Influence of Temperature and Fungicide on Germination, Growth, and Virulence of *Ulocladium cucurbitae* on Cucumber

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ABSTRACT

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Germination of conidia of *Ulocladium cucurbitae* was greatest at 30 C after 1 h, but germination occurred from 9 to 36 C. Growth of cultures from single conidia was greater at 30 C than at 9, 12, or 36 C. Cultures at 8 C, producing alternarioid-type conidia, produced ulocladioid-type conidia within 4 days of transfer to 21 or 27 C. By 10 days, the ulocladioid type predominated. When starting with ulocladioid conidial types, the conidial population shifted to alternarioid types when cultured at 8 C, but maintained the dominant ulocladioid morphology at 21 and 27 C. In pathogenicity tests using conidial suspensions of the two main types, alternarioid suspensions consistently produced more necrotic lesions and larger diameter lesions on susceptible and resistant cucumber leaves and leaf disks than did ulocladioid-type conidia. Increasing ulocladioid-type conidial concentrations resulted in proportionally more infections, but lesion size remained small. The number of germ tubes per conidium was significantly greater for alternarioid and intermediate types than for ulocladioid types. Sensitivity of conidia to benomyl, chlorothalonil, and iprodione was determined in vitro. Conidia were most sensitive to chlorothalonil, followed by iprodione, and were least sensitive to benomyl. Chlorothalonil and iprodione performed equally well as protective sprays, but were no better than benomyl when used as curative sprays on two of the more susceptible cultivars tested.

Additional keywords: conidium morphology, *Cucumis sativus*, Ulocladium leaf spot.

Ulocladium cucurbitae (Letendre and Roumeguere) Simmons causes a leaf spot disease of cucumber, which was first observed in commercial fields in western New York in 1988 (11). Although Simmons (8) identified isolates of *U. cucurbitae* from cucumber (*Cucumis sativus* L.) from New Zealand, Canada, India, and the United States, there have been few previous field reports of this disease. Ulocladium leaf spot may have occurred in cucumber fields in western Czechoslovakia since 1972 and sporadically in glasshouse crops in England since 1974 (1,4).

Simmons (8) recognized variability in morphology of *U. cucurbitae* conidia and felt the fungus may have previously been identified as *Stemphylium ilicis* Tengwall (6), *Alternaria pluriseptata* (Karst. & Har.) Jorstad (2,4), or *Ulocladium atrum* G. Preuss (1). He observed that conidia cultured at 8 or 23 C produced alternarioid- or ulocladioid-type conidia, respectively. Temperature-induced changes in morphology of conidia were also reported for *U. chartarum* (G. Preuss) E. Simmons and *S. floridanum* Hannon and G. Weber (5).

During our initial isolation of *U. cucurbitae*, we consistently encountered conidia that fit the description of *Alternaria alternata* (Fr.:Fr.) Keissl. We subsequently showed that *U. cucurbitae* is responsible for the leaf spot disease in New York and that *A. alternata* occurs as a saprophyte (11). Recently, a leaf spot disease of cucumber and melon caused by a pathotype of *A. alternata* f. sp. *cucurbitae* has been reported from Crete (9,10). Because of the possible confusion surrounding the different conidial types found associated with Ulocladium leaf spot of cucumber, we undertook studies to examine the influence of temperature on germination of conidia, mycelial growth, and conidium morphology. The virulence of conidial types was investigated. The sensitivity of conidia on water agar or cucumber plants to fungicides was examined.

MATERIALS AND METHODS

Inoculum production and preparation. Isolate 313 of *U. cucurbitae* isolated from field-grown cucumber near Ithaca, NY, was used in this study (11). Unless stated otherwise, conidia were obtained from 5- to 7-day-old cultures grown on V8 agar by adding sterile distilled water to the plates and gently scraping the agar surface.

Effect of temperature on conidium germination and mycelium growth. Five milliliters of a conidial suspension containing 2.2×10^4 spores per milliliter was poured onto each water agar plate (WA, Difco Bacto agar, 1.5%, Difco Laboratories, Detroit, MI), the excess liquid was decanted, and the plates were placed upside down in 10 incubators at 9, 12, 15, 18, 21, 24, 27, 30, 33, and 37 C without lights. After 1, 2, 4, 6, 8, 12, and 24 h, petri dishes were removed from each incubator, and 5 ml of 20% formaldehyde solution was added to each plate to inhibit further conidial germination. The germination of approximately 200 conidia for each plate and for each temperature and incubation period was recorded. Two plates were used at each temperature, and the experiment was repeated.

