Factors Affecting the Survival and Spread of *Acidovorax avenae* subsp. *citrulli* in Watermelon Transplant Production Facilities

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ABSTRACT

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Survival of the watermelon fruit blotch pathogen (*Acidovorax avenae* subsp. *citrulli*) on plastic transplant production trays and dissemination of the pathogen in the greenhouse was studied. Bacteria survived 63 days on nontreated trays containing residues of potting substrate and root debris. Longevity of the pathogen decreased with increasing storage temperature. Fruit blotch bacteria were not detected on contaminated plastic trays after the trays were immersed for 5 min in a sodium hypochlorite (10% household bleach) solution. Standard greenhouse sanitation practices should preclude perennation of the pathogen associated with plastic trays used for watermelon seedling production. Overhead irrigation of seedlings resulted in greater levels of pathogen spread. Reduced relative humidities after overhead irrigation resulted in diminished levels of disease. The lowest incidence of disease occurred when trays were subirrigated. Copper hydroxide applied prior to irrigation also reduced disease levels. The threat of serious watermelon fruit blotch epidemics in transplant production facilities can be significantly reduced by eliminating the mechanism for splash dispersal, reducing atmospheric moisture, and using copper sprays as protectants.

Bacterial fruit blotch, caused by *Acidovorax avenae* subsp. *citrulli*, is a seedborne disease that first appeared in commercial watermelon fields in the United States in 1989 (8,12,19). Fruit infection results in expansive lesions on mature watermelons and is responsible for numerous cases of near total losses from 1989 to 1994 (3,8,15). Disease symptoms on foliage generally are inconspicuous (12). Because the pathogen is associated with seed (11, 15), volunteer watermelon seedlings can be a source of inoculum that threatens affected fields during the following season. Also, recovery of the pathogen from sections of rind buried for 10 months (September through June) at a depth of 20 to 30 cm indicated that the pathogen can perennate in association with affected crop residue (R. Latin and K. Rane, *unpublished data*). Resistant watermelon cultivars have yet to be identified, although triploid watermelons apparently sustain less damage than diploid types (9). Protection of young fruit with carefully timed applications of copper hydroxide has been partially successful in reducing yield losses associated with the disease (3).

In many commercial production areas, the high cost of seed of hybrid diploid and triploid (seedless) watermelons has caused a change in planting practices from direct field seeding to field transplanting of 4- to 6-week-old seedlings. Greenhouse facilities used to raise the seedlings often are constructed of clear polyethylene plastic stretched over tubular metal frames. Seeds are planted in soilless media in plastic trays. Temperatures of 20 to 30°C are maintained during early spring, and irrigation is applied primarily through automated overhead sprinkler systems. The warm, humid conditions, overhead irrigation, and susceptibility of the seedling crop contribute to the establishment and spread of seedborne pathogens in transplant facilities.

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The fruit blotch epidemics in Indiana in 1989 were first observed as several distinct foci of symptomatic seedlings in transplant production facilities. The disease was most likely introduced through contaminated seed, and the initial level of contamination was estimated to be 1 infested seed per 9,000 (15). Although the disease causes a seedling blight characterized by necrotic lesions on cotyledons and true leaves (sometimes seedling death), the epidemiology of *A. avenae* subsp. *citrulli* among populations of seedlings has only recently begun receiving attention (10,11). This research was conducted to assess survival of *A. avenae* subsp. *citrulli* on seedling production materials and to investigate factors that affect the spread of fruit blotch on watermelon seedlings.

MATERIALS AND METHODS

Factors affecting pathogen survival. Survival of *A. avenae* subsp. *citrulli* (strain 8908) was assessed on plastic trays (TSC Polyform, Minneapolis, MN) commonly used to raise watermelon seedlings in transplant production facilities. The trays, which were cut to a manageable size (13.8 × 13.8 cm), contained 16 cells; the volume of each cell was approximately 25 cm^3. Both 'new' and 'used' trays were tested. The new trays were unused and contained no apparent organic residue on the plastic surface. Used trays were generated by growing healthy watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) seedlings in a soilless potting medium (Terra Lite Vegetable Plug Mix, W.R. Grace, Cambridge, MA) for 3 weeks and then removing the potting medium and plant material. The used trays were not washed, and residual potting substrate and root debris were evident.

