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Tests of MicroBloc™ for controlling Botrytis blossom blight of roses and for decontaminating spore-soiled hard surfaces.

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Part A. Control of Botrytis Blossom Blight of Roses—inoculation and incubation.

In initial tests, stems of three rose cultivars, 'Madam', 'Aalsmear Gold', and 'Peckuboo' were inoculated in four ways with two isolates of *Botrytis cinerea* to determine how rose buds should be inoculated prior and after treatment with MicroBloc. Stems of each cultivar were cut under water and then placed in standard vases containing "Crystal Clear" flower food. Nine teams of students inoculated the rose buds with an aqueous spore suspension containing 1×10^5 conidia/ml of either an isolate of *B. cinerea* from strawberry or from a blighted rose. The four inoculation treatments were: mist with the spore suspension to near runoff, briefly dip buds into the suspension, place droplets of suspension on the top of the buds, or wound the buds with straight pins that had been dipped into the spore suspension. Half of the inoculated buds were covered with a plastic (polyethylene) bag immediately after inoculation and half after the buds dried for an hour. Control buds in each bag included water alone and no treatment. The vases were held in the laboratory which averaged 22 C. The initial observation of flower blight was recorded after 48 h and then at 24-h intervals thereafter. The results reported here were at the 72-h observation. The scale used was 1=no signs or symptoms, 2=signs and symptoms on 1 to 20%; 3=signs and symptoms on 20 to 40%; 4= signs and symptoms on 40 to 60%; 5= signs and symptoms on 60 to 80% and 6= signs and symptoms on 80 to 100%.

Results: Symptoms as well as a few signs including visible mycelium and sporulation were apparent by 48 h post inoculation. Up to 80% of a few buds were blighted. By 72 h, 4 of 18 buds that had been inoculated by immersion were in the severest blight group. There was no apparent difference in aggressiveness between the isolate from rose or from strawberry. 'Madam' buds were less susceptible than those of 'Aalsmear Gold' or 'Peckuboo.' The most severe disease occurred on buds that had been dip inoculated, followed by mist inoculated, droplet inoculated with wound inoculated ranking lowest. There was no marked increase in disease associated with placing a bag over the inoculated buds immediately as compared with 1-h later. Some symptoms were observed on control buds, particularly if they were near inoculated buds. Buds in contact with the bags were disease prone. Finally, there was an obvious "team" effect on severity of disease after mist inoculation. Certain teams of students misted more inoculum on their buds than did others.

Conclusions: For subsequent tests with MicroBloc treatment of rose buds, use 'Aalsmear Gold' or 'Peckuboo.' Mist inoculate but make sure each group of buds receives the same volume of mist. Cover the inoculated buds with a plastic bag but make sure none of the buds contacted the inside of the bag. Use just the rose isolate of *B. cinerea*. Evaluate the treated roses daily for 1 wk or until a few buds were completely blighted. Use

temperatures below room temperature. Change the evaluation system slightly to give more weight to initial symptoms.

Treatment of rose buds with MicroBloc.

Stems of 'Aalsmear Gold' were received, cut under water and placed in 1-liter vases containing Crystal Clear flower food. The buds were uniformly misted with an aqueous suspension of 1×10^5 conidia/ml either before or after the buds were misted with a solution of MicroBloc. The buds were allowed to dry for 30 min after inoculation or MicroBloc treatment and before being covered with a plastic bag. Microbloc concentrations were as suggested by vender, e.g. 0, 1.0, 2.5, 5.0 and 10.0 ml of the formulated product per liter of tap water (pH 8.0-8.5). This provided solutions containing 0, 200, 500, 1000, or 2000 ppm active ingredient. The pH of the most concentrated solution was 6.7. The buds were misted to runoff. Each vase contained 10 stems with each treatment represented, e.g. 0 to 2000 ppm applied before inoculation and 0 to 2000 ppm applied after inoculation. A separate vase held 10 stems that were not inoculated or treated with MicroBloc. There were 10 vases (=replicates) for each of three temperatures, 5, 15 and 24°C. Each storage unit featured fluorescent lights that were on for 12 h per day. The buds were examined daily and blight was recorded on a 0 to 6 scale based on the discoloration of the bud surfaces where 0 = 0%, 1= 1to 5%, 2= 5 to 15%, 3=15 to 25%, 4 = 25 to 50%, 5 = 50 to 75% and 6=.75%. Buds were discarded when signs of blight (mycelium and sporulation) were observed.

Results

A sample of the stems received for the inoculation, incubation study reported above was misted with MicroBloc at 0 to 2000 ppm to test for phytotoxicity. Discoloration was observed on the Peckuboo buds treated with all concentrations although symptoms were mild with the 200 and 500-ppm concentrations. Scorching was observed among all buds treated with 2000 ppm. Apparent watersoaking was found among 'Aalsmear Gold' treated with 500 ppm and became more severe with the higher concentrations. Buds of 'Madam' had no apparent symptoms of phytotoxicity when treated with 200 to 1000 ppm.

The average blight among 'Aalsmear Gold' buds held at 5°C for 6 days ranged up to nearly 10% for buds treated before inoculation (Fig. 1) or to 5% (Fig. 2) for buds treated after inoculation. Error bars represent the 95% confidence limits. The highest blight severity was among buds treated before inoculation with 2000 ppm MicroBloc. Among buds stored at 15°C for 5 days, the 2000-ppm MicroBloc treatment applied before inoculation (Fig. 3) again led to the most disease with a severity over 30%. In contrast, the 500 and 1000-ppm treatments applied after inoculation (Fig. 4) appeared to reduce disease severity significantly. Among buds stored at 24°C for 4 days, the 2000-ppm MicroBloc treatment applied before inoculation (Fig. 5) again led to the most disease with a severity of nearly 40%. The lowest severity of blight in the 24-C group occurred among the control blooms that had been treated after inoculation (Fig. 6).

