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Heterothallism in *Sphaerotheca fuliginea*

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Abstract: *Sphaerotheca fuliginea* infecting cucurbits was demonstrated to be heterothallic. Isolates were obtained from pumpkin (*Cucurbita pepo*) in New York and summer squash (*C. pepo*) in Florida. Heterothallism was demonstrated by growing isolates singly or in pairs on detached leaves of summer squash in culture. These leaves were in two joined petri dishes with the leaf blade in the upper dish and the petiole in soil in the lower dish. Cleistothecia formed only when two compatible isolates were grown together. Six isolates were assigned to mating type *MAT1-2* and 59 were assigned to *MAT1-1*. Special stimulatory conditions, such as low temperature or senescence of host tissue, were not required for cleistothecial formation on detached leaves or on greenhouse-grown plants. Hyaline cleistothecia were observed as soon as 8 days after inoculation and became brown in 1–5 days. Unequal distribution of mating types may at least partially account for the rare occurrence of cleistothecia worldwide.

Key Words: cleistothecia, cucurbits, powdery mildew, teleomorph

INTRODUCTION

Powdery mildew is a common and important disease of cucurbits that occurs every year throughout most regions of the world where cucurbits are grown. It is caused either by *Erysiphe cichoracearum* DC. or by *Sphaerotheca fuliginea* (Schltdl.) Pollacci. The latter is more common (15).

There have been only five reports of the teleomorph in North America (8, 13, 17, 18, 20) despite the fact that the anamorph occurs every year. Cleistothecia have been observed rarely outside North America as well (6, 16, 19, 23, 30).

The objective of this study was to determine whether *S. fuliginea* infecting cucurbits in the USA is heterothallic or homothallic. This is a first step in elucidating the reasons for the rare occurrence of cleistothecia. In addition, sexuality must be known before genetic studies can be undertaken. A preliminary report of part of this work has been made (21).

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MATERIALS AND METHODS

Sphaerotheca fuliginea *isolates and handling procedures.*—A detached leaf culture technique was developed for maintaining and mating powdery mildew isolates. This technique involved growing detached leaves of summer squash (*Cucurbita pepo* L.) or cucumber (*Cucumis sativus* L.) in a pair of petri dishes. Young, expanding or expanded leaves of summer squash cv. Seneca Prolific were used predominately. Cotyledon disks on 2% water agar also were used occasionally. Two plastic petri dishes (10 cm diam) were joined together in an offset position by using a hot cork borer to melt a hole (about 12 mm diam) in the bottom part of the upper dish and the top part of the lower dish. The bottom dish contained moist Cornell potting mix. A hole (about 6 mm diam) was also made in the top of the lower dish for adding water to the soil as needed. The detached leaf was placed in the upper dish with its petiole extending through the hole into the lower dish. The two parts of each dish were taped together. The watering hole was kept covered with tape. These leaves usually survived for 1 to 2 months and often developed adventitious roots.

Detached leaves were inoculated by transferring conidia or conidial chains with an eyelash or a thick hair inserted in paraffin wax in the shortened tip of a disposable pasteur pipet. To maintain isolates, they were transferred to new leaves routinely every 3–4 wk. About 10 conidial chains were placed at each of 6–12 locations per leaf. The source leaves often were heavily infected and quite possibly had more than one isolate even in an apparently single colony. To ensure that there were no mixtures of powdery mildew isolates, discrete colonies were selected and single conidial-chain transfers were made at least once before isolates were used in mating studies. Cultures were kept in an incubator at 23 C/19 C (day/night) with a 12-h photoperiod.

