Effects of Temperature, Moisture, and Cucumber Cultivar Resistance on Lesion Size Increase and Conidial Production by *Colletotrichum lagenarium*

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ABSTRACT

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Conidial production by *Colletotrichum lagenarium* on *Cucumis sativus* 'Calypso' in the greenhouse, increased linearly over 48 hr when free moisture was maintained on leaves, but remained low if free moisture was not maintained. Lesions appeared 7, 6, 6, 5, and 7 days after inoculation at constant 16, 20, 24, 28, and 32 C, respectively. Initially, lesion size increase was more rapid at 20, 24, or 28 C than at 16 or 32 C. Later, however, lesions were significantly larger at 16 C than at other temperatures and that increase was best described with a quadratic function. Lesion size increase from 20 to 32 C was best described with linear functions. Conidial production commenced when lesions became visible and was greatest at 24 C in the early stages of lesion size increase, but total conidial production was greatest at 16 C and became progressively less at higher temperatures. Quadratic functions best described conidial production. Conidial production was greater on plants that had been misted during the night prior to sampling than on nonmisted controls, however, misting nightly did not increase conidial production or lesion size over plants misted once prior to sampling. Lesion size increase and conidial production were greater on older leaves than on younger leaves. Leaf area increased and the proportion of old necrotic leaves decreased as temperature increased, indicating that higher temperatures were more favorable for growth of the cucumber plants. In seven field environments, lesion appearance and conidial production were initiated 5 or 6 days after inoculation. Lesion size increase and conidial production were 2–4 times greater on the susceptible cultivar Earlipik 14 than on cultivars Calypso or Calico. A third-order polynomial based on time from inoculation explained approximately 50% of the variation in lesion size increase and conidial production; a model composed of environmental variables (degree-days, hours of leaf wetness, hours of RH >85%, daily maximum temperature >32 C, irrigation, and rain) explained only about 20%. Combining time with environmental variables in one model did not increase predictive accuracy. In field experiments, more conidia were present in early morning samples (66,176 per lesion) than in late afternoon (38,678). In all studies, the leaves became senescent or necrotic before lesion size increase and conidial production ceased.

Additional key words: anthracnose, *Cucumis sativus*, disease cycle components.

With improved knowledge of plant disease epidemics it should be possible to develop more efficient and dependable methods for disease management (6). The infection cycle during an epidemic has three major components: sporulation, dissemination, and infection (2) that are amenable to environmental influences and thus are epidemiologically distinct (11). The components can be subdivided and studied quantitatively so that, in principle, a complete analysis of the infection cycle is possible (11).

Anthracnose, caused by *Colletotrichum lagenarium* (Pass.) Ell. & Halst., is one of the main leafspot diseases on *Cucumis sativus* L. in the southeastern United States. *C. lagenarium* produces its conidia singly from conidiophores on lesions and the aggregate of conidia forms a sticky water-soluble matrix (4). In *C. graminicola* this matrix is composed of polysaccharides and proteins (9). The infection cycle of *C. lagenarium* has not been studied quantitatively or in detail, although previous studies have qualitatively evaluated the influence of temperature and moisture on anthracnose development (4,5,7,8). Littrell and Epps (8) found that 12 hr in a mist chamber were necessary for substantial anthracnose development and that 16–24 hr in a mist chamber were required for maximum disease development. Increasing the temperature from 18 to 24 C or 21 to 27 C resulted in higher levels of disease after 10 hr; however, similar disease levels occurred both before and after the rapid increase in disease following 10 hr in the mist chamber. Layton (7) and Littrell and Epps (8) observed no disease development at 10 C and increasing rates of development as temperatures maintained after infection were increased from 17 to 30 C. No quantitative evaluations of lesion size and conidial production by *C. lagenarium* have been done.

The objective of this study was to assess the influence of temperature, moisture, and cultivar resistance on lesion size increase and conidial production by *C. lagenarium* on cucumbers in controlled environments and in the field.

