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

Anthony P. Keinath*, Virginia B. DuBose

Clemson University, Coastal Research and Education Center, 2700 Savannah Highway, Charleston, SC 29414-5329, USA

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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. Quinoxyfen 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.

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

* Corresponding author. Tel.: +1 843 402 5390; fax: +1 843 571 4654.
E-mail address: tknth@clemson.edu (A.P. Keinath).

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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 Milsana) (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 quinoxyfen (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

Table 1
Incidence and severity of powdery mildew on inter-specific squash hybrid *Cucurbita* 'Strong Tosa' after plants received three applications of fungicides and were exposed to powdery mildew after the first protective application.

Treatment	Product	Fungicide type	FRAC code	Fungicide activity	Amount L^{-1}	Incidence (%) repetition 1[a]	Incidence (%) repetition 2	Mean severity (%)[b]
Experiment 1								
Nonsprayed control	(None)	(None)	(None)	(None)	(None)	100.0 a[c]	100.0 a	8.8 a
Water	(None)	(None)	(None)	(None)	(None)	100.0 a	78.9 ab	3.9 b
Skim milk, 10%	GV brand	Organic	(None)	Contact	100 ml	100.0 a	100.0 a	3.6 b
Hydrogen dioxide 27%	Oxidate	Biopesticide	(None)	Contact	10.00 ml	97.7 a	97.7 ab	2.7 bc
Thiophanate methyl 45%	Topsin M	Conventional	1	Systemic	0.60 g	97.7 a	90.8 ab	3.2 b
Reynoutria sachalinensis extract 5%	Regalia	Biopesticide	P	Systemic	10.00 ml	88.2 ab	85.4 ab	1.3 cd
Paraffinic oil 97%	JMS Stylet Oil	Organic	NC	Contact	15.00 ml	41.5 bc	2.3 d	0.18 ef
Edible fish oil 92% + sesame oil 5%	Organocide	Biopesticide	NC	Contact	20.00 ml	41.5 bc	0.0 d	0.13 ef
Quinoxyfen 22.58%	Quintec	Conventional	13	Contact	0.47 ml	39.0 bc	23.4 cd	0.43 de
Copper hydroxide 46.1%	Kocide 3000	Organic	M1	Contact	1.50 g	9.2 cd	73.0 bc	0.21 ef
Potassium bicarbonate 85%	Armicarb	Biopesticide	NC	Contact	6.00 g	5.6 cd	0.0 d	0.01 ef
Sulfur 98.2%	Yellow Jacket Sulfur	Organic	M2	Contact	11.99 g	3.8 cd	0.0 d	0.02 ef
Penthiopyrad 20.6%	Fontelis	Conventional	7	Systemic	1.36 ml	0.0 d	0.0 d	0.00 f
Tebuconazole 38.7%	Monsoon	Conventional	3	Systemic	0.62 ml	0.0 d	0.0 d	0.00 f
Myclobutanil 40%	Rally	Conventional	3	Systemic	0.37 ml	0.0 d	0.0 d	0.00 f
Cyprodinil 37.5% + fludioxonil 25%	Switch	Conventional	9, 12	Systemic, Contact	1.05 g	0.0 d	0.0 d	0.00 f
Experiment 2								
Nonsprayed control	(None)	(None)	(None)	(None)	(None)	100.0 a	85.4 a	6.3 a
Water	(None)	(None)	(None)	(None)	(None)	100.0 a	73.0 a	4.2 a
Skim milk, 10%	(None)	Organic	(None)	Contact	100 ml	94.4 a	65.1 a	1.4 b
Streptomyces lydicus WYEC 108 0.037%	Actinovate AG	Biopesticide	NC	Contact	0.90 g	90.8 a	56.9 a	1.7 b
Bacillus subtilis QST713 1.34%	Serenade ASO	Biopesticide	44	Contact	15.00 ml	73.0 a	0.0 b	0.15 c
Skim milk, 50%	GV brand	Organic	(None)	Contact	500 ml	14.6 b	0.0 b	0.03 c
Potassium bicarbonate 85%	Armicarb	Biopesticide	NC	Contact	6.00 g	2.3 b	0.0 b	0.005 c
Tebuconazole 38.7%	Monsoon	Conventional	3	Systemic	0.62 ml	2.3 b	0.0 b	0.005 c