For three of the four experiments, inoculum was applied only by immersing the trays in a suspension (approximately 10^8 CFU/ml) of *A. avenae* subsp. *citrulli* for 2 min. Inoculum suspensions were prepared by washing 24-h cultures incubated at 35°C from the surface of 9-cm-diameter Petri dishes of King's medium B

(KB) agar (17). Trays were allowed to dry for 24 h at 23°C before proceeding with treatments or isolation attempts. In a fourth survival experiment, 12 trays of 3-week-old watermelon seedlings were inoculated by spraying the seedlings with a 10^8 CFU/ml suspension of *A. avenae* subsp. *citrulli* and then incubating at 24°C and 95 to 98% relative humidity (RH) in a lighted incubation chamber for 24 h. Symptoms developed within a few days; after 21 days, most true leaves and cotyledons showed some necrosis. The plant material and potting substrate were emptied from the trays before immersing the trays for 2 min in a 10^8 CFU/ml suspension of *A. avenae* subsp. *citrulli*. Trays were held overnight at 23°C to dry; treatments and isolation attempts proceeded the next day.

Trays that were only dipped in an inoculum suspension were stored at a constant 22°C, a constant 4°C, or in the greenhouse, where temperatures during the study ranged from 24 to 46°C (March through September). The trays that were contaminated by growing diseased seedlings in them and then immersing them in a suspension (10^8 CFU/ml) of *A. avenae* subsp. *citrulli* were stored in the greenhouse during a period when temperatures ranged from 24 to 36°C (November through February). Trays that were treated with sodium hypochlorite (10% household bleach) were soaked in bleach solution for 5 min before they were rinsed with fresh water and allowed to dry at 22°C for 12 h.

On each sample date, a single 6.4-mm-diameter piece was cut from each tray and placed in a droplet (0.01 ml) of sterile distilled water for 2 min. Three replicate trays were sampled for each isolation attempt. Aliquots (1 loopful) from the droplets (four aliquants from each tray for each sample date) were streaked onto KB medium in 9-cm-diameter Petri dishes, incubated at 35°C, and examined after 48 h. Colonies (usually one colony per Petri dish) characteristic of *A. avenae* subsp. *citrulli* were transferred to KB agar and incubated again at 35°C for 48 h. The pure cultures were tested for pathogenicity by infiltrating 0.1 ml of a 10^8 CFU/ml suspension of *A. avenae* subsp. *citrulli* into cotyledons of 3-week-old watermelon seedlings (cv. Crimson Sweet) with a disposable syringe. Inoculated seedlings were maintained on a greenhouse bench and examined for necrotic lesions after 48 to 72 h. If any of the four aliquants from a sample contained bacteria that induced necrosis on watermelon, the sample was considered to be infested by *A. avenae* subsp. *citrulli*.

