Heterothallism in the Apple Powdery Mildew Fungus, Podosphaera leucotricha

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ABSTRACT

Fourteen cultures of *Podosphaera leucotricha*, each derived from a single conidium, were maintained on *Malus sylvestris* seedlings in special isolation chambers. Cleistothecia developed on leaves, stems, and petioles of plants inoculated with certain randomly paired cultures, but did not form on plants inoculated with single cultures. Therefore, *P. leucotricha* is heterothallic, and two mating types are suggested.

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Homothallism in the Erysiphaceae was first reported in 1933 by Homma (5) who demonstrated that single conidial isolates of *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Pollacci produced ascocarps on *Taraxacum ceratophorum* (Ledeb.) DC. She later showed that this fungus was heterothallic on *T. officinale* Weber (6). Cherewick (1) reported homothallism for *Erysiphe graminis* [DC.] Mérat f. sp. *hordei* Em. Marchal on *Hordeum* sp. and for *E. graminis tritici* Em. Marchal on *Triticum* sp. Both were later reported to be heterothallic (4, 7). In 1970, Smith listed three more heterothallic genera. Of the species he studied, only *E. polygoni* [DC.] St.-Amans on *Ranunculus acris* L. was homothallic. A summary of reported homothallism-heterothallism among the Erysiphaceae is given in Table 1.

In this study, single conidial isolates of *Podosphaera leucotricha* (Ell. & Ev.) Salm. were paired on *Malus sylvestris* Mill. to determine heterothallism of the apple powdery mildew fungus. A preliminary report of the results has been published (2).

MATERIALS AND METHODS.—Twelve isolates of *P. leucotricha* were collected from apple seedlings and two from pear seedlings. These cultures were established on leaves of *M. sylvestris* in isolation chambers (3). A human eyelash was glued to a 1-ml polypropylene pipette to transfer single conidia from spore chains. Conidia adhered readily to the transfer probe and were released on the leaf surface without injury. The isolation chambers were sealed to prevent entry of contaminating conidia and air flow was withheld 24 h after inoculation to increase relative humidity (RH) during the spore germination period, after which air flow was restored gradually. Preliminary tests showed that a sudden decrease in RH caused plants to wilt and spore germination to decrease. Colonies were usually visible in 7-10 days, at which time they were dispersed to other susceptible tissues on the same plant by a camel's-hair brush, which had been sterilized previously in 70% ethanol and air-dried before use. These isolates were maintained as stock cultures.

Crosses were made by combining stock cultures in pairs on previously uninfected plants. A leaf was marked and each half of the leaf was inoculated with a different culture in various combinations. When the colonies were established, conidia were brushed from the leaves to stems and petioles because initial germination and growth of the fungus was more successful on leaves, but cleistothecia formed predominantly on stems. The inoculated plants were placed in a growth room where the temp was 21-25 C and RH was 30-35%. RH within the plant chamber was 45-95%. The room was illuminated 12 h daily with both fluorescent and incandescent lamps at an intensity of approximately 5,400 lux measured at plant level.

RESULTS.—Ascocarps formed on leaves, stems, and petioles of 12 of 23 plants inoculated with paired cultures (Table 2). The shortest interval between inoculation and the appearance of ascocarps was 24 days; the longest was 109 days. Differences in the rate and quantity of mycelial growth among the various cultures were apparent. Although initial growth of both cultures occurred on each plant, it was impossible to determine whether both continued to develop as the plant became more completely invaded by the fungus. When the same culture was used to inoculate both halves of a leaf, cleistothecia failed to develop. Uninoculated plants remained free of powdery mildew infection for 180 days, at which time the experiment was terminated.

The data in Table 2 suggest that two mating types occur in the wild population of *P. leucotricha* in Hood River, Oregon. The random combinations tested showed that cultures 2, 4, 7, 8, and 9 belong to one group while cultures 1, 3, 5, 10, 12, and 13 belong to another. Cultures 6, 11, and 14, while not tested extensively to determine their classification, did not develop cleistothecia when paired with other isolates.

Cleistothecia were abundant on some of the inoculated plants and sparse on others. No relationship between amount of mycelial growth and abundance of ascocarps was evident. Some plants were observed with only a limited amount of mycelium in the asexual state and relatively large numbers of ascocarps. Conversely, some plants produced abundant mycelial growth and relatively small number of ascocarps.