[a] Incidence was calculated as the percentage of seedlings with powdery mildew on hypocotyls or upper or lower sides of the cotyledons. There was a significant repetition-by-treatment interaction in experiment one ($P = 0.048$).
[b] Severity was rated as the percentage of the surface area of the cotyledons covered with powdery mildew. Severity was rated separately on the upper and lower surfaces. The sums of the individual ratings were analyzed.
[c] Means within columns by experiment followed by the same letter are not significantly different (t tests of least squares means, $P = 0.01$).

by calculating the arcsine-square root, and percentage severity was transformed by calculating the square root for experiments one, two, and three and the arcsine-square root for experiment four, based on normal quantile plots and log-likelihood ratios from model fit statistics. In all experiments there was a significant repetition-by-treatment interaction ($P < 0.05$) for incidence but not for severity. Thus, incidence is shown for each repetition, and mean severity is calculated across both repetitions of each experiment.

3. Results

3.1. Incidence of powdery mildew on seedlings

Incidence of powdery mildew on any part of nonsprayed *Cucurbita* and *Lagenaria* seedlings was 100% in six of the eight individual trials (two repetitions of the four experiments) and 94.4% and 85.4% in the other two trials. In the first two experiments, incidence was 85.9% and 85.2% on the upper sides of cotyledons of *Cucurbita*, 57.4% and 1.7% on the undersides of cotyledons, and 30.9% and 0% on hypocotyls. In the humidity chamber in experiment three, occurrence of powdery mildew was approximately equal on the upper and lower cotyledon surfaces of *Cucurbita* and was lower on hypocotyls (Fig. 1). In the last experiment, incidence was 100% on *Lagenaria* plants and cotyledons and 85.4% and 100% on hypocotyls in the two repetitions.

3.2. Preventative applications to hybrid squash

In the first experiment, incidence of powdery mildew did not differ between the nonsprayed and the water controls. Severity of powdery mildew was reduced by half in the water-sprayed control, but this effect of water was seen only in this experiment (Table 1). Skim milk at 10% dilution, hydrogen dioxide, and thiophanate-methyl were ineffective. *Reynoutria* did not reduce incidence but reduced severity. Copper hydroxide reduced severity and reduced incidence in the first but not in the second repetition. Penthiopyrad,

