Effects of Temperature and Duration of Surface Wetness on Spore Production and Infection of Cucumbers by *Didymella bryoniae*

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ABSTRACT

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Effects of temperature on spore germination, sporulation, infection, and symptom development on stem pieces and flowers of cucumber infected with *Didymella bryoniae* were studied under conditions of 100% relative humidity (RH). Infection of leaves and petioles of young, intact cucumber plants was studied at various combinations of temperatures and durations of surface wetness. Processes studied at 100% RH occurred over a wide range of temperatures (20–28 C), with an optimum of 24–25 C. When temperature and duration of surface wetness were varied in combination, wetness duration was the most important factor that affected the proportion of leaves and petioles that became infected.

Gummy stem blight of cucumber is caused by the fungal pathogen *Didymella bryoniae* (Auersw.) Rehm. The disease is characterized by brown, oozing lesions dotted with black perithecia or pycnidia on stems, angular papery lesions on leaves, and malformed fruits. The disease can affect a wide range of cucurbits in field and greenhouse situations. In northeastern Ohio, the disease is a problem that limits greenhouse production of European seedless cucumbers. Rapid disease progression is favored by humid conditions and by free moisture on leaf surfaces (5,8). Temperatures around 24 C are favorable for disease development on leaves and fruits of cucumber and other cucurbits (1,2,5,7). Wounding the leaf increases infection by the pathogen (5). In commercial greenhouse culture, infection sites are readily available because fruit harvesting and leaf pruning continually cause wounding of plants.

Management of gummy stem blight with fungicides has proven inefficient because frequent applications are needed, and control of fruit infection is ineffective (6). Strains of *D. bryoniae* resistant to benzimidazoles have also been reported (3).

The purpose of this study was to more closely investigate the effects of temperature and temperature/wetness duration on various components of the disease development process. This information is critical for developing disease-predictive models, which may be useful in disease management, especially in computer-controlled greenhouse systems.

MATERIALS AND METHODS

Inoculum production. An isolate of *D. bryoniae* (#671) from an infected cucumber fruit, collected in 1986 from a northeastern Ohio greenhouse, was used throughout this study. The isolate was maintained on potato-dextrose agar (PDA) slants at 10 C and on PDA plates at room temperature (20–24 C) in continuous light. Pycnidia were the only fruiting structures observed in cultures grown on PDA at room temperature. For all inoculations, a spore suspension was prepared by growing *D. bryoniae* at room temperature for 5–10 days, flooding the culture with sterile distilled water, and subsequently adjusting the concentration of the suspension to 1×10^6 conidia per milliliter.

Influence of temperature on conidial germination. Drops (100-μl) of a conidial suspension from an automatic pipette were placed on thin, 2-cm^2, water-agar slabs that rested on glass slides in sterile petri dishes. Petri dishes, enclosed in plastic bags lined with moist paper towels, were placed in the dark in chambers with temperatures maintained at 2, 12, 16, 18, 20, 24, 28, 32, and 37 C. After 0, 4, 8, 12, 16, 20, 24, 28, 32, and 48 hr of incubation, two slides were removed from each chamber. Spores were stained with a drop of lactophenol/cotton blue solution. Two sets of 200 spores each were observed on each slide, and the number of spores germinated in each set was recorded for each temperature/time combination.

Influence of temperature on symptom development in excised stem pieces. Stem pieces (3-cm) were cut from internodes of 8- to 12-wk-old, greenhouse-grown cultivar Corona cucumber plants, were surface-disinfested for 30 sec in 0.05% NaOCl, and were rinsed twice in sterile distilled water. Two stem pieces were placed on moistened filter paper in each of 10 petri dishes for each temperature tested. Stem pieces in five petri dishes per temperature were each inoculated with a 50-μl drop of a conidial suspension of *D. bryoniae*. Stem pieces in the remaining five petri dishes were treated with distilled water. Petri dishes that contained stem pieces were enclosed in plastic bags and incubated

in the dark at 12, 16, 18, 20, 24, 28, 30, and 32 C. Symptom development in stem pieces was evaluated daily for 12 days with a scale of 0–4; 0 = no symptoms, 1 = browning at ends, 2 = complete browning, 3 = dark fruiting structures on <50% of the stem surface, and 4 = fruiting structures on >50% of the stem surface.

