Effect of Interrupted Wetness Periods on Spore Germination and Apple Infection by *Botryosphaeria obtusa*

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ABSTRACT

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Conidia of *Botryosphaeria obtusa* were allowed to germinate in water for 4 hr at 24 C, were air-dried, and were rewetted following dry periods ranging from 0 to 8 hr. No increase in germ tube length was observed 4 hr after rewetting in any treatment. Mean germ tube length was similar (approximately 126 μm) for all drying treatments. Mean germ tube length was 532 μm when conidia were allowed to germinate in water for 8 hr without interruption. Apple seedlings were inoculated with conidia of *B. obtusa* in aqueous suspension and were exposed to 24-hr wetness periods, which were interrupted after 12 hr for 1, 2, 3, and 4 hr. Infection of apple foliage stopped irreversibly with interruptions of 1 or more hr in the wetness period. Interruptions as short as 1 hr also resulted in reduced infection of fruit.

The fungus *Botryosphaeria obtusa* (Schwein.) Shoem. (synonym, *Physalospora obtusa* (Schwein.) Cooke) causes black rot on apple (*Malus* × *domestica* Borkh.) fruit, frogeye leafspot, and a limb canker (5). These diseases can cause considerable losses (7,10), especially in warm, humid areas.

Arauz and Sutton (2) studied temperature and wetness duration requirements for infection of apple foliage and fruit by *B. obtusa* and developed preliminary models for predicting infection periods. Optimum temperature for leaf infection was 26.6 C, and, at this temperature, 4.5 and 13 hr of continuous wetting resulted in light and severe infection, respectively. Optimum temperature for fruit infection was 22.5 C; 9 hr of continuous wetting was required for infection at this temperature.

An interruption in the wetness period that is required for infection by a pathogen can lead to reduced disease severity. This has been shown, for example, for apple scab caused by *Venturia inaequalis* (Cke.) Wint. (8,9).

The effect of the interruption of wetting periods on apple infection by *B. obtusa* is not known. The present investigation was undertaken to obtain this information, which could complement the models for infection prediction based on continuous wetting 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. A conidial suspension containing 10^5 spores per milliliter was prepared as described previously (2).

In vitro experiment. To study the effect of interrupted wetness periods on conidial germ tube elongation, one 2-μl drop of spore suspension was placed on each of 32 cover glasses, which were placed in groups of four in inverted water-agar petri dishes to ensure 100% relative humidity (RH) (4). Dishes were sealed with Parafilm (American Can Company, Greenwich, CT) and placed in an incubator at 24 C for 4 hr to allow germination in free

water to take place. Cover slips were removed from the water-agar moisture chambers and allowed to dry at ambient conditions (approximately 23 C and 28% RH [measured with a hygrothermograph]) until no water was detected visually (about 20 min). Cover glasses were randomly allocated to either empty petri plates, corresponding to ambient humidity (28% RH), or to water-agar dishes amended with 2.84 M NaCl, corresponding to 90% RH (6). Dishes were sealed and placed in an incubator at 30 C for dry periods of 1, 2, 4, and 8 hr. This temperature was chosen to simulate the conditions during a dry period in the summer in North Carolina. After the prescribed dry period, conidia were rewetted with sterile water that had been aerated by agitation for 5 sec in a blender. Cover glasses with the rewetted conidia were placed in the 100% RH chambers at 24 C for another 4-hr period. After the second wet period, cover glasses were inverted on a drop of cotton blue in lactophenol on a glass slide to stop germ tube elongation and preserve the samples for future observation. Percent spore germination was determined by observing 50 conidia on each cover glass. A conidium was considered to have germinated if the germ tube was at least one half of the length of the spore. Germ tube length was determined by measuring 10 germ tubes selected randomly. Two controls were used: one in which conidia were allowed to germinate for 8 hr without interruption of the wetting period and one in which conidia germinated for 4 hr only. Each set of treatments was replicated four times in a completely randomized design, and the experiment was conducted twice.

Leaf infection experiment. Seedlings of open-pollinated apple cultivar Delicious bearing seven to nine fully unfolded leaves were inoculated with a conidial suspension by spraying the lower surface of the leaves to runoff with an airbrush. Each plant was immediately placed in a polyethylene bag, which contained a wet paper towel to ensure high RH after the bag was sealed. This procedure allowed the foliage to remain wet for the prescribed wetting period. After a 12-hr wetting period at room temperature (20 C in the first run of the experiment, 24 C in the second), bags were removed and the foliage was allowed to dry. Leaves were kept dry for 1, 2, 3, or 4 hr and then were rewetted with sterile distilled water applied to runoff with an air brush. Seedlings were placed in plastic bags as described previously, for another 12-hr period. One control group of plants was kept in the bags for 24 hr after inoculation and a second control group was allowed to dry and was not rewetted following the initial 12-hr wetness period. Plants were transferred to a greenhouse at approximately 25 C to allow development of symptoms.