Single conidia from actively growing cultures were transferred to separate plates of potato-dextrose agar (PDA, Difco) and held in incubators at 9, 12, 15, 18, 21, 24, 27, 30, 33, or 37 C. Growth was measured in two perpendicular directions at 2-day intervals through 8 days. Three replicate plates were used for each treatment, and the experiment was repeated.

Effect of temperature on conidium morphology. Conidia of ulocladioid or alternarioid morphology were individually transferred to each of nine petri plates containing V8 agar and incubated under continuous light at 8, 21, and 27 C. Four plates were removed from each chamber after 4 or 10 days, and 100 conidia per plate were examined at random under a microscope at ×320 and categorized as alternarioid, intermediate, or ulocladioid. The experiment was repeated.

Effect of conidial type on pathogenicity to cucumber. Single conidial isolates of *U. cucurbitae* of the two conidial types (A =

alternarioid and U = ulocladioid) grown at 8 and 27 C, respectively, were used in each of four combinations: alternarioid-type conidia grown at 8 C and transferred to 8 or 27 C, or ulocladioid-type conidia grown at 27 C and transferred to 8 or 27 C. All cultures were grown for 10 days on V8 agar under continuous light in constant temperature chambers at 8 or 27 C. The delay in harvesting the cultures was required to allow the alternarioid-type cultures to produce sufficient conidia at 8 C. Two tests were performed with conidial concentrations adjusted to 8×10^4 conidia per milliliter except for the A to A sequence grown at 8 C, which provided only 24,690 conidia per milliliter in the first test, and the U to A combination, which yielded 45,625 conidia per milliliter in the second test. For each test, four pots of the cucumber cultivars Poinsett 76 and Marketmore 76 (both susceptible to Ulocladium leaf spot) were inoculated in the three-leaf stage with the four conidial suspensions by atomizing the conidia onto the upper leaf surface at 10 psi until runoff (a 20-ml suspension was sufficient to spray eight potted plants). In both tests, two pots of each cultivar sprayed with water served as controls. An aliquot of each conidial suspension was saved for determination of conidial type. After inoculation, plants were moved to a mist chamber for 48 h at 25 C with 12 h of fluorescent light. Lesions were counted on the first two true leaves 2 days after incubation.

Leaf disk inoculation. Ten leaf disks 14 mm in diameter were cut at random with a cork borer from fully expanded first true leaves two and three (five disks each) from susceptible Poinsett 76 and Marketmore 76 and resistant Poinsett 87-8 and Marketmore 87 (11). The five disks were placed with the adaxial surface up on each plate of 2% WA. Each disk was inoculated with a 10-μl droplet of *U. cucurbitae* conidial suspension from cultures maintained at 8 C (alternarioid type) or 27 C (ulocladioid type). Two tests (two plates each) were performed using low (4×10^4) and high (8×10^4) conidial suspensions of both conidial types. After inoculation, the petri plates were placed in clear plastic garment boxes lined with moist paper towels and incubated for 7 days at 27 C under fluorescent light (14-hr photoperiod beginning with dark). Measurements were taken at 3 and 7 days. Results are based on the final readings. Treatment means were compared by calculating LSD at $P = 0.05$.

Germ tube development for alternarioid-, intermediate-, and ulocladioid-type conidia. Fourteen-day-old cultures of *U. cucurbitae* grown continually at 8 and 27 C were used to determine germ tube development. A 2×10^4 dilution was prepared for each culture, and both were added separately to WA plates. After 1 min, the liquid was decanted, and the plates were incubated at 21 C under constant light. After 24 h of incubation, 5 ml of 20% formaldehyde was added to stop further germination. The number of germ tubes produced for 100 conidia for each of the three types was recorded. Two replicate plates were used for each suspension. The experiment was conducted twice, and the data were analyzed by analysis of variance.

