

**Effect of Temperature, Wetness Duration, and Inoculum Density  
on Infection and Lesion Development of *Colletotrichum coccodes* on Tomato Fruit**

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Appreciation is extended to A. Cobb for technical assistance and to J. Barnard for helpful suggestions.  
Accepted for publication 7 June 1989 (submitted for electronic processing).

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**ABSTRACT**

Dillard, H. R. 1989. Effect of temperature, wetness duration, and inoculum density on infection and lesion development of *Colletotrichum coccodes* on tomato fruit. *Phytopathology* 79:1063-1066.

The influence of temperature on lesion development and sporulation of *Colletotrichum coccodes* was determined on detached tomato fruit. Lesions did not develop after 8 days of incubation on spray- or puncture-inoculated fruit incubated at 7 C. Lesion diameter of spray- or puncture-inoculated fruit was greatest at 25 or 31 C. Spray-inoculated tomatoes incubated at 25 or 31 C developed more lesions than those incubated at 16 C. The number of conidia produced per lesion increased with increasing temperature from 16 to 28 C and decreased at 31 C. Lesions did not develop on tomato fruit that received 0, 3, or 5 hr of continuous wetness. Disease incidence was 35, 75, 92, 100, 100, 100, and 100% on

tomato fruit that received 10, 20, 24, 29, 44, 48, and 53 hr of continuous wetness, respectively. Disease severity increased with increasing hours of wetness duration and peaked at 48 hr. Disease severity increased with increasing inoculum density from  $10^1$  to  $10^6$  conidia/ml, and was greatest at  $10^6$  conidia/ml. Germination of conidia obtained from 7-day-old lesions on tomato fruit incubated in near 100% relative humidity (25 C) was 73.4%. Germination was 35.7, 36.3, or 24.6%, respectively, after lesions on tomato fruit were exposed to 2, 8, or 24 hr of drying at room temperature (27–30 C) and 55–57% relative humidity.

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Anthrachnose of tomato (*Lycopersicon esculentum* Mill.), caused by *Colletotrichum coccodes* (Wallr.) Hughes, is an important disease on processing tomatoes in the northeastern United States (6,8,10,14–16). *C. coccodes* has been recorded on more than 35 hosts from 13 families, primarily in the Leguminosae, Solanaceae, and Cucurbitaceae (5,16). Green and ripe tomatoes can be infected, but symptoms are expressed on ripe fruit (2,8,10,11,14,15). Latency of infections associated with green fruit can be overcome by a period of low temperature storage (10). Symptoms on ripe fruit include circular, depressed lesions with darkened centers. As the fungus colonizes the fruit, a semisoft decay occurs. Anthracnose lesions often merge and result in large rotted areas, which render the fruit unfit for processing (9).

Infections of tomato fruit are initiated by conidia that are produced in an acervulus (8,18). Moisture is necessary for release of the conidia from the gelatinous matrix in the acervulus. Conidia are spread by splashing rain to susceptible fruit, and lesions on ripe fruit become visible within 5–6 days after infection with favorable temperatures.

Severe outbreaks of tomato anthracnose are most often associated with high rainfall during the growing season (2,6,8,10,11); however, the relationship between wetness duration and infection has not been quantitatively defined. Optimum lesion development was reported by Fulton (8) as 26.6 C, with slow lesion development at 10 C and no lesion development at 37.7 C. Sporulation of *C. coccodes* on agar media was shown to increase with increasing light intensity (1) and was favored by temperatures of 24–28 C (11).

Because of changes in the taxonomy of the causal agent and

reports of isolations of other anthracnose fungi from tomato fruit (1,4), it is not clear whether early research was consistently conducted with *C. coccodes*. The objective of this study was to determine the effect of temperature, wetness duration, and inoculum density on infection and lesion development of *C. coccodes* on tomato fruit.

## MATERIALS AND METHODS

**Production of inoculum and preparation of fruit.** Isolate 210 of *C. coccodes* that originated from tomatoes grown in Lockport, NY, was used in this study. Conidia were obtained from 10–14-day-old cultures grown on V-8 juice agar (300 ml/L of V-8 juice, 30 g/L of Difco Bacto agar in distilled water, pH 4.6–5.0). Sterile distilled water was added to the plates and the agar surface was gently scraped to dislodge the spores. The suspension was centrifuged and the supernatant discarded. Conidia were resuspended in sterile distilled water.