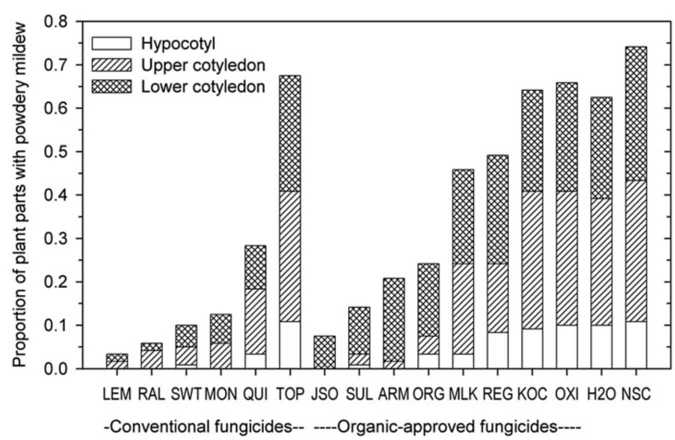

Fig. 1. Distribution of powdery mildew colonies on hypocotyls and upper and lower surfaces of cotyledons on hybrid *Cucurbita* 'Strong Tosa' seedlings after they were exposed to powdery mildew for 7 days, treated once with fungicides or biofungicides, and placed in a humidity chamber for 7 days to simulate healing conditions after grafting. Proportions were calculated based on the number of diseased plant parts (one hypocotyl and four cotyledon surfaces per seedling) in each experimental unit (one pot with three seedlings). LEM = penthiopyrad, RAL = mycobutanil, SWT = cyprodinil + fludioxonil, MON = tebuconazole, QUI = quinoxyfen, TOP = thiophanate-methyl, JSO = paraffinic oil, SUL = sulfur, ARM = potassium bicarbonate, ORG = fish oil + sesame oil, MLK = 50% skim milk, REG = *Reynoutria* extract, KOC = copper hydroxide, OXI = hydrogen dioxide, H_2O = water, NSC = nonsprayed control.

tebuconazole, myclobutanil, and cyprodinil plus fludioxonil completely suppressed powdery mildew in both repetitions. The other six treatments reduced incidence and severity compared to the nonsprayed and water controls.

In the second experiment, skim milk (10% dilution) and *S. lydicus* reduced severity but not incidence of powdery mildew. *B. subtilis* reduced severity and reduced incidence in only the second repetition. Skim milk at 50% dilution was as effective as potassium bicarbonate and tebuconazole, which were included as positive controls in this experiment. Because a 50% dilution of skim milk was more effective than a 10% dilution, the higher concentration was used in the subsequent experiments.

3.3. Curative applications of fungicides and biofungicides

In the humidity chamber, powdery mildew severity was approximately three times greater on the water and nonsprayed controls than in the previous experiments (Table 2). Fewer treatments were effective in this experiment than in experiments one and two (Tables 1 and 2). Treatments that reduced incidence of powdery mildew on cotyledons also reduced or prevented powdery mildew on hypocotyls (Fig. 1). Copper hydroxide, hydrogen dioxide, and thiophanate-methyl were ineffective. Skim milk (50% dilution), *Reynoutria*, and fish oil + sesame oil reduced severity on the upper cotyledon surfaces but not on the cotyledon undersides and had no impact on incidence. Quinoxyfen, potassium bicarbonate, sulfur, tebuconazole, and paraffinic oil reduced severity on upper and lower cotyledon surfaces in both repetitions and reduced incidence in one repetition. The most effective treatments that reduced incidence and severity in both repetitions were the conventional fungicides penthiopyrad, myclobutanil, and cyprodinil plus fludioxonil, which were three of the four fungicides that suppressed powdery mildew in experiments one and two. In experiment three, however, incidence across these three fungicides ranged from 2.3% to 35%. Severity across these fungicides in experiment three was very low, $\leq$0.1%, similar to experiments one and two.

3.4. Verifying the best treatments on bottle gourd

The eight fungicides that performed the best in the first three experiments plus three of the biofungicides that performed well in experiments two or three were applied preventatively to *L. siceraria* ‘Emphasis’ to verify their performance on a different rootstock species. Incidence and severity of powdery mildew were higher in this experiment than in the previous three experiments (Table 3). The lowest incidence was 5.3% and 27% in repetitions one and two, respectively, and the lowest mean severity was 0.7%. Quinoxyfen caused such severe phytotoxicity in the first repetition that all cotyledons were dead by the second rating; therefore, this treatment was excluded from the combined analysis. Paraffinic oil, potassium bicarbonate, and *B. subtilis* were ineffective in this experiment. As in experiment three, skim milk (50%) reduced severity on the upper but not on the lower cotyledon surfaces and had no effect on incidence. Cyprodinil plus fludioxonil reduced severity but not incidence. Myclobutanil, sulfur, and fish oil + sesame oil reduced severity in both repetitions and reduced incidence in one repetition. Penthiopyrad and tebuconazole reduced incidence and severity in both repetitions.

The costs of the products applied in the final experiment differed greatly (Table 3). Milk was the most expensive product tested; at a 50% dilution it cost $5.29 to apply 10 L. The next most expensive product was fish oil + sesame oil at $2.95 10 L^{-1} of diluted spray suspension. The least expensive product was sulfur ($0.09), and the least expensive conventional fungicide was a generic formulation of tebuconazole ($0.16).

