Ecology and Epidemiology

Temperature and Wetness Duration Requirements for Apple Infection by *Botryosphaeria obtusa*

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ABSTRACT

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The combined effect of temperature and wetness duration on infection of apple by *Botryosphaeria obtusa* was studied on Delicious seedlings and Golden Delicious apple fruit. The optimum temperature for leaf infection was 26.6 C; at this temperature, 4.5 and 13 hr were required for the pathogen to cause light and severe infection, respectively. Lower temperatures required longer wetting periods for infection to occur, and no infection was observed at 8 C with wetness periods shorter than 48 hr. At 32 C, infection was reduced and a longer wetting period was required for infection than at 28 C. The optimum temperatures for fruit infection ranged from 20 to 24 C; 9 hr of wetting were required for light infection to occur. Infection of fruit required 38 hr of wetting at 8 C, whereas 28 and 32 C resulted in reduced fruit infection. Models were derived empirically to indicate the duration of leaf wetness (W) necessary, at a given temperature (T), for a specified level of infection to occur. For light leaf infection ($<$ 1 lesion/ 100^2 cm of leaf tissue), $W = 3527.7T^{-2}$, and for severe leaf infection ($>$ 10 lesions/ 100 cm^2), $W = 116 - 5380.7T^{-1} + 70257.5T^{-2}$. For fruit infection, $W = 14.8 - 265.2T^{-1} + 2988.4T^{2}$. No infection occurred under field conditions in those instances where no infection was predicted. The leaf infection models accurately predicted the level of 84.7% of the infections obtained under field conditions. In 8.5% of the cases, less disease than expected for the particular combination of W and T was obtained; more infection than predicted was observed in 6.8% of the cases. Most of the incorrect predictions occurred for wetting periods where moderate infection was anticipated.

The fungus *Botryosphaeria obtusa* (Schw.) Shoem. (synonym, *Physalospora obtusa* (Schw.) Cooke) causes black rot on apple fruit, frogeye leaf spot, and a limb canker (4). Losses due to this pathogen can be considerable (5), especially in warm, humid areas. The management of these diseases is based on the elimination of inoculum sources (6) and fungicide sprays (4).

Timing of the chemical treatments and development of cultural control tactics can be improved through a better knowledge of factors that influence the infection process. Foster (3) investigated

the effect of temperature on frogeye leaf spot development for a 24-hr wetness period and studied the effect of different wetness duration periods at 20 C. However, he did not study the combined effect of temperature and wetness duration on infection. A study of the combined effect of temperature and wetness duration on infection has provided information for the development of forecasting models that are valuable aids in the timing of fungicide sprays for other apple diseases such as scab (7) and cedar-apple rust (1). Such models may lead to more efficient pesticide use in disease control.

The objectives of this study were to determine the effects of temperature and wetness duration and their interaction on apple leaf and fruit infection by *B. obtusa* and to develop preliminary models for predicting infection periods.

MATERIALS AND METHODS

Inoculum production. Isolate 087 of *B. obtusa*, isolated from apple fruit from the Central Crops Research Station, Clayton, NC, was grown on cellulose films placed on top of oatmeal-agar medium (Difco Laboratories, Detroit, MI), under continuous fluorescent illumination. Conidia were obtained from 12- to 13-day-old cultures by scraping off the nonsporulating mycelia and blending (Waring Model 31BL92, Waring Products Division, New Hartford, CT) the cellulose films, on which pycnidia were present, for 20 sec in sterile distilled water. The conidia were strained twice through two layers of cheesecloth, and the suspension was standarized by means of a hemacytometer. A suspension of 2×10^5 conidia/ml was used for foliage inoculations, and 1×10^5 was used for fruit inoculations.

Leaf infection experiment. Seedlings of open-pollinated apple (*Malus domestica* Bork. 'Delicious'), bearing seven to nine fully unfolded leaves, were preconditioned in controlled-temperature incubators (Percival Manufacturing Co., Boone, IA) for 12 hr at temperatures ranging from 8 to 32 C with 4-C increments. Plants were inoculated with a conidial suspension by spraying to runoff the lower surfaces of the leaves with an airbrush. Each plant was immediately placed in a polyethylene bag which contained a wet paper towel to ensure high relative humidity once the bag was sealed. This procedure allowed the foliage to remain wet for the prescribed wetting period. Plants were returned to the same controlled-temperature incubators for 2, 4, 8, 12, 16, 20, 24, 32, 40, and 48 hr. After the wetting period, plants were removed from the bags and allowed to dry at room temperature (about 24 C) for approximately 20 min. Plants were then transferred to an air-conditioned chamber in a greenhouse at approximately 20 C for symptom development.