Eleven isolates of *S. fuliginea* were obtained from pumpkin (*Cucurbita pepo*) leaves with cleistothecia in

September 1989. They were designated MM1–MM11 (33). Three isolates (MM1–MM3) were obtained from separate leaves collected from an experimental field at the Long Island Horticultural Research Laboratory (LIHRL). Isolates MM4–MM11 came from two commercial fields on Long Island. Of the six isolates from commercial field no. 1, MM4 came from one leaf, MM5–MM7 came from another leaf and MM8 and MM9 came from a third leaf. The two isolates from commercial field no. 2 (MM10, MM11) came from the same leaf. Whenever more than one isolate came from the same leaf, they always came from well separated and distinct mildew colonies. Some of these isolates were subcultured once or twice by using single conidial chains, thus obtaining subisolates. A letter was added to the end of the isolate name to designate such subisolates (e.g., MM1a).

Additional *S. fuliginea* isolates were obtained in September 1991 from individual pumpkin leaves without cleistothecia collected from LIHRL research plots (MM12–MM19), from a Long Island commercial field (MM20–MM26) and from a Michigan commercial field (MM27–MM30).

Isolates also were obtained from Florida, one of the states where cleistothecia have never been observed. In March 1992, isolates MM31–MM44 were obtained from yellow summer squash leaves and MM45–MM48 from zucchini leaves collected from a commercial field in Homestead, and in April 1992, isolates MM49–MM66 were obtained from experimental summer squash plantings in Belle Glade.

Fungus identification.—Conidia of each isolate were examined for fibrosin bodies to confirm the identity of the fungus as *S. fuliginea* (14).

Mating type determination.—Isolates collected in 1989 were grown alone or in pairs at 23 C/19 C (day/night) to determine whether *S. fuliginea* is heterothallic or homothallic and will form cleistothecia without special conditions. Pairs of isolates were transferred in close proximity (2–5 mm apart) at three to six locations (usually six) per three (sometimes two) detached leaves in culture. Thus there usually were a total of 18 sites. Small triangular pieces of labeling tape were affixed to leaves to facilitate proper placement of conidia by marking each inoculation site. Sites were located at maximum possible distances from each other but avoiding leaf margins and big veins so that each pair of isolates remained separated from all other pairs for as long as possible. The two isolates of each pair were transferred (about 10 conidial chains of each) at least 3 h apart or on 2 consecutive days to minimize the potential for cross-contamination. Conidia of the two isolates were not placed on the same spot to permit determining whether inoculation of both isolates was successful and to allow the two isolates to become established before growing together. Infection success for each isolate was determined by examining leaves 3–5 days after the inoculation of the second isolate. Later on, inoculated leaves were examined periodically until cleistothecia were formed or until the leaf died or for at least 1 month after the inoculation of the second isolate, whichever came first.

Mating type was determined for the isolates collected in 1991 and 1992 by pairing them with isolates of mating type *MAT1-1* (either MM2, MM9 or MM11) and with an isolate of mating type *MAT1-2* (MM7). The method of mating was the same as with the 1989 isolates except that each 1991 and 1992 isolate was paired separately with isolates of each mating type.

Cleistothecial formation under greenhouse conditions.—Plants were inoculated with pairs of isolates of the two mating types to determine whether cleistothecia would form on intact plants in the absence of special conditions. Inoculation was done using the procedures described above on 19 and 20 November 1992, which was 29 days after planting.

Assessment of ascospores as infecting agents.—Cleistothecia produced under field and controlled conditions were examined for ascospores. Two procedures were used to determine if ascospores produced under field conditions were infectious: i) cleistothecia were mounted in a tiny drop of water on a cover slip which was inverted over a paper ring affixed to a detached leaf in culture, and ii) segments of leaves with cleistothecia were soaked for about 1 h and then taped to the inside of the lid of the upper dish over a detached cucurbit leaf in culture.

RESULTS

The detached leaf-culture technique as well as cotyledon disks on water agar proved fully adequate for maintaining powdery mildew cultures in isolation and for investigating cleistothecial formation. Cotyledon disks supported a slightly faster growth rate of *S. fuliginea* than the detached leaves; however, detached leaves remained viable for a longer time than disks.