3.5. Phytotoxicity on rootstock seedlings

Phytotoxicity was observed with five of the six synthetic fungicides registered for use on all cucurbits. No phytotoxicity occurred with thiophanate-methyl. Quinoxyfen caused severe chlorosis or necrosis on cotyledons of *Cucurbita* and *Lagenaria* seedlings in all four replications in all six trials in which it was tested. (Cotyledons also were chlorotic in one pot of nonsprayed *Lagenaria* seedlings in one trial.) The other four fungicides stunted seedlings to various degrees. Affected seedlings were shorter and had smaller cotyledons than the nonsprayed or water-sprayed seedlings. Tebuconazole, which was included in all eight trials, stunted *Cucurbita* and *Lagenaria* seedlings in all trials. Approximately 71% of the *Cucurbita* seedlings (17 of 24 pots) and 100% of *Lagenaria* seedlings (eight of eight pots) were reduced in size by 33%–50% compared to nonsprayed seedlings. Cyprodinil plus

Table 2
Incidence and severity of powdery mildew on inter-specific hybrid squash *Cucurbita* ‘Strong Tosa’ after plants were exposed to powdery mildew for 7 days, treated once with a fungicide application and held in a humidity chamber for 7 days.

Treatment	Incidence (%) repetition 1[a]	Incidence (%) repetition 2	Mean severity (%)[b]	Mean severity top (%)[b]	Mean severity underside (%)[b]
Nonsprayed	100.0 a[c]	94.4 ab	23.0 a	14.9 a	7.1 a
Water	100.0 a	100.0 a	17.5 ab	10.2 ab	5.1 a
Hydrogen dioxide	100.0 a	100.0 a	12.5 abc	5.5 bcd	4.8 a
Copper hydroxide	100.0 a	97.7 a	13.6 abc	8.2 abc	4.3 a
Skim milk, 50%	100.0 a	97.7 a	7.8 bcd	2.2 cde	3.4 ab
Thiophanate methyl	100.0 a	85.4 abc	7.4 bcd	3.9 bcd	3.0 ab
Reynoutria sachalinensis extract	90.8 ab	85.4 abc	4.8 cde	1.1 de	2.9 abc
Edible fish oil + sesame oil	65.1 abc	97.7 a	3.3 def	0.2 e	2.8 abc
Quinoxyfen	90.8 ab	14.6 d	1.8 defg	1.2 de	0.3 d
Potassium bicarbonate	97.7 a	27.0 cd	1.0 efg	0.02 e	0.7 bcd
Sulfur	85.4 ab	14.6 d	0.9 efg	0.03 e	0.7 bcd
Tebuconazole	34.9 bcd	43.1 bcd	0.5 efg	0.2 e	0.2 d
Paraffinic oil	2.3 d	58.5 abcd	0.5 fg	0.0 e	0.4 cd
Cyprodinil + fludioxonil	14.6 cd	27.0 cd	0.2 fg	0.07 e	0.07 d
Myclobutanil	34.9 bcd	2.3 d	0.1 fg	0.08 e	0.02 d
Penthiopyrad	2.3 d	14.6 d	0.05 g	0.0 e	0.03 d

[a] Incidence was calculated as the percentage of seedlings with powdery mildew on hypocotyls or upper or lower sides of the cotyledons. There was a significant repetition-by-treatment interaction ($P = 0.0087$).
[b] Severity was rated as the percentage of the upper and lower surface areas of the cotyledons covered with powdery mildew. The sum and the individual ratings were analyzed.
[c] Means within columns followed by the same letter are not significantly different (t tests of least squares means, $P = 0.01$).

Table 3
Incidence and severity of powdery mildew on *Lagenaria siceraria* 'Emphasis' after plants received three applications of fungicides and were exposed to powdery mildew after the first (protective) application.

