Residual Efficacy of Fungicides Used in the Management of *Botrytis cinerea* on Greenhouse-Grown Geraniums

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ABSTRACT

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Fungicides were applied singly or in mixtures to seed geraniums (*Pelargonium* × *hortorum* 'Red Elite'). Immediately after sprays dried (day 0) and weekly for 3 wk, 1-cm-diameter disks excised from treated leaves were inoculated with spores from a benomyl-resistant, vinclozolin-sensitive strain of *Botrytis cinerea*. After 10 days of incubation, the number of leaf disks exhibiting the browning characteristic of Botrytis blight was recorded. Of the materials applied singly, vinclozolin provided the best protection, followed by chlorothalonil and mancozeb. Zineb, dicloran, and cupric hydroxide allowed more than 50% of the treated leaf disks to become infected. The relative performance of fungicides used singly was similar whether plants were watered directly in the pots or irrigated overhead during the experiment. Two, three, or four-component mixtures of vinclozolin, chlorothalonil, cupric hydroxide, and mancozeb at reduced rates were more effective and had longer residual efficacy than the fungicides applied singly. Because reduced-rate mixtures controlled *B. cinerea* as well as full-strength mixtures, the cost of applying a mixture can be lowered by using reduced rates without sacrificing residual efficacy. To reduce the exposure of *B. cinerea* populations to vinclozolin and, thereby, lessen the selection pressure toward vinclozolin resistance, results indicate that the reduced-rate, four-component mixture of vinclozolin, chlorothalonil, cupric hydroxide, and mancozeb or vinclozolin-free, reduced-rate, two- or three-component mixtures of chlorothalonil, cupric hydroxide, and mancozeb provided good protection of geranium leaf tissue for 21 days.

Leaf spots, flower blights, stem rots, and branch diebacks (2), caused by *Botrytis cinerea* Pers.:Fr., pose a constant threat to greenhouse-grown plants. To protect plants, a relatively few fungicides effective against *B. cinerea* are used repeatedly. Continuous use of systemic fungicides may result in the selection of resistance in *B. cinerea* populations. Resistance to benzimidazoles, such as benomyl, and dicarboximides, such as vinclozolin, has been documented in the United Kingdom (6,7), Italy (4), Israel (5), Greece (11), and Canada (10). We have isolated strains of *B. cinerea* that are resistant to benzimidazoles from many greenhouses in Pennsylvania and no longer recommend benzimidazoles for Botrytis blight control. Dicarboximide fungicides are still recommended because only a few greenhouses harbored strains with resistance to both benzimidazoles and dicarboximides (9).

The use of protectant fungicides is recommended (1,3) as an alternative to systemics (benzimidazoles and dicarboximides) because these multisite inhibitors do not appear to be at great risk for the development of resistance. Although protectant fungicides are important tools in managing fungicide resistance in *B. cinerea* populations, growers have noted that they are less effective than systemic fungicides. Therefore, growers may want to use both systemics and protectants in the management of Botrytis blight. It is important to know the duration that an effective concentration of a chemical remains on the plant so that subsequent sprays can be timed to maintain protection. In managing fungicide resistance, the residual activity of the chemical that is at risk for resistance development should be equal to or less than that of the partner components so that *B. cinerea* populations are not exposed only to the at-risk chemical. The research reported here was conducted to compare the residual efficacy of fungicides used alone and in mixtures for the control of *B. cinerea* on greenhouse-grown geraniums.

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MATERIALS AND METHODS

Seedling geraniums (*Pelargonium* × *hortorum* L. H. Bailey 'Red Elite') grown for 12 wk in a greenhouse were sprayed with water, a single fungicide, or a mixture of fungicides. After treatment, plants were watered directly in the pot or overhead. A hand-held CO_2-powered (30–35 psi) sprayer with a flat-fan nozzle was used to apply fungicides to runoff. The full-strength (1×) concentrations of fungicides tested in all experiments were as follows: vinclozolin at 0.6 g a.i./L (Ornalin 50WP), chlorothalonil at 1.2 g a.i./L (Daconil 2787, 4.17F), mancozeb at 0.5 g a.i./L (Dithane M-45), cupric hydroxide at 0.9 g a.i./L (Kocide 101, 77WP), zineb at 1.3 g a.i./L (Zineb 75W), and dicloran at 0.4 g a.i/L (Botran 75WP).

