Effect of Temperature and Moisture on Cristulariella pyramidalis

A. J. Latham

Department of Botany and Microbiology, Auburn University, Auburn, Alabama 36830.
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ABSTRACT

Development of *Cristulariella pyramidalis* from sporophores on agar and pecan leaves was similar at various temp. Lesions caused by *C. pyramidalis* on pecan leaves increased in size from 6 C through 27 C, with maximal development at 21 C. Sporophores formed on lesions at 9 C through 24 C with maximal production at 21 C. Maximal sporophore production occurred on wet leaves. Low levels of sporophore production occurred at 96 ± 2% relative humidity (RH). Below 94% RH, peripheral development from established lesions did not occur after 24 h nor were sporophores produced. *C. pyramidalis* remained viable in 60-day-old lesions on pecan leaves and produced sporophores after being moist 96 h.

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Additional key words: epiphytology, humidity chamber, zonate leafspot.

Cristulariella pyramidalis Waterman and Marshall is pathogenic to pecan, peanut (4, 10), and several ornamental and deciduous hosts (1, 2, 5, 8, 11). Sporadic epiphytotics of zonate leafspot caused by *C. pyramidalis* have occurred in pecan orchards during recent years in the southeastern United States. Reports on disease relationships with environmental factors have been associated with high moisture (5), cool weather (1), or above-average humidity (7).

This study investigates the effects of temp and moisture on growth and sporophore production by *C. pyramidalis* on an agar medium and on pecan leaves.

Sporophores were used as source of inoculum since spores rarely occur (11).

MATERIALS AND METHODS.–*Effect of temperature on growth from single sporophores.- C. pyramidalis* sporophores were dislodged from fruiting lesions onto petri plates of Czapek Dox broth plus 20 g agar and 4 g Difco yeast extract/liter (CDY). Six h after germination, sporophores were aseptically transferred to other CDY plates and incubated at various temp from 6 to 30 C. Sporophores came from the same lesions as those used for inoculating pecan leaves. Data were taken on colony diam from single sporophores.

Effect of light and temperature on lesion development and fruiting. – Potted 6-to 8-wk old pecan seedlings (from Schley nuts) were inoculated with 2-day-old sporophores of *C. pyramidalis*. Sporophores were removed from lesions with a moist camel's-hair brush and 25 placed randomly on moist leaves of the plants. Inoculated plants were covered with polyethylene bags (humidity chambers) and placed in a tray of water in a constant temp incubator. Plants were subjected to a 9-h photoperiod each 24 h and incubated 96 h at 21 C. Lesion diameters and sporophore production (fruiting) were recorded at the end of incubation.

Temperature investigations were made at 3 C intervals from 6 to 30 C. After 48 h, bagged plants were removed from the incubators, lesion diam recorded, plants sprayed to run off with demineralized water, and plants incubated 120 h. Lesion diam were recorded at 24-h intervals after the initial 48-h incubation period. Eight plants were inoculated and tested at 30 C and 12 at other temp. Lesion diam were averaged for 24 h intervals. Sporophores produced on lesions were counted and then washed off. Subsequent lesion growth and sporophore numbers were recorded.

Effect of RH on lesion growth and sporophore production.–Plants were inoculated and incubated in plastic bags at 21 C for 96 h. Lesions were measured and sporophores were counted and brushed off dry leaves with a camel's-hair brush. Subsequently, the plants were placed in a Vapor-Temp Blue M controlled temp-humidity chamber (Blue M Electric Co., Model VP-400 AT-1, Blue Island, Ill.). A hygrothermograph was also placed in the chamber to record temp and humidity data. The Vapor-Temp was programmed to the desired RH and plants incubated for specific time intervals. Lesion diam and number of sporophores were recorded according to RH regime. Finally, the same plants were placed in

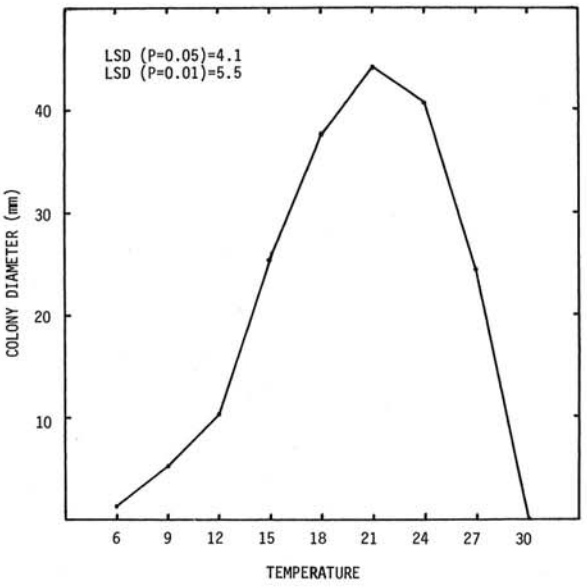

Fig. 1. Growth of *Cristulariella pyramidalis* on Czapek Dox plus yeast extract agar after 96 h. Each mean is the avg of the colony diam growth from 180 sporophores in four experiments.