Influence of temperature on sporulation. Sporulation of *D. bryoniae* on cucumber stems was assessed over time at 16, 20, 24, 28, and 30 C. Stem pieces (2-cm) were cut from internodes of 8- to 12-wk-old, greenhouse-grown Corona cucumber plants, were surface-disinfested as before, and were placed in petri dishes (four stems per dish) on moistened filter paper. A 50-μl drop of a conidial suspension of *D. bryoniae* was placed on each stem piece. Uninoculated control stem pieces were treated with sterile distilled water. Petri dishes, enclosed in plastic bags lined with moist paper towels, were placed in controlled-temperature growth chambers with continuous light; in preliminary studies, sporulation did not occur in continuous darkness. After 0, 3, 6, 9, and 12 days of incubation, 16 inoculated and four uninoculated stem pieces were removed from chambers at each temperature being tested. Each stem piece was agitated for 15 sec in 2 ml of 50% ethanol in distilled water. Spores were counted (two counts per piece) with the aid of a hemacytometer. Symptom development and prevalence of visible fruiting structures also were recorded. Two or three temperatures were tested in each of many experimental runs until each temperature had been tested three times.

Influence of temperature on infection of cucumber flowers. Newly expanded flowers with attached juvenile fruits were excised from mature greenhouse-grown plants. Each flower and attached fruit was placed in a sterile petri dish lined with moistened filter paper. Flowers were inoculated by placing a 50-μl drop of a conidial suspension of *D. bryoniae* in the center of each flower corolla. Uninoculated control flowers were treated with the same amount of sterile distilled water. For each temperature, 20 inoculated and five uninoculated flowers were prepared and placed in plastic bags lined with moist paper towels. Bags were placed in the dark in chambers with temperatures maintained at 12, 16, 18, 20, 24, 28, and 32 C. To access infection, daily observations of flowers were made for 10 days. Blackening of the flower tissue and visible mycelial growth in the flower corolla were recorded as positive signs of infection.

Influence of temperature and postinoculation wetness duration on infection of intact leaves and petiole stubs. Corona cucumber plants were grown in 6.4-cm-diameter pots in the greenhouse for 4–5 wk until the second true leaf had fully expanded. Before inoculation, the growing point of each plant was removed. The first true leaf of each inoculated plant was excised with a sterile scalpel dipped in a conidial suspension of *D. bryoniae*; a petiole stub approximately 7-cm-long remained. The second true leaf was inoculated by cutting a hole in the leaf with a sterile 12-mm-diameter cork borer dipped in the same conidial suspension. Uninoculated control plants were treated with sterile distilled water by identical procedures. After inoculation, all plants were placed in a growth chamber that contained a mist generator (Hermidifier Co., Lancaster, PA) enclosed within a 0.7-m^3 clear plastic chamber. Every 5 min, the mist generator delivered a 1.5-min mist-period, which provided constant moisture on all plant surfaces. All tests were conducted with continuous light. At 0, 0.5, 1, 2, 4, 8, 12, 24, and 48 hr after inoculation, eight inoculated plants and one uninoculated control plant were removed from the mist chamber and placed in a chamber with the same light and temperature conditions for the rest of the experiment. Although plant surfaces remained dry in this chamber, relative humidity (RH) was maintained at 80–95% with a mist generator and by placing the pots in shallow pans of water. Seven days after inoculation, all plants were removed from the second chamber and evaluated for infection. Petiole stub-browning and browning or chlorosis at the cut leaf edge were considered positive signs of infection with *D. bryoniae*. Some isolations were made to confirm that infection had occurred. Temperatures tested were 16, 20, 24, 28, and 30 C. Each temperature was tested twice, and the order in which temperatures were tested was random.

Statistical analyses. All experiments were done twice. A completely randomized design was used in all cases. Regression analysis was used to determine the relationship between temperature and conidial germination, sporulation, symptom development on stem pieces, and infection of flowers. With SAS (4), first-, second-, and third-order polynomials were tested. Regression analysis was also used to ascertain relationships between wetness duration, temperature, and infection of petioles and leaves of intact plants. The regression analyses were done, not to develop a mechanistic model of the infection and sporulation, but to determine if there was a significant relationship between environmental variables and disease development. Proportions of plants with positive infections were transformed with the arcsine-square root transformation. Minitab software (Minitab Statistical Software, State College, PA) was used for initial analysis of variance and subsequent testing of polynomial models.