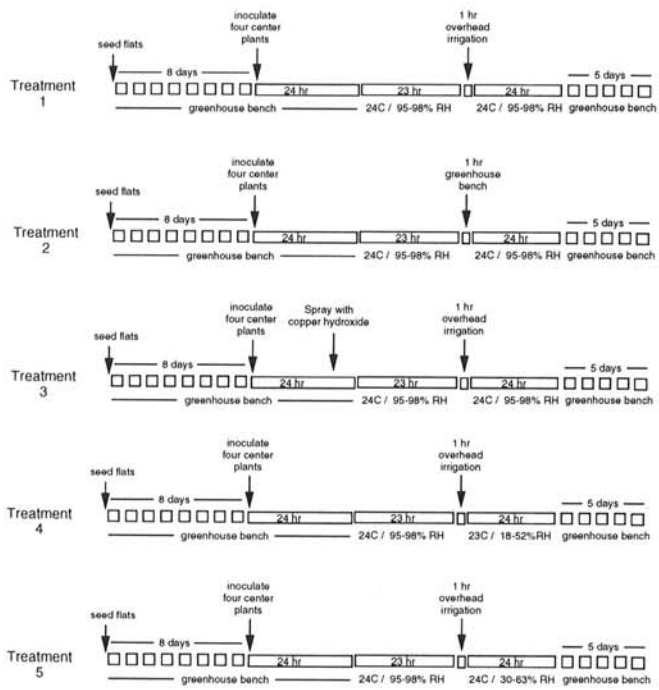

Fig. 1. Description of treatments applied to trays of watermelon seedlings to investigate the effect of certain factors on watermelon fruit blotch disease spread.

Factors affecting pathogen spread. Secondary disease development was evaluated in the greenhouse and controlled environment chambers. Watermelon (cv. Crimson Sweet) seeds were planted in a soilless potting medium (Terra Lite Vegetable Plug Mix) in plastic growing trays (TSC Polyform) that measured 27 × 54 cm and contained 128 cells. Four replicate trays were planted for each experiment, and each experiment was conducted twice. Seedlings were raised on a greenhouse bench where temperature and RH ranged from 21 to 25°C and 30 to 68%, respectively. Environmental conditions in the greenhouse varied with time of day and amount of cloud cover. Treatments began approximately 9 days after planting when cotyledons were fully expanded and the first true leaves were emerging.

Four seedlings in the center of each tray were inoculated 8 days after planting by infiltrating cotyledons with 0.1 ml of a suspension (10^8 CFU/ml) of *A. avenae* subsp. *citrulli*. The trays were maintained on a greenhouse bench for 24 h after inoculation. The bench was modified to water plants through the bottoms of the trays (subirrigation), thereby avoiding any overhead irrigation unless prescribed by the experimental design. After the 24-h postinoculation period, the trays were placed in a plant growth chamber (16 h light, 8 h dark) at 24°C and 95 to 98% RH for 24 h (Fig. 1).

Treatment 1. Trays were irrigated from overhead using Delavan RA-5 raindrop nozzles (Delavan-Delta, Lexington, TN) mounted on a boom over a greenhouse bench. Nozzle pressure was 2.11 kg/cm^2, and output was 0.53 liters/min. The irrigation event consisted of a 5-s shower every 3 min for 1 h. Total water delivery was approximately 1.06 cm/h. After irrigation, trays were moved to the lighted dew chamber for 24 h (24°C and 95 to 98% RH) before being returned to the greenhouse bench.

Treatment 2. Plants were exposed to the same incubation environments as in treatment 1. However, instead of overhead irrigation, plants were placed on a greenhouse bench for 1 h at 23 to 25°C and 40 to 50% RH.

Treatment 3. Plants were exposed to the same irrigation/incubation routine as in treatment 1. However, approximately 20 h after the four center plants were inoculated, the seedlings were sprayed with a suspension of copper hydroxide (Kocide DF, 61.4% a.i., 40% metallic copper equivalent, Griffin Corporation, Valdosta, GA) equivalent to 12 g/liter. The copper application was applied with a hand-held, CO_2-pressurized boom sprayer fitted with a Tee-Jet 8002VS (Spraying Systems Co., Wheaton, IL) flat fan nozzle. The deposit was allowed to dry for several hours before the preirrigation incubation period was initiated.

Treatment 4. Plants were irrigated as in treatment 1 and were exposed to the same preirrigation incubation routine. However, postirrigation incubation differed in that the seedlings were placed on a greenhouse bench where the mean temperature was 23°C and RH ranged from 18 to 52%.

Treatment 5. This treatment was the same as treatment 4, except that plants were incubated after irrigation in a growth chamber where the mean temperature was 24°C and RH ranged from 30 to 63%.