MATERIALS AND METHODS

Greenhouse experiments. Cultivar Calypso cucumber plants 3 to 5 wk old with well developed, 10–20 mm^2 anthracnose lesions were sprayed with distilled water from an atomizer. Misted plants were covered with polyethylene bags, and placed in a mist chamber where free moisture was maintained on the leaves. Similar plants were left on the greenhouse bench where free moisture was not maintained on the leaves. Mist chamber and greenhouse temperatures were similar and ranged from 20 to 26 C. Ten lesions were randomly sampled on three plants in each moisture regime after 6, 9, 12, 18, 24, and 48 hr. Lesions were excised from the leaves and the conidia were washed from them into distilled water in a vortex test tube mixer. The estimated numbers of conidia per lesion were calculated by averaging four hemacytometer counts per sample. All experiments were done twice.

Controlled-environment experiments. Calypso cucumber plants 3 to 4 wk old were sprayed to run off with an atomized suspension of 25,000 conidia per milliliter and placed in a mist chamber at 24 C for 24 hr. Plants were then moved to individual A-chambers (1) at constant 16, 20, 24, 28, or 32 C with a 12-hr photoperiod. The standard light sources (fluorescent and incandescent), the standard soil mix, and fertilizer have been previously described (1). Fertilizer solution was applied daily. The night before plants were to be

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sampled, they were sprayed with distilled water and covered with a large polyethylene bag; care was taken to not touch the leaves.

When lesions became visible, ten lesions were removed from each of six plants per temperature treatment. Conidia were collected and counted as previously described. Lesion diameters were measured and lesion areas calculated. Lesions were sampled daily for the first 16 days, after which they were sampled at 2–3 day intervals. Plants sprayed with distilled water and placed in polyethylene bags the night prior to sampling were compared to nonmisted or dry plants. In the second experiment, to simulate nightly dew in the field, a group of plants was sprayed with distilled water and placed in polyethylene bags nightly for 16 days after inoculation. Other plants were sprayed with distilled water and placed in polyethylene bags the night prior to sampling. Lesion size increase and conidial production was compared on older (first through third from base of the plant) and younger (fifth through seventh) leaves. Leaf area per plant was measured once in the first experiment and twice in the second experiment, with an Automatic Area Meter (Hayashi Denko Co., Ltd., Tokyo, Japan). The experiments were performed twice over time with random reassignment of a specific temperature to each controlled-environment chamber in each experiment. The experimental design was a randomized complete block with temperatures as blocks and sampling times as subplots.

Field experiments. Lesion size increase and conidial production were evaluated during 1982 and 1983 in seven field plots at Clinton or Castle Hayne, NC. Spring crops were planted in May, and fall crops were planted in late July or early August. The cucumber cultivars Earlipik 14, Calypso, and Calico, that are highly susceptible, moderately resistant, and highly resistant to anthracnose, respectively, were used. The cultivars were overseeded into rows 1.0 m apart, on raised, shaped beds 0.5 m across the top and thinned to a density of 49,000-59,000 plants per hectare. Plots were at least four rows wide and 3.7 m long. Fertilizer applications were based on soil analysis and herbicides and insecticides were applied as needed (3). Overhead sprinkler irrigation was applied as needed to provide 2.5 cm of water per week. Sampling intervals varied from 1 to 8 days but were close together during the early stages of anthracnose development.