Leaves were evaluated for typical "frogeye" spots 12 days after inoculation. Leaf area was estimated visually with the aid of a leaf area diagram, and the number of lesions per unit of leaf area was recorded. For the second evaluation after 12 days, less than 1 lesion/100 cm^2 of leaf tissue was considered light infection, and more than 10 lesions/100 cm^2 of leaf tissue was considered severe. Infection between these two values was considered moderate. Surface area of most of the leaves ranged from 15 to 30 cm^2. Only the five most heavily infected leaves of each seedling were used in the statistical analysis. A preliminary experiment had shown no difference in susceptibility among the first seven unfolded leaves.

Each combination of temperature and wetness duration was applied to three seedlings per experiment, and the experiment was performed three times. A split plot design was used, in which runs were replications, temperatures were whole plots, and wetness periods were subplots.

Fruit infection experiment. Unsprayed Golden Delicious apple fruit, collected about 2–3 wk before commercial harvest, were washed with 0.5% NaClO, rinsed in tap water, and inoculated the same day they were harvested. The inoculation was performed by dipping a 2.5×2.5 cm square of four-ply laboratory paper towel (Tidi, Uni/Disco, Inc., Troy, MI) into a conidial suspension and placing it on the uninjured surface of the fruit. Inoculated fruit were placed in plastic boxes ($30.5 \times 12.7 \times 6.4$ cm) and covered with a layer of aluminum foil, on top of which three layers of wet paper towel were placed. The boxes were closed and placed in incubators at the same temperatures and for the same periods as described for seedlings. Each combination of temperature and wetness period was applied to four fruit. Fruit that received sterile water instead of spore suspension were kept for 48 hr at each temperature to assess natural infection levels.

After the assigned period, fruit were wiped with 95% ethanol to eliminate superficial inoculum, allowed to dry, and placed in moist chambers at room temperature for 6 days. Initial lesions were counted with the unaided eye in a 5-cm circle on the inoculated surface of each fruit. The total number of lesions on the four-fruit experimental unit was recorded as number of lesions per 20 cm^2.

This experiment was conducted three times in a split plot design with temperatures as whole plots and wetness durations as subplots.

Statistical analysis. Severity data were transformed by $\log_{10}(y + 1)$, in which y = the number of lesions per 100 cm^2 of leaf area or 20 cm^2 of fruit surface area, and analyzed by linear regression. The regression models obtained were used to draw curves relating wetness periods and temperature to infection. The predicted temperature and wetness combinations necessary to yield light (1 lesion/100 cm^2) and severe (10 lesions/100 cm^2) leaf infections were obtained by interpolation from these curves. These interpolated data were used to obtain new regression equations in which the duration of leaf wetness necessary to result in light or severe level of infection was determined as a function of temperature.