Except for the prescribed overhead irrigations, plants were subirrigated on the modified greenhouse bench to avoid unintended splash dispersal. Symptomatic seedlings were counted 6 days after overhead irrigation. Disease incidence was tallied for five classes based on the distance from the center of each cell in the tray to the nearest seedling with inoculated cotyledons. The five distance classes (5, 10, 15, 20, and 25 cm) were defined such that symptomatic seedlings in cells located 5 cm or less from the inoculum source were grouped in the 5-cm class, symptomatic seedlings in cells located 6 to 10 cm from the inoculum source were grouped in the 10-cm class, etc.

Results for the four replicate trays for each treatment were averaged for each of the two trials. Distance and disease incidence data were fit to Gregory's model for disease spread (6). Prior to $\log_{10}$ transformation of the data, a numerical constant (0.1) was

added to all of the disease incidence means. This created a slightly biased estimate of disease incidence but allowed cases in which no symptomatic seedlings in one or more distance classes occurred to be included in the model. Regression of log-transformed incidence data on log-transformed distance classes resulted in a linear expression of spread (disease gradient) for each treatment. A general linear test approach was used to test the equality of the regression lines, thereby determining treatment differences (14). Regressions were performed using the GLM procedure of the Statistical Analysis System (SAS Institute, Cary, NC) (16).

RESULTS AND DISCUSSION

Survival experiments. The fruit blotch pathogen *A. avenae* subsp. *citrulli* was not recovered from any of the trays soaked in bleach solution, regardless of storage temperature. *A. avenae* subsp. *citrulli* was isolated on days 1 and 7 for the new trays and on days 1, 7, and 14 for the used trays stored at a constant 22°C (Fig. 2A). Survival of *A. avenae* subsp. *citrulli* was substantially longer (63 days) on infested used trays stored at 4°C (Fig. 2B), but the pathogen was only recovered at 14 days on new trays. *A. avenae* subsp. *citrulli* was recovered only at 1 day after infestation when used trays were stored on a greenhouse bench where temperatures fluctuated during the spring and summer months. Also, the pathogen was not detected beyond 1 day on trays infested by growing diseased seedlings in the trays and a 2-min dip in a suspension of *A. avenae* subsp. *citrulli*.

The most likely site for survival of *A. avenae* subsp. *citrulli* in transplant production facilities was the surface of plastic trays that contained infected seedlings. Trays are commonly reused in subsequent seedling production cycles. However, our results are evidence that the pathogen is not likely to survive from one season to the next on residue attached to plastic trays. If trays are stored in warm (22°C) or fluctuating (26 to 40°C) temperatures, *A. avenae* subsp. *citrulli* should not survive beyond a few weeks. Also, treatment of trays with a disinfestant, such as sodium hypochlorite, should further reduce the pathogen population. Since reused trays normally are not stored under refrigeration and usually are treated with a bleach solution before reuse, it appears that growers should be safe in reusing their trays during the next season.

Our results may underestimate the survival period because we used sterile distilled water as a recovery medium. It is possible that a sterile buffer recovery medium would have increased the chances for detecting lower levels of surviving bacteria. Alternatively, our methods may have resulted in an overestimation of the fruit blotch threat because we only determined the presence or absence of the pathogen. The minimum inoculum level necessary for disease development remains uncertain and perhaps warrants further study.

Survival of *A. avenae* subsp. *citrulli* is similar to that of other phytopathogenic bacteria (13). Schaad and White (18) demonstrated that *Xanthomonas campestris* survived for more than 200 days in host debris. However, when a bacterial suspension alone was added to soil, the pathogen was recovered at a maximum of 42 days after infestation. They also showed that survival is likely to be longer in cool temperatures than in warm ones. Basu (1) showed a similar relationship between survival of *Clavibacter michiganensis* subsp. *michiganensis* and temperature.

