Management of Transplant House Spread of Acidovorax avenae subsp. citrulli on Cucurbits with Bactericidal Chemicals in Irrigation Water

Donald L. Hopkins, Professor, and Constance M. Thompson, Biologist, University of Florida, Mid-Florida Research and Education Center, 2725 Binion Road, Apopka, FL 32703; and Branko Lovic, Pio Baroja 5-2H, 30011 Murcia, Spain

Corresponding author: D. L. Hopkins. dhop@ufl.edu


Abstract
Bacterial fruit blotch (BFB), caused by Acidovorax avenae subsp. citrulli, is a seed-borne disease of cucurbits that spreads rapidly in the warm, humid environment of the transplant house, often resulting in high numbers of infected plants going into the field. The only control options for BFB once it gets into a transplant house are crop destruction or multiple applications of a copper-containing bactericide/fungicide. In this study, various treatments were compared with the standard foliar spray application of cupric hydroxide for BFB control under transplant house conditions. Peroxyacetic acid at 80 µg/ml and ionized copper at 1.0 and 1.5 µg/ml applied through the daily irrigation water were more effective than cupric hydroxide in reducing spread of A. avenae subsp. citrulli. Combining ionized copper or peroxyacetic acid in the irrigation water with a weekly foliar application of acibenzolar-S-methyl was most effective in reducing spread. The utilization of these transplant house treatments along with the elimination of all transplants with symptoms or near plants with symptoms should greatly reduce the chances of introducing BFB into fields on transplants.

Introduction
Bacterial fruit blotch (BFB) of cucurbits, caused by Acidovorax avenae subsp. citrulli (Willems et al.) (Schaad et al.) (12,15), can be a devastating disease. BFB on watermelon has occurred in one or more watermelon-producing states in the eastern United States every year since it first occurred in 1989 (9,11,13) (D. L. Hopkins, unpublished data). In some fields, losses have been more than 90% of the total marketable fruit (11). The characteristic symptom of BFB that renders the fruit unmarketable is the water-soaked, dark, olive-green stain, or blotch, that develops on the upper surface of infected fruit (8). Eventually, the lesions turn brown, crack, and ooze a sticky, amber substance. Secondary organisms invade and rot the fruit.

Currently, the most effective control of BFB is the exclusion of the bacterium (7). The intensive efforts of the watermelon seed and transplant industries to produce seeds and transplants free of A. avenae subsp. citrulli have reduced the incidence of BFB significantly over the last 5 to 6 seasons. These efforts include improved production practices by the seed producer, fermentation, and acid treatment of diploid seeds (2,4), use of seeds from lots that have assayed negative for A. avenae subsp. citrulli by grow-out of at least 10,000 seedlings and/or PCR assay (14), and careful inspection of seedlings by transplant producers. In spite of these efforts, the bacterium still appears in a few fields every year.

Most of the severe losses from BFB that have occurred since 1989 have involved infected transplants (3). Contaminated cucurbit seed lots is one way in which the bacterium may be introduced into a transplant house (3). A. avenae subsp. citrulli has been reported to cause disease on cucurbit crop hosts other than watermelon including muskmelon (Cucumis melo L.) (8), honeydew (Cucumis melo L.) (5), and pumpkin (Cucurbita pepo L.) (6). Seed transmission
of *A. avenae* subsp. *citrulli* was observed in 9 different cucurbits, even when there were no fruit symptoms, illustrating that all cucurbit crops must be considered for BFB management (3).

In cucurbit seedlings, *A. avenae* subsp. *citrulli* produces water-soaked lesions on hypocotyls, cotyledons, and leaves. The warm, humid environment of a transplant house is favorable for bacteria from infected seeds to produce disease and spread on the developing seedling (Fig. 1) (8,10). Most transplant facilities have overhead irrigation, which is an effective method of splash-dispersing bacteria to neighboring seedlings. Secondary spread in the transplant house can result in high numbers of infected plants going into the field. Some of these transplants may harbor the bacterium, but show no symptoms. Often different seed lots and even different cucurbits are grown in the same transplant house and *A. avenae* subsp. *citrulli* can spread to seedlings that came from pathogen-free seed lots. The only control options for BFB once it gets into a transplant house are crop destruction or multiple applications of a copper-containing bactericide/fungicide (1). The objective of this study was to evaluate chemical treatments for the control of *A. avenae* subsp. *citrulli* spread on watermelon seedlings in the transplant house.