A similar procedure was used to obtain a model for fruit

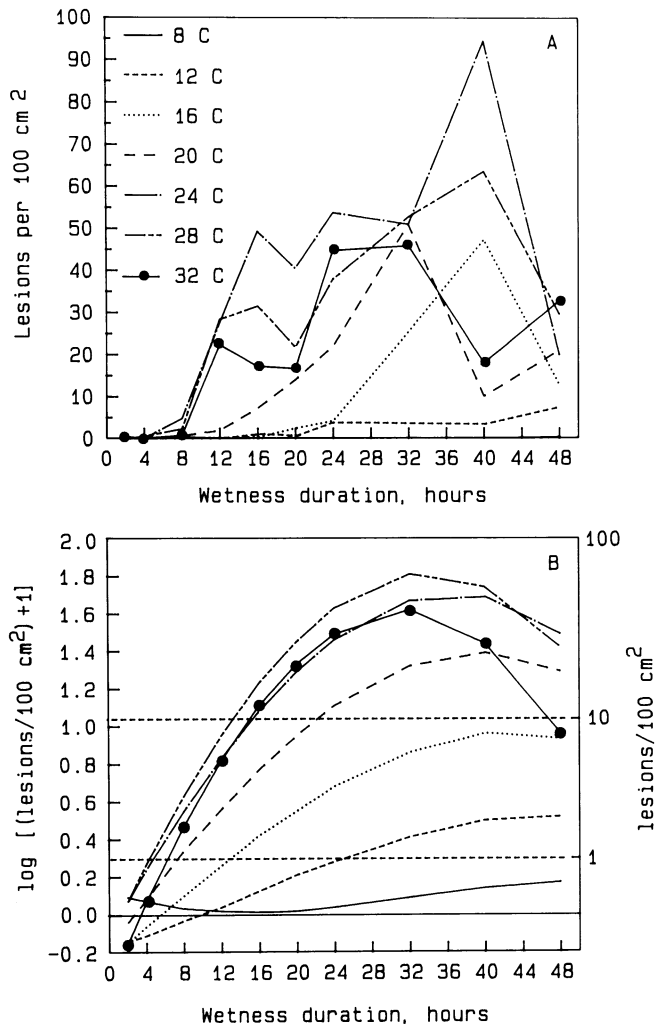

Fig. 1. Effect of temperature and wetness duration on apple leaf infection by *Botryosphaeria obtusa*. **A,** Experimental data. **B,** Predicted infection levels; the horizontal dotted lines represent the threshold values defined for light and severe infection.

infection. The threshold value for this variable was fixed at 1 lesion per fruit, that is, 4 lesions/20 cm^2, considering that 1 lesion is enough to cause loss of the fruit.

Model validation. Eight open-pollinated Delicious seedlings were placed outdoors under a cage with apple twigs bearing pycnidia of *B. obtusa*. Seedlings were exposed to spore deposition only for the duration of a specific wetting period; then they were allowed to dry, placed in a greenhouse, and observed 2 to 3 wk later for symptom development. Temperature, relative humidity, leaf wetness duration, and total rainfall were recorded for each wetness period. Temperature and relative humidity were measured with a hygrothermograph (Belfort Instrument Co., Baltimore, MD); rainfall was measured with a top-weighing rain gauge (Belfort Instrument Co., Baltimore, MD); and wetness duration was measured with a DeWit leaf wetness meter (Valley Streams Farm, Orono, Ont.). The amount of inoculum released during each wetting period was estimated by collecting rainfall water beneath the cage with two funnel traps. Each trap consisted of a 10.5-cm-diameter funnel that was inserted in a 1-L plastic bottle. Twenty milliliters of 10% $CuSO_4 \cdot 5H_2O$ was placed in each bottle to prevent spore germination and bacterial growth. Bottles were changed at the end of each wetness period. The number of spores in each bottle was determined by filtering a 1- or 5-ml sample through a 25-mm-diameter (1.2-μm pore size) gridded filter and counting the number of spores in each of three grids selected at random. This experiment was conducted on the campus of North Carolina State University in Raleigh and at a site in Buncombe County, North Carolina.

RESULTS AND DISCUSSION

Leaf infection. A preliminary analysis of variance showed significant effects of temperature and wetness duration and their interaction on leaf infection. Seedling infection increased with increasing temperature up to 24–28 C; infection decreased at 32 C. In general, increased wetting periods resulted in higher infection, but a decrease in infection was observed at 48 hr of wetting at the higher temperatures (Fig. 1A). This trend has been observed in similar studies on apples and other crops (1,2), although no satisfactory explanation has been given for this phenomenon.

Symptoms began to appear as early as 48 hr after inoculation with long wetting periods (>24 hr) at 24–28 C. Four days were needed for initial symptom development if wetting periods were shorter or temperatures lower. Initial symptoms appeared as brown pinpoint lesions on the lower surface of the leaf.

The influence of temperature and wetness period on infection of apple leaves by *B. obtusa* was described by the following equation:

$$Y = 1.86 - 0.34T - 0.09W + 0.018T^2 - 0.0003T^3 + 0.0015W^2 + 0.01TW - 0.00009TW^2 - 0.0001T^2W \quad (1)$$

where $Y = \log_{10}$ [(number of lesions/100 cm^2) + 1], T is the temperature (C), and W is the wetness period (hours) required for infection. The R^2 value for this regression was 0.74; R^2 adjusted for degrees of freedom was 0.73. Infection values predicted by this regression are shown in Figure 1B.

