Factors Affecting the Induction of Sexual Reproduction in *Phytophthora parasitica* by *P. colocasiae*

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When *Phytophthora colocasiae* (A²) was used as a hormone producer and *P. parasitica* (A¹) as a hormone receptor, no sex organs of *P. parasitica* were observed in matings lasting for 7 h, but the amount of hormone produced was sufficient to stimulate the production of 341 oospores cm⁻² 6 d later. The maximum induction of sex organs was reached in matings lasting 48 h. Hormone production was inhibited by light, but the effect of light on oospore development was small. Temperatures of 10 and 15 °C inhibited the growth and hormone production by *P. colocasiae*, and prevented *P. parasitica* from forming new sex organs after stimulation by hormone. The effect of temperature on hormone production differed from that on oospore formation. Hormone production was poor at 30 °C, but oospore development was good at this temperature.

INTRODUCTION

Direct evidence for the hormonal regulation of sexual reproduction in both cross-inducing and self-inducing species of *Phytophthora* was obtained by Ko (1978, 1980) using a polycarbonate membrane method. The results of membrane matings demonstrate that the sexual reproduction in *Phytophthora* is controlled by hormones and receptors. For the cross-inducing species of *Phytophthora* it was postulated that the initiation of oospore formation by A² isolates depends on the presence of an active receptor to the sex hormone α^1 secreted by A¹ isolates. Oospore formation by A¹ isolates depends on the presence of the receptor to the hormone α^2 which is formed by A² isolates. The purpose of the present research was to study the effect on hormone production and reception of length of mating, light and temperature.

METHODS

Phytophthora colocasiae (Pco-1T, A²) isolated from taro [*Colocasia esculenta* (L.) Schott] was used as a hormone producer and *P. parasitica* (P991, A¹) supplied by G. A. Zentmyer was used as a hormone receptor. Blocks (20 × 10 × 3 mm) of V-8 agar, which consisted of distilled water, 10% (v/v) V-8 juice, 0.02% (w/v) CaCO₃ and 1.8% (w/v) Bacto-agar, were each inoculated with a piece (about 1 × 1 × 1 mm) of mycelium and incubated in a dark moist chamber at 25 °C. As young cultures of *Phytophthora* are better hormone producers and old cultures are more responsive to hormones (Ko, 1978), the colonies of *P. colocasiae* and *P. parasitica* were grown for 3 and 6 d, respectively. A culture block of *P. parasitica* placed in the centre of a Petri dish (100 × 15 mm) was covered with a polycarbonate membrane (CPR, 0.2 µm, 90 mm diam.; Nuclepore Corporation, Pleasanton, Calif., U.S.A.) and paired with *P. colocasiae* of the same size on the opposite side of the membrane. After incubation in a moist chamber for 3 to 72 h at 25 °C in darkness, the colonies of *P. colocasiae* and membranes were removed, but *P. parasitica* was further incubated for oospore development. The number of

oospores produced by *P. parasitica* was counted under a microscope, after 4 to 6 d incubation. In each test the number of oospores produced in 10 microscopic fields per block was counted using a 10× or 40× objective. Two replicates per treatment were used and all the experiments were repeated at least twice.

RESULTS AND DISCUSSION

Mating time. Culture blocks of *P. colocasiae* together with the membranes were removed from four plates after mating for 3, 5, 7, 9, 12, 24, 48 or 72 h. At each period of mating the numbers of sex organs produced by *P. parasitica* on two plates were counted immediately, and the numbers of oospores produced on the other two plates were counted 6 d after the beginning of mating. All plates were incubated in darkness. No sex organs were observed in matings lasting 7 h or less (Table 1). However, the amount of hormone produced by *P. colocasiae* in 7 h was sufficient to stimulate *P. parasitica* to produce 341 oospores cm⁻² 6 d after the beginning of mating. The results also show that after stimulation by the hormone, the fungus will form sex organs and mature oospores in the absence of the hormone producer. The maximum induction of sex organs occurred in matings lasting 48 h.

Light. To determine the effect of light on hormone production and oospore formation, half the plates were kept under fluorescent light and the other half in darkness during 24 h matings. Culture blocks of *P. colocasiae* and membranes were removed after mating, and the plates containing culture blocks of *P. parasitica* were further incubated at 25 °C for 5 d under light or in darkness for the development of oospores. Results showed that exposure to light during mating greatly reduced the amount of oospores produced, and that the effect of light on oospore formation after hormone stimulation was relatively small (Table 2). In other experiments using a 12 h mating period, oospore formation after hormone stimulation was significantly decreased by light but the inhibitory effect of light on hormone production was

Table 1. *Relationship between the number of sex organs formed by P. parasitica (A¹) and the duration of mating with P. colocasiae (A²)*

Duration of mating (h) (A ¹ /A ²)	Oogonia (no. cm ⁻²) at the end of mating (A ¹)	Oospores (no. cm ⁻²) 6 d after the beginning of mating (A ¹)
3	0	1
5	0	32
7	0	341
9	21	2 672
12	1 634	4 681
24	6 350	9 517
48	11 991	12 000
72	10 616	11 836

Table 2. *Effect of light on oospore formation by P. parasitica (A¹) after induction by P. colocasiae (A²)*

Treatment during mating (A ¹ /A ²)	Treatment during oospore formation (A ¹)	Oospores (no. cm ⁻²) (A ¹)
Dark	Dark	9987 A*
Dark	Light	8784 A
Light	Dark	123 B
Light	Light	45 C

* Numbers followed by the same letter are not significantly different at $P = 0.05$.