TABLE 1. Lesion growth and sporophore production by *Cristulariella pyramidalis* on pecan leaves at several temp and incubation intervals

Temp (C)	Lesion diam (mm) by hours of incubation				Sporophores/lesion by hours of incubation			Lesion range incubation
	48[a]	72	96	120	72	96	120	96 h
6	0	0	0	0[b]	0	0	0	0
9	0	0.8[c]	1.4	1.9	0	0	0[d]	0.5- 2.0
12	0.9	1.6	2.5	3.3	0	0	0[d]	0.5- 6.0
15	1.6	3.0	4.4	6.0	0	0	3.0	0.5- 9.0
18	2.5	4.8	7.9	11.5	1.5	6.1	8.2	1.5-23.0
21	3.2	6.9	11.0	15.3	5.5	8.3	11.2	0.5-22.0
24	3.3	6.5	10.1	14.0	2.7	5.9	8.1	0.5-18.5
27	1.8	2.8	3.7	4.5	0	0	0	0.5-13.5
30	0	0	0	0	0	0	0	0

[a]Incubation period in hours.

[b]Lesions grew 1.1 mm avg in 240 h.

[c]Average diameter in mm for 12 replications.

[d]Sporophores produced in 288 h at 9 C and 192 h at 12 C.

plastic-bag humidity chambers, sprayed to run off, and incubated an additional 48 h after which lesion diam and number of sporophores were recorded.

Rewetting effects on lesion growth and fungal fruiting.—Plants with *C. pyramidalis* lesions were placed on a greenhouse bench where RH ranged from 30 to 98%. After 16 and 60 days, plants were covered with plastic bags, sprayed to run off, and incubated 96 h at 21 C. Lesion growth was recorded from six plants.

RESULTS.—*Effect of temperatures on growth from single sporophores.*—The growth curve of *C. pyramidalis* on agar plates is shown in Fig. 1 with cardinal temp at 6, 21, and 30 C. *C. pyramidalis* colonies were white at temperatures from 6 through 24 C, but a greenish pigment formed in cultures at 27 C.

Effect of light and temperature on lesion development and fruiting.—Lesions were either honey-brown or darker in color. Both types of lesions appeared on the same plant. Honey-brown lesions developed more rapidly and produced more sporophores. Darker-colored lesions grew slowly and resembled lesions on plants incubated at high temp; these lesions grew slowly or not at all.

Light had little or no effect on lesion diam or sporophore production by *C. pyramidalis* on pecan leaves. After several days of continuous sporophore production, sclerotia frequently formed on lesions of plants incubated in the dark. When plants were exposed to artificial light or daylight, sporophore production resumed.

No infection was apparent by lesion formation at 30 C. Lesion enlargement at 27 C was slow, averaging only 4.5 mm in diam after 120 h. Growth of *C. pyramidalis* approached maximal levels at 24 and 18 C; however, maximal growth occurred on leaves at 21 C (Table 1). At 6 C lesions grew an average of 1.1 mm in 240 h.

Lesions were 8-mm diam, or larger and 72-h-old when sporophores were observed; however, lesion size ranged to 21-mm diam in the 18 to 24 C range (Table 2). Average lesion size at 21 C was 12.2-mm diam. Average increases in lesion diam from 72 to 96 h was 3.7, 3.4, and 1.7 mm for 24, 21, and 18 C, respectively (Table 2). No sporophore production occurred at 27 C although incubation was prolonged beyond 120 h. Sporophore production by *C. pyramidalis* occurred within 72 h at 18, 21, and 24 C. The greatest number of sporophores were produced at 21 C. At 15 C, the first sporophores were produced at 120 h. Sporophores were produced at 12 and 9 C after 192 and 288 h, respectively. At 6 C, no sporophores developed in 288 h. Sporophore numbers doubled in 24 h at the optimal temp of 21 C and nearly a 7-fold increase occurred at 24 C and a 48-fold increase at 18 C.

Influence of RH on lesion growth and sporophore production.—Lesions of *C. pyramidalis* that produced sporophores on wet leaves did not continue to do so when vapor content of the atmosphere in the Vapor-Temp chamber was dried to 87 ± 3% RH (Table 3). Drying lesions increased in diam only during the first 24 h. Increase in size under drying conditions was usually along leaf veins and resulted in an irregular growth which deviated from the typical circular pattern. Lesions increased in diam and sporophores formed when RH was raised from 92.5 ± 2.5% to 97 ± 1% range in the Vapor-Temp humidity chamber. In one test, sporophores formed when RH was reduced from 96 to 94%. However, the total number of sporophores produced at 97 ± 1% RH was only 13% of the number produced on moist leaves. Leaves sprayed to run off and incubated in plastic bags for an additional 48 h supported fruiting equal to 80% of the original counts.

At 97 ± 1% and 100% RH, growth of lesions and sporophore production continued several days (Table 3, 4). Sporophores were produced as long as lesions enlarged, or until all leaf tissue became necrotic. Subsequently, fruiting continued a few days until the infected leaf fell from the plant.