The curves derived for the effect of temperature on the wetness period required for light and severe infection and the equations describing them are presented in Figure 2. The optimum temperature for infection, that is, the temperature at which the least time was required for infection to occur, was predicted to be 26.6 C. Foster (3) noted that leaf infection of Yellow Transparent and Northwestern Greening apples by *B. obtusa* was maximum at 20 C for the isolate (8a) he used for infection studies. However, other isolates had a higher optimum temperature than 8a for germ tube

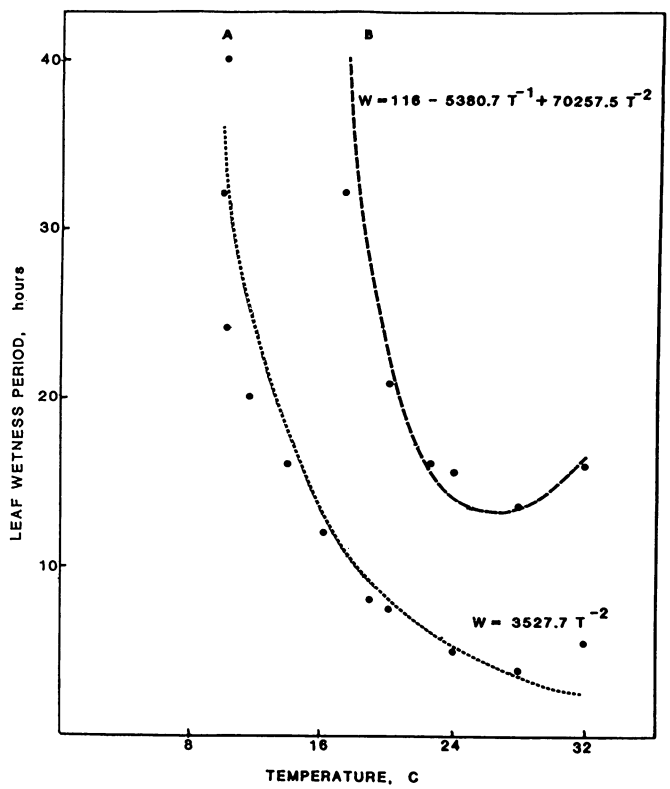

Fig. 2. Effect of temperature (T) on the wetness period (W) required for **A,** light infection, and **B**, severe infection of apple leaves by *Botryosphaeria obtusa*.