Spread experiments. The effects of various irrigation/incubation treatments on pathogen spread are described in Figure 3. Spread was greatest in treatment 1, which was characterized by overhead irrigation followed by 24 h of incubation at 24°C and 95 to 98% RH. In treatment 1, more than 80% of the seedlings immediately surrounding the source of inoculum (5-cm distance class) developed symptoms typical of fruit blotch 6 days after irrigation. Also, unlike other treatments, symptomatic seedlings occurred in all distance classes. Treatment 1 resulted in the flattest disease gradient (slope = –1.79) and was significantly different from other disease gradients (Fig. 3A).

The treatment that did not include overhead irrigation (treatment 2) resulted in the lowest incidence of disease, despite trays having been incubated in a 95 to 98% RH environment (Fig. 3B). Symptomatic plants occurred in only the 5- and 10-cm distance classes. Pathogen spread also was limited in treatment 3, in which seedlings were sprayed with copper hydroxide 27 h before overhead irrigation (Fig. 3C). A test for linear equality showed that the regression lines for treatments 2 and 3 did not differ in either slope or intercept. Disease gradients for treatments 2 and 3 were significantly different from those of the other treatments.

Treatments 4 and 5 included overhead irrigation, but differed in their postirrigation incubation environment. Disease distribution was similar, although symptomatic plants were not observed beyond the 15-cm distance class for treatment 4. Symptomatic plants were observed in the 20-cm distance class, but not the 25-cm distance class for treatment 5. The test for linear equality showed no statistically significant ($P = 0.05$) difference between the regression lines for treatments 4 and 5. Disease gradients were less severe than in treatment 1 but were more severe than in treatments 2 and 3. Without careful consideration of disease incidence close to the inoculum source, it could be interpreted that treatments 4 and 5 resulted in less pathogen spread than treatments 2 and 3 because of their more negative slope values (–3.62 and –3.05 for treatments 4 and 5, respectively). However, the flatter slopes (–2.75 and –2.71 for treatments 2 and 3, respectively) were due to lower disease incidence around the inoculum source.

Overhead irrigation appeared to be the primary cause of pathogen dispersal and subsequent disease spread. Elimination of overhead irrigation (treatment 2) lowered disease incidence and resulted in no disease beyond the 10-cm distance class despite incubation at optimum temperature and a nearly saturated atmosphere. Avoidance of overhead irrigation in commercial operations is likely to reduce the threat of serious seedling epidemics. Although most transplant production facilities utilize overhead systems, some facilities use subirrigation. Given the precedence for success in vege-

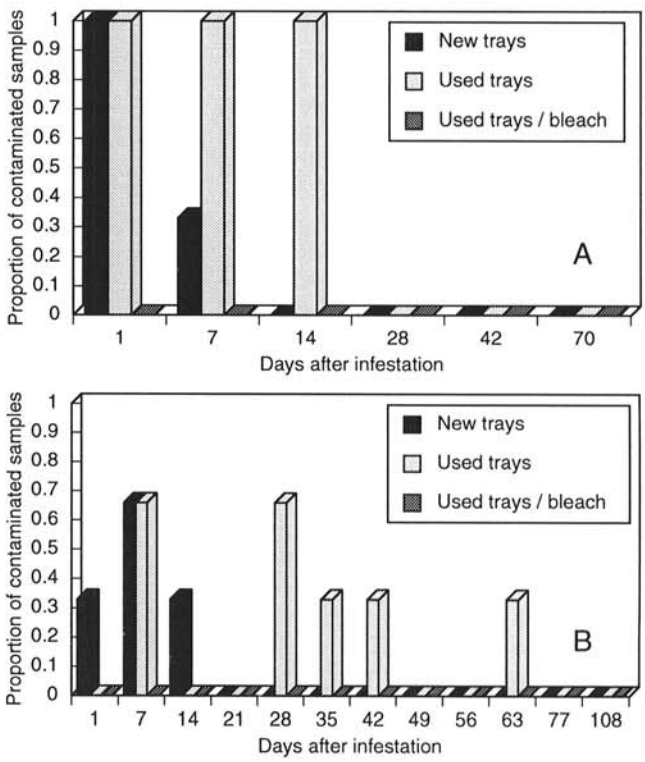

Fig. 2. Proportion of samples of new, used (containing residual potting substrate and root debris), and bleach-treated plastic trays infested with *Acidovorax avenae* subsp. *citrulli* that resulted in successful isolation of the pathogen over time. During the course of the sampling period, trays were stored at **A,** 22 and **B,** 4°C.

table transplant operations, it appears that subirrigation systems are a viable option for managing splash-dispersed pathogens such as *A. avenae* subsp. *citrulli*.