The isolates used in this study were identified as *S. fuliginea* based on the presence of fibrosin bodies in conidia (14). In addition, their conidia were oval whereas conidia of *E. cichoracearum* were more barrel-shaped.

Sexuality and mating type determination.—When unpaired, none of the 11 isolates (MM1–MM11) ever produced cleistothecia. During the course of this experiment, each of these isolates was transferred at least 10 times over a 30–40 wk period. Two isolates (MM9, MM11) were maintained for 26 months. MM2 and

MM7 have been maintained for over 48 months. Thus, ample opportunity was available for individual isolates to form cleistothecia if they were capable.

Cleistothecia developed only when a pair involving MM1–MM11 included MM7; all other crosses were unsuccessful. MM7 was designated as *MAT1-2* (33). Isolates that were sexually compatible with MM7 were designated as *MAT1-1*. There were seven sexually compatible pairs of isolates out of the 24 tested pairs (nine compatible out of 28 pairs when subisolates are considered) (TABLE I).

Isolates MM14, MM18, and MM23 collected in 1991 produced cleistothecia only when grown with a *MAT1-1* isolate (TABLE I). These isolates were designated, therefore, as *MAT1-2*. The remaining isolates (MM12 MM13, MM15–MM17, MM19–MM22, MM24–MM30) produced cleistothecia with *MAT1-2* and hence were designated as *MAT1-1*. Out of the 36 isolates obtained from Florida in 1992, only two, MM58 and MM64, produced cleistothecia when grown with a *MAT1-1* isolate and, therefore, were designated as *MAT1-2*. The rest of these isolates (MM31–MM51, MM53–MM57, MM59–MM63, MM65, MM66) produced cleistothecia with *MAT1-2* and, hence, were designated as *MAT1-1*. Isolate MM52 produced cleistothecia by itself and with both *MAT1-1* and *MAT1-2* isolates (TABLE I). This isolate may have been a mixture of isolates of opposite mating type as a result of the inadvertant transfer of conidia from more than one conidiophore. To assess whether MM52 was actually a mixture of isolates, 11 single conidial chains were transferred from a site on a detached leaf in culture where MM52 had developed cleistothecia by itself. Three of these single conidial-chain transfers were successful and resulted in infection. When these three subisolates were mated separately with *MAT1-1* and with *MAT1-2*, each of them produced cleistothecia only with *MAT1-1*. The subisolates obtained from MM52 each produced cleistothecia at all 12 sites where grown with *MAT1-1*. In contrast, when grown alone, the original culture of MM52 formed cleistothecia at less than 25% of the inoculated sites. This suggests that this was a mixture, and that both mating types were present at only a few sites.

Cleistothecial formation.—Conidiophores with conidia typically were first observed about 6 days after inoculation, and cleistothecia were first observed about 9 days later. The earliest detection of conidia and of cleistothecia was 4 and 8 days after inoculation, respectively. Cleistothecia were initially hyaline. By 4 days after cleistothecia were first observed, they became dark enough to be visible to the unaided eye. Cleistothecia also formed on intact plants under greenhouse conditions in December. They formed within 13 days of inoculation at 70 of 78 inoculation sites on both adaxial and abaxial leaf surfaces. Powdery mildew colonies resulting from these inoculations provided inoculum for infections on leaves that were not inoculated. Cleistothecia were observed on these leaves 24 days after inoculation.

Assessment of ascospores as infecting agents.—Many of the cleistothecia from the field and from successful mating experiment leaves contained ascospores. However, no infection was obtained with these cleistothecia. Some of the cleistothecia on cover slips had dehisced.