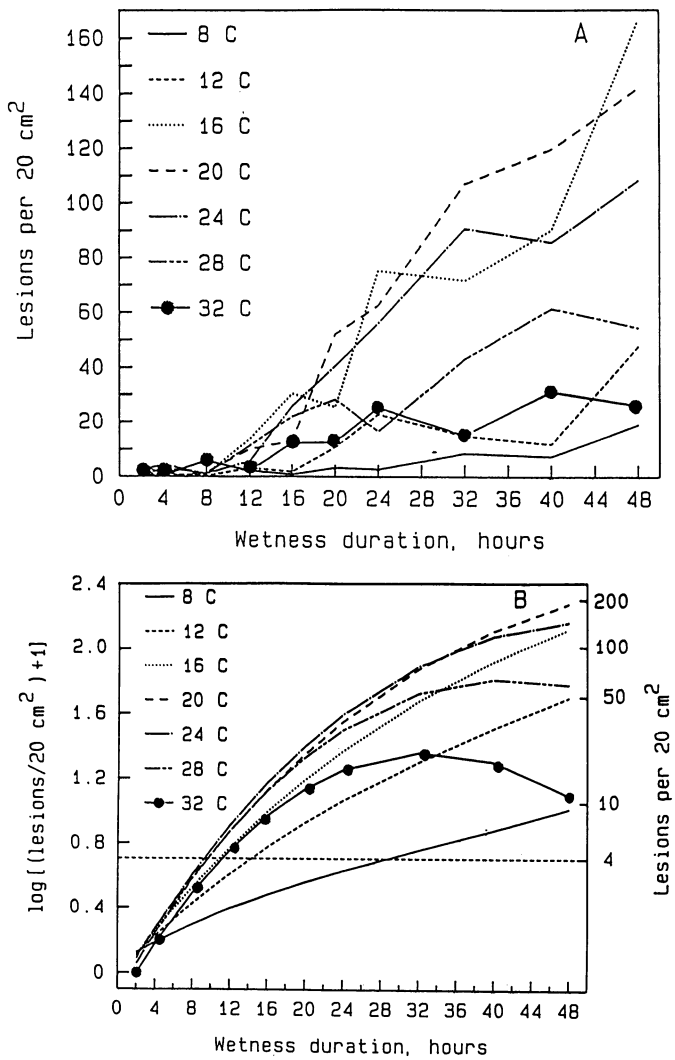

Fig. 3. Effect of temperature and wetness duration on apple fruit infection by *Botryosphaeria obtusa*. **A,** Experimental data. **B,** Predicted infection levels; the horizontal line represents the threshold infection value of 4 lesions/20 cm^2 or 1 lesion/fruit.

elongation in vitro, which suggests a higher optimum temperature for infection as well.

Fruit infection. An increase in the number of lesions was observed with increasing temperatures up to 20–24 C; infection declined slightly at 28 C and sharply at 32 C (Fig. 3A). Increased wetting periods resulted in higher infection.

The influence of temperature and wetness time on infection was described by the following equation:

$$Y = -0.1 + 0.005T - 0.016W + 0.009TW - 0.0002T^2 - 0.0005W^2 - 0.0002T^2W - 0.00004TW^2 \quad (2)$$

where $Y = \log[(\text{number of lesions}/20\ \text{cm}^2) + 1]$, and T and W are defined as before. The R^2 value for this regression was 0.77; the R^2 adjusted for degrees of freedom was 0.76 (Fig. 3B).

The equation derived for the effect of temperature on the wetness period necessary for fruit infection, and the curve associated with it, are shown in Figure 4. This equation predicts an optimum temperature for fruit infection at 22.5 C; at this temperature, almost 9 hr of wetness would be required for infection.

Model validation. The model was evaluated for 58 separate wetting periods at the two locations (Fig. 5). The following possibilities were considered acceptable predictions: light infection (L) below or to the left of the lower confidence band; light or moderate infection (L or M) within the lower confidence band; moderate infection (M) between the lower and upper confidence bands; moderate or severe infection (M or S) within the upper confidence band; and severe infection above the upper confidence band. No infection occurred under field conditions in those instances where no infection was predicted. When infection occurred, the model (Fig. 2) predicted correctly the level of infection obtained in 84.7% of the cases (Fig. 5). Most of the incorrect predictions occurred for wetting periods where moderate infection was anticipated. The model was more efficient in predicting light or severe infection than in separating intermediate values of infection. Factors such as inoculum density, ability to monitor precisely the microclimate on the leaf surface, and individual differences among seedlings can cause random variation that would tend to affect intermediate predictions more than extreme cases of temperature and duration of wetness. No apparent relation was observed between the amount of inoculum trapped and the accuracy of the predictions.

The possibility of using the model proposed here to improve the timing of fungicide applications depends on the climatic characteristics of a particular growing area as well as the availability of eradicant fungicides. Because of the short time required for infection to occur at temperatures greater than 20 C, the time frame to apply an eradicant spray is very short. Thus, during the summer in the southeastern United States, the use of postinfection or eradicant spray probably would not be practical. However, in the spring, when temperatures are lower in the Southeast, or in cooler growing areas of the United States, such as Michigan and New York, the opportunities for postinfection fungicide application are greater.

Another factor that will influence the practical implementation of this model is the availability of fungicides with eradicant properties against *B. obtusa*. Currently none have been identified under field conditions, but Sutton et al (8) have observed strong inhibition of this pathogen in vitro by some sterol-inhibiting fungicides. These fungicides exhibit eradicant activity against other apple diseases such as scab, powdery mildew, and cedar-apple rust. Therefore, it is important to investigate the possible use of these chemicals as management tools for frogeye leaf spot and black rot on apples.

