A Leaf Spot of Cucumber Caused by *Ulocladium cucurbitae* in New York

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ABSTRACT

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Ulocladium cucurbitae, a leaf spot pathogen of cucumber (*Cucumis sativus*), was recovered from commercial fields and breeding plots in two widely separated areas of New York. The fungus was pathogenic to many commonly grown cucumber cultivars in the Northeast, including Poinsett 76, Marketmore 76, Sweet-Slice, Dasher II, Pacer, and Raider. In addition, *Alternaria alternata* was recovered from all of the lesions examined. In pathogenicity tests, only *U. cucurbitae* produced symptoms identical to those observed in the field. *A. alternata* failed to infect cucumber directly, but when plants were inoculated with a mixture of conidia of both fungi, *A. alternata* readily colonized the necrotic tissue produced by the *U. cucurbitae* infections. *U. cucurbitae* did not infect other cucurbits. Several isogenic cucumber lines bred for resistance to target spot, caused by *Corynespora cassiicola*, also showed excellent resistance to Ulocladium leaf spot.

A survey of cucumber (*Cucumis sativus* L.) fields in two counties in western New York in 1988 and experimental plant breeding plots located near Ithaca during 1988 and 1989 revealed the presence of a severe leaf spot affecting both young and old plantings during late August and early September. In commercial fields, all cultivars grown appeared equally susceptible whereas differences in susceptibility were obvious among the experimental lines. A similar-appearing leaf spot of cucumber had been observed by T. A. Zitter in cucumber breeding plots near Ithaca since 1981 but had not been previously observed in commercial fields (*unpublished*). The disease in Ithaca was previously reported to be caused by the fungus *Ulocladium cucurbitae* (Letendre & Roumeguere) Simmons based upon mycological examinations of a culture and leaf tissue by S. M. Francis of the Commonwealth Mycological Institute, Kew, England (6,7). However, Lane (6) was unsuccessful in all attempts at reproducing the disease under controlled conditions. Previous reports of a leaf spot of cucumber caused by *Ulocladium* are few and have come from outside the United States (2,5). In addition to finding the ulocladioid-type conidia described by Lane (6), we found in both 1988 and 1989 that for all lesions examined, a second conidial type was consistently recovered that closely fit the description of *Alternaria alternata* (Fr.) Keissler (3,4,10,12). Because under certain laboratory culture conditions both ulocladioid- and alternarioid-type conidia can occur with *Ulocladium* spp. (8,11) and because *A. alternata* has recently been reported to cause a leaf spot disease of cucumber (12), we decided to examine the pathogenicity of both organisms toward cucumber. In this paper we describe the disease symptoms, methods used for infectivity tests and completing Koch's postulates, the experimental host range of *U. cucurbitae*, and the resistance to this pathogen in several Cornell cucumber breeding lines. A preliminary report of this work has been published (13).

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MATERIALS AND METHODS

Field collections and isolations. Mature and young cucumber leaves were collected from eight fields in Genesee County, from four fields in Orleans County, and from several rows within H. M. Munger's cucumber breeding plots near Ithaca (Varna, Tompkins County). Individual leaf spots were removed, surface-sterilized in 0.5% NaOCl for 1–2 min, rinsed with sterile distilled water, and incubated in petri plates containing potato-dextrose agar (PDA) or water agar (WA). Eighteen isolates of both *Ulocladium* and *Alternaria* (seven from Genesee County, six from Orleans County, and five from Tompkins County) were obtained from lesions of varying size in order to conduct an initial pathogenicity test. After these initial isolations, all studies relied on single-spore isolates. Leaf collections were also made from an *Amaranthus* weed species commonly growing in the alleyways of the commercial fields and heavily infected by a severe leaf blight.

Greenhouse inoculations. Eighteen mature, pot-grown cucumber plants saved from a plant breeding progeny increase of families of Marketmore 76 and 80 and Poinsett 83 were available for the initial inoculation. The upper part of the plants was removed so that only five or six lower, mature leaves remained. Individual leaves were inoculated with *Ulocladium, Alternaria*, or a mixture of *Ulocladium* and *Alternaria*, along with a water control. Spore suspensions of conidia prepared from 6- to 8-day-old cultures were adjusted to 100,000 conidia per milliliter before inoculation. The suspensions were atomized onto the upper leaf surface at 10 psi until runoff. After inoculation, the plants were held in a mist chamber for 48 hr before being moved back to a greenhouse at 29 C and supplemented with 16 hr of fluorescent light. Leaves were inspected for lesions 5–7 days after inoculation.