Microclimatic data were collected with standard one-scan-per-minute CR 21 Microloggers (Campbell Scientific, Logan, UT). Most parameters were stored on audio cassette tapes as 60-min average, sum, or sample. Temperatures were measured with Fenwal model UUTR51J1 thermistors (obtained as Model 101 from Campbell Scientific). Free moisture was detected with wetness sensors (Dewsensor; obtained as Model 731 from Campbell Scientific). Wetness sensors were painted three or four times with flat green acrylic latex paint (Weatherbeater, Meadow Green #077; Sears, Roebuck and Co., Chicago, IL) and mounted on wooden stakes. They were placed in horizontal orientation at heights of 20 and 30 cm within and outside of the plant canopy, respectively. Rains and sprinkler irrigations were monitored with tipping-bucket rain gauges (Sierra-Misco; obtained from Campbell Scientific) at a height of 1.50 m. Wet- and dry-bulb temperatures were measured at a height of 40 cm with aspirated (2–3 m/sec) thermistors placed in an insulated polyvinylchloride pipe. Aspiration was done with an electric motor (Dayton model 2M271, Dayton Mfg. Co., Chicago, IL) attached to a 7.6-cm-diameter fan blade and powered by 12 VDC. Within-canopy temperatures were measured with nonaspirated thermistors at a height of 10 cm, under plant leaves. Relative humidity (RH) was calculated from wet and dry bulb temperatures. Leaf wetness and wet bulb temperatures were not monitored at Castle Hayne, NC. Tests in the spring of 1982 at Clinton, NC, were 300 m from a weather station.

All plants were inoculated with *C. lagenarium* race 1 (No. 52609, American Type Culture Collection, Rockville, MD) (4) by spraying a suspension containing 25,000 conidia per milliliter, 15 ml per plot, onto plants at the two- to four-leaf stage except the 1983 spring crop in which plants were inoculated when they were beginning to flower. At least 10 lesions were removed from two plots at each

TABLE 1. Influence of free moisture and temperature on conidial production per lesion by *Collectotrichum lagenarium* on *Cucumis sativus* cultivar Calypso in controlled-environment chambers

Temperature (C)	Free moisture[a]	Conidia per lesion at postinoculation day: 11	21	24
16	No	2,292	4,236	5,069
	Yes	6,435	18,511	50,000
20	No	625	2,639	1,945
	Yes	8,750	22,732	25,069
24	No	0	347	347
	Yes	7,639	14,792	4,653
28	No	694	1,597	0
	Yes	2,639	3,264	2,222
32	No	0	243	556
	Yes	70	0	1,042
C.V.		129	151	178
FLSD, $P = 0.05$		715	4,709	7,376

[a]Plants were atomized with water until the point of run off and then covered with a large polyethylene bag for a 12-hr dark period prior to sampling for conidial production.

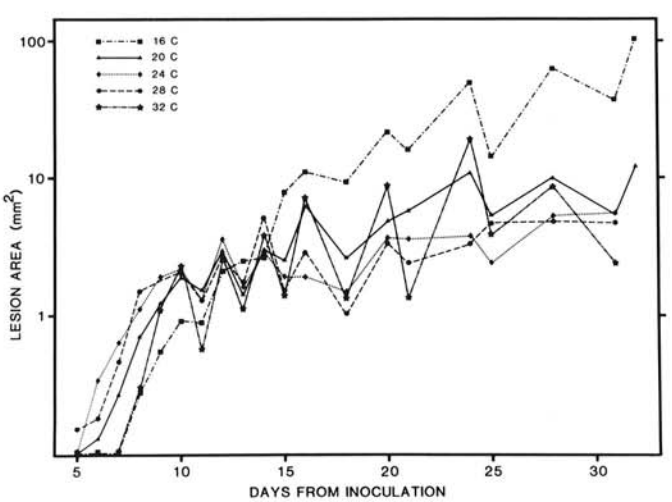

Fig. 1. Effect of temperature on average size increase of lesions by *Colletotrichum lagenarium* on *Cucumis sativus* cultivar Calypso in controlled environment chambers. Lesion area is plotted on a log scale.

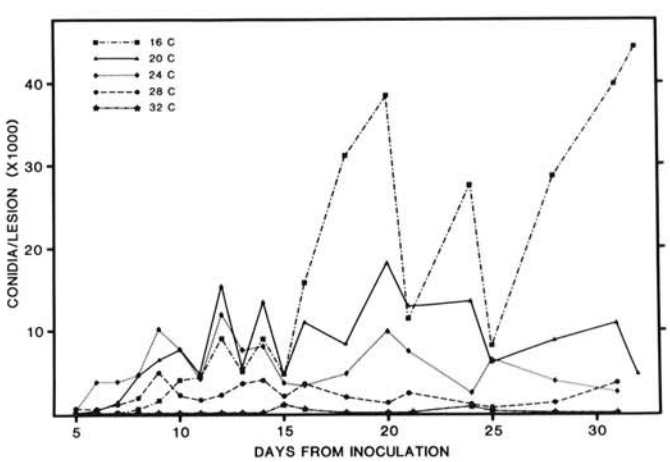

Fig. 2. Conidial production on lesions caused by *Colletotrichum lagenarium* on *Cucumis sativus* cultivar Calypso in constant temperature controlled-environment chambers.

