P. palmivora* were grown on V-8 agarose medium for 6 d at 24 °C. This medium consisted of distilled water, 10 % (v/v) V-8 juice, 0.02 % (w/v) CaCO₃ and 0.8 % (w/v) agarose (SeaKem HGT-P Agarose; Maine Colloids, Rockland, Maine, U.S.A.) which is used as a solidifying agent. A piece of A² mating type culture (15 × 10 × 3 mm) in the centre of a Petri plate (100 × 15 mm) was covered with a polycarbonate membrane (CPR, 0.2 µm, 90 mm diam.; Nuclepore Corporation, Pleasanton, California, U.S.A.) and paired with an A¹ isolate of the same size on the opposite side

Table 1. *Induction of oospore formation by diffusible sex hormones originating in opposite mating type among isolates of P. cinnamomi (Pci), P. parasitica (Par) and P. palmivora (Pal)*

Combination*		Oospores (no. cm ⁻²)	
A ¹ (Top)	× A ² (Bottom)	A ¹	A ²
Pci	Pci	5	0
Par	Pci	6122	0
Pal	Pci	18	0
Pci	Par	216	1633
Par	Par	16837	469
Pal	Par	21224	830
Pci	Pal	0	27
Par	Pal	3265	61
Pal	Pal	563	28

* Paired isolates were separated by interposed polycarbonate membranes. The isolates used were UCR97 (Pci A¹), 64F (Pci A²), P991 (Par A¹), P731 (Par A²), P611 (Pal A¹) and P255 (Pal A²).

Table 2. *Effect of age on production of sex hormones by paired isolates of P. cinnamomi (Pci), P. parasitica (Par) and P. palmivora (Pal)*

Age combination*		Oospores (no. cm ⁻²)	
A ¹ (Top)	× A ² (Bottom)	A ¹	A ²
Pci (y)	Pci (o)	0	8
Pci (o)	Pci (y)	41	0
Par (y)	Par (o)	1372	1083
Par (o)	Par (y)	80102	0
Pal (y)	Pal (o)	181	9
Pal (o)	Pal (y)	4592	0
Pci (y)	Par (o)	361	561
Pci (o)	Par (y)	2041	0
Par (y)	Pci (o)	7	22
Par (o)	Pci (y)	20918	0

* Paired isolates were separated by interposed polycarbonate membranes. o, Six-d-old culture; y, newly inoculated culture.

of the membrane. After incubation in a moist chamber for 7 d at 24 °C in darkness, the number of oospores produced by each mating type was counted under a microscope. The mycelia of the test fungi did not reach the edge of the membranes, nor did they penetrate the membranes during the incubation period. When only the top or bottom layer was inoculated with a single isolate of the fungi, the opposite layer remained sterile after 7 d incubation. Sterility was tested by incubating the non-inoculated layers on nutrient agar for 7 d at 24 °C.

RESULTS AND DISCUSSION

All isolates except the A² type of *P. cinnamomi* formed oospores by selfing when they were paired, but separated by membranes, with the opposite mating type of the same or different species (Table 1). All the matings resulted in oospore formation predominantly by one mating type. Similar results were obtained when pairings were made with A² isolates on top and A¹ underneath. No oospores were formed when A¹ and A² isolates of *P. parasitica* were similarly paired with the same mating types of *P. parasitica*, *P. cinnamomi* or *P. palmivora*. In other experiments, A¹ and A² isolates of *P. parasitica* were paired with autoclaved cultures of opposite mating types of the same species or with live cultures of heterothallic *Pythium splendens*, but no oospores were observed.

The membrane matings demonstrate the control of sexual reproduction by substances originating in the opposite mating type and reaching the site of activity by diffusion through the membrane. The sex hormone produced by A¹ isolates of *Phytophthora* (designated hormone α^1 to differentiate it from hormone A¹ of *Achlya*) can induce sexual reproduction of A² isolates but not A¹ isolates. On the other hand, the sexual reproduction of A¹ isolates can only be regulated by hormone α^2 produced by A² isolates. The substances produced by *Trichoderma* (Brasier, 1971; Pratt *et al.*, 1972) and those present in avocado roots (Ho & Zentmyer, 1977) stimulated oospore formation by A² but not A¹ isolates of *Phytophthora*; they were therefore similar to hormone α^1 in action. These results strongly support Ashby's suggestion (1929) of the chemical nature of heterothallism among species of *Phytophthora*.

When 6-d-old and newly inoculated cultures were paired as described above, it was found that, in general, oospores were produced predominantly by old cultures in both intra- and interspecific pairings (Table 2). A¹ isolates also produced more oospores than A² isolates in paired cultures. The results indicated that young cultures and A² isolates were better hormone producers, whereas old cultures and A¹ isolates were more responsive to hormones.

The demonstration of the regulation of sexual reproduction by hormones in heterothallic species of *Phytophthora* is mainly due to the use of a polycarbonate membrane instead of cellophane which does not prevent penetration by species of *Phytophthora* (Brasier, 1972; W. H. Ko, unpublished data). The use of agarose instead of agar also led to the production of increased numbers of oospores. A number of chemicals are inactivated in agar by binding (Ko, Kliejunas & Shimooka, 1976), and inactivation of chemicals can be minimized or prevented by the use of SeaKem agarose (unpublished data). This method facilitates oospore production by single isolates of heterothallic species of *Phytophthora* which is important in the identification and genetic studies of this group of fungi.

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