In two subsequent pathogenicity tests, two isolates each of *Ulocladium* and *Alternaria* obtained from Orleans and Tompkins counties and designated U11 and A11 and U313 and A313, respectively, were used. The same general procedures mentioned above were followed except that the cultivar Poinsett was chosen because of its proven susceptibility to natural infection by *Ulocladium* (6). Individual potted plants in the five- to nine-leaf stage were inoculated with the three conidial suspensions for each isolate. Each conidial concentration was adjusted to 30,000 spores per milliliter. In the first trial, plants were kept in a mist chamber set at 24 C for 48 and 96 hr, with two or three pots for each inoculum and incubation period. After inoculation, the plants were moved to a greenhouse as previously described and observed for lesion development. In trial 2, four pots were inoculated with each conidial suspension and were placed in the mist chamber set at 24 C for 24, 48, and 96 hr before being returned to the greenhouse. Six days after the plants were inoculated, three pots from each group were returned to the mist chamber for an additional 50 hr of incubation. These pots were again returned to the greenhouse, and the lesion sizes for all groups were subsequently measured. A minimum of 25 lesions from each inoculation were measured microscopically, always from the underside of leaves.

In order to test the susceptibility of cucumber fruit to *Ulocladium*, Poinsett fruit saved from the first trial and stored during the interim period in a refrigerator were inoculated by spraying a conidial suspension of the U11 and U313 isolates. Fruit were held in a moist chamber consisting of a plastic garment box for approximately a week and then observed for infection.

Comparison of conidial sizes for isolates of *U. cucurbitae* and *A. alternata*. Single-spore isolates obtained from naturally infected leaves collected in western New York and from Ithaca were cultured on PDA at 21 C and measured for total length and width of conidia. Seven isolates of *A. alternata* and four of *U. cucurbitae* were examined.

Susceptibility of cucumber cultivars and breeding lines to *U. cucurbitae*. Because of the difficulty experienced by Lane in achieving any infection of cucumber progeny under controlled conditions (6), we attempted to increase the susceptibility of young seedlings (one- to four-leaf stage) by varying the starting soil medium. The amount of disease on transplants can be very dependent on plant vigor (9). Seeds were started in flats using either regular greenhouse soil mix or a 50:50 mixture of sand and peat. Plants were inoculated in the three- to four-leaf stage with a suspension of approximately 8.0×10^4 conidia per milliliter. The flats were held in a mist chamber for 96 hr and scored for disease severity 1 wk later. Eight to 10 seedlings of the following commercial cultivars were tested on two occasions: Marketmore 76, Poinsett 76, Sweet-Slice, Dasher II, Pacer, and Raider. Similar tests were performed on two occasions with the following isogenic lines provided by H. M. Munger, which vary in resistance to target spot caused by *Corynespora cassiicola* (Berk. & Curt.) Wei: Marketmore 70, Marketmore 87, Poinsett 87, Dryden (an SMR 58 type), Dryden 87, SR 551, PMR 551 (Albion), and Albion 87. Poinsett seedlings served as the standard control, and uninoculated plants served as checks.

Host range of *U. cucurbitae*. A limited number of species and cultivars of Cucurbitaceae were tested for susceptibility to *U. cucurbitae*, including summer squash (*Cucurbita pepo* L.) cultivars Zucchini Elite and Multipik and pumpkin cultivar Howden; muskmelon (*Cucumis melo* L. var. *reticulatus* Naudin) cultivar Gold Star; squash (*Cucurbita moschata* (Duchesne) Duchesne ex Poir.) cultivar Waltham Butternut; and watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) cultivar Sugar Baby. Seedlings were inoculated as described for cucumbers. Control plants for each cultivar were sprayed with water and covered with plastic bags to prevent infection in the mist chamber. Plants were examined for leaf spots 1–2 wk after inoculation. Any suspicious leaf spots were surface-sterilized in 0.5% NaOCl for 1–2 min, rinsed in sterile distilled water, and then incubated in a moist chamber before examination.