DISCUSSION

Sphaerotheca fuliginea infecting cucurbits is heterothallic because cleistothecia formed only when two isolates were grown together and one of them was MM7, MM14, MM18, MM23, MM58 or MM64, while the other was any isolate other than one of these six isolates. Mating type *MAT1-2* was assigned to these six isolates and *MAT1-1* was assigned to the rest of the isolates. One isolate, MM52, did produce cleistothecia when grown alone; however, this isolate probably was a mixture of isolates of compatible mating types rather than a homothallic strain. This conclusion is supported by the following observations: when grown alone, this isolate produced cleistothecia at less than 25% of the inoculation sites on detached leaves whereas cleistothecia usually formed at all sites inoculated with a pair of isolates of compatible mating types. The most likely explanation is that only one of the two mating types in this isolate was transferred to most sites on a leaf. The three subisolates obtained from MM52 by transferring single conidial chains produced cleistothecia only when grown with *MAT1-1*. Isolate MM52 had produced cleistothecia when grown with either *MAT1-2* or *MAT1-1*.

Cleistothecia formed at almost all sites where two isolates of compatible mating types grew together for the isolates collected in 1991 and 1992, whereas cleistothecia formed at only an average of 31% of the sites for crosses made in 1990. This difference may reflect improved technique considering that for two of the isolates crossed in 1990, MM2 and MM7, the frequency of cleistothecial formation has been almost 100% when these have been crossed subsequently for other experiments using similar procedures and conditions (28).

Inoculations with ascospores of *S. fuliginea* were not successful, conceivably because they were not viable or the environment was not conducive. Several cleistothecia had dehisced. In contrast, pea leaves were infected by ascospores of *Erysiphe polygoni* DC. (*sensu*

E. S. Salmon) when pieces of leaf with cleistothecia were first placed on moist blotting-paper in the lid of a polystyrene box (26).

Within the family Erysiphaceae heterothallism seems to be more common than homothallism. Heterothallism has been demonstrated for *S. pannosa* (Wallr.) Lév. var. *rosae* Wor. on rose (*Rosa rugosa* Thunb. and *R. multiflora* Thunb.) (3); *S. mors-uvae* (Schw.) Berk. on blackcurrant (*Ribes nigrum* L.) (12); *Erysiphe polygoni* DC. on *Lupinus* sp. (cv. Russell), *Heracleum sphondylium* L., *Lathyrus pratensis* L., and *Pisum sativum* L. (27); *E. cichoracearum* on *Aster laevis* L. (27); *E. cichoracearum* on sunflower (*Helianthus annuus* L.) (22, 31); *E. cichoracearum* on wild lettuce (*Lactuca serriola* L.) (25); *E. cichoracearum* on zinnia (*Zinnia elegans* Jacq.) (22); *Blumeria graminis* (DC.) Speer = *E. graminis* DC. f.sp. *tritici* on barley (*Hordeum vulgare* L.) (27) and on wheat (*Triticum aestivum* L.) (24); *Microsphaera penicillata* (Wallr.) Lév. on *Lathyrus ochroleucus* Hook. (27); and *Uncinula necator* (Schw.) Burrill on *Parthenocissus* sp. (27). Species that may be heterothallic, since cleistothecia did not form when single conidia were used for inoculation, are *Cystotheca lanestris* (Harkn.) Miyabe on *Quercus serrata* Thunb. (10), *E. cichoracearum* on *Plantago major* L. (10), and *Podosphaera tridactyla* (Wallr.) de Bary on *Prunus triflora* Roxb. (10). In sharp contrast with the results from the present study, Homma concluded that *S. fuliginea* infecting *Taraxacum ceratophorum* (Ledeb.) DC. is homothallic based on the following results: when single conidia were transferred to leaves of 23 plants enclosed in glass containers, cleistothecia developed on all four of the 23 leaves where monosporic inoculation was successful (9, 10). Homothallism also has been demonstrated for *E. polygoni* DC. on *Ranunculus acris* L. (27). Each conidium of a powdery mildew fungus usually contains one nucleus (32); therefore, these fungi on these hosts probably are truly homothallic (able to produce the teleomorph starting from a single haploid nucleus). However, if these fungi produce multinucleate conidia and the nuclei in a conidium are of opposite mating type, then apparent homothallism could really be masked heterothallism.

