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CHEMICAL REGULATION OF SEXUAL REPRODUCTION IN *PHYTOPHTHORA COLOCASIAE*[1,2]

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Ability of heterothallic *Phytophthora* to produce oospores when paired with different species of the same genus inspired the speculation that sexual reproduction of these fungi may be due to chemical stimulation (Ashby, 1929). Many efforts have since been made to obtain evidence in support of this hypothesis. Although stimulation of oospore formation by filtrates, passed through Chamberland filter, of a paired culture (Galloway, 1936) and a single culture of opposite mating type has been reported earlier, other researchers were not able to confirm these reports using similar or different methods (Apple, 1959; Brasier, 1972; Chang, *et al.*, 1974; Haasis and Nelson, 1963; Marx, *et al.*, 1965; and Stamps, 1953). Recently, evidence for hormonal regulation of sexual reporduction in *Phytophthora parasitica*, *P. palmivora* and *P. cinnamomi* was provided by Ko (1978) who used polycarbonate membrane to separate paired cultures of the same or different species and induced oospore formation of both mating types. In our study of sexual reproduction in *P. colocasiae* the polycarbonate membrane technique was used to determine if similar phenomenon exists in this fungus which is also heterothallic (Ko, 1979; Savage *et al.*, 1968).

Species of *Phytophthora* uses were *P. colocasiae* Raciborski (#17, A^1 and #1, A^2), *P. parasitica* Dastur (P 991, A^1 and #27, A^2), *P. palmivora* (Butler) Butler (P 611, A^1 and P 255, A^2) and *P. cinnamomi* Rands (UCR 97, A^1 and 64 F, A^2). Isolates P 991, P 611, P 255 and UCR 97 were supplied by G. A. Zentmyer, and isolates #17 and 64 F were supplied by W. H. Ko. Isolates #1 and #27 were isolated in Taiwan by H. S. Chang and P. J. Ann, respectively.

Both A^1 and A^2 types of *P. colocasiae*, *P. parasitica*, *P. palmivora* and *P. cinnamomi* were grown for 7 days at 25°C on V-8 agar (10% V-8 juice, 0.02% $CaCO_3$ and 1.8% agar). A piece of A^2 culture (20 × 10 × 3mm) placed in the

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center of a petri plate was covered with a 90 mm Nuclepore membrane (0.2μm) and paired with an A^1 culture of the same size on the opposite side of the membrane. The combinations included pairing of *P. colocasiae* A^1 or A^2 with compatible type of the same or different species. Pairings with A^2 isolates on top and A^1 isolates underneath were also made. The number of oospores produced by each mating type was determined under a Lestz microscope at ×125 magnification after 7-day incubation at 20°C in a moist chamber in darkness. When pieces of sterile V-8 agar were paired with single isolates of the test fungi either on top or on bottom, they remained sterile after 7-day incubation. This indicates that no penetration of membranes by test fungi occurs during the incubation period.

With A^1 on top of the membranes, A^1 isolate of *P. colocasiae* did not produce oospores, but it induced oospore formation of A^2 isolates of all four species tested (Table 1). The number of oospores produced by A^2 isolates ranged from 1 per cm^2 by *P. palmivora* to 319 per cm^2 by *P. parasitica*. Very few oospores were produced by A^2 isolate of *P. colocasiae*. The number of oospores produced ranged from 0 when paired with *P. parasitica* A^1 to 7 per cm^2 when paired with *P. palmivora*. However, *P. colocasiae* A^2 appeared to be a good hormone producer. More than 1,000 oospores per cm^2 were produced by A^1 isolates of *P. parasitica* and *P. palmivora* in response to hormone(s) produced by *P. colocasiae* A^2. Similar results were obtained when the mating position was reversed.

Table 1. *Induction of oospore formation between compatible isolates of* ***Phytophthora colocasiae, Phytophthora parasitica, Phytophthora palmivora and Phytophthora cinnamomi*** *by pairing on opposite sides of polycarbonate membranes*

Pairing combination		Oospores (No./cm^2)			
		Treatment I		Treatment II	
A^1	A^2	A^1 (Top)	A^2 (Bottom)	A_1 (Bottom)	A^2 (Top)
P. colocasiae	*P. colocasiae*	0	15	0	1
P. colocasiae	*P. parasitica*	0	319	0	81
P. colocasiae	*P. palmivora*	0	1	0	2
P. colocasiae	*P. cinnamomi*	0	48	0	70
P. parasitica	*P. colocasiae*	1,482	0	615	9
P. palmivora	*P. colocasiae*	1,049	7	607	10
P. cinnamomi	*P. colocasiae*	52	3	241	6

Using the polycarbonate membrane method we were able to demonstrate chemical regulation of sexual reproduction in *P. colocasiae* with both intra-

specific and interspecific pairings between compatible types. Our results showed that both A^1 and A^2 isolates of *P. colocasiae* were capable of producing substance(s) which are to initiate the formation of sexual organs of the isolates of *P. parasitica, P. palmivora* and *P. cinnamomi* tested, but were relatively insensitive in response to hormone(s) produced by opposite mating types.

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芋疫病菌分泌物調節疫病菌之有性生殖

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利用柯氏夾膜 (polycarbonate membrane) 配對法，顯示 *Phytophthora colocasiae* 之 A^1 菌株隔了一層膜與所試之 *P. colocasiae, P. cinnamomi, P. palmivora* 及 *P. parasitica* 之 A^2 菌株配對，一星期後，能使 A^2 菌株分別形成1～319個/cm^2 不等之卵孢子，但其本身不形成有性器官，利用同樣方法，*P. colocasiae* 之 A^2菌株能使 *P. cinnamomi P. palmivora* 及 *P. parasitica* 之 A^1 菌株分別形成 52～1482個/cm^2 不等之卵孢子，其本身最多只形成 10個/cm^2 之卵孢子，由此而知 *P. colocasiae* 能產生某些未經鑑定的化學物質，引致上述幾種所試疫病菌之相對配對型菌株形成卵孢子，但本身對此類物質之作用反應却很遲鈍。