Tomato fruit were obtained from greenhouse-grown plants of the cultivar Perfecto. Greenhouse-produced fruit were used to eliminate the risk of latent infections of *C. coccodes*, which was a problem in preliminary experiments with fruit produced in the field. Detached, ripe fruit were used in all the experiments. The fruit were surface disinfested in 0.5% NaOCl for 2 min, rinsed in sterile distilled water, and allowed to dry in a laminar flow hood (The Baker Company, Inc., Sanford, ME).

**Effect of temperature on infection and lesion development.** Before inoculation, six tomatoes were acclimated at 7, 16, 25, or 31 C in controlled temperature incubators for 5 hr. Fruit were inoculated by atomizing a spore suspension ( $1.2 \times 10^4$  conidia/ml) onto them until runoff or by puncturing the fruit at three sites with a hypodermic needle containing the spore suspension. A puncture inoculation treatment was included to reduce the chance of fruit escaping infection (3,17). Inoculated tomatoes were placed on wire racks in closed plastic boxes and returned to appropriate incubators. Hot water was added to the bottom of the plastic box to promote condensation and high relative humidity. The number of lesions produced on each fruit and their diameters were recorded after 8 days. The experiment was repeated once. Lesion number and lesion diameter from pooled data from two experimental runs were analyzed by analysis of variance.

**Effect of temperature on sporulation.** Eight tomatoes were acclimated at 16, 19, 22, 25, 28, and 31 C for 3.5 hr in controlled temperature incubators. After acclimation, each tomato was inoculated by puncturing the epidermis once with a hypodermic needle containing a spore suspension ( $1.5 \times 10^4$  conidia/ml). Less than one drop of suspension was retained in the fruit, as most was forced out of the wound by turgor pressure. Inoculated tomatoes were placed on a wire rack and inserted in a closed plastic box for each temperature treatment. Hot water was added to the bottom of the plastic box to promote condensation and high relative humidity. Plastic covers were placed over the boxes and the closed containers were secured in plastic bags to prevent moisture loss. After 5, 7, and 10 days, lesion diameters were recorded. Depending on lesion size, 7–20 ml of sterile distilled water was used to rinse the conidia from each lesion. The conidia were counted using a hemacytometer. The experiment was repeated once. Total conidia produced per lesion from pooled data from two experimental runs were transformed by  $\log_{10}$  and analyzed by analysis of variance. Standard errors were calculated for the treatment means.

**Effect of wetness duration on infection and lesion development.** Six tomatoes were placed on a wire rack in an open plastic box, sprayed with a spore suspension of *C. coccodes* ( $1.2 \times 10^4$  conidia/ml) until runoff, and incubated in a moist chamber in a greenhouse for 0, 3, 5, 10, 20, 24, 29, 44, 48, and 53 hr. The moist chamber consisted of a greenhouse bench completely enclosed by clear, 4-mil plastic. Two humidifiers (Welbilt Ultrasonic Humidifier, Welbilt Corp., Korea) were placed inside the chamber to provide near 100% relative humidity. The temperature in the chambers ranged from 21 to 26 C. After each time period, a plastic box containing six inoculated tomatoes

was removed and placed on an open-air greenhouse bench to allow the fruit to dry. The temperature on the greenhouse bench ranged from 17 to 25 C. Disease severity was recorded 7, 14, and 16 days after inoculation on a scale where 0 = no apparent disease; 1 = 1–12.5%; 2 = 12.5–25%; 3 = 25–37.5%; 4 = 37.5–50%; 5 = 50–62.5%; 6 = 62.5–75%; 7 = 75–87.5%; 8 = 87.5–100% fruit surface area diseased. The experiment was repeated once. Disease severity from pooled data from two experimental runs was analyzed by analysis of variance. Standard errors were calculated for the treatment means.