RESULTS

Description of the disease. Symptoms were first noted in commercial cucumber fields on 24 August. At this stage, the lesions were relatively small, although some were large enough to begin to crack as the lower leaves reached their maximum size. When leaf tissue was collected on 7 September, the disease had dramatically increased in terms of both the number of fields affected and plants infected and the severity on the lower leaves. Immature lesions, usually appearing on younger leaves one-third to one-half of the way out on the vines, were reddish brown and averaged 1–2 mm in diameter. A few of the lesions on the lower leaves near the center of the plant were young but most were mature, typically with beige centers surrounded by a dark brown ring and brown halo and measuring 6–7 mm in diameter (Fig. 1A and B). Some lesions were larger; many were beginning to coalesce and the centers were beginning to tear, giving a similar appearance to angular leaf spot. Growers by this time had applied several copper sprays without any apparent reduction in spread of disease. Measurements of 75 lesions found on each of three medium-sized cucumber leaves collected from Orleans and Tompkins counties showed that lesion diameters ranged

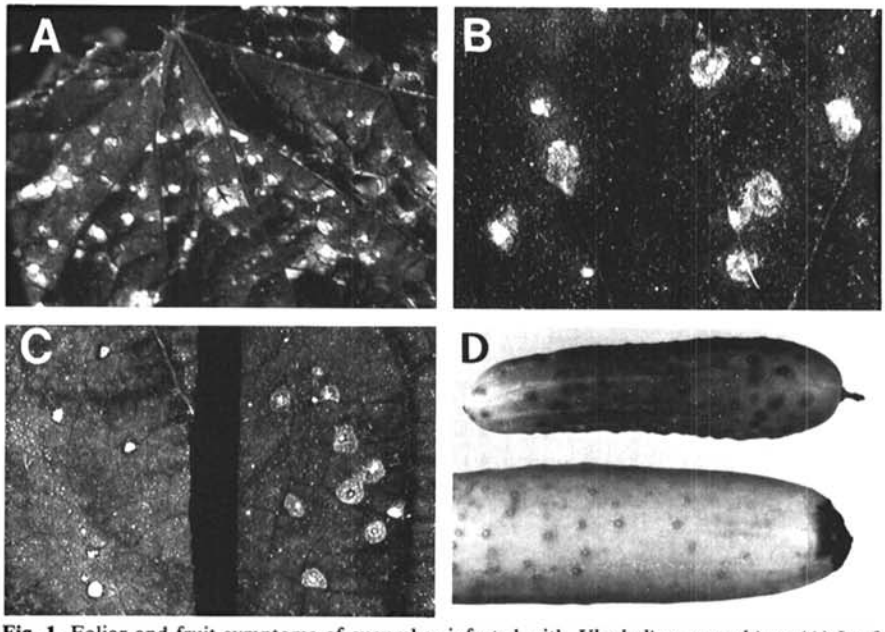

Fig. 1. Foliar and fruit symptoms of cucumber infected with *Ulocladium cucurbitae:* **(A)** Leaf spot on naturally infected leaf of the cultivar Marketmore 76. **(B)** Detail of individual leaf spots. **(C)** Leaves of the cultivar Poinsett inoculated with *U. cucurbitae* isolate 313 (left) after incubation in a mist chamber for 96 hr and (right) after an additional 50 hr of incubation. **(D)** Poinsett fruit inoculated with *U. cucurbitae* showing sporulation at the stem and blossom ends and water-soaked areas surrounding the spines.

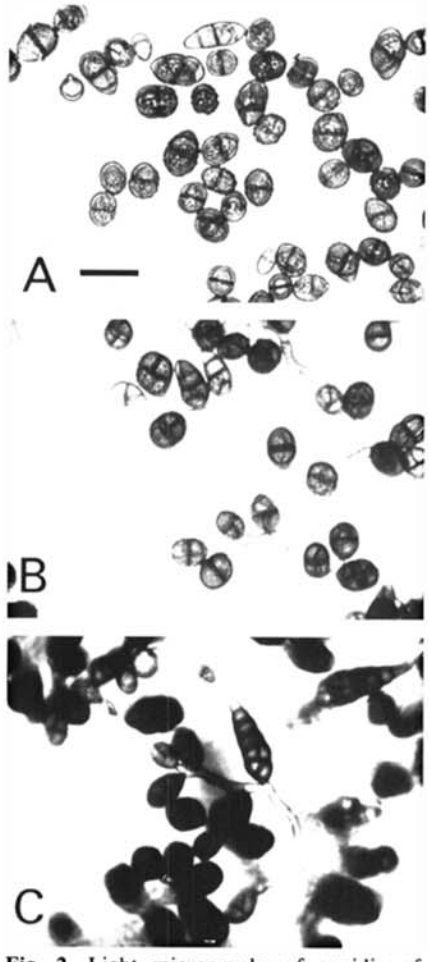

Fig. 2. Light micrographs of conidia of *Ulocladium cucurbitae:* **(A)** Conidia from a 10-day-old culture grown on V-8 agar at 27 C are obovoid to ellipsoid and have smooth to rough walls. **(B)** Conidia from a 10-day-old culture grown on PDA at 27 C are attached to conidiophores. **(C)** Ulocladioid-type conidia mix with alterarioid-type conidia on V-8 agar at 24 C. Scale bar = 10 μm.

from 1.00 to 5.32 mm and 0.88 to 6.52 mm, respectively (av. diameter of 2.96 mm for both locations). No fruit infection was found either in the field or at the packinghouses.