Treatment	Incidence (%) repetition 1[a]	Incidence (%) repetition 2	Mean severity (%)[b]	Mean severity top (%)[b]	Mean severity underside (%)[b]	Cost ($10 L^{-1})
Nonsprayed	100.0 a[c]	100.0 a	56.7 a	44.4 a	23.4 a	(None)
Water	100.0 a	100.0 a	53.5 a	36.8 a	13.9 ab	(None)
Paraffinic oil	100.0 a	100.0 a	31.3 ab	28.8 a	9.8 ab	0.79
Potassium bicarbonate	100.0 a	100.0 a	23.0 b	22.4 ab	11.7 ab	1.66
Bacillus subtilis	100.0 a	100.0 a	19.5 bc	24.5 ab	11.2 ab	0.48
Skim milk, 50%	94.4 a	97.7 a	10.7 bcd	6.8 bc	3.2 bc	5.29
Myclobutanil	65.1 b	85.4 ab	4.1 cd	3.1 c	0.9 c	0.21
Cyprodinil + fludioxonil	90.8 a	80.2 ab	3.6 cd	3.1 c	0.4 c	1.56
Sulfur	34.9 bc	97.7 a	3.7 cd	3.0 c	0.7 c	0.09
Edible fish oil + sesame oil	27.0 bc	97.7 a	3.6 cd	2.6 c	0.9 c	2.95
Penthiopyrad	14.6 bc	43.1 bc	1.3 d	0.9 c	0.3 c	Unknown
Tebuconazole	5.6 c	27.0 c	0.7 d	0.5 c	0.0 c	0.16

[a] Incidence was calculated as the percentage of seedlings with powdery mildew on hypocotyls or upper or lower sides of the cotyledons. There was a significant repetition-by-treatment interaction ($P = 0.0265$).
[b] Severity was rated as the percentage of the surface area of the cotyledons covered with powdery mildew. Severity was rated separately on the upper and lower surfaces. The sum and the individual ratings were analyzed.
[c] Means within columns followed by the same letter are not significantly different (*t* tests of least squares means, $P = 0.01$).

fludioxonil stunted *Cucurbita* seedlings in two of four trials (four of 16 pots) and *Lagenaria* seedlings in one of two trials (one of eight pots). Penthiopyrad stunted *Lagenaria* seedlings in both repetitions of experiment four in two and one of four pots, respectively. Myclobutanil stunted seedlings only in one pot in one experiment (one of 24 pots across six trials). No phytotoxicity or stunting was observed with any other treatment in any experiment.

4. Discussion

The new fungicide penthiopyrad was very effective in these experiments, as seen previously on *C. pepo* in the field (Keinath et al., 2010b; Langston and Sanders, 2010). Cyprodinil plus fludioxonil, myclobutanil, and tebuconazole, three fungicides that currently are registered to control cucurbit powdery mildew, also were among the most effective treatments in the first three experiments. Although resistance to myclobutanil has been found in *P. xanthii* isolates in New York, Pennsylvania, Ohio, and Indiana (although not in New Jersey), powdery mildew in the current study was sensitive (McGrath et al., 2009; Wyenandt et al., 2010). Quinoxyfen often was less effective than these four fungicides, even though it has been effective in the field (Keinath et al., 2010b). This difference in efficacy may be due to the fact that quinoxyfen is a protectant but cyprodinil, myclobutanil, and tebuconazole have systemic activity. However, quinoxyfen has a high vapor pressure, which presumably allows it to reach the underside of some leaves (McGrath, 2005). The difference between the protectants penthiopyrad and quinoxyfen might be due to high sensitivity of cucurbit powdery mildew to penthiopyrad because of a lack of previous exposure to this fungicide, which was registered in early 2012. *P. xanthii* isolates monitored in 2006–2008 in the northeastern, mid-Atlantic, and Midwestern US were sensitive to quinoxyfen (McGrath et al., 2009; Wyenandt et al., 2010). The lack of activity by thiophanate-methyl was not unexpected. Resistance to methyl-benzimidazole-carbamate fungicides in cucurbit powdery mildew is widespread, and there is cross-resistance among fungicides in this group (FRAC Group 1) (FRAC, 2011; McGrath, 2001; McGrath et al., 2009).