**Effect of desiccation on spore viability.** Twenty-four tomatoes were inoculated by puncturing the epidermis with a hypodermic needle containing a spore suspension of *C. coccodes* ( $1.6 \times 10^4$  conidia/ml). The tomatoes were placed on wire racks in plastic boxes (six fruit per box). Hot water was added to the bottom of the boxes, and the boxes were sealed to maintain high relative humidity (near 100%). After 7 days of incubation at 25 C in a growth chamber, the inoculated fruit were held at room temperature (25–30 C) and a relative humidity of 55–57%. The viability of a minimum of 400 conidia from each of six fruit was assessed 0, 2, 8, and 24 hr after the initial 7-day incubation period by applying one drop of sterile distilled water containing one drop of Tween 20 per liter to each lesion. The moistened lesion was pressed against a thin layer of water agar in a 9-cm-diameter plastic petri dish to dislodge the spores. The conidia were distributed on the water agar with a bent glass rod, and germination was determined after 20 hr. A conidium was considered germinated if the germ tube length was >50% of the length of the conidium. The experiment was repeated once. Data were analyzed by analysis of variance.

**Influence of inoculum density on disease severity.** Spore concentrations were adjusted to  $1.2 \times 10^1$ ,  $10^2$ ,  $10^3$ ,  $10^4$ ,  $10^5$ , or  $10^6$  conidia/ml. Six tomato fruit were inoculated with each spore density by spraying a spore suspension until runoff. The tomatoes were incubated in a growth chamber (Model 160DL, Percival Manufacturing Co., Boone, IA) at 20–22 C and 80% relative humidity. Disease severity was recorded after 6, 8, and 13 days with the scale previously described. The experiment was repeated once and the pooled data from two experimental runs were analyzed by linear regression.

## RESULTS

**Effect of temperature on infection and lesion development.** Lesions did not develop after 8 days of incubation on spray- or puncture-inoculated fruit incubated at 7 C (Table 1). There was no significant difference in the number of lesions that developed on puncture-inoculated fruit after 8 days of incubation at 16, 25, or 31 C. Lesion diameter of puncture-inoculated fruit

TABLE 1. Effect of temperature on development of lesions on tomato fruit caused by *Colletotrichum coccodes* by using two inoculation methods<sup>a</sup>

Temperature (C)	Mean no. lesions per fruit		Mean lesion diameter (mm)	
	Puncture <sup>b</sup>	Spray <sup>c</sup>	Puncture	Spray
7	0	0	0	0
16	2.9	0.1	9.0	0.3
25	3.0	3.3	24.4	8.9
31	2.8	2.4	31.3	10.9
Standard error	0.1	0.7	3.2	1.6
<i>P</i> <sup>d</sup>	<0.001	0.081	0.017	0.033

<sup>a</sup> Evaluated after 8 days of incubation. Data presented are the means of two individual experiments with six subsamples per experiment.

<sup>b</sup> Fruit were inoculated by puncturing each fruit at three locations with a hypodermic needle containing a spore suspension ( $1.2 \times 10^4$  conidia/ml).

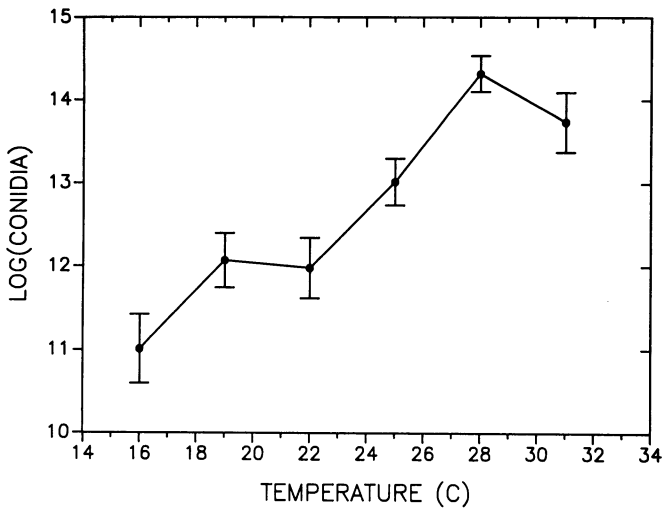
<sup>c</sup> Fruit were inoculated by spraying a spore suspension ( $1.2 \times 10^4$  conidia/ml) onto the fruit until runoff.

<sup>d</sup> Achieved significance level of *F*.