Fungicide sensitivity. Inhibition of alternarioid and ulocladioid conidial germination was determined in vitro using fungicide-amended agar. Chlorothalonil, iprodione, and benomyl were used to amend autoclaved WA to give final suspensions of 10, 100, 1,000, and 5,000 ppm (to achieve final concentrations of 0.01, 0.1, 1, and 5 μg a.i./ml). The media were prepared 1 day before use. A conidial suspension at 22,500 conidia per milliliter was atomized onto the fungicide-amended media and incubated at 21 C for 24 h under constant light. After the incubation period, further spore germination was inhibited by adding 5 ml of 20% formaldehyde solution to each plate. Spore germination was determined by counting the germinated spores out of 100 on each replicate plate. A conidium was considered germinated if its germ tube length was equal to the width of the conidium. Four replicate plates were used for each treatment, and the experiment was repeated. The mean percentage of germination for each treatment in the two experiments was expressed as a percentage of the results obtained from the control plates.

The same fungicides were used to study their effectiveness in controlling *U. cucurbitae* applied as either protective or curative sprays. Iprodione and benomyl were used at 0.5 g a.i./L and chlorothalonil at 1.5 g a.i./L. The cucumber cultivars Dasher II, Marketmore 76, Raider, and Pacer in the three-to-four leaf stage were used. Two replicate plants were inoculated for each treatment, and both fungicide experiments were repeated. For protective sprays, the fungicides were applied with a hand sprayer, and the plants were allowed to dry for 24 h before inoculation. A conidial suspension containing 8×10^4 conidia per milliliter was atomized onto the adaxial surface at 10 psi until runoff. Plants sprayed with water and later inoculated served as controls. After inoculation, the plants were held in a mist chamber at 24 C for 48 h before returning them to a greenhouse at 29 C. The

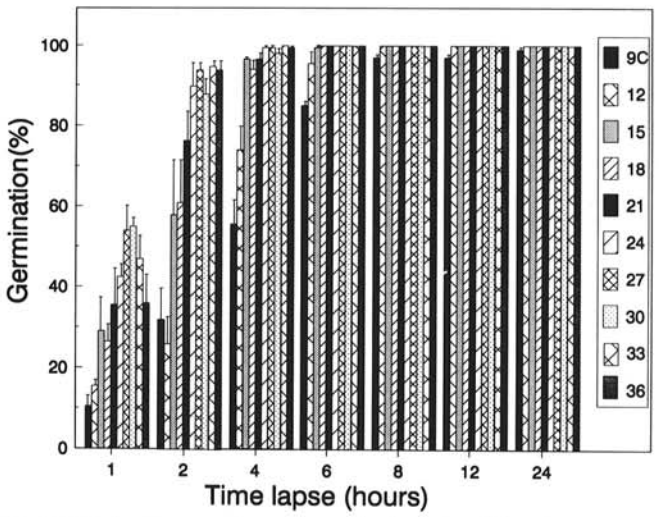

Fig. 1. Effect of temperature on germination of conidia of *Ulocladium cucurbitae* on Difco Bacto water agar. Error bars represent the standard errors of individual means.

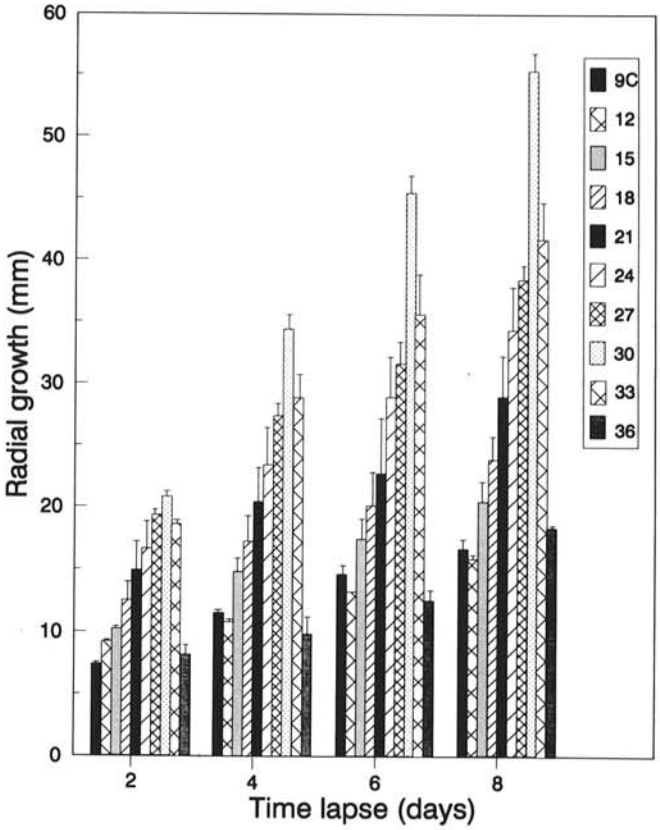

Fig. 2. Effect of temperature on mycelial growth of *Ulocladium cucurbitae* on Difco potato-dextrose agar. Error bars represent the standard errors of individual means.

curative effects of the fungicides were tested by inoculating plants 24 h in advance of spraying with fungicide. Plants were allowed to dry before placing them in a mist chamber for 48 h. Lesions were counted on the first three true leaves 11 days after inoculation, and the average number of lesions was calculated.

