A Model Defining the Relationship Between Temperature and Leaf Wetness Duration, and Infection of Watermelon by *Colletotrichum orbiculare*

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ABSTRACT

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Controlled environment experiments were conducted to determine the relationship between temperature, leaf wetness duration, and infection of watermelon by *Colletotrichum orbiculare*. Flats of watermelon seedlings were inoculated and exposed to various combinations of temperature (12, 15, 18, 21, 24, 27, and 30°C) and leaf wetness duration (2, 4, 8, 12, 16, and 24 h). The experimental design was a split-plot, with whole units represented by temperature and subunits represented by leaf wetness duration. Anthracnose incidence, defined as the percentage of symptomatic seedlings in each flat 10 days after inoculation, increased with increasing leaf wetness duration at all levels of temperature. The optimum temperature for infection ranged from 21 to 24°C. At most temperatures, as little as 2 h of leaf wetness was required for infection. Analysis of variance with orthogonal polynomial contrasts and multiple regression procedures was used to define the relationship of anthracnose incidence to temperature and leaf wetness duration.

Additional keywords: cucurbit anthracnose

Anthracnose, caused by *Colletotrichum orbiculare* (Berk & Mont.) Arx (=*C. lagenarium* (Pass.) Ellis & Halst.), is an important disease of watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai) in the midwestern and eastern United States. The disease produces symptoms on leaves, stems, and fruit. Defoliation caused by stem and foliar infections can result in substantial reductions in bulk yield (1,15). Also, because symptomatic fruit are unmarketable, outbreaks of anthracnose can result in total losses in some fields (9).

Control options for management of anthracnose are limited. Host resistance to *C. orbiculare* is not available in preferred watermelon cultivars, but farmers can reduce the threat of serious anthracnose outbreaks through standard cultural practices, such as rotation with nonhost crops. However, because of the very low tolerance for fruit infection, growers must rely upon repeated applications of protective fungicides for acceptable levels of disease control. After years of marginal control with other fungicides, commercial farmers in Indiana use chlorothalonil and mancozeb applied at 7- or 10-day intervals throughout the growing season.

There are several published reports concerning the effect of environmental conditions on anthracnose development on cucurbits and other hosts. Gardner and Gilbert (7) observed that anthracnose of watermelon was not a problem at temperatures greater than 30°C. A period of leaf wetness following conidial deposition was necessary for infection of cucumber by the anthracnose pathogen (11). Also, a direct relationship was established between length of dew period and anthracnose development on seedlings of *Xanthium spinosum* (13). Temperatures between 20 and 25°C during the dew period resulted in highest disease ratings, but extended dew periods of 48 h are necessary for disease development at temperatures below 16°C.

Empirically determined relationships between environmental factors and anthracnose have been used to help time fungicide sprays. It has been suggested that rainfall accumulations can be used to schedule fungicide sprays for cucumber anthracnose control (17). More recently, Duthie et al. (4) reported a decision rule based on temperature and precipitation for scheduling fungicide sprays for controlling watermelon anthracnose. A weather-based system was recently developed for scheduling fungicide sprays for control of Alternaria leaf blight of muskmelon (5,10). Since most muskmelon growers in the midwestern United States also raise watermelons, development of a forecaster for watermelon disease would be a logical next step toward implementation of modern disease management practices. Objectives of this research were to define the relationship between temperature and leaf wetness duration and infection of watermelon by *C. orbiculare*, and to develop a model for indexing environmental conditions in terms of favorability for anthracnose development.

MATERIALS AND METHODS

Plant production. Watermelon seeds (cv. Crimson Sweet, Asgrow Seeds, Kalamazoo, MI) were sown into 16-cell flats at one seed per cell. Flats were created by cutting commercially manufactured plastic growing trays with 128 cells (TLC Polyform, Inc., Plymouth, MN) into 16-cell squares. Each cell volume was approximately 25 ml and was filled with a soilless potting medium (Sunshine Mix LC1, Fison's Horticulture, Bellevue, WA). Plants were irrigated from above prior to inoculation and subirrigated after inoculation. Seedlings were inoculated when cotyledons were fully expanded and the first true leaf was emerging. Time from planting to inoculation ranged from 7 to 14 days. Greenhouse temperature and relative humidity ranged from 18 to 33°C and 37 to 85%, respectively, and were recorded with a WeatherLog Relative Humidity and Temperature Log (RainWise, Inc., Bar Harbor, ME).