**Bacterial Point Source for Spread Studies**

Naturally-infected ‘Crimson Sweet’ or ‘Charleston Gray’ watermelon seeds were used to introduce *A. avenae* subsp. *citrulli* into all the greenhouse spread experiments. The ‘Crimson Sweet’ seeds were from a commercial lot and the ‘Charleston Gray’ seeds were produced on the Mid-Florida Research and Education Center in Apopka, FL. The strains that infected the seeds were not identified and could have been mixtures of strains.

**Greenhouse Spread Tests**

In all of the experiments, seeds were planted in plastic trays consisting of 400 2.5-cm-square planting cells per replication filled with commercial potting mix. Each treatment was replicated three times in a complete randomized block design. Each treatment replication was a 50-cm by 50-cm (20-cell by 20-cell) plastic tray. The four center cells were planted with the naturally-infected seeds and the remaining 396 cells were planted with commercial ‘Charleston Gray’ seeds. After planting, trays were watered from the top with a fan nozzle whenever the surface of the potting mix was dry. After emergence, overhead watering was applied to the seedlings daily. Chemical treatments were first applied as seedlings began to emerge (5 to 7 days after planting). Four to five days after seedling emergence, locations of symptomatic seedlings in the 20-cell by 20-cell treatment replications were mapped and counted; this was usually 10 to 12 days after planting (DAP). New symptoms were mapped on 2- or 3-day intervals. Seedlings were not touched during all mappings except the final one, when seedlings were examined carefully for any symptoms that may have been
missed. At the end of the experiments, total emerged seedlings were determined and this number was used to calculate the percent symptomatic seedlings in the various replications. The percent infection for a treatment was the average of three replications. Analysis of variance was run on the percentage infected seedlings and Duncan’s new multiple range test was run to determine statistical differences among treatments on a given date.

Chemicals and Application Methods
Cupric hydroxide (Kocide 101; Griffin Chemical Co., Valdosta, GA) and acibenzolar-S-methyl (ABM) (Actigard; Syngenta Crop Protection Inc., Greensboro, NC) were applied weekly to the seedlings to runoff with a hand-sprayer bottle. Peroxyacetic acid (PA) (Tsunami 100; Ecolab Inc. Mendota Heights, MN) and hydrogen dioxide (ZeroTol; BioSafe Systems, Glastonbury, CT) were applied in the irrigation water with a Dial-a-Spray hose attachment. For ionized copper treatments, a direct current was applied to copper electrodes to electrically generate copper ions and disperse them into the irrigation water that flows over the electrodes (Superior Aqua Enterprises Inc., Sarasota, FL). The direct current to the copper electrodes and the resulting dose rate was maintained by a solid state control unit. The actual copper concentration in µg/ml at the irrigation nozzle was confirmed with a colorimetric test (Taylor Technologies Inc., Kingsford, MI) that measured concentrations between 0.05 and 1.0 µg/ml. An ozone generator produced ozone by splitting oxygen molecules into oxygen atoms. Some of these atoms quickly react with oxygen to form ozone. This ozone was bubbled through a column in the irrigation system, thus adding ozone to the irrigation water (Superior Aqua Enterprises Inc.). The ozone generator was adjusted to produce the desired concentration of ozone at the irrigation nozzle, which was confirmed with a colorimetric ozone test kit (Hach Co., Loveland, CO).

Comparison of Daily Irrigation Water Treatments With a Weekly Copper Treatment
Cupric hydroxide at 840 µg/ml applied weekly was used as the standard copper control treatment for BFB. The other treatments included PA at 80 µg/ml applied daily in irrigation water, PA at 80 µg/ml applied daily in irrigation water plus cupric hydroxide at 840 µg/ml applied weekly, PA at 80 µg/ml applied daily in irrigation water plus ABM at 0.05 g/liter applied weekly, and a water control treatment. The test ran 3 weeks and 2 weekly applications of the cupric hydroxide and ABM were made.