Fig. 1. (a) Effect of temperature on the mycelial growth rate of *P. colocasiae* A². (b) Effect of temperature on hormone production by *P. colocasiae* A² as judged by oospore formation in *P. parasitica* A¹.

Fig. 2. (a) Effect of temperature on the mycelial growth rate of *P. parasitica* A¹. (b) Effect of temperature on oospore development of *P. parasitica* A¹ after induction by *P. colocasiae* A².

still much greater than that on oospore development. The reported inhibition of oospore production of *Phytophthora* by light (Brasier, 1969; Harnish, 1965; Honour & Tsao, 1974; Huguenin & Boccas, 1971; Klisiewicz, 1970; Ko & Chang, 1979) is apparently due primarily to suppression of hormone production and secondarily to oospore development.

Temperature. To determine the effect of temperature on hormone production, plates were incubated in darkness at 10, 15, 20, 25, 28 or 30 °C during 24 h matings. Culture blocks of *P. colocasiae* and membranes were removed after mating, and plates containing culture blocks of *P. parasitica* were further incubated in darkness at 25 °C for 3 d to allow the development of oospores. The effect of temperature on the growth of the mycelium of *P. colocasiae* was determined by incubating at different temperatures V-8 agar plates each containing a culture disc (5 mm) of *P. colocasiae* in the centre. The linear growth from the disc was measured after 5 d. Growth and hormone production of *P. colocasiae* at 10 and 15 °C were very poor (Fig. 1). The optimum temperature for growth and hormone production was 25 °C. Although *P. colocasiae* grew well at 30 °C, its ability to produce hormone was very low at this temperature. To determine the effect of temperature on oospore

formation, plates were incubated in darkness at 25 °C during 24 h mating. Culture blocks of *P. colocasiae* and membranes were removed after mating, and plates containing culture blocks of *P. parasitica* were further incubated in darkness at 10, 15, 20, 25, 28 or 30 °C for 3 d to allow the development of oospores. The effect of temperature on the growth of *P. parasitica* was determined as described above. Low temperatures prevented the fungus from forming new sex organs after stimulation by hormone, as more oospores were produced at 20, 25, 28 and 30 °C than at 10 and 15 °C (Fig. 2). The maturation of oospores proceeded at all temperatures tested except 10 °C which also prevented the growth of *P. colocasiae*. Similar results were obtained in other experiments using a 12 h mating period. The effect of temperature on hormone production was different from its effect on oospore formation. Hormone production was very poor at 30 °C (Fig. 1*b*), but oospore development was favoured at that temperature (Fig. 2*b*). The reported temperature effect on sexual reproduction (Brasier, 1969; Honour & Tsao, 1974; Huguenin & Boccas, 1971; Klisiewicz, 1970) is apparently the net result of its effect on hormone production and oospore development.

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REFERENCES

- BRASIER, C. M. (1969). The effect of light and temperature on reproduction *in vitro* in two tropical species of *Phytophthora*. *Transactions of the British Mycological Society* **52**, 105–113.
- HARNISH, W. H. (1965). Effect of light on production of oospores and sporangia in species of *Phytophthora*. *Mycologia* **57**, 85–90.
- HONOUR, R. C. & TSAO, P. H. (1974). Production of oospores by *Phytophthora parasitica* in liquid medium. *Mycologia* **66**, 1030–1038.
- HUGUENIN, B. & BOCCAS, B. (1971). Influence of some factors on the formation and germination of oospores in strains of *Phytophthora palmivora* Butl. *Annales de Phytopathologie* **3**, 353–371.
- KLISIEWICZ, J. M. (1970). Factors affecting production and germination of oospores of *Phytophthora drechsleri*. *Phytopathology* **60**, 1738–1742.
- KO, W. H. (1978). Heterothallic *Phytophthora*: evidence for hormonal regulation of sexual reproduction. *Journal of General Microbiology* **107**, 15–18.
- KO, W. H. (1980). Hormonal regulation of sexual reproduction in *Phytophthora*. *Journal of General Microbiology* **116**, 459–463.
- KO, W. H. & CHANG, H. S. (1979). *Phytophthora katsurae*, a new name for *Phytophthora castaneae*. *Mycologia* **71**, 840–844.