RESULTS

Effect of temperature on conidial germination and mycelium growth. Conidia germinated on WA at 9–36 C within 24 h. One hundred percent germination occurred by 6 h at 24–36 C (Fig. 1). Radial growth from single conidia increased from 2 through 8 days at 9–36 C (Fig. 2). Growth was slowed at 9, 12, and 36 C, and optimal growth occurred at 30 C at each recording time.

Effect of temperature on conidium morphology. After 4 days at 8 C, no alternarioid or ulocladioid conidia were present. By 10 days, alternarioid conidia predominated (Table 1). Ulocladioid-type conidia predominated at 27 C. Alternarioid and intermediate types predominated after 4 days at 21 C. After 10 days, the majority of the conidia were ulocladioid. Ulocladioid conidia predominated within 4 days at 21 C and continued to increase in number through 10 days. The conidia produced at 27 C were uniformly blackish-brown in color. The alternarioid conidia at 8 C were mainly dark brown but some appeared light yellow. For many of the alternarioid conidia the apical cell continued to grow, becoming conidiogenous at the tip of the false beak (see Ref. 7, Fig. 25, upper drawing).

Effect of conidium type on pathogenicity to cucumber. More necrotic lesions resulted from inoculation with alternarioid and intermediate types than with ulocladioid types (Table 2). This difference occurred whether the alternarioid-type conidia originated from cultures continually maintained at 8 C or reverted to the alternarioid type when ulocladioid-type cultures were moved from 27 C to the lower temperature. Fewer lesions occurred when the ulocladioid-type conidial suspensions were used as inoculum (ulocladioid conidia accounted for 95% of the suspension). In both instances in which less than 8×10^4 conidia of the alternarioid type were available for inoculation, the number of lesions that developed was still greater than the number resulting with ulocladioid inoculum and was equivalent to the amount that developed when the higher concentration of alternarioid-type inoculum was used. None of the control plants developed symptoms.

Leaf disk inoculations. Stronger infectivity as determined by

TABLE 1. Effect of temperature on conidium morphology of *Ulocladium cucurbitae* after 4 and 10 days

Temperature (C)	Initial conidial type	Percentage of conidium by morphological type[a]					
		Alternarioid[b]		Intermediate		Ulocladioid	
		4 days	10 days	4 days	10 days	4 days	10 days
8	Alternarioid	NA[c]	87 ± 1.3	NA	13 ± 1.3	NA	0 ± 0.0
	Ulocladioid	NA	80 ± 1.5	NA	19 ± 1.8	NA	1 ± 0.7
21	Alternarioid	19 ± 3.7	8 ± 0.4	63 ± 3.9	12 ± 1.3	18 ± 2.4	80 ± 1.4
	Ulocladioid	14 ± 3.2	6 ± 0.6	16 ± 2.1	9 ± 1.7	70 ± 4.4	85 ± 3.1
27	Alternarioid	7 ± 2.7	13 ± 1.1	18 ± 2.4	14 ± 3.8	75 ± 3.6	73 ± 4.8
	Ulocladioid	7 ± 0.5	9 ± 1.8	6 ± 1.1	10 ± 2.1	87 ± 1.6	81 ± 3.7

[a] Conidial types are the means with standard error of means of eight plates (four per experiment) with 100 conidia recorded per plate.
[b] Refer to Figure 3 for conidial appearance.
[c] Conidia with this morphology were not available.