After about 10 days, infected seedlings had emerged in the four center cells, symptoms had developed in these point source plants, *A. avenae subsp. citrulli* had spread to test plants, and symptoms began to develop. In the non-treated group, BFB had spread to 87% of the seedlings by 19 DAP (Fig. 1 and 2). Cupric hydroxide at 840 µg/ml applied weekly, a standard treatment for BFB control, reduced the spread by 50% compared to the non-treated at 17 DAP, but only by 25% after 19 days. Applied daily in irrigation water, PA at 80 µg/ml was significantly more effective than cupric hydroxide 14 and 17 DAP, reducing spread by 80%. By 19 days, PA was not significantly different than cupric hydroxide. The combination of PA at 80 µg/ml applied daily in irrigation water plus cupric hydroxide at 840 µg/ml applied weekly provided significantly better control of BFB than either treatment alone, reducing spread when compared with the non-treated by 90% at 17 days and by 65% at 19 days. By 19 DAP, PA plus ABM at 0.05g/liter applied weekly provided significantly better control of BFB spread than all other treatments, except the PA plus cupric hydroxide combination. Disease spread was reduced by 87% and 80% at 17 and 19 days, respectively. The experiment was repeated with similar results.
Fig. 2. Control of spread of bacterial fruit blotch of watermelon in the transplant house with peroxyacetic acid (PA) in the irrigation water, PA in the irrigation water plus weekly sprays of cupric hydroxide or acibenzolar-S-methyl (ABM), or weekly sprays of cupric hydroxide alone. PA treatments were applied at 80 µg/ml in the daily irrigation water; cupric hydroxide was applied weekly as a 840 µg/ml spray solution; and ABM at 0.05 g/liter was also applied as a weekly spray. Treatments labeled with the same letter on a given date were not significantly different at $P = 0.05$ by analysis of variance and Duncan’s new multiple range test.

### Comparison of Dose Rates of Ionized Copper in Daily Irrigation Water

Ionized copper treatments in the daily irrigation water at 0.5, 1.0, or 1.5 µg/ml were evaluated for the control of spread of BFB in June when conditions were ideal for spread (hot and humid). Daily irrigation with water only was the control treatment. The test ran 2 weeks and was repeated twice with similar results.

By 16 DAP, more than 60% of the untreated control seedlings had symptoms of BFB (Fig. 3). Under these conditions, all three rates of ionized copper significantly controlled spread of BFB through 13 DAP when compared with the non-treated, but only the 1.5 µg/ml rate significantly controlled disease spread 16 DAP. In the irrigation water, ionized copper at 1.5 µg/ml reduced spread of BFB by 97% thirteen DAP when compared to the non-treated control and by 92% at 16 days. Over the 16-day experiment, ionized copper at 0.5 and 1.0 µg/ml reduced spread, by 45% and 55%, respectively.
Comparison of Various Treatments in the Daily Irrigation Water

In this experiment, ozone and ozone plus copper were dispersed in the daily irrigation water and compared with ionized copper and hydrogen dioxide for the control of spread of *A. avenae* subsp. *citrulli*. Treatments included (i) ionized copper at 1.0 µg/ml, (ii) ozone at 0.05 µg/ml, (iii) ionized copper at 0.5 µg/ml plus ozone at 0.05 µg/ml, and (iv) hydrogen dioxide at 900 µg/ml. When this experiment was repeated, hydrogen dioxide was decreased to 540 µg/ml, because 900 µg/ml was phytotoxic. Copper ions were generated as previously described. Daily irrigation with water was the control.