Cleistothecia were formed as soon as two isolates of compatible mating types grew together on green leaf tissue under controlled conditions or in a greenhouse. The earliest observation of cleistothecia was 8 days after inoculation. Therefore, special stimulatory conditions, such as low temperature or senescence of host tissue, were not required for cleistothecial formation. Detached leaves in culture do not appear to be an abnormal system in regard to the lack of need for special conditions, since cleistothecia formed on leaves of intact plants under greenhouse conditions. Cleistothecial formation by several other fungi likewise was found to require only the pairing of compatible mating types under controlled conditions by Smith (27), Gadoury and Pearson (7), and Schnathorst (25). These investigators reported first seeing cleistothecia 4–21 days after inoculation. Formation by *U. necator* starts within 48 h of hyphal contact and is not affected by temperature, day length, humidity, leaf age, or host resistance (7). Cleistothecia were produced more abundantly under longer day lengths (27), between 10 and 20 C (27), and when leaf tissue was incubated on 0.3 M sucrose solution than on distilled water (22).

In contrast with these results, however, special conditions for cleistothecial formation are required based on observations and previous research results. Formation of cleistothecia may be influenced by host-related factors. Whenever cleistothecia of *S. fuliginea* have been found in northern India, they have been only on certain cucurbits (16). This observation was verified by dusting conidia onto plants in the 4- to 6-leaf stage. Cleistothecia formed on only three of seven cultivars of *Lagenaria leucantha* (Duch.) Rusby and five of 12 cultivars of cucumber (16). Cleistothecia have been found in the Sudan only on vegetable marrows (*C. pepo*), though the conidial stage was observed on other cucurbits (29). Two of 16 isolates of *E. cichoracearum* from *Zinnia elegans* did not cross with any of 13 isolates from *Helianthus annuus* (22). Environmental conditions also seem to be influential because the time required for cleistothecia to form after inoculation varied during a study conducted in India: only 20–25 days during December to February, 60–65 days during September to November, whereas none formed during March to August (16). Factors that have been suggested to be triggers for cleistothecial formation by *U. necator* based on field studies or observations include severe powdery mildew, drought, cold, heat, or an unfavorable environment for the fungus (7). Both poor nutrition and healthy growth have been reported to be stimulatory. Furthermore, special conditions seem to be required for the Erysiphaceae in general. Cleistothecia begin to form late in the season when temperatures are cool and conidial production slows down or ceases (1, 2). They form in the older areas of infection (1). Host senescence, low nutritive condition of the host, dry atmosphere, and low temperature are cited commonly as conditions favoring cleistothecial formation (31). Dryness, in combination with hot temperatures, has been attributed to being the most important stimulatory factor (4). Additional conditions described as stimulatory are unsuitable environmental conditions, particular age of the host, and pauperization of the host by the action of other parasitic fungi or insects (4).

Occurrence of cleistothecia of *S. fuliginea* in nature may be affected by distribution and frequency of mating types. Distribution alone cannot account for cleis-

TABLE I. Mating type of *Sphaerotheca fuliginea* isolates collected from Long Island, New York, in 1989 and 1991, from Michigan in 1991 and from Florida in 1992

