



Controlling powdery mildew on cucurbit rootstock seedlings in the greenhouse with fungicides and biofungicides

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ABSTRACT

Powdery mildew (*Podosphaera xanthii*) affects seedlings of inter-specific hybrid squash (*Cucurbita moschata* × *Cucurbita maxima*) and bottle gourd (*Lagenaria siceraria*) used as rootstocks to graft seedless watermelon (*Citrullus lanatus* var. *lanatus*). Because powdery mildew grows primarily on the leaf surface where contact fungicides are effective, biofungicides may be effective preventative treatments for powdery mildew. The objectives of this study were to determine which biofungicides, organic fungicides, and conventional synthetic fungicides provided the best control of powdery mildew and least phytotoxicity on cucurbit rootstock seedlings in the greenhouse. Sixteen treatments (six biopesticides, four additional organic-approved fungicides, and six conventional synthetic fungicides) were tested. Four experiments were conducted and all were repeated once. Hybrid squash 'Strong Tosa' seedlings were used in the first three experiments, and bottle gourd 'Emphasis' seedlings were used in experiment four. In experiments one, two, and four, seedlings were sprayed three times at 5-day intervals and exposed to powdery mildew continuously after the first application. In the third experiment, seedlings were exposed to inoculum for 7 days, sprayed once, and held in a humidity chamber for 7 days under conditions used for healing after grafting. The most effective organic-approved fungicides were sulfur and fish oil + sesame oil, and the most effective conventional fungicides were penthiopyrad, myclobutanil, and cyprodinil plus fludioxonil. Quinoxifen was phytotoxic to cotyledons of both species, and tebuconazole stunted both species. To manage powdery mildew, one or two preventative applications of sulfur or fish oil + sesame oil and one application of myclobutanil or penthiopyrad, if needed, are recommended.

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1. Introduction

Grafting cucurbits is done in various countries, notably Korea, Japan, and Israel, to combat soilborne pathogens, provide cold tolerance, and increase yield (Cohen et al., 2007; Kubota et al., 2008). For grafting watermelon (*Citrullus lanatus* var. *lanatus*), the two most common rootstocks used are cultivars of a squash inter-specific hybrid (*Cucurbita moschata* × *Cucurbita maxima*) and cultivars of bottle gourd (*Lagenaria siceraria*) that were bred specifically for use as rootstocks (King et al., 2008; Thies et al., 2010; Yetisir and Sari, 2003).

Powdery mildew is a common problem in greenhouses where relative humidity is kept low to create environmental conditions unfavorable for other foliar pathogens (Jarvis, 1992). Cucurbit powdery mildew (*Podosphaera xanthii*) may affect rootstock and watermelon seedlings before grafting and after grafting during the

healing phase, when plants are held for 7 days in a mist chamber at 100% relative humidity or on greenhouse benches with frequent overhead misting to keep seedlings turgid while the vascular bundles of the scion and rootstock connect to each other (Hassell et al., 2008; Keinath et al., 2010a). Because seedlings and trays are spaced closely together in greenhouses in which cucurbit seedlings are produced, the potential for rapid spread of powdery mildew from a few infected seedlings is likely (Jarvis, 1992).

Because powdery mildew grows primarily on the outside of leaves, contact (or protectant) fungicides are effective for managing powdery mildew on the upper leaf surfaces (McGrath, 2002). Since most biofungicides and other fungicides approved for organic production are contact fungicides, these products have been effective against *P. xanthii* on cucurbits. In various field trials on *Cucurbita pepo*, the most effective materials have been sulfur (McGrath, 2002), paraffinic oil (McGrath, 2002; McGrath and Shishkoff, 1999), and potassium bicarbonate (McGrath, 2002; McGrath and Shishkoff, 1999). Likewise, these three fungicides were among the most effective products tested on greenhouse

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cucumber (*Cucumis sativus*) (Smither-Kopperl et al., 2005). Other biofungicides have been effective in some trials but not in others; these include 92% edible fish oil + 5% sesame oil (Organocide) (McGrath, 2005; McGrath and Davey, 2007), *Streptomyces lydicus* (Actinovate) (McGrath and Davey, 2007; Zhang et al., 2011), *Reynoutria sachalinensis* extract (Regalia, formerly marketed as Mil-sana) (Langston and Sanders, 2010; McGrath, 2002), and *Bacillus subtilis* (Serenade) (Langston and Kelley, 2002; McGrath, 2002). Cow's milk and whey also have controlled powdery mildew on *C. pepo* and cucumber in some trials but not in others (Bettiol et al., 2008; Ferrandino and Smith, 2007; Smither-Kopperl et al., 2005). It is possible that some biopesticides may be more effective in the controlled environment of a greenhouse than in the field. However, protectant fungicides often provide poor control of powdery mildew, which also grows on abaxial leaf surfaces (McGrath, 2002; Keinath et al., 2010a,b). Thus, effective management of powdery mildew on the whole plant often requires application of systemic fungicides. In addition, systemic fungicides with curative activity may be needed if the fungicide is not applied until symptoms of powdery mildew are noticed.