TABLE 2. Effect of ulocladioid- and alternarioid-type conidia of *Ulocladium cucurbitae* produced at 27 and 8 C, respectively, on pathogenicity to two cucumber cultivars

Initial conidial type	Temperature (C)	Subsequent conidial type	Temperature (C)	Number of necrotic lesions[a]		Percentage of conidial suspension composition		
				Poinsett 76	Marketmore 76	Ulocladioid	Intermediate	Alternarioid
Ulocladioid	27	Ulocladioid	27	45 ± 5.8[b]	26 ± 6.1	95 ± 0.0	5 ± 0.0	0 ± 0.0
Ulocladioid	27	Alternarioid	8	114 ± 21.7	104 ± 20.0	2 ± 1.5	28 ± 7.5	70 ± 6.0
Alternarioid	8	Ulocladioid	27	56 ± 6.8	30 ± 6.5	95 ± 0.5	5 ± 0.5	0 ± 0.0
Alternarioid	8	Alternarioid	8	119 ± 19.5	113 ± 21.5	10 ± 0.0	52 ± 19.5	38 ± 14.5

[a] Lesion counts were made 2 days after incubation.
[b] Data represent the mean number of lesions with standard error of means for leaves one and two from eight plants for each cultivar (four plants for each experiment).

TABLE 3. Effect of alternarioid- and ulocladioid-type conidial suspensions at two concentrations on development of leaf spot in *Ulocladium cucurbitae* susceptible and resistant cucumber leaf disks

Inoculum	Concentration[b]	Diameter of necrotic lesions (mm)[a]			
		Susceptible		Resistant	
		Poinsett 76	Marketmore 76	Poinsett 87-8	Marketmore 87
Alternarioid conidia	Low	3.70	3.75	1.30[c]	2.75[c]
	High	3.95	4.15	1.64[c]	2.75
Ulocladioid conidia	Low	1.23[c]	1.60[c]	0.85[c]	1.52[c]
	High	1.77[c]	2.50[c]	1.23[c]	1.83[c]
Water control		0	0	0	0
LSD		0.98	1.22	0.71	0.84

[a] Lesions were measured 7 days after inoculation of leaf disks.
[b] Concentrations: low = 4×10^4; high = 8×10^4.
[c] Consists of one or more small necrotic specks (0.1–0.5 mm diameter), which were totaled to give the approximate lesion size.

lesion size was also shown when cucumber leaf disks were inoculated. Lesions developed when the two inoculum types were tested at the low concentration (4×10^4), but lesions were significantly smaller on Poinsett 76, Marketmore 76, and Marketmore 87 when inoculated with ulocladioid suspensions (Table 3). Lesions appeared as a general chlorotic spot that, when examined under magnification (×200), revealed distinct necrotic specks 0.1–0.5 mm in diameter. At the high concentration, alternarioid conidia produced significantly larger diameter lesions on the two susceptible cultivars and on Marketmore 87 than the ulocladioid inoculum. No lesions or water-soaked spots developed on any of the disks inoculated with water.

Germ tube development. A significant difference in the number of germ tubes was noted with each conidial type (Fig. 3). The means and ranges were as follows: ulocladioid, 2.5 (range 1–5 germ tubes); intermediate, 4.3 (range 2–8); alternarioid, 5.1 (range 1–9). The LSD for mean separation ($P = 0.05$) was 0.25. Intermediate-type conidia were only found on plates originating from the alternarioid type at 8 C.

Fungicide sensitivity. Chlorothalonil-amended WA gave reduced conidium germination at the two lowest concentrations (Fig. 4). Iprodione resulted in a significantly greater reduction in germination at 1.0 μg a.i./ml when compared with chlorothalonil. Benomyl had no effect on conidium germination when tested at the three lowest concentrations. In general, little difference in fungicide performance was noted between the conidial types, although chlorothalonil tended to show slightly greater activity against alternarioid conidia.

Chlorothalonil and iprodione applied as protectants were more effective than benomyl in controlling Ulocladium leaf spot (Table 4). When used as a curative spray, chlorothalonil and iprodione were no better than benomyl when tested on the two most susceptible cultivars (Dasher II and Marketmore 76), but were better than benomyl on Raider and Pacer cultivars.