Inoculum production and inoculation technique. Inoculum was increased from an isolate of *C. orbiculare* (no. 9319, R. Latin, Purdue University) collected from infected watermelon in southwestern Indiana. Cultures were maintained on acidified potato dextrose agar (APDA) in 9-cm petri dishes under a 12-h photoperiod at 24 ± 2°C. Sections of the sporulating cultures were added to vials containing 5 ml of a sterile 1-g/liter suspension of streptomycin sulfate (Agristrep, 21.2 WP, Merck, Inc., Rahway, NJ). Approximately 1 ml of the suspension was pipetted onto the surface of new 9-cm petri dishes with APDA and incubated under the conditions just described for 14 days.

Conidia were washed from the cultures with deionized water and filtered through a

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double layer of surgical gauze. The conidial suspension was adjusted to a concentration of 50,000 ± 5,000 conidia per ml with the aid of a hemacytometer. It had been determined from previous experiments that 50,000 conidia per ml was an effective concentration to use for inoculation (14). Seedlings in flats were inoculated by misting plants with the inoculum suspension using a hand-held plastic spray bottle. Each flat received an average of 3.2 ml of conidial suspension. Seedlings were not misted to runoff.

Experimental technique and design. Experiments were conducted at seven temperatures (12, 15, 18, 21, 24, 27, and 30°C) and six leaf wetness durations (2, 4, 8, 12, 16, and 24 h). The saturated environment for the leaf wetness periods was created in a dew chamber (Percival Model I-35 D, Boone, IA). At each temperature, 18 inoculated flats were placed in the dew chamber. Three replicate flats were removed at each prescribed leaf wetness duration and transferred to a growth chamber (Environmental Growth Chambers Model M-31, Chagrin Falls, OH) where the same temperature was maintained as in the dew chamber. Relative humidity in the growth chamber ranged from 18 to 35%. After 24 h, all flats were returned to the greenhouse bench. Temperature and relative humidity in the chambers and the greenhouse were monitored and recorded with an electronic temperature and relative humidity logger (RainWise, Inc., Bar Harbor, ME). Temperatures to be tested were selected in random order.

Each temperature–leaf wetness duration combination was repeated twice. The experimental design was a split-plot with whole units arranged in a randomized complete block (16). Whole units were represented by temperature, and the three replications of temperature in time served as blocks. Subunits were represented by leaf wetness duration. Three replicate flats of each wetness duration within every whole unit gave a pure subunit error.

Disease was assessed as the percentage of seedlings per flat that exhibited characteristic anthracnose lesions on stems or leaves 10 days after inoculation.

Data analysis and model development. Disease percentages (Y) were transformed to angles by use of the arcsine transformation ($\sin^{-1}Y^{1/2}$) prior to analysis of variance. Analysis of variance and multiple regression procedures were used to define the relationship between environmental factors and infection by *C. orbiculare*. The analysis of variance model (16) was the following (eq. 1): $\sin^{-1}(Y_{ijkl}) = \mu + R_i + T_j + a_{(ij)} + W_k + TW_{ik} + b_{(ijk)} + b'_{(ijk)l}$, where Y_{ijkl} is the anthracnose percentage of the $ijkl$th individual flat; μ is the overall population mean; R_i is the effect of the ith replication in time; T_j is the effect of the jth temperature; $a_{(ij)}$ is the random effect of the ijth whole unit; W_k is the effect of the kth leaf wetness duration; TW_{jk} is the interaction of the jth temperature and the kth leaf wetness duration; $b_{(ijk)}$ is the random effect of the ijth block by wetness and ijkth block by temperature by wetness interactions; and $b'_{(ijk)l}$ is the random effect of the lth subunit replicate within the ijkth combination.

Statistical procedures were performed with SAS (SAS Institute, Cary, NC) for all data analyses. Sums of squares for temperature, leaf wetness duration, and the temperature by leaf wetness duration interaction were partitioned using orthogonal polynomial coefficients computed by the SAS (IML) ORPOL function. A polynomial model was constructed using significant sources of variation from the analysis of variance results and all lower order terms.

Data from the second run (replication) of the 21°C temperature leaf wetness duration experiment were discarded as outliers after a dew chamber malfunction resulted in variable ambient moisture and temperature during the 24-h period. Missing values were generated in part by the SAS GLM procedure using analysis of covariance (16). The degrees of freedom were adjusted accordingly.