By 16 DAP, 41% of the non-treated control seedlings had symptoms of BFB (Fig. 4). Ozone at 0.05 µg/ml did not significantly reduce BFB spread when compared to the non-treated control. Hydrogen dioxide at 900 µg/ml significantly reduced spread at 14 days, but did not significantly reduce spread at 16 days. It also was phytotoxic to the seedlings. Ionized copper at 1.0 µg/ml, hydrogen dioxide at 900 µg/ml, and ionized copper at 0.5 µg/ml + ozone at 0.05 µg/ml provided significant control of disease spread through 14 DAP when compared to the non-treated control. Only the ionized copper at 1.0 µg/ml provided significant control through 16 days, reducing spread by 70% over the 16-day experiment. In the repeat of this experiment, hydrogen dioxide was ineffective at 540 µg/ml (*data not shown*).
Fig. 4. Comparison of ionized copper, ozone, and hydrogen dioxide in the irrigation water for the control of spread of bacterial fruit blotch of watermelon in the transplant house. Treatments included were ionized copper alone at 1.0 µg/ml or 0.5 µg/ml in combination with ozone, ozone at 0.05 µg/ml, and hydrogen dioxide at 900 µg/ml. Treatments labeled with the same letter on a given date were not significantly different at $P = 0.05$ by analysis of variance and Duncan’s new multiple range test.

Best treatments for the control of greenhouse spread of BFB

Some of the better treatments from the three experiments reported above were compared for efficacy. Treatments included (i) PA at 80 µg/ml applied daily in irrigation water, (ii) PA at 80 µg/ml applied daily in irrigation water plus ABM at 0.05 g/liter applied weekly, (iii) ionized copper at 1.0 µg/ml, (iv) ionized copper at 0.5 µg/ml plus ABM at 0.05 g/liter applied weekly, and (v) daily irrigation water as the control. The experiment was repeated once.

In the non-treated seedlings, BFB had spread to 70% of the seedlings 14 DAP and to 84% of the seedlings at 16 days (Fig. 5). All four of the treatments were effective on the seedlings at 14 DAP, reducing spread by 83 to 97%. At 16 DAP, Cu at 1.0 µg/ml plus ABM at 0.05 g/liter applied weekly reduced spread by 95%. PA at 80 µg/ml plus ABM at 0.05 g/liter applied weekly and ionized copper at 1.0 µg/ml also were effective through transplanting, reducing spread by 80%. PA at 1.0 µg/ml lost effectiveness as the seedling became larger; however it was still significantly better than the non-treated control, but not as effective as the other treatments.
Fig. 5. Evaluation of the more effective treatments for the control of spread of bacterial fruit blotch of watermelon in the transplant house. Treatments included were peroxyacetic acid (PA) at 80 µg/ml applied daily in irrigation water, PA plus acibenzolar-S-methyl (ABM) at 0.05 g/liter applied weekly, ionized copper in irrigation water alone at 1.0 µg/ml, and ionized copper in irrigation water at 0.5 µg/ml in combination with weekly ABM applications. Treatments labeled with the same letter on a given date were not significantly different at \( P = 0.05 \) by analysis of variance and Duncan's new multiple range test.

**Discussion**

Application of PA in the daily irrigation water provided better control of *A. avenae* subsp. *citrulli* spread than the standard weekly spray with cupric hydroxide (1). While PA was very effective in the young seedlings, the level of control declined as the seedlings grew and reached transplanting size. Perhaps the coverage was not as good with overhead irrigation in larger plants. Ionized copper in the irrigation water maintained a high level of control of BFB even as the seedlings grew larger. Ionized copper provided control at a copper concentration that was 800-fold lower than with the cupric hydroxide. This was probably because of the high solubility of the ionized copper and the positive charge of the ion that attracted the bacteria. With the ionized copper, there also is much less copper applied, which should reduce run-off of copper onto the soil. Ionized copper easily could be incorporated into the overhead irrigation systems used by many transplant growers.

Weekly applications of ABM along with the PA or ionized copper in the daily irrigation water improved the control of BFB spread over PA or ionized copper alone, especially as the plants approached transplanting size. The systemic acquired resistance that results from ABM treatments can carry over to help protect the plants in the field (D. L. Hopkins, *unpublished*). We consider the ionized copper + ABM treatment to best control of transplant house spread of BFB and to have the best chance of preventing the introduction of BFB into the field. Registration and labeling may preclude the use of some of the chemical treatments used in this study.

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Literature Cited