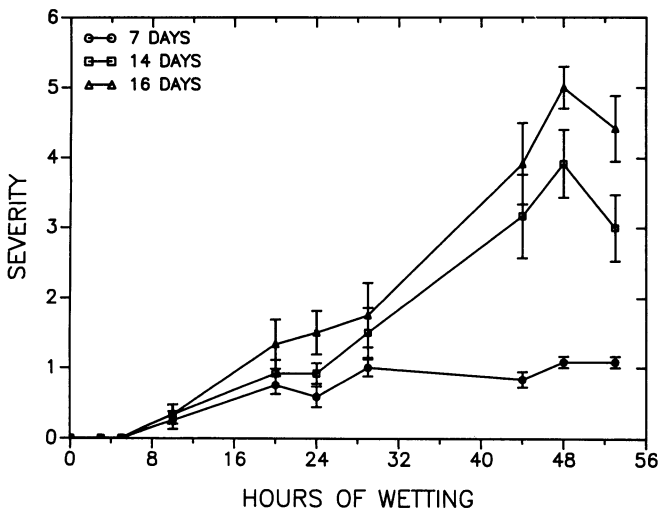
was greater at 25 or 31 C than at 16 C. Initial symptoms on spray-inoculated tomatoes incubated at 16, 25, or 31 C were expressed within 5 days as light brown flecking. Spray-inoculated fruit incubated at 25 or 31 C developed more lesions with larger diameters than spray-inoculated fruit incubated at 16 C.

**Effect of temperature on sporulation.** The total number of conidia produced per lesion after 10 days increased with increasing temperature (Fig. 1). There was no significant difference in the number of conidia produced from lesions incubated at 19 or 22 C. Production of conidia was greatest at 28 C and decreased at 31 C. The achieved significance level of *F* in the analysis of variance test was *P* = 0.018.

**Effect of wetness duration on infection and lesion development.** Lesions did not develop on tomato fruit receiving 0, 3, or 5 hr of free moisture after 16 days of incubation (Fig. 2). At 7 days of incubation, lesions were present on fruit that had been exposed to 10 or more hours of continuous wetness. At 16 days of incubation, 33, 75, 92, 100, 100, 100, and 100% of the fruit were diseased after exposure to 10, 20, 24, 29, 44, 48, and 53 hr of



**Fig. 1.** Effect of temperature on sporulation of *Colletotrichum coccodes* on tomato fruit after 10 days of incubation. Values represent the mean number of conidia produced per lesion of 12 fruit. Bars represent standard error of the mean.



**Fig. 2.** Influence of wetness duration on infection of tomato fruit by *Colletotrichum coccodes*. Values represent the mean disease severity rating of 12 fruit inoculated with a spore suspension ( $1.2 \times 10^4$  conidia/ml). Disease severity rating scale is defined as 0 = no apparent disease, 1 = 1–12.5%, 2 = 12.5–25%, 3 = 25–37.5%, 4 = 37.5–50%, 5 = 50–62.5%, 6 = 62.5–75%, 7 = 75–87.5%, 8 = 87.5–100% fruit surface area diseased. Bars represent standard error of the mean.

continuous wetness, respectively. At 14 and 16 days of incubation, disease severity increased with increasing hours of wetness duration and peaked at 48 hr. There was a decrease in severity at 53 hr of wetness, but the decrease was not significant.

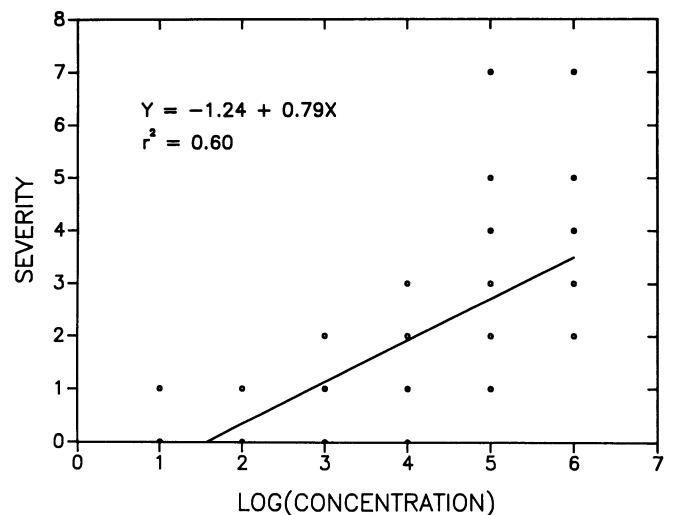
**Effect of desiccation on spore viability.** Germination of conidia obtained from 7-day-old lesions from fruit incubated at 25 C in high relative humidity (near 100%) was 73.4% on water agar. After 2, 8, or 24 hr of exposure to room temperature (27–30 C) and 55–57% relative humidity, germination decreased to 35.7, 36.3, or 24.6%, respectively. There was no significant difference in germination of conidia with 2, 8, or 24 hr of desiccation.