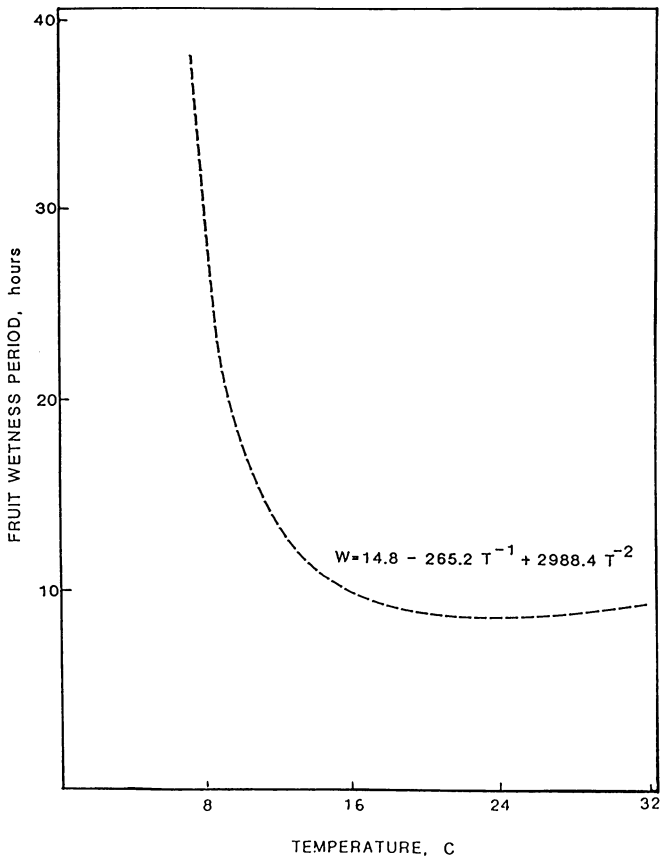

Fig. 4. Effect of temperature (T) on the wetness period (W) required for infection (1 lesion/5 cm^2) of apple fruit by *Botryosphaeria obtusa*.

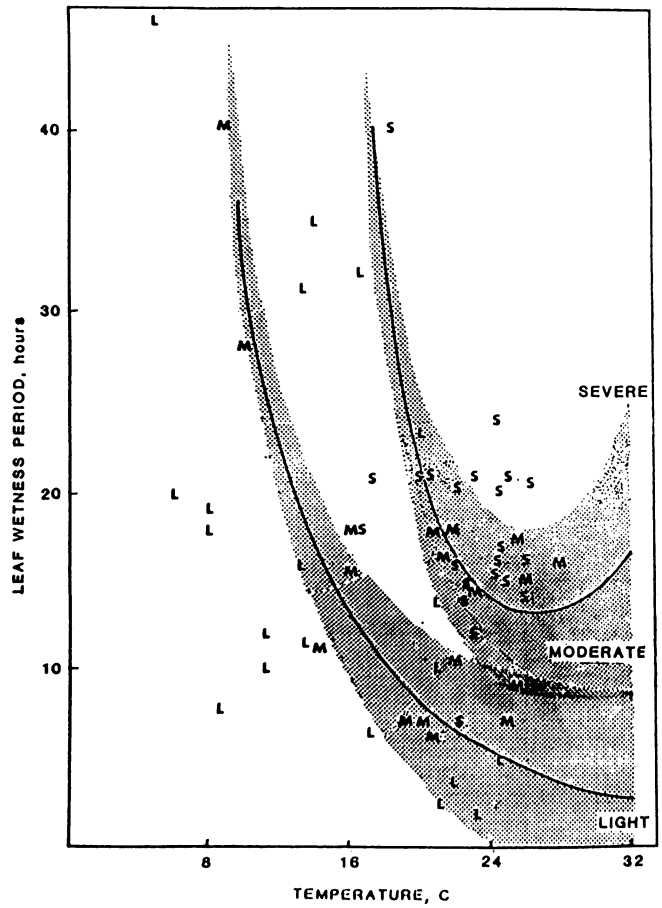

Fig. 5. Levels of apple leaf infection by *Botryosphaeria obtusa* obtained under field conditions. L = light infection; M = moderate infection; S = severe infection. Each letter position indicates the wetness duration and mean temperature during the wetness period in which infection occurred. Shaded areas are 90% confidence intervals for the leaf infection models obtained in controlled conditions.

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characters (1).