In every case, cleistothecia were found on stems, petioles, or leaf mid-ribs. In no instance were they formed on the interveinal areas of the leaf lamina. Cleistothecia

TABLE 1. Reports of homothallism and heterothallism among members of the Erysiphaceae

Fungus	Host	Sexuality: Homothallic	Sexuality: Heterothallic	Authority
Sphaerotheca fuliginea (Schlecht. ex Fr.)Pollacci	*Taraxacum ceratophorum* (Ledeb.) DC.	X		Homma1933
	T. officinale Weber		X	Homma1937
Erysiphe cichoracearum DC. ex Mérat	*Helianthus annuus* L.		X	Yarwood1935
	Lactuca serriola L.		X	Schnathorst1959
	Aster laevis L.		X	Smith 1970
E. graminis [DC.] Mérat f. sp. *hordei* Em. Marchal	*Hordeum* sp.	X		Cherewick1944
			X	Hiura & Tomoda1959
E. graminis tritici Em. Marchal	*Triticum* sp.	X		Cherewick1944
			X	Powers & Moseman1956
E. polygoni [DC.] St.-Amans	*Ranunculus acris* L.	X		Smith 1970
	Lupinus sp. ('Russell')		X	Smith 1970
	Heracleum sphondylium L.		X	Smith 1970
	Pisum sativum L.		X	Smith 1970
Microsphaera penicillata (Wallr. ex Fr.) Lev.	*Lathyrus ochroleucus* Hook.		X	Smith 1970
Uncinula necator (Schw.) Burr.	*Parthenocissus* sp.		X	Smith 1970
Podosphaera leucotricha (Ell. & Ev.) Salm.	*Malus sylvestris* Mill.		X	Coyier 1972

were most prevalent on stem tissue. An examination of individual mature ascocarps revealed the presence of a single ascus with eight ascospores.

DISCUSSION.—Previous reports on homothallism and heterothallism among the Erysiphaceae have been confined to powdery mildews on herbaceous hosts (1, 4, 5, 6, 7, 8, 11, 13). This is the first report of heterothallism on a woody host.

Development of ascocarps on more than half of the 23 plants inoculated with paired cultures and none of the 14 plants inoculated with only a single conidial isolate established heterothallism for *P. leucotricha* on *M. sylvestris*. Failure of ascocarp development might be due to one or a combination of the following factors: (i) incompatibility; (ii) similarity of cultures; (iii) lack of intermingling of compatible mycelial strands on the host surface; or (iv) failure of continued growth of one of the cultures. When isolates 4 and 5 (Table 2) were combined, ascocarps developed on two plants but failed to develop on a third. All were inoculated on the same day, and maintained under similar conditions. Because ascocarps formed on two of three plants inoculated with cultures 4 and 5, factors (i) and (ii) were eliminated as probable causes for lack of ascocarp development on the third plant.

The data indicate that some cultures form ascocarps more readily and in less time than others. However, additional data are needed to verify this point.

In each successful mating reported here, the cleistothecia were identified as those of *P. leucotricha*. Formation of the sexual structures predominantly on stems was consistent with observations of naturally infected apple seedlings and cultivars grown in the greenhouse or in the field. *Podosphaera clandestina* (Wallr. ex Fr.) Lev. was also reported on *M. sylvestris* (9, 10, 12). Attempts to establish single conidial isolates of *P. clandestina* on *M. sylvestris* were unsuccessful. When

TABLE 2. Development time for cleistothecia in *Podosphaera leucotricha* in several combinations of single conidial isolates

Isolates crossed	Days[a]
1 × 2	65
1 × 4	52
1 × 5	NA
1 × 7	64
1 × 8	63
1 × 13[b]	NA
2 × 12	NA
2 × 13	NA
2 × 14[b]	NA
3 × 8	70
3 × 9	109
4 × 5	43
4 × 5	30
4 × 5	NA
4 × 12	38
5 × 13	NA
6 × 7	NA
6 × 9	NA
7 × 13	24
7 × 13	40
9 × 10	43
10 × 12	NA
11 × 12	NA

[a]Days between inoculation and observation of cleistothecia. NA indicates no cleistothecia formed.

[b]Originally isolated from *Pyrus communis*. All other isolates from *Malus sylvestris*.

single conidia were collected from *Prunus avium* L. and placed on leaves of *M. sylvestris,* germination was poor. Several conidia germinated and developed secondary branched mycelia before cessation of growth, but none developed secondary conidia. This suggests that these species are host-specific or that host-specific strains exist within each species. When *P. avium* is naturally infected with *Podosphaera clandestina,* the ascocarps occur primarily on the interveinal areas of the leaf lamina rather than on stems and petioles, as is the habit for *P. leucotricha* on *M. sylvestris.*

Further studies should investigate host specificity based on single conidial cultures and expand the knowledge of homothallism-heterothallism among species of the Erysiphaceae which attack other woody hosts.

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