After the spray dried (day 0), leaves numbered 3, 4, and 5 (counting down from leaf 1, which was at least 1 cm in diameter) were excised from plants selected at random. The plants from which leaves had been excised were discarded. Seven, 14, and 21 days later, leaves that had been designated numbers 3, 4, and 5 on day 0 were excised from the remaining plants selected at random. A cork borer was used to cut 1-cm-diameter disks from leaves (8). Ten leaf disks were placed on moist filter paper in each of five petri plates per treatment on each date. The *B. cinerea* strain used in this work was isolated from an infected geranium collected from a Pennsylvania greenhouse and was shown to be resistant to benomyl and sensitive to vinclozolin in vivo (9). One hundred spores suspended in 25 μl of sterile 0.1 M dextrose were applied to the adaxial side of each leaf disk. One plate per treatment with 10 leaf disks received 25 μl of sterile 0.1 M dextrose. After inoculation, the plates were sealed with Parafilm and placed in a lighted incubator (16-hr photoperiod supplied by 20W cool-white fluorescent lights at 5.8 W/m^2 at 20 C) for 10 days. After incubation, the number of disks exhibiting browning typical of Botrytis blight was recorded.

In experiment 1, plants were grown in the greenhouse, watered directly in the pot, and sprayed with water or single fungicides on 20 March 1988 the first time the experiment was conducted and on 17 November 1989 the second time it was conducted. In experiment 2, plants were sprayed similarly on 10 July 1990 the first time the experiment was conducted and on 24 July 1990 the second time it was carried out, but plants were watered overhead with a hand-held hose whenever moisture was required. Zineb was not tested in experiment 2. In experiment 3, plants were sprayed with water or with one of all possible two-, three-, or four-component mixtures of vinclozolin, chlorothalonil, mancozeb, and cupric hydroxide at full- (1×), half- (0.5×), third- (0.33×), or quarter-strength (0.25×) concentrations on 12 December 1989 the first time conducted and on 10 January 1990 the second time the experiment was conducted. After treatment, these plants were watered directly in the pots. In each

experiment, the arcsine transformation of the number of leaf disks infected was subjected to analysis of variance, and the Waller-Duncan k-ratio t test was applied (12).

RESULTS AND DISCUSSION

Vinclozolin applied alone provided excellent control of the vinclozolin-sensitive *B. cinerea* strain for 7 days (Tables 1 and 2), allowing less than one of 10 leaf disks per plate to become infected. Chlorothalonil allowed, on average, fewer than three of the 10 inoculated leaf disks to become infected after 14 days. Mancozeb allowed slightly fewer than five of the leaf disks inoculated 14 days after treatment to become infected. In these tests, cupric hydroxide performed poorly, allowing more than six of the disks inoculated 7 days after treatment to become infected. The number of disks treated with dicloran or zineb that became infected was not significantly different from the number infected when no fungicide was used. In experiments 1 and 2 involving single fungicides, the relative performance of the materials was similar whether plants were watered directly in the pot or irrigated overhead.

In experiment 3, fungicide mixtures provided excellent protection of the leaf disks inoculated 21 days after treatment (Table 3). In comparing mixtures of the same materials, the number of infected disks treated with reduced-rate mixtures was, in most cases, not significantly different from the number infected after treatment with full-strength mixtures. Therefore, because no disease control advantage is gained by applying full-rate mixtures, the cost of the mixture can be lowered by using reduced rates without sacrificing protection. Although statistical comparisons of single fungicides and mixtures were not made, the data suggest that fungicide mixtures gave better protection and had greater residual efficacy than materials applied singly.