Isolate number	Source[a]	Cross with *MAT-1*[b]			Cross with *Mat1-2*[b]			Mating type
		Sites inoculated	Successful pairs[c]	Pairs with cleistothecia	Sites inoculated	Successful pairs	Pairs with cleistothecia	
MM1a	NY-R	51	40	0	18	11	1	*MAT1-1*
MM1b	NY-R	33	27	0	16	15	4	*MAT1-1*
MM1d	NY-R	15	14	0	18	13	3	*MAT1-1*
MM2	NY-R	31	31	0	16	16	5	*MAT1-1*
MM3	NY-R	32	24	0	16	16	3	*MAT1-1*
MM4c	NY-CF	31	30	0	16	16	4	*MAT1-1*
MM8	NY-CF	31	29	0	18	12	4	*MAT1-1*
MM9a	NY-CF	30	28	0	10	6	2	*MAT1-1*
MM11	NY-CF	32	25	0	10	7	3	*MAT1-1*
MM12	NY-R	18	16	0	18	18	18	*MAT1-1*
MM13	NY-R	12	11	0	18	18	18	*MAT1-1*
MM14	NY-R	12	12	9	18	0	0	*MAT1-2*
MM15	NY-R	18	18	0	12	12	12	*MAT1-1*
MM16	NY-R	12	9	0	18	18	18	*MAT1-1*
MM17	NY-R	12	12	0	18	18	18	*MAT1-1*
MM18	NY-R	12	12	12	12	12	0	*MAT1-2*
MM19	NY-R	18	18	0	12	11	11	*MAT1-1*
MM20	NY-CF	18	17	0	18	18	18	*MAT1-1*
MM21	NY-CF	18	18	0	18	17	17	*MAT1-1*
MM22	NY-CF	18	18	0	18	18	18	*MAT1-1*
MM23	NY-CF	12[d]	11	11	18	14	0	*MAT1-2*
MM24	NY-CF	12	12	0	12	12	12	*MAT1-1*
MM25	NY-CF	12	10	0	12	12	12	*MAT1-1*
MM26	NY-CF	12	11	0	12	12	12	*MAT1-1*
MM27	M-CF	12	12	0	18	17	17	*MAT1-1*
MM28	M-CF	12	12	0	12	12	12	*MAT1-1*
MM29	M-CF	12	11	0	12	12	12	*MAT1-1*
MM30	M-CF	12	7[e]	0	18	9[f]	7[f]	*MAT1-1*
MM31	FL-CF	12	12	0	12	12	12	*MAT1-1*
MM32	FL-CF	12	12	0	12	12	12	*MAT1-1*
MM33	FL-CF	12	12	0	12	12	12	*MAT1-1*
MM34	FL-CF	12	12	0	12	12	12	*MAT1-1*
MM35	FL-CF	12	12	0	12	12	9	*MAT1-1*
MM36	FL-CF	12	12	0	12	12	12	*MAT1-1*
MM37	FL-CF	12	12	0	12	12	12	*MAT1-1*
MM38	FL-CF	12	12	0	12	12	12	*MAT1-1*
MM39	FL-CF	12	12	0	12	12	12	*MAT1-1*
MM40	FL-CF	12	12	0	12	12	12	*MAT1-1*
MM41	FL-CF	12	12	0	12	11	11	*MAT1-1*
MM42	FL-CF	12	12	0	12	12	11	*MAT1-1*
MM43	FL-CF	12	12	0	12	12	12	*MAT1-1*
MM44	FL-CF	12	12	0	12	12	11	*MAT1-1*
MM45	FL-CF	12	12	0	12	12	12	*MAT1-1*
MM46	FL-CF	12	12	0	18	18	18	*MAT1-1*
MM47	FL-CF	12	12	0	12	12	12	*MAT1-1*
MM48	FL-CF	12	12	0	12	12	12	*MAT1-1*
MM49	FL-R	12	12	0	12	12	12	*MAT1-1*
MM50	FL-R	12	12	0	12	12	12	*MAT1-1*
MM51	FL-R	12	12	0	12	12	12	*MAT1-1*
MM52	FL-R	12	11	4[e]	12	12	12	mixture
MM53	FL-R	12	12	0	12	12	12	*MAT1-1*
MM54	FL-R	12	12	0	12	12	12	*MAT1-1*
MM55	FL-R	12	12	0	12	12	12	*MAT1-1*