Organocide and potassium bicarbonate were the two most effective biofungicides tested; in the last experiment, Organocide was superior to bicarbonate. In two field experiments on pumpkin, Organocide provided 85% control on upper leaf surfaces and also controlled powdery mildew on the lower leaf surfaces in one of the experiments (McGrath, 2005; McGrath and Davey, 2007). In the latter experiment, it was more effective on the upper leaf surface than potassium bicarbonate (McGrath and Davey, 2007). In another series of studies on melon (*Cucumis melo*) and pumpkin, potassium bicarbonate controlled powdery mildew on upper and lower leaf surfaces in two and three of three field trials, respectively (McGrath and Shishkoff, 1999). Among the non-biopesticide, organic-approved fungicides tested, sulfur was more effective than paraffinic oil, which was more effective than copper hydroxide. In a previous study on greenhouse cucumber, sulfur and potassium bicarbonate were equally effective; potassium bicarbonate but not sulfur was equivalent to a synthetic fungicide standard (Smither-Kopperl et al., 2005).

Powdery mildew incidence was determined in this study because any powdery mildew on rootstock seedlings renders them unusable. The risk of pathogen spread to noninfected seedlings is high (Jarvis, 1992). There also is a potential risk of introducing the pathogen to the field on grafted seedlings. In general, *S. lydicus*, hydrogen dioxide, *R. sachalinensis* extract, and *B. subtilis* did not reduce incidence although they reduced severity. Milk at 50% dilution reduced incidence only in the first two experiments, although it also reduced severity. Because of the low threshold for powdery mildew on rootstock seedlings, these five products would not provide a sufficient level of control. The biopesticides *S. lydicus*, *R. sachalinensis* extract, and *B. subtilis* and milk also were partially effective or variably effective in reducing severity of powdery mildew in previous field and greenhouse tests (Bettiol et al., 2008; Ferrandino and Smith, 2007; Langston and Kelley, 2002; Langston and Sanders, 2010; McGrath, 2002; McGrath and Davey, 2007; Smither-Kopperl et al., 2005; Zhang et al., 2011.).

In general, the controlled greenhouse environment did not increase the effectiveness or consistency of biopesticides and milk. These products were more variable among experiments than conventional fungicides were. This environment also provided a favorable environment for powdery mildew, as severity on nonsprayed seedlings in the last experiment was as great as or greater than severities in some field experiments (Keinath et al., 2010b; Langston and Kelley, 2002; McGrath and Shishkoff, 1999). One exception was that hydrogen dioxide reduced severity in experiment one, even though it was ineffective in the field (McGrath, 2005).

Powdery mildew inoculum level, measured as percentage severity on nonsprayed plants, affected the performance of products. Powdery mildew severity on the nonsprayed control was approximately one-quarter as great in the first two experiments and one-half as great in the third experiment as in the fourth experiment. Consequently, potassium bicarbonate was very effective in the first two experiments, moderately effective in the third experiment, and not very effective in the fourth experiment, where it only reduced severity by half. The effect of inoculum level on performance of potassium bicarbonate also was seen in previous field experiments on melon, where potassium bicarbonate significantly reduced powdery mildew when disease severity on non-sprayed plants ranged from 2% to 13% across rating dates but did not reduce disease when severity ranged from 26 to 67% (McGrath and Shishkoff, 1999). Likewise, sulfur was very effective in the first two experiments in the current study and moderately effective in the third and fourth experiments, and paraffinic oil was effective in the first three but not the last experiment. In contrast, conventional synthetic fungicides, particularly tebuconazole and myclobutanil, performed more consistently across experiments than biofungicides or organic-approved fungicides.