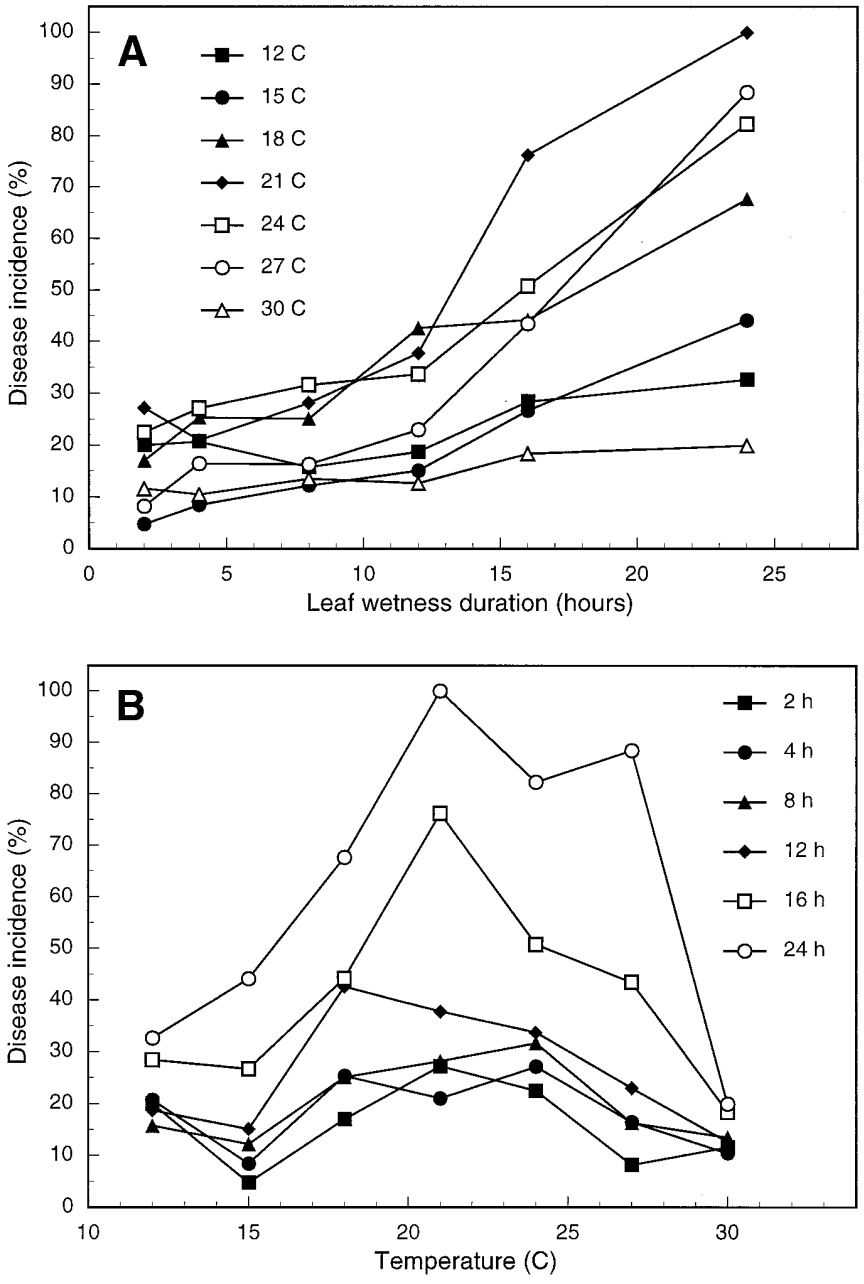

Fig. 1. Observed mean anthracnose incidence at various **(A)** leaf wetness durations and **(B)** temperatures.

RESULTS

In general, disease incidence increased with leaf wetness duration at all levels of temperature. Incidence ranged from 0 to 100%, with the maximum disease occurring at 24-h leaf wetness duration. For all

but the highest and lowest temperatures tested, significant increases in disease resulted between 12- to 16-h wetness and 16- to 24-h wetness (Fig. 1A). Disease incidence was greatest at 21°C. High (30°C) and low (12 and 15°C) temperatures resulted in the least amounts of infection (Fig. 1B).

The analysis of variance indicated a significant quadratic relationship between $\sin^{-1}Y^{1/2}$ and temperature, and highly significant linear and significant quadratic relationships between $\sin^{-1}Y^{1/2}$ and leaf wetness duration (Table 1). The analysis of variance also identified two significant temperature by leaf wetness duration interactions. Blocks were nonsignificant and pooled with the whole unit error.