The use of copper hydroxide appeared to negate the effect of overhead irrigation. In the interpretation of these results, it must be considered that only a single irrigation event occurred, and at the time of irrigation, the level of copper protection was at or near its maximum. It is clear that copper sprays will afford some protection. However, their utility should be studied more comprehensively in situations in which daily irrigation contributes to a loss of deposit, seedling growth results in a greater proportion of unprotected plant surfaces, and repeated sprays may select copper-tolerant pathogen strains.

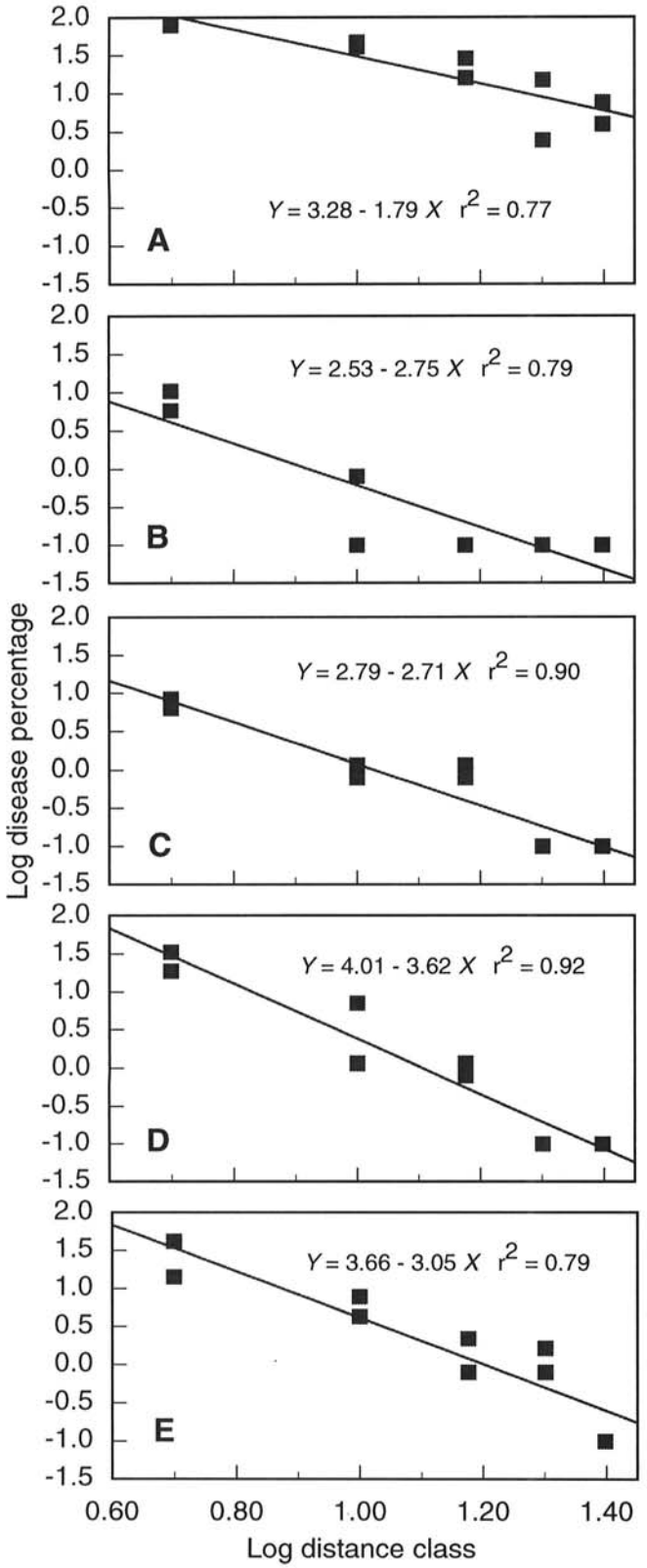

Fig. 3A–E. Disease gradients describing the effects of five treatments on the spread of fruit blotch among watermelon seedlings. **A–E,** treatments 1–5, respectively.