RESULTS

Conidia of *D. bryoniae* germinated in vitro readily in the 20–28 C range (Fig. 1). At 4–8 hr, 30–60% of the conidia tested had germinated in this temperature range. After 12 hr at these temperatures, 80–95% had germinated. After 20 hr, 80–100% of conidia at 12–32 C had germinated. The regression model selected for these data was

$$Y = B_0 + B_1T + B_2T^3$$

in which Y = percentage of conidia germinated, and T = temperature. Because, in some cases, germination at most tempera-

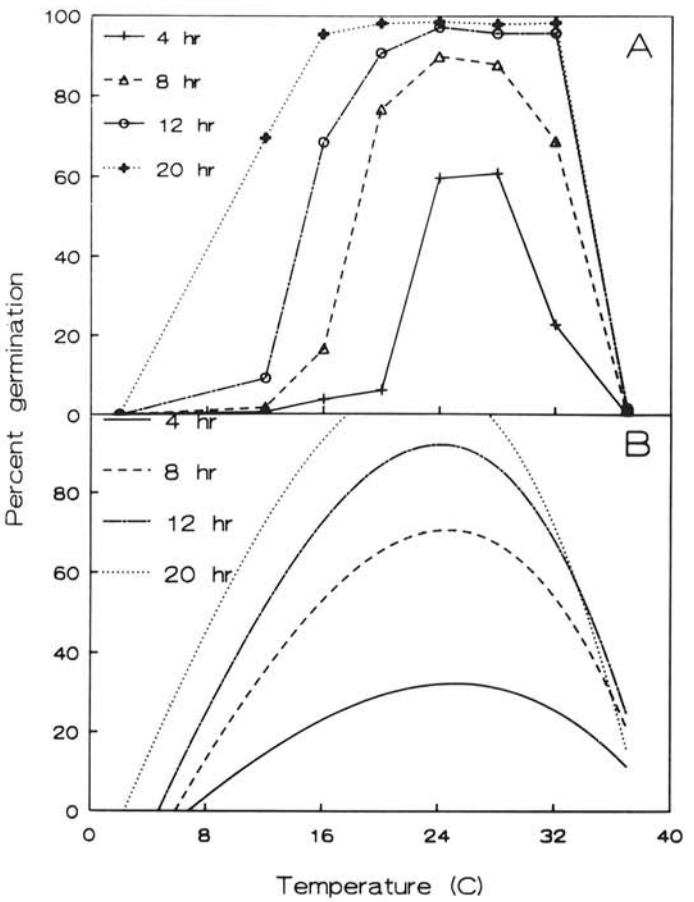

Fig. 1. Effect of temperature (T) on in vitro conidial germination of *Didymella bryoniae*. **A,** Mean percentage of conidial germination versus temperature. Data points represent pooled means of repeated experiments. **B,** Regression equations obtained: 4 hr, $Y = -21.37 + 3.21T - 0.0017T^3$, $R^2 = 0.31$; 8 hr, $Y = -38.86 + 6.69T - 0.0037T^3$, $R^2 = 0.59$; 12 hr, $Y = -38.33 + 8.14T - 0.0047T^3$, $R^2 = 0.73$; 20 hr, $Y = -20.23 + 8.5T - 0.0055T^3$, $R^2 = 0.91$. Y = percentage of conidia germinated.

tures was 100% (Fig. 1A), regression lines reflect this ceiling effect by rising above the 100% line (Fig. 1B).

Spore production of *D. bryoniae* on excised stem tissue was optimal in the range 24–27 C (Fig. 2). Spore production on day 3, although greater than zero, was not distinguishable from the *x*-axis in Figure 2. Spore production peaked at 9 days after inoculation, then declined. Symptoms on inoculated stem pieces at temperatures above or below the optimal 24–27 C range consisted of blackening and the presence of fruiting structures, with no measurable spore-production surge. For these data, the model,

$$Y = B_0 + B_1T + B_2T^2,$$

fit the data most closely (Fig. 2B). In this equation, Y = spore production.

Inoculated cucumber flowers developed initial symptoms and signs of infection (blackened flower tissues and visible fungal hyphae) most rapidly when held at 24 C (Fig. 3). However, by day 7 and 10, 80–100% of flowers held at 18–28 C were visibly infected. The regression model chosen for these data was

$$Y = B_0 + B_1T + B_2T^2$$

in which Y = percentage of flowers with symptoms, and T = temperature (Fig. 3B).

Development of gummy stem blight symptoms occurred most rapidly on inoculated stem pieces at 20–28 C (Fig. 4). Six days after inoculation, stem pieces at 24–28 C were completely covered with dark fruiting structures. After 8–10 days, stems held at 20–28 C were covered with black fruiting structures. The regression model that fit these data was

$$Y = B_0 + B_1T + B_2T^3$$

in which Y = disease index, and T = temperature (Fig. 4B).