Rewetting effects on lesion growth and fungal fruiting.—Lesions dried 16-days and subsequently rewetted and incubated in plastic bags increased in size only around part of the lesion; lesions dried 60-days did not. New growth usually occurred in a small sector on the edge of a lesion. Sporophores had developed in 12% of the lesions that had dried for 16-days before rewetting and 48 h of incubation. After an additional 48 h incubation, sporophores developed on 49.3% of the lesions. Although no peripheral growth occurred from lesions incubated 96 h after a 60-day drying period, an average of 2.2

TABLE 2. Lesion diameters and sporophore numbers produced by *Cristulariella pyramidalis* on pecan leaves after 72 and 96 h incubation

Temp (C)	Incubation: 72 h				Incubation: 96 h			
	Lesion			Sporophores	Lesion			Sporophores
	mm[a]	no.[b]	range	avg/lesion	mm	no.	range	avg/lesion
18	12.5	3	10-15.5	2.0	14.2	50	8.5-23	97.8
21	12.2	35	8-17.0	56.5	15.6	144	9.0-31	122.2
24	14.2	19	9-21.0	5.4	17.9	49	9.0-31	37.2

[a]Average diam.

[b]no. = number of observations.

TABLE 3. Growth of *Cristulariella pyramidalis* at 21 C and various RH levels on moist pecan leaves

Incubation procedure	Moisture	Lesion		Sporophores/lesion
Time (h)	level or RH	mm	no.[a]	avg no.
96 h bagged	100.0%[b]	15.9[c]	54	131.9
144 h Vapor-Temp[d]	87.0 ± 3.0%	16.3	54	0
192 h Vapor-Temp	92.5 ± 2.5%	16.3	54	0
240 h Vapor-Temp	97.0 ± 1.0%	19.0	37	17.6
288 h bagged	100.0%	27.4	26	106.0

[a]no. = number of observations.

[b]Leaves moist.

[c]Average diam.

[d]Vapor-Temp = Blue M controlled temp-humidity chamber.

TABLE 4. Sporophores produced by *Cristulariella pyramidalis* on pecan lesions during 48 h incubation intervals at 97 ± 1% relative humidity and 21 C

Incubation time	Sporophores		
(h)	total	no./lesion	avg/lesion
48	389	20	19.5
96	345	22	15.7
144	231	21	11.0
192	7	20[a]	0.3

[a]Lesions coalesced to cover whole leaf.

sporophores per lesion developed randomly over 35% of the lesions.

DISCUSSION.–Within a population of *C. pyramidalis* sporophores, there is wide variability in potential for growth and parasitism. Sporophores functioned as inoculum similar to single spores on agar and pecan leaves. On pecan leaves, some infections resulted in small, pin-point lesions similar to those observed on sassafras (6); other infections developed into large lesions accompanied by prolific fruiting. This variability shown by *C. pyramidalis* sporophores appeared similar to that of *Venturia inaequalis* biochemical mutants reported by Kline et al (3). The mutants caused only a fleck reaction on apple leaves with pathogenicity considered nil or virtually nil. Pathogenicity was restored temporarily, wholly or in part, by application of an appropriate amino acid or vitamin solution to the leaf surface. *C. pyramidalis* sporophores that initiated pin-point lesions on leaves might also have been nutritionally deficient for some amino acid or growth factor.

Wide variation in parasitism within a population of sporophores possibly was demonstrated by the fact that some infections resulted in sporophore production in relatively small lesions (8 mm in diam); whereas, with others fruiting did not occur until lesions were 18 to 21 mm in diam. Increase in lesion size and sporulation by *C. pyramidalis* appeared similar to that of *Alternaria solani* on potato as reported by Rands (9) who found that spore production did not begin on lesions until they were 3-4 mm in diam.

Fruiting by *C. pyramidalis* usually did not occur experimentally below 96% RH, but at this and higher RH before leaves became wet, sporophores developed in low numbers. Maximal growth and fruiting occurred when leaves were wet. This is similar to Rands' (9) finding that *A. solani* sporulated in ever-increasing amounts on wet leaves. These data indicate that *C. pyramidalis* sporophores develop abundantly in association with rain as previously suggested (1, 5, 7). Also, development of relatively few sporophores in high relative humidity explains why only a few sporophores may be found on a pecan leaf lesion in the orchard (4). Under conditions of high temp (27 C and above) and dry atmosphere (90% RH and below), an epiphytotic of zonate leafspot of pecan would be terminated rapidly. When rainy weather returns, sporophores develop on old lesions and are disseminated to susceptible foliage.

Infection and increase in lesion size at 6 C through 27 C and development of sporophores at 9 C through 24 C confirmed observations that *C. pyramidalis* is favored by moderate to cool temp (1). The prolific development of sporophores under optimal conditions of temp and moisture revealed how rapidly inoculum for a zonate leafspot epiphytotic may build up (5).

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