Field isolations and greenhouse inoculations of *U. cucurbitae* and *A. alternata*. Following incubation of the leaf tissue on agar, sporulation was observed on all 18 lesions examined representing both large and small spots collected from the field. Without exception, all sporulating lesions yielded a mixture of two conidial types representing *U. cucurbitae* and *A. alternata*. Only an *Alternaria* spp. was isolated from the *Amaranthus* weed species.

In initial pathogenicity tests, all 18 plants inoculated with the *Ulocladium* isolates produced many small necrotic lesions, which coalesced and caused many leaves to die prematurely. The fungus was readily reisolated and sporulated profusely on many of the lesions. In contrast, when plants were inoculated with the 18 *Alternaria* isolates, a few faint necrotic specks appeared on the leaf surface. *Alternaria* conidia were sparingly found on the leaf surface, with no apparent colonization. Leaves inoculated with a mixture of *Ulocladium* and *Alternaria* conidia developed lesions identical in size and number to those inoculated with *Ulocladium* alone. Reisolation from these leaf spots showed moderate to profuse sporulation of both fungi for many of the lesions examined.

In pathogenicity trials 1 and 2 using the cultivar Poinsett, no disease developed with the A11 and A313 isolates from cucumber. Although faint lesions appeared on the inoculated leaves, they also could be found on some water-sprayed checks. Many of these specks could be attributed to physical injury from weekly greenhouse fumigation and spraying. Both *Ulocladium* isolates produced lesions in approximately equal numbers whether incubated for 48 or 96 hr, although the isolate originating from the plant breeding plots (U313) produced more lesions. Plants that were incubated for 96 hr developed larger lesions (trial 1). In trial 2, plants incubated for 24, 48, or 96 hr and inoculated with the two *Ulocladium* isolates all developed lesions. When plants were subjected to an additional 50 hr of incubation, the resulting lesions were larger and resembled leaf spots in the field (Fig. 1C). Although the two isolates produced lesions similar in size, U313 produced more lesions per square centimeter. In trial 2, lesions developed on leaves two through seven with isolate U313 when plants in the nine-leaf stage were incubated for 96 hr followed by an additional 50 hr of moisture.

Fruit was infected with *U. cucurbitae* by inoculating overmature fruit and incubating them for over 1 wk in a moist chamber. Conidia could be recovered from both ends of the fruit and from areas associated with spines (Fig. 1D).

Pathogen description and comparison of conidia sizes for isolates of *U. cucurbitae* and *A. alternata*. The following description of isolate 313 of *U. cucurbitae* is based on a 10-day-old culture grown at 27 C on V-8 agar under continuous light. The conidiophores were thick-walled, septate, and light yellowish brown. They ranged from 19 to 80 μm or longer but most commonly were 19–35 μm. The conidia were solitary or, rarely, in chains of two, attached to the tip and closely along the sides of the conidiophores, forming clusters of conidia. The walls of the conidia were smooth to rough, obovoid to ellipsoidal or, more commonly, subspherical to spherical, together with a few alternarioid-shaped conidia, with one to five transverse and one to four longitudinal or oblique septa, and measured 12–30 μm long × 12–21 μm wide (Fig. 2). The size of the conidiophores and conidia fit the general description reported by Simmons (11).

On PDA, the overall body length of *U. cucurbitae* conidia of four isolates from the two widely separated areas of the state averaged 21.5 μm (range, 10.8–35.1 μm) and had a body width of 12.5 μm (range, 8.1–18.9 μm), again agreeing favorably with published reports (11).

The following measurements of *A. alternata* are based on 25 conidia from seven isolates as initially cultured on PDA at 21 C. The overall body length was 21.2 μm (range, 6.8–54.0 μm), the body width was 11.2 μm (range, 5.4–18.9 μm), and the beak length was 4.5 μm (range, 1.4–16.2 μm). These measurements are within the general ranges of previous reports (3,4,10,12).