The postirrigation environment for treatment 1 was characterized by temperatures of 22.7 to 26.0°C (mean = 23.9°C) and high RH (96 to 98%). Comparison of the results in Figure 3A with Figure 3D and E revealed that disease development was influenced by the postirrigation environment. For both trials of treatment 4, postirrigation incubation temperatures ranged from 15.5 to 29.0°C (mean = 23.0°C), whereas RH ranged from 18 to 52% (mean = 31.6%). For both trials of treatment 5, incubation temperatures ranged from 22.9 to 25.8°C (mean = 24.1°C), whereas RH ranged from 30 to 63% (mean = 46.5%). The diminished levels of disease at low RH supports conclusions drawn by Hopkins (10). Our work also demonstrated that considerable spread will occur where overhead irrigation is applied to unprotected seedlings, despite low RH.

The disease gradients clearly demonstrate the importance of certain cultural practices in promoting spread of the watermelon fruit blotch pathogen. Significant reductions in spread were evident when overhead irrigation was eliminated, copper hydroxide was used to protect young seedlings, or postirrigation conditions included reduced RH. It appears that a combination of protective sprays and environmental and management modifications may substantially reduce the threat of epidemics in transplant production facilities.

Unless growers can be assured of an uncontaminated seed supply, survival and spread of seedborne pathogens in transplant production facilities will remain a serious concern. Our results support a multifaceted approach to managing the threat of watermelon fruit blotch epidemics in transplant operations. Standard sanitation practices should nearly eliminate the threat of pathogen survival in transplant facilities. Maintaining adequate air circulation to reduce atmospheric moisture will contribute to the management of other diseases as well as benefit seedling growth. During a 4-week period in a commercial transplant operation in Indiana, nightly dew periods of 4 to 9 h were not uncommon (R. Latin, *unpublished data*). Improving air circulation should reduce the duration of periods of leaf wetness.

Overhead irrigation remains a primary determinant of disease spread. Transforming transplant production facilities from overhead to subirrigation will be costly and complicated. There are various approaches to subirrigation, each with a different challenge to transplant producers. However, where the risk of watermelon fruit blotch is great, conversion to subirrigation systems should be considered. In lieu of abandoning overhead irrigation, protection of plant surfaces with a copper bactericide remains a viable option. However, factors such as application rate and interval, loss of deposit over time, and possible phytotoxicity must be studied further before copper applications can be incorporated into routine seeding production operations. Regardless, copper will make its greatest contribution to limiting spread if efforts also are made to reduce RH and possibly limit overhead irrigation.

This research did not address epiphytic survival of the pathogen. It is likely that bacteria were dispersed to seedlings other than those that were symptomatic and that they survived on plant surfaces without causing lesions (4,7). It also is possible that epiphytic populations could later result in infection and symptom expression, thereby increasing the disease incidence resulting from a single irrigation event. The contribution of latent infections to the severity of watermelon fruit blotch epidemics among seedlings remains uncertain. However, because of the relatively short incubation period of the pathogen under these conditions, it is likely that the most significant sources of secondary inoculum are lesions

on symptomatic plants. Environmental and nutritional factors may affect the length of the incubation period, and epiphytic populations apparently contribute significantly to epidemics of other bacterial diseases (2,5,20). Therefore, in our efforts to learn more about the nature of watermelon fruit blotch, it is clear that the role of epiphytic residents warrants further study.

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