The final study was an evaluation of the effects of temperature and postinoculation wetness duration on symptom development. The most significant factor that affected infection was the length of time plant surfaces were wet after inoculation. At 16–30 C, temperature did not have a significant effect on the proportion of inoculated plants that became infected. When the mean transformed proportion of plants infected was regressed on wetness duration over all temperatures (Fig. 5), the following equations were obtained:

$$\text{leaf infection, } Y = 0.306 + 0.012\ W\ (R^2 = 0.93)$$
$$\text{petiole infection, } Y = 0.942 + 0.010\ W\ (R^2 = 0.96)$$

in which Y = the transformed proportion of plants infected, and W = the duration of wetness in hours.

DISCUSSION

Conidial germination, sporulation, development of symptoms on stems, and infection of cucumber leaves, petioles, and flowers by *D. bryoniae* occurred over a wide range of temperatures. At 100% RH, those processes continuously occurred at various rates at temperatures from 20 to 28 C, with an optimum of 24–25 C.

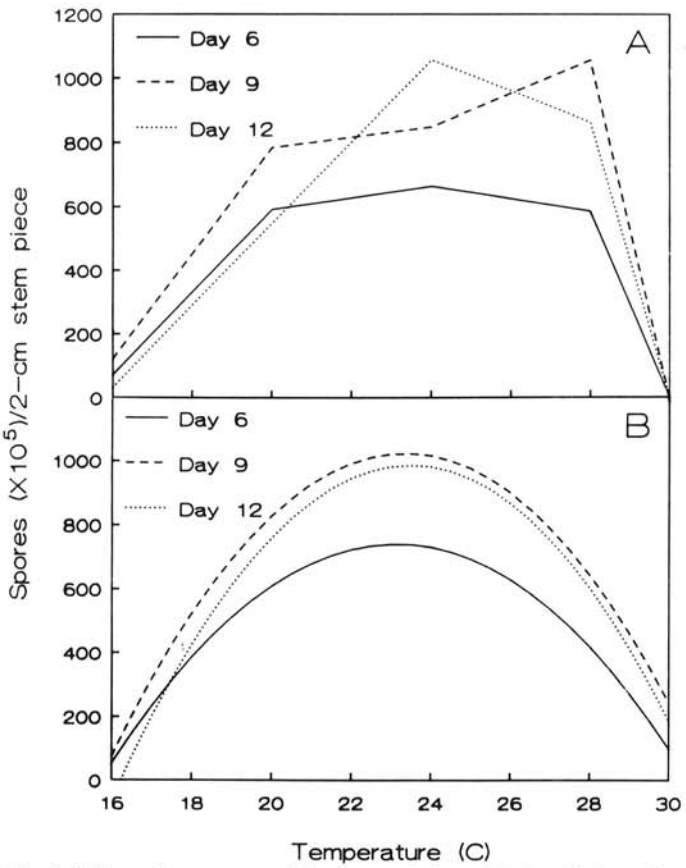

Fig. 2. Effects of temperature (T) on sporulation of *Didymella bryoniae* on excised cucumber stem pieces. **A,** Mean number of spores per stem piece versus temperature. Data points represent pooled means of repeated experiments. **B,** Regression equations obtained: Day 6, $Y = -6{,}548 + 630.6T - 13.5T^2$, $R^2 = 0.21$; day 9, $Y = -8{,}585 + 823.4T - 17.64$, $R^2 = 0.23$; day 12, $Y = -9{,}384 + 883.6T - 18.82T^2$, $R^2 = 0.21$. Y = number of spores.