Susceptibility of cucumber cultivars and breeding lines. All of the commercial cucumber cultivars tested proved to be equally susceptible to *U. cucurbitae*. Inoculation of Dasher II resulted in numerous large lesions on the cotyledons, resulting in their premature death. The time in which the lesions developed and their general appearance were similar to those with the early Poinsett inoculations, and disease resembled that in the field. Similar trends were established whether the plants were grown in regular greenhouse soil and inoculated in the four- to five-leaf stage or grown in regular soil or a mixture of sand and peat and inoculated in the early two- to three-leaf stage.

Results of inoculating selected cucumber cultivars and breeding lines with *U. cucurbitae* are summarized in Table 1. Strong susceptible reactions were noted for Poinsett, Poinsett 76, Marketmore 76, PMR 551 (Albion), and PSMR18WS (Dryden), consisting of leaf death and many necrotic leaf spots. An intermediate reaction was observed for lines SR 551 and Marketmore 70. Albion 87, Marketmore 87, Dryden 87, and Poinsett 87-8 were classified as resistant, based on fewer number of dead leaves, fewer necrotic leaf spots, and lower overall disease rating.

Host range of *U. cucurbitae*. No lesions developed and no *U. cucurbitae* conidia were recovered from the following test plants: Gold Star musk-

Table 1. Reaction of seedlings of selected cucumber cultivars and isogenic breeding lines to *Ulocladium cucurbitae*[a]

Cultivar or line	No. of plants	Number of dead leaves and total number of lesions on live leaves for: Leaf 1 Dead ± SE[d]	Leaf 1 Lesions ± SE	Leaf 2 Dead ± SE	Leaf 2 Lesions ± SE	Leaf 3 Dead ± SE	Leaf 3 Lesions ± SE	Average rating[b]	Disease reaction[c]
Poinsett	18	17 ± 0.2	13 ± 0.5	10 ± 0.3	37 ± 0.9	1 ± 0.2	30 ± 0.5	7.2	S
Poinsett 76	16	15 ± 0.4	10 ± 1.7	12 ± 0.0	115 ± 4.2	3 ± 0.5	84 ± 2.0	7.4	S
Marketmore 76	18	17 ± 0.2	17 ± 2.8	10 ± 0.7	84 ± 2.3	4 ± 0.7	81 ± 1.8	7.3	S
PMR 551 (Albion)	18	13 ± 0.8	14 ± 0.6	15 ± 0.5	26 ± 0.6	11 ± 1.2	54 ± 0.9	7.5	S
PSMR18WS (Dryden)	18	18 ± 0.0	30 ± 2.1	17 ± 0.2	40 ± 6.6	9 ± 1.3	91 ± 4.1	6.2	S
SR 551	18	18 ± 0.0	0 ± 0.0	12 ± 0.0	22 ± 0.6	1 ± 0.2	15 ± 0.5	5.0	MR
Marketmore 70	17	15 ± 0.2	10 ± 0.9	8 ± 1.4	161 ± 2.6	6 ± 1.0	17 ± 0.6	4.9	MR
Albion 87	18	2 ± 0.3	120 ± 1.0	0 ± 0.0	105 ± 1.4	0 ± 0.0	5 ± 0.1	2.5	R
Marketmore 87	18	1 ± 0.2	100 ± 0.9	0 ± 0.0	91 ± 1.7	0 ± 0.0	12 ± 0.7	2.3	R
Dryden 87	18	6 ± 0.9	45 ± 1.8	0 ± 0.0	79 ± 1.3	0 ± 0.0	16 ± 1.1	2.4	R
Poinsett 87-8	17	7 ± 0.3	13 ± 0.2	0 ± 0.0	12 ± 0.3	0 ± 0.0	0 ± 0.0	1.7	R

[a] Average of two trials (nine plants per trial maximum). Leaves were atomized with 8 × 10^4 conidia per milliliter and plants were incubated in a mist chamber for 96 hr at 25 C.
[b] Based on a 0–9 scale, where 0 = no symptoms and 9 = numerous leaf spots leading to leaf collapse and death.
[c] S = susceptible, MR = moderately resistant, R = resistant.
[d] Standard error of means.

melon, Zucchini Elite summer squash, and Waltham Butternut squash. The Sugar Baby watermelon seedlings grew very slowly in either the regular soil mix or the 50:50 mixture but did not appear to develop any leaf spots. Necrotic spots developed on the inoculated leaves of Multipik summer squash; however, when the leaves were incubated and examined in detail *U. cucurbitae* sporulated very well along the edges of leaves with necrotic areas but not on any necrotic spots. This reaction was particularly noted on seedlings that grew poorly in the 50:50 soil mixture.