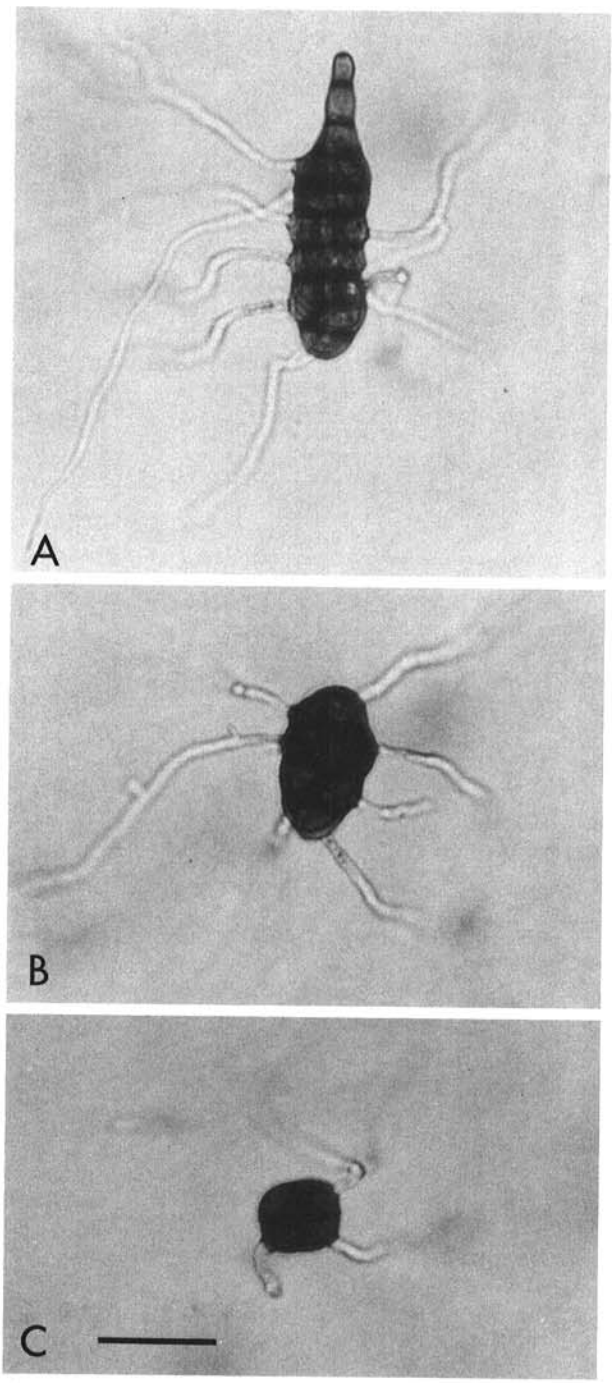

Fig. 3. Morphology of three germinated *Ulocladium cucurbitae* conidia after 24 h at 21 C on water agar. **A,** alternarioid; **B,** intermediate; **C,** ulocladioid. Scale bar = 10 μm.

DISCUSSION

In this study, conidial germination and growth are optimal at 30 C. When compared with similar diseases of cucumber, these values are only slightly higher than those reported for *A. alternata* f. sp. *cucurbitae* (10), and similar to optimal conditions reported for *A. cucumerina* (Ellis & Everh.) J. A. Elliott (3). Ulocladium leaf spot was first found in commercial fields in western New York in 1988, a growing season noted for unseasonably high temperatures (11). The high temperature requirement may explain in part its prominence that season, although the disease has recurred at varying levels of infection each season in breeding plots near Ithaca (11).

This study corroborates reports of marked changes in conidial morphology of *U. cucurbitae* and other *Ulocladium* spp. as influenced by temperature (5,7,8). Low temperatures (8 C) favored alternarioid-type conidia, whereas at higher temperatures (27 C),