Infection model. The relationship between temperature, leaf wetness duration, and infection of watermelon by *C. orbiculare* was best defined by the following model (eq. 2): $\sin^{-1}Y^{1/2} = b_0 + b_1T + b_2T^2 + b_3T^3 + b_4W + b_5W^2 + b_6TW + b_7T^2W + b_8T^3W + E$, where Y is disease incidence, T is temperature, and W is leaf wetness duration. The parameter estimates and their standard errors are listed in Table 2. The lack of significance of many of the parameter estimates is not surprising due to high correlations with other independent variables in the model. The response surface of the above model is shown in Figure 2. The unadjusted and adjusted coefficients of determination for the model in equation 2 were 0.90 and 0.87, respectively. A random pattern of residuals occurred over a range of predicted means.

Table 1. Analysis of variance of the arcsine transformation in degrees of watermelon anthracnose incidence for various combinations of temperature and leaf wetness duration

Source	df	Mean square	F^a
Temperature (T)	6	5,394.06	1.207
T-linear	1	60.23	0.014
T-quadratic	1	22,882.00	5.123 *
T-cubic	1	5,206.26	1.166
Lack of fit	3	1,405.18	0.315
Whole unit error	13	4,466.84	
Leaf wetness (W)	5	11,184.30	39.471 **
W-linear	1	52,716.00	186.041 **
W-quadratic	1	2,464.53	8.698 *
Lack of fit	3	247.01	0.872
T × W	30	609.53	2.151 *
T-linear × W-linear	1	374.57	1.332
T-linear × W-quad	1	168.15	0.593
T-quad × W-linear	1	10,550.00	37.232 **
T-quad × W-quad	1	512.23	1.808
T-cub × W-linear	1	1,553.59	5.483 *
T-cub × W-quad	1	841.18	2.969
Lack of fit	24	178.66	0.630
Subunit error	65	283.31	
Flat-to-flat error	240	103.50	

[a] * and ** = statistical significance at probability levels $P = 0.05$, and $P = 0.01$, respectively.

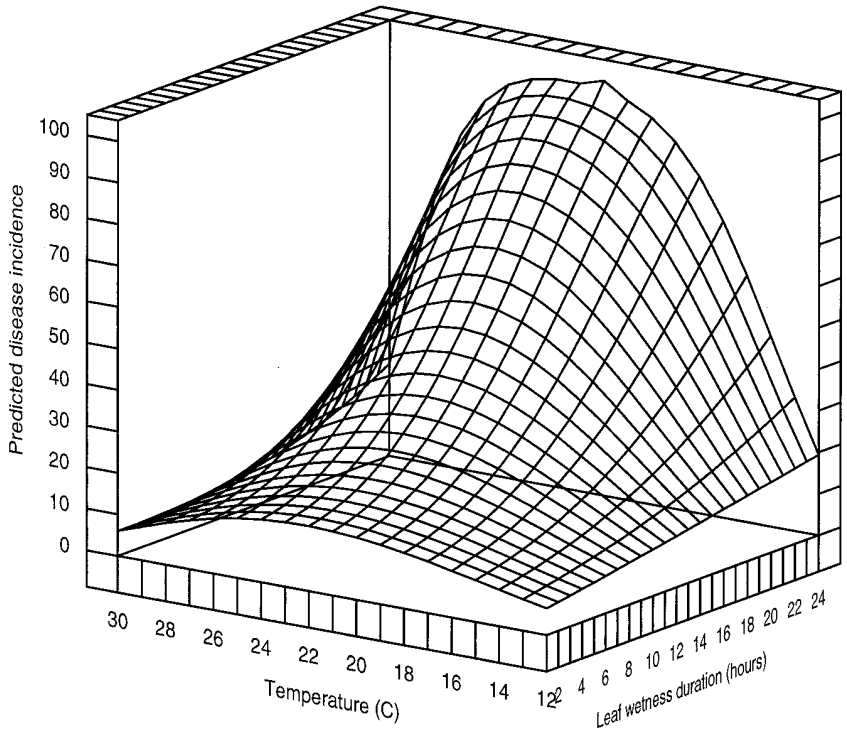

Fig. 2. A response surface based on the polynomial model (eq. 2) describing the influence of temperature and leaf wetness duration on infection of watermelon by *Colletotrichum orbiculare*.