**Influence of inoculum density on disease severity.** Disease severity increased with increasing inoculum density from  $10^1$  to  $10^6$  conidia/ml, and was greatest at  $10^6$  conidia/ml (Fig. 3). After 6 days of incubation, lesions began to coalesce on tomatoes inoculated with  $10^5$  and  $10^6$  conidia/ml; after 13 days of incubation, lesions also coalesced on tomatoes inoculated with  $10^4$  conidia/ml. The proportion of diseased fruit after 13 days of incubation was 17, 17, 58, 92, 100, and 100% on fruit inoculated with  $10^1$ ,  $10^2$ ,  $10^3$ ,  $10^4$ ,  $10^5$ , and  $10^6$  conidia/ml.

## DISCUSSION

Temperature significantly influenced infection and lesion development of *C. coccodes* on tomato fruit. The temperatures found favorable for production and expansion of lesions were similar to day temperatures in tomato fields during the growing season in New York State. The temperature requirements for infection, lesion development, and sporulation closely paralleled the effects of temperature on growth and germination of conidia of *C. coccodes* (7). The results of this study are in agreement with Fulton (8) who found that lesions were produced most rapidly at 27 C and with Kendrick and Walker (11) who determined that 24–28 C was most favorable for sporulation, germination of spores, and growth on agar media.

Available moisture was critical for infection of tomato fruit by *C. coccodes*. Inoculated tomato fruit receiving 0, 3, or 5 hr of continuous free moisture did not develop anthracnose lesions, but fruit exposed to 10 or more hours of continuous wetness consistently developed lesions. The longer the period of high moisture, the greater the disease severity. These data are consistent with previously published observations that severe outbreaks of tomato anthracnose are commonly associated with prolonged rainy weather (2,6,15). Conidia of *C. coccodes* are produced in



**Fig. 3.** Effect of inoculum concentration on infection of tomato fruit by *Colletotrichum coccodes* after 13 days of incubation. Values represent mean disease severity rating of 12 fruit inoculated with a spore suspension and incubated at 20–22 C and 80% relative humidity. Disease severity rating scale is defined as 0 = no apparent disease, 1 = 1–12.5%, 2 = 12.5–25%, 3 = 25–37.5%, 4 = 37.5–50%, 5 = 50–62.5%, 6 = 62.5–75%, 7 = 75–87.5%, 8 = 87.5–100% fruit surface area diseased.

an acervulus and are covered by a mucilaginous water soluble film (18). Research conducted with other *Colletotrichum* spp. has shown that the mucilaginous matrix aids in the preservation of the viability of the spores (12,13). Although desiccation experiments were conducted in this study in the presence of natural matrix, only 24.6% of the conidia germinated after exposure to 27–30 C and 55–57% relative humidity for 24 hr. Further research is needed to characterize the relationships between temperature and moisture on development of anthracnose on tomato plants at various stages of growth in the field.

Results from this study demonstrate the central role that temperature, moisture, and inoculum density contribute to the development of tomato anthracnose caused by *C. coccodes*. In New York State, prolonged wet periods and mild temperatures are common when fruit are ripening in the field. Further research is needed to quantify levels of overwintering inoculum (sclerotia, infested debris) and concentrations of conidia of *C. coccodes* in tomato fields. Future plans include incorporation of the environmental variables examined in this study into an integrated pest management program currently being developed for tomatoes grown in New York State.

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