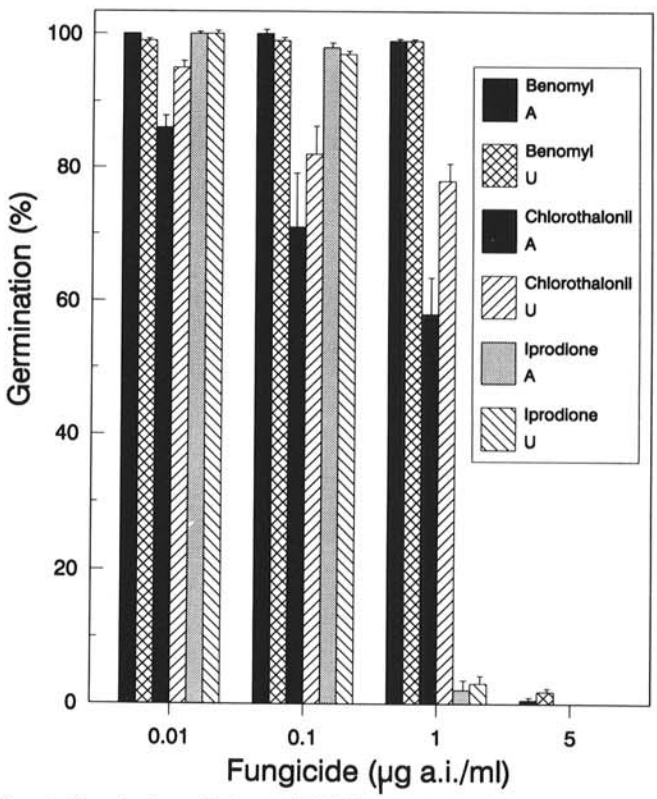

Fig. 4. Germination of alternarioid (A) and ulocladioid (U) conidia of *Ulocladium cucurbitae* on fungicide-amended water agar. Error bars represent the standard errors of individual means.

TABLE 4. Effectiveness of fungicides applied as protective and curative sprays to cucumber against *Ulocladium cucurbitae*

Treatment	Average number of lesions[a]							
	Protective				Curative			
	Dasher II	Marketmore 76	Raider	Pacer	Dasher II	Marketmore 76	Raider	Pacer
Chlorothalonil (Bravo 500)	6 ± 2.3[b]	0.5 ± 0.2	0.9 ± 0.5	1.4 ± 0.9	43 ± 10.8	30 ± 8.4	23 ± 4.3	7 ± 2.1
Iprodione (Rovral 50WP)	10 ± 2.8	3 ± 0.9	6 ± 2.4	1.4 ± 0.6	48 ± 9.6	28 ± 7.1	14 ± 4.5	8 ± 2.3
Benomyl (Benlate 50DF)	57 ± 23.5	43 ± 16.5	36 ± 7.7	19 ± 5.0	49 ± 9.0	30 ± 6.9	66 ± 21.3	29 ± 5.6
Control	63 ± 13.9	42 ± 8.9	43 ± 14.1	18 ± 4.5	53 ± 10.7	33 ± 6.8	34 ± 5.7	14 ± 3.4

[a] Results of two trials (two plants per trial) with lesions counted on the first three true leaves.
[b] Standard error of means.

ulocladioid conidia predominated (Table 1). The changes in conidial morphology are not restricted to culture plates. When leaves inoculated with alternarioid and ulocladioid conidial suspensions were examined after 2 days of incubation in a moist chamber at 21 C, the sporulating lesions had predominately alternarioid and intermediate conidial types.

Conidial morphology was correlated with greater pathogenicity for cucumber in two ways. A significantly greater number of necrotic lesions developed on whole plants when inoculated with alternarioid conidia. The resulting lesions covered most of the leaf surface. Ulocladioid inocula produced fewer lesions that frequently appeared near the margins. When alternarioid and ulocladioid conidial suspensions were tested on individual leaf disks, alternarioid conidia caused larger diameter lesions on both susceptible and resistant cultivars. Poinsett 87-8 exhibited good resistance, which agrees with earlier findings (11).

An obvious difference in pathogenicity between the two main conidial types appears to be related to conidial cell number and the number of germ tubes that develop. Both the alternarioid and intermediate types produced significantly more germ tubes than did the ulocladioid types. Although germ tube number was not directly correlated with infection efficiency or lesion size, this condition should provide a competitive advantage for producing earlier and a greater number of successful infections, even under varying weather conditions.

As far as we are aware, this is the first report demonstrating increased pathogenicity associated with conidial cell number. Practical implications include seasonal disease occurrence, attempting to reproduce disease symptoms depending on which conidial types are selected, and influencing the evaluation of cultivars as to their susceptibility or determining the level of resistance in breeding lines. In nature, conidia would be subjected to the range of temperature fluctuations as examined in this study. Temperate growing conditions in New York State (warm dry days and cool moist nights) would favor development of alternarioid and intermediate conidial types and would result in the large and frequently coalescing necrotic lesions as found in this study and observed in the field (11). The specific moisture and temperature requirements for conidial development in nature have not been established.

Chlorothalonil is currently not labeled for the control of Ulocladium leaf spot. The results of this study and previous field tests (12) demonstrate that chlorothalonil is effective when used as a protective spray. These results agree with the known activity of this product against *Alternaria* and closely related fungi (10).

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