TABLE I. Continued

Isolate number	Source[a]	Cross with *MAT-1*[b] Sites inoculated	Successful pairs[c]	Pairs with cleistothecia	Cross with *Mat1-2*[b] Sites inoculated	Successful pairs	Pairs with cleistothecia	Mating type
MM56	FL-R	12	12	0	12	11	11	*MAT1-1*
MM57	FL-R	12	12	0	12	12	11	*MAT1-1*
MM58	FL-R	12	12	12	12	12	0	*MAT1-2*
MM59	FL-R	12	12	0	12	12	12	*MAT1-1*
MM60	FL-R	12	12	0	12	12	12	*MAT1-1*
MM61	FL-R	12	12	0	12	12	12	*MAT1-1*
MM62	FL-R	12	12	0	12	12	10	*MAT1-1*
MM63	FL-R	12	12	0	12	12	12	*MAT1-1*
MM64	FL-R	12	12	12	12	12	0	*MAT1-2*
MM65	FL-R	12	12	0	12	12	12	*MAT1-1*
MM66	FL-R	12	12	0	12	12	12	*MAT1-1*

[a] NY-R: Research plots in Riverhead, New York; NY-CF: Commercial fields on Long Island, New York; FL-CF: Commercial field in Homestead, Florida; FL-R: Research plots in Belle Glade, Florida; M-C: Commercial field in Michigan. Isolates MM1-MM11 were collected in 1989.

[b] Mating type of isolates MM1–MM11 was determined by intercrossing them; therefore, the mating type of the isolate that each was crossed with often was not determined until after several additional crosses were made. Isolates MM12-MM66 were crossed with isolates identified through the previous crosses to be mating type *MAT1-1* (MM2, MM9, and MM11) and *MAT1-2* (MM7).

[c] The pair at a site was considered to be successful if both isolates grew.

[d] One leaf died in 9 days and was ignored.

[e] Powdery mildew development was compromised because one of two leaves was partially wilted.

[f] Powdery mildew development was compromised because two of three leaves were partially wilted.

tothecia having never been found in Florida (K. L. Pohronezny, pers. comm.) because both mating types were found in a research field. Mating type *MAT1-2* is less common than *MAT1-1* based on the results from this study. Only one of the 11 isolates obtained in 1989 was of mating type *MAT1-2*. Similarly, only three of the 19 isolates collected in 1991 from New York and Michigan and only two of the 35 isolates collected in 1992 from Florida were of mating type *MAT1-2*. Because of this, cleistothecia of cucurbit powdery mildew may occur rarely in nature. In addition, cleistothecia would not be expected to form as quickly in nature as in the laboratory because of the time required for individuals of compatible mating type to come into contact, especially when one mating type occurs at low frequency. Under field conditions in New York, cleistothecia of *S. fuliginea* were not found until 32 days after powdery mildew was first observed on summer squash in 1991 (McGrath and Ghemawat, unpubl.). This delay could be misinterpreted as an apparent correlation with some host or environmental condition. The recent findings of cleistothecia in California, Indiana, North Carolina, and Canada indicate that both mating types are at least now present in climatically diverse regions of North America.

Disparity in the occurrence of the cleistothecial stage and the conidial stage is not unique to *S. fuliginea*. Cleistothecia of *S. macularis* (Wallr.) Lind also have been found seldomly, although the conidial stage occurs in most areas of the world where strawberries are grown (11). There are many successful mildews that do not produce fertile cleistothecia (5).

Occurrence of the sexual stage of a pathogen is significant and worthy of investigation. Cleistothecia could enable *S. fuliginea* to survive harsh environmental conditions and absence of hosts (over winter or summer), and they could function as a primary inoculum source. Interregional movement of conidia is thought to be the source of inoculum for cucurbit powdery mildew in many areas. This would account for the disease not being a problem on spring crops. A local source, such as cleistothecia, could result in earlier disease onset. In addition, increased genetic diversity resulting from sexual reproduction could include, for example, new combinations of virulence genes and fungicide resistance.

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