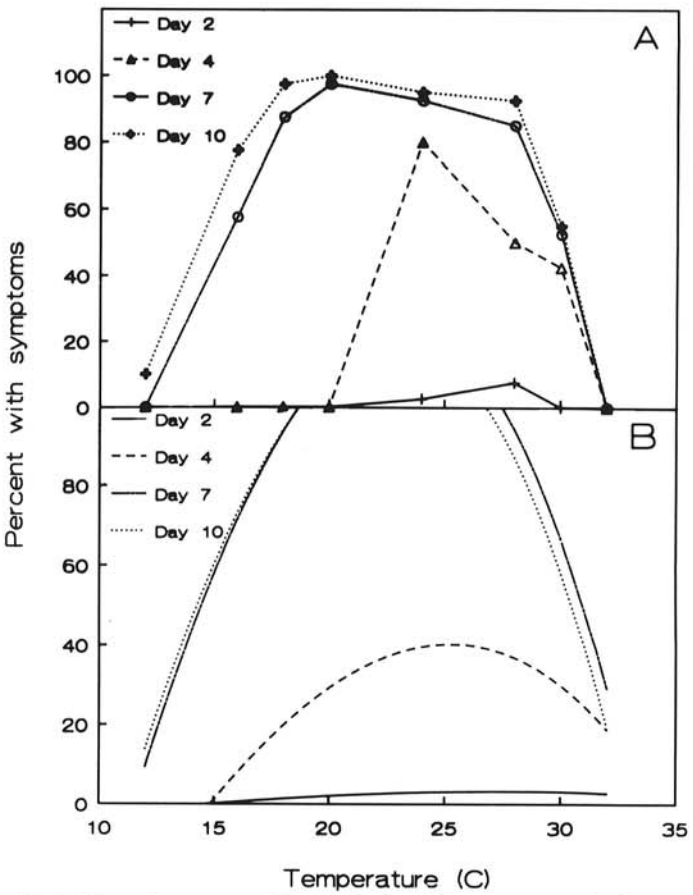

Fig. 3. Effect of temperature (T) on infection of detached cucumber flowers by *Didymella bryoniae*. **A,** Mean percentage of flowers with symptoms versus temperature. Data points represent pooled means of repeated experiments. **B,** Regression equations obtained: Day 2, $Y = -8.89 + 0.67T - 0.0003T^3$, $R^2 = 0.09$; day 4, $Y = -143.26 + 10.97T - 0.0057T^3$, $R^2 = 0.29$; day 7, $Y = -256.01 + 24.27T - 0.015T^3$, $R^2 = 0.79$; day 10, $Y = -242.79 + 23.55T - 0.015T^3$, $R^2 = 0.82$. Y = percentage of flowers with symptoms.

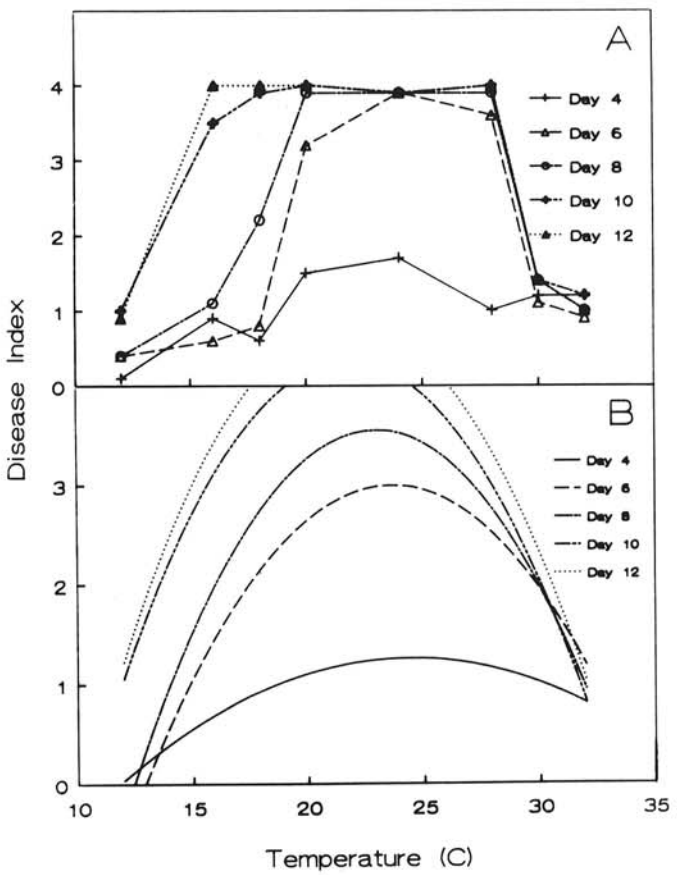

Fig. 4. Effects of temperature (T) on symptom development of gummy stem blight on excised cucumber stem pieces. Symptoms evaluated on scale of 0 (no symptoms) to 4 (severe infection). **A,** Mean disease indices versus temperature. Data points represent pooled means of repeated experiments. **B,** Regression equations obtained: Day 4, $Y = -3.49 + 0.39T - 0.008T^2$, $R^2 = 0.36$; day 6, $Y = -11.55 + 1.23T - 0.026T^2$, $R^2 = 0.49$; day 8, $Y = -13.33 + 1.47T - 0.032T^2$, $R^2 = 0.65$; day 10, $Y = -11.47 + 1.44T - 0.033T^2$, $R^2 = 0.80$; day 12, $Y = -12.09 + 1.53T - 0.035T^2$, $R^2 = 0.82$. Y = disease index.