TABLE 2. Influence of leaf age and temperature on lesion area and conidial production per lesion by *Colletotrichum lagenarium* on *Cucumis sativus* cultivar Calypso in controlled-environment chambers

Temperature (C)	Leaf age[a]	Lesion area (mm^2) at postinoculation day:				Conidia per lesion at postinoculation day:			
		10	13	20	25	10	13	20	25
16	Old	1.61	2.51	29.51	23.01	7,431	8,333	46,852	15,463
	Young	0.26	2.53	14.34	7.07	695	1,458	18,820	2,222
20	Old	3.01	1.94	6.26	7.19	14,097	9,653	29,097	8,264
	Young	0.69	0.79	3.41	3.36	1,320	1,042	7,570	3,889
24	Old	3.89	2.93	6.40	2.53	14,306	15,000	15,972	12,837
	Young	0.51	0.20	1.07	2.26	1,111	278	4,074	208
28	Old	4.14	3.42	6.26	5.08	4,236	7,245	2,152	0
	Young	0.09	0.06	0.55	4.46	70	0	185	1,042
32	Old	4.03	2.20	14.56	5.74	139	77	0	540
	Young	0.54	0.06	3.10	2.32	0	0	69	0
FLSD, $P = 0.05$		0.34	0.58	4.47	1.86	1,810	1.622	8,479	6,165

[a]Old = first to third leaf, and young = fifth to seventh leaf from base of the plant.