The Fungicide Resistance Action Committee (FRAC) has assigned a medium risk of resistance development to many of the fungicide active ingredients currently registered for use against cucurbit powdery mildew (Brent and Holloman, 2007b; FRAC, 2011). These include myclobutanil (Rally), tebuconazole (Monsoon), cyprodinil (Switch), fludioxonil (Switch), and quinoxifen (Quintec). Penthiopyrad, a new active ingredient registered as the fungicide Luna in early 2012, is rated as having a medium to high risk of resistance development (FRAC, 2011). *P. xanthii* develops resistance (insensitivity) to fungicides more readily than other fungal pathogens do (Russell, 2004). This is particularly true in greenhouses, where once the fungus is introduced, the same population is repeatedly exposed to fungicides with successive applications (Brent and Holloman, 2007a,b; McGrath, 2001). One advantage of using biofungicides is that no pathogens are resistant to any biofungicide (FRAC, 2011). Similarly, organic-approved copper fungicides (FRAC group M1) and sulfur (FRAC Group M2) have multiple modes of action, which prevent development of resistance (FRAC, 2011).

The objective of this study was to determine which biofungicides, organic fungicides, and conventional synthetic fungicides provided the best control of powdery mildew on cucurbit rootstock seedlings in the greenhouse. Because bottle gourd and hybrid squash are not routinely tested when fungicides are evaluated for crop safety, seedlings also were checked for phytotoxicity symptoms.

2. Materials and methods

2.1. General procedures

The experimental design for all experiments was a randomized complete block with four replications. All experiments were done twice. Each experimental unit was a 10-cm plastic pot with three 10-day-old seedlings. Seedlings were raised in a greenhouse room with natural lighting and 58% relative humidity that was free of powdery mildew. Treatments were started when cotyledons were fully expanded before the first true leaf emerged. Fungicides prepared at labeled rates in 935 L of water were applied to seedlings with a hand-held, pump-pressurized sprayer. Non-sprayed and water-sprayed controls were included in all experiments. Because seedlings are used for grafting before the first true leaf emerges, the true leaf or leaves were removed immediately before the second and third fungicide applications, and powdery mildew that developed on true leaves that emerged between spraying and rating was ignored (Memmott and Hassell, 2010). The numbers of plants that had symptoms (colonies) of powdery mildew on

hypocotyls or upper or lower cotyledon surfaces were counted separately. Severity was rated using a 16-point Horsfall-Barratt-type scale to estimate the percentages of the surface areas of the upper and lower cotyledons with symptoms (Keinath and DuBose, 2004). Symptoms of phytotoxicity were noted and recorded as present or absent for each experimental unit at each rating period.

2.2. Applications of fungicides and biofungicides

The first two experiments included a total of 16 products: six biopesticides, four additional active ingredients allowed in organic production (paraffinic oil, sulfur, copper hydroxide, and skim milk), and six conventional synthetic fungicides effective against powdery mildew and registered for use in greenhouses (Table 1). Seedlings of *Cucurbita* hybrid 'Strong Tosa' were sprayed once and then exposed to powdery mildew by moving plants into a greenhouse room in which powdery mildew was present on other cucurbit plants and placing eight plants of squash (*C. pepo*) (experiment one) or 'Strong Tosa' (experiment two) with sporulating colonies of powdery mildew among the pots. Seedlings were treated two more times at 5-day intervals while they were continuously exposed to powdery mildew. Plants were examined and rated immediately before and 5 days after the third application. In this experiment only, the number of colonies of powdery mildew was counted as an alternative method of assessing severity; because the treatment means were ranked in exactly the same order and were separated the same using the severity rating and colony counts, only severity ratings were used subsequently (data not shown).

In the third experiment, curative (post-infection) applications of fungicides and biofungicides were tested. Seedlings of *Cucurbita* hybrid 'Strong Tosa' were exposed to powdery mildew inoculum for 7 days before treatment by moving plants into a greenhouse room in which powdery mildew was present on other cucurbit plants and placing eight nonsprayed control plants of 'Strong Tosa' from experiment two with sporulating colonies of powdery mildew among the pots. They were then sprayed once with one of 14 products and moved to an enclosed chamber with 100% relative humidity for 7 days to simulate healing conditions as if they had been grafted. Powdery mildew was rated 7 days after treatment when seedlings were removed from the humidity chamber.

In the fourth experiment, seedlings of *L. siceraria* 'Emphasis' were sprayed three times at 5-day intervals with 11 products that performed well in the previous experiments. Seedlings were exposed to powdery mildew inoculum continuously after the first application as described previously. Plants were examined and rated immediately before and 5 days after the third application. The cost of the treatments included in this experiment was calculated from retail prices supplied by a local agrochemical dealer.

2.3. Data analysis

Because the level of powdery mildew always increased between the first and second ratings, only data from the second ratings were analyzed. Powdery mildew incidence was calculated as the proportion of seedlings that had symptoms (colonies) of powdery mildew on any plant part. The midpoint of the percentage ranges used to assess severity was used in place of the numerical rating for data analysis. The midpoint percentage severities for the upper and lower cotyledon surfaces were summed before analysis. Percentage severity on the top and underside of cotyledons in experiments three and four also was analyzed separately. (To avoid problems with inequality of variance, only combined severity was analyzed in experiments one and two when many experimental units had no disease.) Data were analyzed with PROC MIXED of SAS version 9.1 (SAS Inc., Cary, NC). Before analysis, incidence expressed as a percentage was transformed

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