When infection of leaves and petioles was studied at various combinations of temperatures and surface wetness durations, wetness duration was the most important factor. Temperature in the range studied did not appreciably affect the proportion of plant leaves or petioles that became infected.

The rapid rate of spore germination observed at 16–28 C correlates closely with results of other researchers. Chiu and Walker (1) found 90–100% germination in 24 hr at 20–28 C in orange or cucurbit seed extracts. Optimal temperature for spore germination was 24 C.

In our study, sporulation on stem pieces was greatest at 25 C, with measurable production at 22–28 C. Sporulation increased up to 9 days after inoculation, then decreased by day 12. Even above and below the range of measurable sporulation, blackened stem tissues and fruiting structures were observed on stem pieces. Growth of *D. bryoniae* in culture occurs at 12–32 C with an optimum at approximately 24 C (1,7,9). Temperature requirements for sporulation appear to be more restrictive than for vegetative growth.

Flower infection and symptom development on stem pieces both occurred rapidly at 16–28 C, with optimal development at 24–25 C. Other researchers have reported similar results. The greatest disease progression occured in inoculated watermelon seedlings at 24 C, although considerable disease developed in the range of 20–28 C (1). VanSteekelenburg (7) reported that fruit rot of cucumbers occurred most rapidly at 23 C, whereas Luepschen (2) showed that watermelon fruit rot was most severe at 24 C. Svedelius (5) found cucumber leaf infection to be greater

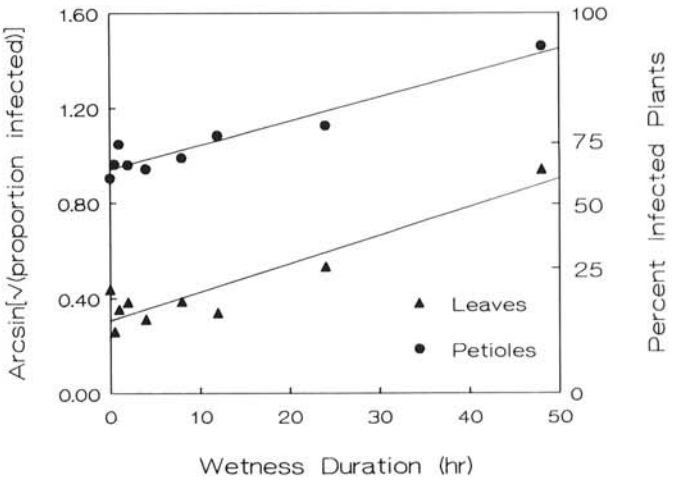

Fig. 5. Effect of tissue wetness duration on infection of leaf petioles and leaves by *Didymella bryoniae*. Regression equations: Leaves, arcsine ($\sqrt{Y}$) $= 0.306 + 0.012\ W$, $R^2 = 0.93$; petioles, arcsine ($\sqrt{Y}$) $= 0.942 + 0.010\ W$, $R^2 = 0.96$. Y = transformed proportion of plants infected, W = duration of wetness in hours.

at 25 C than at 18 or 38 C; injury of leaves increased the level of infection.

In our study, leaf and petiole infection were most influenced by the length of time plant surfaces were wet. Temperature did not have a significant effect on the proportion of plants infected. Svedelius (5) found that free water on leaf surfaces was necessary for initial infection, and that continuous 100% RH was required for subsequent lesion expansion. VanSteekelenburg (8) reported that leaves were rarely infected when held at 65% RH, more frequently infected at 95% RH, and most severely infected when leaf surfaces were always wet. One hour of free water on leaves was sufficient for infection; however, lesion expansion required continued leaf wetness.

The implications of our study for commercial greenhouse cucumber growers are that manipulation of greenhouse temperatures would likely be ineffective in limiting development of gummy stem blight, because *D. bryoniae* is active over such a wide range of temperatures. Conversely, limiting the duration of free moisture on plant surfaces is much more important for reducing the development and spread of this disease. Growers should adopt practices that minimize or eliminate free moisture on plant surfaces with controlled ventilation and strict irrigation management.

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