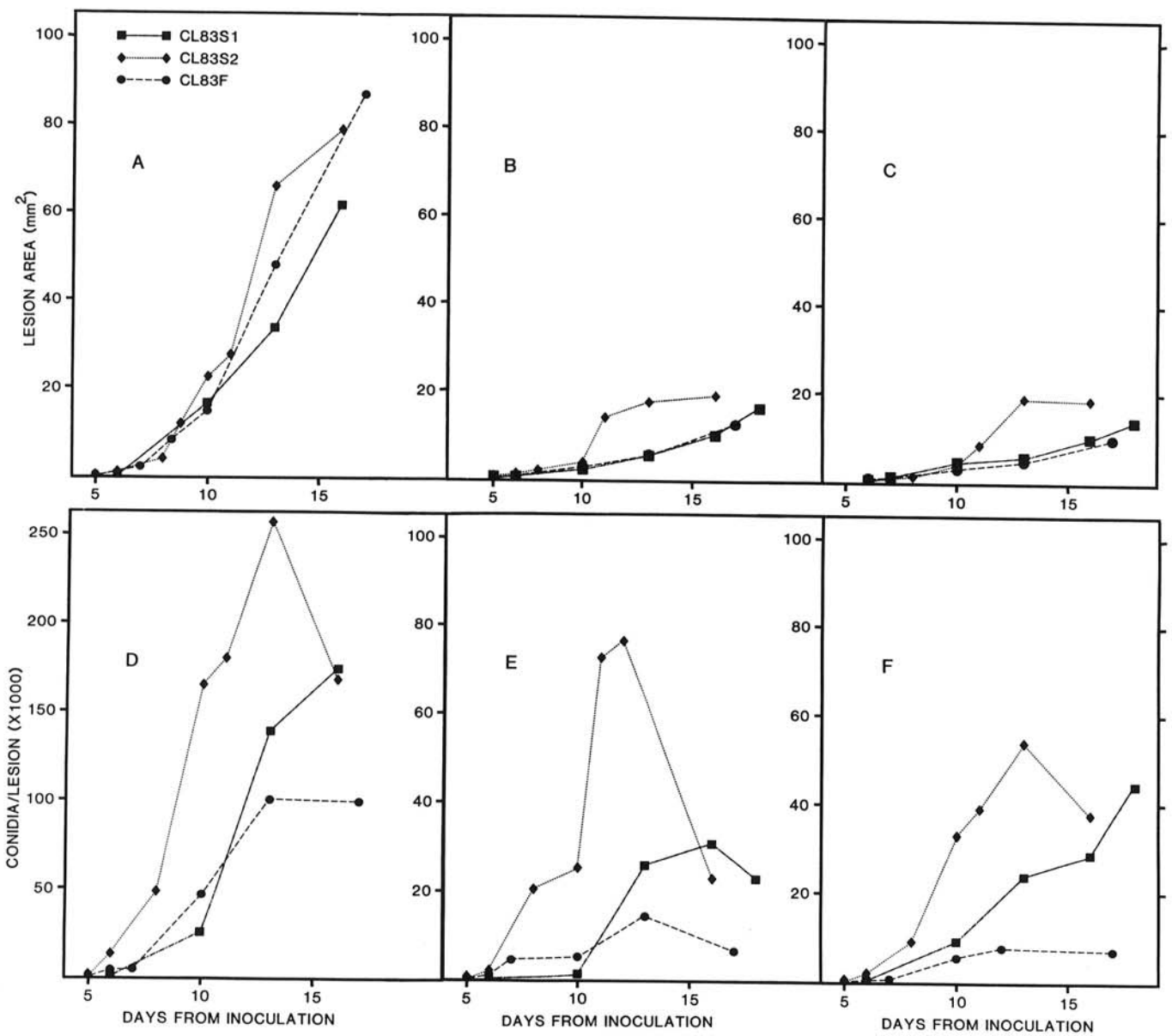

Fig. 3. Lesion area and conidial production by *Colletotrichum lagenarium* on three cultivars of *Cucumis sativus* in the field during 1983. Lesion areas on cultivars **A,** Earlipik 14; **B,** Calypso; and **C,** Calico and conidial production on **D,** Earlipik 14; **E,** Calypso; and **F,** Calico. Legend: CL = cultivar Clinton, NC 83 = North Carolina 1983; S = spring crop; F = fall crop; 1 = Test 1; and 2 = Test 2.

sample time and evaluated for lesion size increase and conidial production as in the controlled-environment studies. Sampling was discontinued after 15–26 days either because inoculated leaves became senescent or necrotic or because infections from other pathogens confounded sampling. The number of conidia collected from lesions in the early morning was compared with the number collected in the late afternoon on 14 separate days.

The experimental design was a completely randomized design with three cultivars and four replications. Data were subjected to analysis of variance and means were compared by using Fisher's least significant difference (FLSD) values at $P = 0.05$. Multiple regression and contrasts were used to compare lesion size increase and conidial production development over time. A variable was considered to have a significant effect if the partial regression coefficient was significantly greater than zero at $P = 0.05$.

RESULTS

Greenhouse experiments. Few conidia were produced when free moisture was not maintained on the leaves during the entire 48-hr period. Conidial production increased linearly over the 48-hr period when free moisture was maintained, and differences between free and no free moisture treatments were observed beginning at 6 hr.

Controlled-environment experiments. Lesions were observed after 5 days at 28 C, 6 days at 20 and 24 C, and 7 days at 16 and 32 C (Fig. 1 [note logarithmic scale]). Lesion size increase was initially more rapid at 20, 24, or 28 C than at 16 or 32 C. After the first few days, lesion size increase was similar at 24, 28, and 32 C. At 16 C, the lesions became significantly ($P = 0.05$) larger than those at other temperatures. Lesion size increase at 16 C was best described with a quadratic function. Lesion size increase at 20, 24, 28, or 32 C was best described with linear functions.