Leaves were evaluated for typical "frogeye" lesions 14 days after inoculation. Leaf area was estimated visually with the aid of a leaf area diagram, and the number of lesions (per square centimeter of leaf area) was recorded in the first three fully expanded leaves that received inoculum on each seedling.

This experiment was conducted in a randomized complete block design with four replications and was run twice. The experimental unit consisted of three seedlings. Following an analysis of variance, treatment means were compared by the Waller-Duncan k-ratio multiple comparison procedure, using a 5% probability level ($k = 100$).

Fruit infection experiment. Apple fruit of the cultivar Golden Delicious that had not been sprayed with fungicides were collected about 4–5 wk before commercial harvest. Fruit were washed with 0.5% NaClO, rinsed in tap water, and inoculated the same day they were harvested. Groups of four apples were placed in plastic boxes (7 × 12 × 17 cm) with wet paper towels in the bottom. The inoculation was performed by spraying to runoff a marked area on the side of the fruit facing up with a conidial suspension (10^5 conidia per milliliter), by means of an airbrush. Boxes were covered and sealed with Parafilm. After a 12-hr wetting period at room temperature (approximately 24 C), boxes were opened and the fruit surface was allowed to dry. Dry periods and the subsequent wetting period were the same as for the foliage infection experiment. The rewetting procedure and the control treatments were also similar. A noninoculated control was included to account for natural infection levels. After the second wetness 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^2 circle on the inoculated surface of

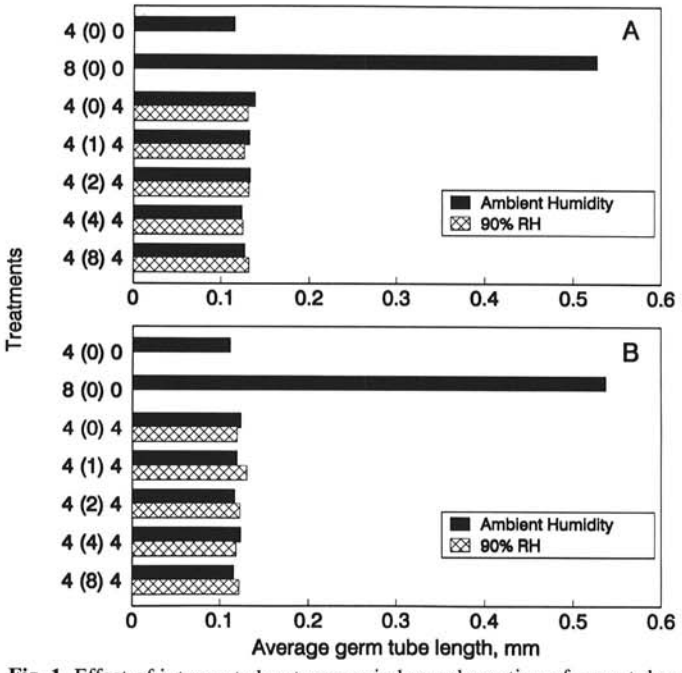

Fig. 1. Effect of interrupted wetness periods on elongation of germ tubes of conidia of *Botryosphaeria obtusa*. Treatment sequence, in hours: wet period after spore release—(dry period)—wet period after dry period. **A,** Run 1; **B,** run 2 of the experiment. In treatment 4(0)4 in both runs, conidia were rewetted immediately after drying.

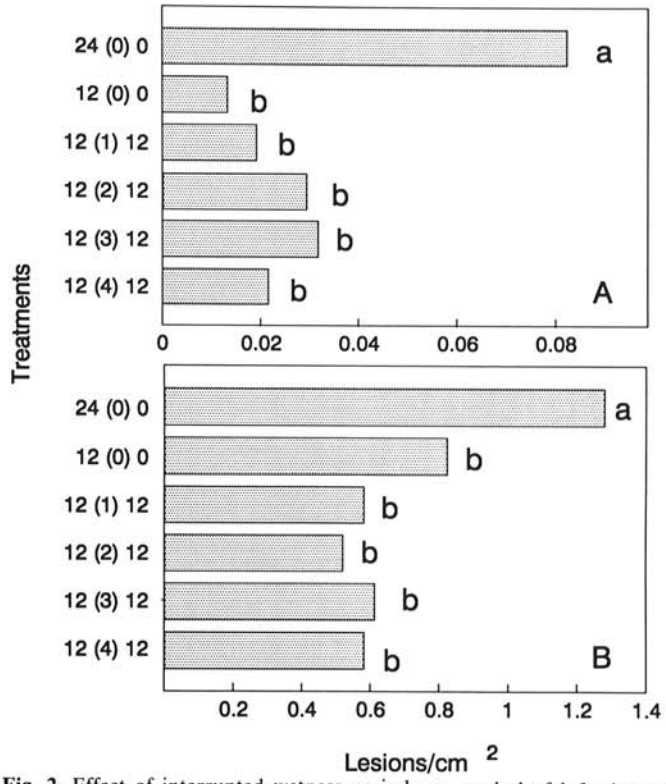

Fig. 2. Effect of interrupted wetness periods on apple leaf infection by *Botryosphaeria obtusa*. Treatment sequence, in hours: wet period following inoculation—(dry period)—wet period after dry period. **A,** Run 1; **B,** run 2 of the experiment. Bars followed by the same letter do not differ significantly from each other (Waller-Duncan k-ratio t-test, $P = 0.05$ [$k = 100$]).