Powdery mildew severity was higher on *L. siceraria* 'Emphasis' than on hybrid squash 'Strong Tosa.' In a field evaluation, 'Strong Tosa' was more susceptible to powdery mildew than 'Emphasis' (Kousik and Hassell, 2009). However, in the current study, the two species were not tested together in the same test and so were not exposed to exactly the same inoculum level.

Water sprays have been recommended to control powdery mildew on greenhouse rose caused by *Podosphaera pannosa* (Jarvis, 1992). In the current study, applying water reduced cucurbit powdery mildew severity only in the first experiment, when overall severity was the lowest among the four experiments. Water sprays never affected incidence of cucurbit powdery mildew. Sporulation by *P. xanthii* has been reported to be tolerant of the effects of high relative humidity and rain, which may explain why water had no negative effect and why disease developed as readily in the humidity chamber as on a greenhouse bench (Nagy, 1976). The humidity chamber experiment illustrates one of the problems with powdery mildew on grafted plants. Even though there may be no symptoms or signs of powdery mildew when plants are placed into the humidity chamber, latent infections by powdery mildew can develop and the pathogen can spread during the 7-day period the healing chambers are closed.

Three of the four most effective products, all synthetic fungicides, were phytotoxic to one or both rootstocks in these greenhouse experiments. Quinoxyfen consistently and severely injured or killed cotyledons on *Cucurbita* and *Lagenaria*. Rootstocks must have at least one healthy cotyledon to provide carbohydrates for formation of new connective and vascular tissues across the graft union with the scion (Memmott and Hassell, 2010). Tebuconazole consistently stunted *Cucurbita* and *Lagenaria* seedlings to a degree where they could not have been used in grafting, as the hypocotyls were too short to hold the clips connecting the scion and rootstock hypocotyls (Hassell et al., 2008). Cyprodinil plus fludioxonil stunted an average of 21% of both rootstock seedlings. Penthiopyrad stunted *Lagenaria* seedlings but not *Cucurbita* seedlings., Myclobutanil, however, should be safe on both cucurbit rootstocks tested in this study if curative control of powdery mildew is needed, since the stunting was inconsistent and affected no more than 4% of the plants.

The fungicides used in this study were applied at the maximum labeled rates in the equivalent of 935 L water ha^{-1}, which is the recommended application volume for many fungicides and for cucurbits. The exception was tebuconazole, which was applied at 0.58 L ha^{-1}, which is the rate recommended to control gummy stem blight, instead of 0.44 L ha^{-1}, which is the rate recommended to control powdery mildew. It may be feasible to reduce the rates of cyprodinil plus fludioxonil, penthiopyrad, and myclobutanil to reduce phytotoxicity. However, a reduced rate of active ingredient would increase the risk of fungicide resistance. One of the widely recommended practices to reduce development of fungicide resistance is to apply fungicides sparingly but at the maximum labeled rates (Brent and Holloman, 2007a).

To manage powdery mildew effectively on cucurbit rootstock seedlings in the greenhouse, the risks of powdery mildew development, phytotoxicity to seedlings, and development of fungicide resistance must be balanced. Quinoxyfen and tebuconazole should not be used on hybrid *Cucurbita* and *Lagenaria* rootstock seedlings at the concentrations tested. To prevent powdery mildew and fungicide resistance and avoid phytotoxicity, biofungicides or organic-approved fungicides should be the products used first. Sulfur and fish oil + sesame oil were the most consistently effective organic-approved fungicides, particularly when powdery mildew severity was high. The best choices for curative fungicides are myclobutanil and penthiopyrad, which should only be applied once each per crop of seedlings to reduce the risk of resistance to these fungicides. Penthiopyrad should be used only on *Cucurbita* seedlings. Because seedlings are in a greenhouse for a relatively short time (4–5 weeks), one or two preventative applications of a biopesticide followed by one application of myclobutanil or penthiopyrad, if powdery mildew appears, should be sufficient to manage powdery mildew.

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