Conclusions

The phytotoxicity symptoms observed after MicroBloc treatment of samples of the first buds received were apparently not due to age of the buds. This phytotoxicity appeared to confound the effects of the a.i. on the spores. The best results in terms of control occurred when MicroBloc was applied after inoculation suggesting a contact rather the protection phenomenon. In contrast, when MicroBloc was applied before inoculation, disease severity mostly increased with increased concentration of a.i.

Treatment of spore contaminated surfaces.

Materials and Methods:

Cleaned glass slides were contaminated with spores of B. cinerea and then treated with MicroBloc solutions. The solutions were removed and the spores covered with a liquid growth medium. Germination was counted. On each slide, 8 µL of an aqueous suspension of B. cinerea containing 400 conidia was applied to each of three locations and then allowed to dry for 20 min in a fume hood. Each spot was treated with 20 µL of MicroBloc for 10 min. Residual MicroBloc was blotted with Kimwipe tissue paper. Next, 20 µL of potato dextrose broth was placed in each spot and the slides were enclosed in humidity chamber on a raised platform. The spores were examined after 18 24, 48 and in certain tests 72 h of incubation at 22 C. At each spot 100 spores were counted. Spores were considered germinated if the germ tube equaled or exceed the diameter of the spore. There were three 100-spore spots(=replication) counted for 0, 9, 19, 47 or 94 ppm a.i. MicroBloc. These concentrations correspond to dilutions of 0, 0.03, 0.06, 0.15 or 0.30 oz of formulated product per 5 gallon of tap water. The entire experiment was repeated three times. Spore germination, reported as percentage of control germination, was averaged for the three experiments and reported on a bar graph with 95% confidence intervals (Sigma Plot version 8.0). In a second series of tests, a wax pencil was used to make a ring on the cleaned glass slide. A spore suspension was applied to the ring as before and allowed to dry for 20 min. MicroBloc solutions were applied to the spore deposit and allowed to stand for 10 min. The excess liquid was shaken from the slides, which where then allowed to completely dry. Potato dextrose broth was placed in the rings and the slides were incubated and observed as before. In a final series of tests, MicroBloc solutions were added to molten agar at 45°C, which was then added to 15 by 60 mm Petri Dishes and allowed to harden. Either a 5.6mm diameter plug of a plate culture or 8 µL spore suspension was placed in the center of the agar plate. Growth of a colony in the center of the plate was recorded over a 7-day period.

Results:
In preliminary tests, the recommended doses of MicroBloc at 1 through 10 ml/L or 100 through 2000 ppm a.i. completely inhibited germination. Consequently, the dosages were cut back to 9 through 94 ppm. The germination of the control treatment was recorded after 18 h. Further counts were difficult because of hyphal development. Treated spores, however, were observed after 24 and 48 h to find if recovery occurred.

Spore germination after 18 h was completely prevented by 47 ppm a.i. (Figs. 7 and 8). The 19-ppm concentration reduced germination to less than 20% of the control. Prolonged incubation did not reveal recovery of the inhibited spores except in certain samples there appeared to be evidence that mycelial fragments were growing even in the 94-ppm treatment. When MicroBloc was added to potato dextrose agar which was then inoculated growing colonies appeared within 7 days from spores placed on 47 but not 94 ppm (Fig. 9) In contrast, the highest concentration tested did not completely prevent growth from the plug of mycelium, which was roughly 40% of the growth of the control (Fig 10).

Discussion and Conclusions:

Non-porous, hard surfaces that are contaminated with spores of *B. cinerea* would be reliably disinfested of that fungus if flooded with a solution of MicroBloc containing 47 ppm a.i. (a dilution of 0.15 ounces of the formulated product in 5 gal of tap water) for 10 min. This efficacy was determined in the presence of 20% infusion of potatoes (in potato dextrose broth) A somewhat higher concentration (≥94 ppm) would be required if the surfaces were somewhat permeable or were contaminated with plant debris. However, growth of *B. cinerea* from established mycelium (diseased petal fragments, colonies already established on hard surfaces) was not completely inhibited by the highest concentration tested (94 ppm a.i.).

Suggestions for further work.

Spores of *B. cinerea* are reliably inhibited by quaternary amine concentrations (47 ppm) less than 1 quarter of lowest amount (200 ppm) applied to the flower buds. The latter was borderline phytotoxic to the buds in preliminary tests (occasionally linked to discoloration particularly where petals contacted the plastic cover). The next highest concentration recommended for our tests (500 ppm) was frequently associated with browned petal tips and other discoloration). Some additional tests on bloom applications with concentrations ranging from 50 to 500 ppm a.i. might be warranted. Also the surface disinfection tests did not include any tests with petal fragments that might be colonized by *B. cinerea*. Although florists are instructed to wash surfaces thoroughly before applying MicroBloc, air-borne fragments might not be reliably removed from the premises. These fragments might be a source of contamination much as bacterial aerosols can cause problems in fresh cut produce preparation areas. Since the tests reported here clearly point out that prevention of the growth of mycelium is more difficult that spore germination, tests to find concentrations that prevent all development of *B. cinerea* might be worth while.