Conidial production commenced when lesions became visible. Total conidial production over 32 days was greatest at 16 C, followed by decreasing production at 20, 24, and 28 C (Fig. 2). Few conidia were produced at 32 C. The measurement of lesion area and conidial production was discontinued after 32 days because the older inoculated leaves became senescent or necrotic, and few to no lesions were available for sampling. Conidial production over time was best described with a quadratic function.

Conidial production was greater in lesions on plants misted during the night before sampling than on nonmisted plants at 16, 20, and 24 C (Table 1). However, the difference at 24 days after inoculation was not significant at 24 C. Conidial production at 28 C at 11 days after inoculation was also significantly greater on misted plants. Daily misting compared to single misting of plants had no consistent effect on lesion size increase or conidial production at 20–32 C except that daily misting significantly ($P = 0.05$) increased conidial production at 16 C at 12 days after inoculation.

Lesions were larger on older leaves than on younger leaves at all temperatures on days 10, 13, and 20. The difference became less pronounced on day 25 at 20–32 C, but not at 16 C (Table 2). In general, conidial production was greater on older leaves than on younger leaves at 16–24 C and at 28 C, 10 and 13 days after inoculation.

Leaves of plants grown at the higher temperatures (24–32 C) were darker green and generally larger than those grown at 16 or 20 C. Leaf area increased as the temperature increased (Table 3). The number of older leaves that became senescent or necrotic from lesions appeared to be greater at the lower temperatures. A random sample of plants on day 28 of the second experiment showed that there were significantly more necrotic leaves at 16 C than at 24–32 C (Table 3).

Field experiments. Lesions appeared and conidial production was initiated 5–7 days after inoculation in all field tests (Figs. 3). Lesion size increase and conidial production were not significantly greater on Calypso than on Calico, but were two to four times greater on Earlipik 14 ($P = 0.05$). Final sample lesion sizes at 15 days after inoculation were 65–90, 15–35, and 10–30 mm for Earlipik 14, Calypso, and Calico, respectively. The maximum number of conidia found per lesion was 260,000, 100,000, and 85,000 for Earlipik 14, Calypso, and Calico, respectively. Lesion size increase and conidial production were generally lower in 1983 than in 1982. Lesion size increase was less variable than conidial production in the seven different test environments.

Regression models indicated that a third-order polynomial was necessary to describe lesion size increase and conidial production in the field when only time from inoculation was considered (Table 4). When only environmental conditions were considered, lesion size increase was not significantly influenced by any of the environmental variables. Leaf wetness, rain, and irrigation had a negative influence on conidial production in the field. High RH and temperatures exceeding 32 C were positively correlated with conidial production.

Combining time from inoculation and the environment in one model resulted in an increased negative relationship of both high RH and days exceeding 32 C and the positive relationship of rain with lesion area (Table 4). Combining time from inoculation and the environment in one model for conidia per lesion decreased the relationship of degree days and temperatures that exceeded 32 C and changed the relationship of irrigation with conidia per lesion. Residual patterns did not differ for the two separate or the combined models of lesion size increase and conidial production. Coefficients of determination (R^2) were greater in the models using time from inoculation alone compared to environmental variables alone. The models combining time from inoculation and environmental variables had the highest R^2 values.

The numbers of conidia collected from lesions from 0700 to 0900 hours were greater in 12 of 14 days than those collected from 1600 to 1800 hours on the same days. An average of 66,176 conidia per lesion were collected in the morning and 38,678 in the afternoon.

DISCUSSION

Anthracnose development on cucumber involves a series of processes including conidial germination, appressorial formation, host penetration, host colonization, and conidial production (4). Lesion sizes in controlled-environment studies did not significantly differ between 20 and 32 C. Lesion size was largest at 16 C. Lesion size increase in the field was correlated with an increase in degree days, calculated between 10 and 32 C (Fig. 3). Lesion size increase was inversely correlated with temperatures exceeding 32 C. Conversely, in the controlled environments lesion areas at 32 C were frequently greater than at 20–28 C and only slightly less than at 16 C (Fig. 1). Temperatures of 16 C were not common in these field studies, occurring only at the beginning of spring and the end of fall crop seasons.