DISCUSSION

On the basis of pathogenicity studies using both commercial cultivars and breeding lines, we have satisfied Koch's postulates showing that *U. cucurbitae* is the causal organism responsible for the severe leaf spot occurring in both commercial fields and experimental plots in New York. Although *A. alternata* was commonly associated with Ulocladium leaf spot of cucumber, our results suggest that this organism is not a primary pathogen but readily colonizes necrotic tissue. When mixed cultures of *U. cucurbitae* and *A. alternata* were tested on cucumber leaves, *A. alternata* sporulated very profusely on the resulting lesions. The recent report of a pathotype of *A. alternata* causing a severe leaf spot of cucumbers grown in plastic houses in Crete indicates that this organism has the potential to directly infect cucumber when the appropriate pathotype occurs (12). *A. alternata* is apparently common to many agricultural settings and has previously been reported to cause pod-flecking of snap beans in New York (1).

Ulocladium leaf spot of cucumber has probably occurred in cucumber breeding plots located near Ithaca throughout the 1980s (6; Zitter, *unpublished*), although to our knowledge it has not previously been reported from commercial cucumber fields in the United States. Simmons (11) failed to recover *U. cucurbitae* from cucumbers grown in central Massachusetts after many attempts but invariably recovered *A. alternata* and related species. Our own findings showed that *A. alternata* was routinely recovered during two seasons from leaf spots initiated by *U. cucurbitae*. Unless single-spore cultures were consistently used, the chances for mixed cultures was increased. It is unclear as to why Lane (6) was unsuccessful in infectivity tests with *U. cucurbitae* despite using some of the same susceptible cultivars and lines we tested in various growth stages, very large spore concentrations, and a number of inoculation procedures. He included an illustration of a culture of alternarioid-type spores isolated directly from infected cucumber tissue and a second illustration of a culture of *U. cucurbitae* grown on V-8 agar that is identical to our own cultures (6). Because of the confusion that exists from the occurrence of alternarioid- and ulocladioid-type conidia depending on culturing conditions of *Ulocladium* spp. (8,11), it would be easy to suspect that one was using a culture of *U. cucurbitae*, not knowing that *A. alternata* might also be present.

Although we were successful in inoculating cucumber fruit with *U. cucurbitae*, these conditions were atypical, and no naturally infected fruit have been recovered from the field. From our attempts to infect other cucurbit species, we conclude that only cucumber is susceptible to *U. cucurbitae*.

Ulocladium lesions can develop with only 24 hr of incubation in a mist chamber, but both number and size of lesions increase the longer the plants are incubated with near 100% relative humidity. When plants are exposed to alternating periods of leaf wetting and drying, larger lesions result that are identical to those occurring in the field. Plants were susceptible in all stages of growth and did not require added stress provided by using either different soil mixtures or plants in the fruit-bearing stage.

Previous work with *Ulocladium* in plant breeding plots has relied on naturally occurring inoculum, which indicates that this organism can successfully overwinter in New York. The disease has usually been detected in mid-August and has persisted until killing frosts occurred in the fall. In preliminary studies on the effects of temperature on spore germination and mycelial growth, both sporulation and fungal growth occurred over a wide range of temperatures (9–36 C), but the optimum was 27–33 C. This may explain why this disease has only been observed late in the season following periods of high temperature and high relative humidities. Although the commercial cucumber cultivars we tested are known to be resistant or tolerant to a number of foliar diseases, they are not known to be resistant to Ulocladium leaf spot. Thus it was not surprising that all were susceptible to *U. cucurbitae*. The breeding lines tested were part of an effort to incorporate resistance to target spot and develop cultivars with wider acceptance for use in southern states (6). Target spot has not been found in New York, and thus all screening work for incorporating target spot resistance has utilized artificial inoculations (6,7). Albion 87, Dryden 87, Marketmore 87, and Poinsett 87-8 represent the fifth backcross generation of material resistant to target spot. Although these lines were not specifically selected for resistance to Ulocladium leaf spot, our tests indicate that they are also resistant to *U. cucurbitae* and confirm previous observations (6; Zitter, *unpublished*). Although no inheritance studies have been made, it would appear that the same gene or a closely related gene confers resistance to both diseases.

Studies are currently under way to determine under what conditions alternarioid- and ulocladioid-type conidia of *U. cucurbitae* are produced.

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