DISCUSSION

Based on our results, temperature and leaf wetness duration were clearly important factors in infection of watermelon by *C. orbiculare*. The largest contributors to the response surface were *T*-quadratic, *W*-linear, and the interaction between them. The significance of these terms explained the linear increase in incidence when plotted against leaf wetness duration (Fig. 1A) and the parabolic nature of disease incidence when plotted against temperature (Fig. 1B).

The optimum temperature range for infection determined by our research (21 to 24°C) compares favorably with other reports on the influence of environment on anthracnose caused by *C. orbiculare* (6,13,17). The optimum temperature range for anthracnose development on spiny cocklebur (*X. spinosum*) was 20 to 25°C (13). Thompson and Jenkins reported that anthracnose lesions on cucumber expanded rapidly at 20 to 28°C and conidia production was greatest at 24°C during the early stages of lesion development (17). Ishida and Akai (8) reported that conidia of *C. orbiculare* germinated readily within a range of 20 to 32°C, and that appressoria formation was optimum at 20 to 26°C. The lower and upper temperature limits for infection also appear to be described accurately by the model given in equation 2. Littrell and Epps (12) observed no anthracnose infection on cucumber below 10°C, and MacRae and Auld (13) showed only a minimum of disease development at 35°C, despite a 24-h dew period. That these temperature ranges for infection are

Table 2. Estimates of parameters from equation 2 for temperature and leaf wetness duration effects on infection of watermelon seedlings by *Colletotrichum orbiculare*

Parameter	Parameter estimate	Standard error
b_0	59.8619	96.0062
b_1	–6.1378	14.9549
b_2	0.3008	0.7403
b_3	–0.0048	0.0117
b_4	4.3679	7.2389
b_5	0.0547	0.0196
b_6	–1.2413	1.1251
b_7	0.0899	0.0557
b_8	–0.0018	0.0009

correctly identified is supported by observed anthracnose outbreaks in Indiana watermelon fields during midsummer, when evening temperatures are near or at their highest. Also, during a 3-week period of extremely rapid anthracnose increase on watermelon in experimental field plots in 1995, temperatures during wet periods ranged from 20.2 to 25.1°C, with a mean of 23.4°C (R. Latin, *unpublished*).

As expected, anthracnose incidence increased with the length of the period of leaf wetness. Our results are supported by other research on cucurbit anthracnose, where maximum disease development occurred with ≥16 h of leaf wetness (13,17). This type of relationship between disease and moisture has been well documented for a variety of plant diseases (2).

The relatively high amount of disease that occurred at 12°C, especially after only 2 h and 4 h of leaf wetness, warrants explanation. Evans et al. (5) observed similar results with Alternaria leaf blight of muskmelon. They attributed unexpectedly high levels of disease to infections enhanced by chilling injury suffered by plants after being transferred from a 25 to 27°C greenhouse environment to the 12°C growth chamber. Chandler and Thomas (3) had already demonstrated the role of mechanical injury in enhancing development of the disease. However, no such relationship has been reported for infection of watermelon seedlings by *C. orbiculare*. There also is no evidence to suggest that any of the disease processes characteristic of cucurbit anthracnose are favored at or near 12°C. It is likely that prolonged drying times of the soilless potting medium, especially at lower temperatures, may be responsible. Drying time on foliage (determined visually) ranged from less than 5 min (30°C) to around 25 min (12°C) but probably had little effect on resulting amounts of infection. However, the drying time of the soilless potting medium could have been as long as several hours or more. We suppose that at 12°C, the substrate remained moist for a prolonged period, affording conidia accumulated at the base of the stem additional time to germinate and infect.

An empirical disease warning system has been developed by Duthie et al. (4), indicating that the relationship between anthracnose development and weather can be used to time fungicide sprays and achieve some degree of control (compared with unsprayed plots) with fewer applications than the weekly spray schedule. However, defoliation percentages in plots sprayed according to their weather-based program were four times greater than the defoliation percentages observed in plots sprayed weekly, suggesting the need for an improved model. Results of our research will be used in the development of a fundamental forecaster for anthracnose on watermelon. Decision rules for scheduling fungicide applications currently are being tested in the field. It is intended that this model for indexing environmental conditions in terms of favorability for anthracnose development will be incorporated into the Melcast system (10) already in use by Indiana melon growers.

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