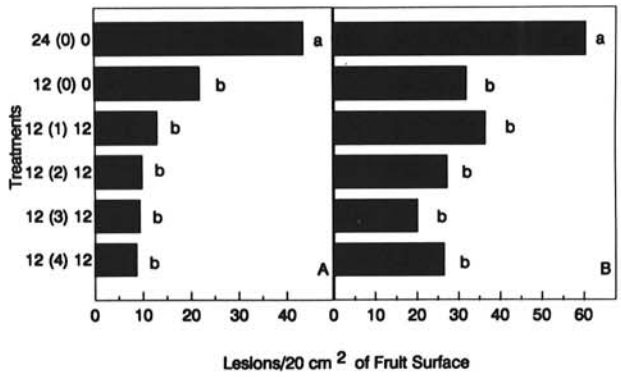

Fig. 3. Effect of interrupted wetness periods on apple fruit infection by *Botryosphaeria obtusa*. Treatment sequence, in hours: wet period following inoculation—(dry period)—wet period after dry period. **A,** Run 1; **B,** run 2 of the experiment. Bars followed by the same letter do not differ significantly from each other (Waller-Duncan k-ratio t-test, $P = 0.05$ [$k = 100$]).

the fruit. The total number of lesions on the four-fruit experimental unit was recorded as number of lesions per 20 cm^2. The mean disease severity value obtained in the noninoculated control was subtracted from the severity obtained on each of the treatments before statistical analysis of the data. Each four-apple box was an experimental unit. The experiment was conducted as a randomized complete block design with four replications and was run twice. Data were analyzed as described for the experiments involving foliage.

RESULTS

Spore germination. Following an initial wetness period of 4 hr, 94% of the conidia of *B. obtusa* had germinated. Mean germ tube length was 122 μm after 4 hr (Fig. 1). No increase in germ tube length was observed after any of the dry and subsequent wet periods. No difference was observed in germ tube length between dry treatments at high (90%) RH and dry treatments at ambient humidity. Average germ tube length after 8 hr of continuous wetting was 532 μm.

Foliage infection. Overall disease severity was higher in the second experimental run than in the first one when lesions and foliage were evaluated (Fig. 2). In each run, disease severity was similar for all the discontinuous 24-hr wetness treatments and between these and the 12-hr control. Disease severity was significantly higher in the 24-hr control than in any other treatments in both runs. The difference in magnitude of disease severity between runs did not allow for a combined analysis of the data.

Fruit infection. The number of lesions (per 20 cm^2) was higher in the 24-hr control than in any other treatment (Fig 3). No significant differences in severity were observed among dry period durations nor between any one dry period and the 12-hr control.

DISCUSSION

When environmental conditions were provided to simulate typical weather during alternating wet and dry periods during the summer in North Carolina, germ tubes of *B. obtusa* were very sensitive to drying in vitro. In no instance did the germ tubes resume growth after being rewetted following dryness periods of any duration. The RH during the dry period did not have any effect on the ability of the germ tubes to continue their elongation upon rewetting. Previous work (3) indicated that nongerminated conidia of *B. obtusa* were also sensitive to drying, with their viability reduced by more than 60% following a 20-min drying period.

Severities of the symptoms caused by *B. obtusa* were similar in the discontinuous wetness treatments and in the 12-hr control on both fruit and foliage. This indicates that infection was interrupted irreversibly following dry periods as short as 1 hr. These results are consistent with those obtained in vitro.

Models for predicting infection periods for *B. obtusa* on apples based on temperature and duration of continuous wetness have been developed (2). Given the drastic effect that short dry periods have on germ tube elongation and on infection, it is apparent that a dry period of 1 hr or longer in duration will end an infection period. Thus, wetness periods interrupted by more than 1 hr can be considered separately when used to predict infection periods.

For disease management purposes, the prediction models for frogeye leafspot and black rot would probably be used in combination with forecasting systems for other early-season apple diseases such as scab (9) and cedar-apple rust (1), which are based also on temperature and wetness duration. The extent to which any particular disease might influence postinfection spraying decisions would be dictated by wetness duration, temperature, and the length of dry intervals between wetness periods. For example, short (< 8 hr), cool (12–16 C) wetness periods separated by a few hours of drying would be added together in the case of apple scab predictions (8) but not to predict the occurrence of frogeye leafspot. Such periods would be more favorable for apple scab. Under conditions of temperature favorable to both diseases, it is possible that spraying decisions would be driven by the apple scab model, because infection by *V. inaequalis* can continue following a short dry period.

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