Size increase of lesions caused by *C. lagenarium* on cucumbers in the field was determined mostly by the length of time from inoculation and was less affected by variaion in environmental conditions. Combining time from inoculation and environmental parameters in one model did not increase the amount of variation in lesion size increase. Regression models using time from inoculation, environmental variables, or their combination explained 48–57% of the variation in lesion size increase.

TABLE 3. Influence of temperature on leaf area production and the senescence of older leaves of *Cucumis sativus* cultivar Calypso in controlled-environment chambers

Temperature (C)	Leaf areas[a] at day: 13	22	24	Necrotic old leaves[a] (no.) at day 28
16	1,092	1,242	1,606	3.5
20	1,185	1,356	1,696	3.0
24	1,311	1,697	1,911	1.8
28	1,434	1,571	1,872	2.8
32	1,384	1,770	2,323	2.3
FLSD, $P = 0.05$	77	161	157	0.7

[a]Average of four plants.

TABLE 4. Regression of lesion area and conidial production on days from inoculation and cumulative environmental variables of seven field environments

	Multiple regression models[a]										
Dependent variable[b]	Intercept	(DFI)[b]	$(DFI)^{2b}$	$(DFI)^{3b}$	Degree days[c]	Hours of: leaf wetness	Hours of: RH>85%	Days >32C	Rain (mm)	Irrig. (mm)	R^2
Lesion area	18.4	−6.16	0.63	−0.02							0.56
	9.06	2.12	0.15	0.003							
	−9.61				0.09	0.02	0.004	−0.07	0.01	−0.01	0.48
	1.81				0.06	0.04	0.09	0.33	0.07	0.04	
	20.8	−7.17	0.74	−0.02	0.11	0.04	−0.19	−0.66	0.15	0.007	0.57
	9.17	2.83	0.16	0.004	0.10	0.03	0.12	0.37	0.07	0.004	
Conidia/ lesion	−100,230	23,739	−1,325	23							0.18
	31,460	7,350	522	11							
	10,926				−140	−213	545	2,463	−633	−377	0.15
	5,879				197	113	301	1,069	229	115	
	−99,429	21,930	−1,480	27	−45	−187	558	1,595	−539	372	0.23
	31,357	9,681	540	12	332	115	396	1,267	244	131	

[a] Partial regression coefficient/standard error of estimate.
[b] *Colletotrichum lagenarium* on the cucumber cultivars Calypso and Calico.
[c] DFI = days from inoculation.
[d] Degree days calculated from hourly temperature values with base 10 C and maximum 32 C.

Conidial production in controlled environments increased linearly over time in high moisture conditions. Only minimal conidial production occurred at low moisture conditions. When temperatures were increased in controlled environments, conidial production decreased. Within a 24-hr period, long periods of and free water on leaves in the field at night resulted in high conidial production. A reduction in the number of conidia produced during the day was associated with a reduction in free moisture and an increase in temperature. Further research is needed to determine whether high temperature, low moisture, radiation, or their interaction is responsible for the decrease in conidial production during the day.

Conidial production in the field was determined more by the length of time from inoculation than by variation in environmental conditions. Combining time from inoculation and environmental parameters in one model did not increase the amount of variation in conidial production. Regression models utilizing time from inoculation, environmental variables, or their combination explained 15–23% of the variation in conidial numbers.

Lesion development and sporulation can be affected by dilatory resistance (10). The polygenic resistance to *C. lagenarium* in Calypso, Calico, and other cultivars has not been studied in epidemiological detail previously. Lesion size increase and conidial production in the two resistant cultivars were two to four times lower than in the susceptible Earlipik 14. Slight differences in lesion size increase and conidial production occurred between the more resistant cultivars Calico and Calypso; however, more significant differences in infection occurred between the two cultivars (D. C. Thompson, *unpublished*).

Monitoring the environment appears to be unnecessary for the prediction of lesion size increase and conidial production. Further studies are needed to determine the influence of environmental factors on the ability of conidia to establish lesions.

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