Vinclozolin applied alone and vinclozolin mixed with one, two, or three

Table 1. Mean number of leaf disks (per 10 inoculated) from greenhouse-grown geraniums[y] infected by *Botrytis cinerea* after spraying with water or single fungicides

Treatment	Days after spraying			
	0	7	14	21
No fungicide (water)	9.3 Aa[z]	7.2 Ba	9.4 Aa	9.5 Aa
Chlorothalonil	1.3 Cd	2.3 BCc	2.4 ABc	3.5 Ac
Cupric hydroxide	5.8 Ab	6.4 Aab	7.2 Ab	8.2 Aa
Dicloran	8.4 Aa	5.7 Bab	8.9 Aab	9.6 Aa
Mancozeb	3.3 Bc	4.9 ABb	4.4 ABc	5.6 Ab
Vinclozolin	0.0 Bd	0.1 Bd	2.9 Ac	4.0 Ac
Zineb	8.2 Aa	7.3 Aa	8.6 Aab	8.7 Aa

[y] Plants were watered directly in the pots.
[z] The arcsine transformation of the number of infected leaf disks was subjected to analysis of variance and the Waller-Duncan k-ratio t test was applied. Numbers within a row followed by the same uppercase letter or within a column followed by the same lowercase letter are not significantly different ($P = 0.05$).

Table 2. Mean number of leaf disks (per 10 inoculated) from greenhouse-grown geraniums[y] infected by *Botrytis cinerea* after spraying with water or single fungicides

Treatment	Days after spraying			
	0	7	14	21
No fungicide (water)	8.1 Aa[z]	6.5 Aa	8.0 Aa	7.5 Aa
Chlorothalonil	0.6 Bcd	1.6 Bb	1.3 Bd	4.8 Abc
Cupric hydroxide	4.0 Ab	6.0 Aa	5.9 Abc	6.4 Aab
Dicloran	3.4 Bb	6.8 Aa	6.7 Aab	8.4 Aa
Mancozeb	2.1 Cbc	2.3 BCb	4.1 Bc	6.6 Aa
Vinclozolin	0.0 Bd	0.3 Bc	4.8 Ac	3.6 Ac

[y] Plants were watered overhead with a hand-held hose.
[z] The arcsine transformation of the number of infected leaf disks was subjected to analysis of variance and the Waller-Duncan k-ratio t test was applied. Numbers within a row followed by the same uppercase letter or within a column followed by the same lowercase letter are not significantly different ($P = 0.05$).

Table 3. Mean number of leaf disks (per 10 inoculated) from greenhouse-grown geraniums[y] infected by *Botrytis cinerea* after spraying with water or two-, three-, or four-component mixtures of fungicides at the full-strength (1×), 0.5×, 0.33×, and 0.25× concentrations

Treatment	Days after spraying			
	0	7	14	21
No fungicide (water)	7.9 Ba[z]	9.9 Aa	9.7 ABa	8.8 ABa
Chlorothalonil + cupric hydroxide—1×	0.6 BCcd	1.8 Acd	1.1 Bd-f	0.4 Cf-j
Chlorothalonil + cupric hydroxide—0.5×	1.2 BCbc	0.7 Cf	5.7 Ab	2.0 Bc
Chlorothalonil + mancozeb—1×	0.7 Abc	1.2 Ade	1.6 Ac-e	0.5 Ae-j
Chlorothalonil + mancozeb—0.5×	1.2 Abc	2.6 Ac	1.5 Acd	1.7 Acd
Cupric hydroxide + mancozeb—1×	1.6 Ab	2.4 Ac	0.9 Bf-h	1.4 ABc-f
Cupric hydroxide + mancozeb—0.5×	0.9 Cbc	3.4 ABb	1.9 BCc	4.5 Ab
Vinclozolin + chlorothalonil—1×	0.0 Bd	0.0 Bg	0.2 Bij	1.3 Ac-g
Vinclozolin + chlorothalonil—0.5×	0.0 Bd	0.9 Af	0.3 ABh-j	0.6 ABf-j
Vinclozolin + cupric hydroxide—1×	0.0 Ad	0.0 Ag	0.0 Aj	0.2 Aij
Vinclozolin + cupric hydroxide—0.5×	0.0 Bd	0.0 Bg	0.0 Bj	0.8 Ae-i
Vinclozolin + mancozeb—1×	0.0 Ad	0.0 Ag	0.0 Aj	0.0 Aj
Vinclozolin + mancozeb—0.5×	0.0 Bd	0.1 Bg	0.0 Bj	1.7 Ac-e
Chlorothalonil + cupric hydroxide + mancozeb—1×	0.7 Bbc	0.8 Bef	1.7 Acd	0.3 Bh-j
Chlorothalonil + cupric hydroxide + mancozeb—0.33×	1.4 Ab	1.0 Aef	0.9 Ae-g	1.1 Ad-h
Vinclozolin + chlorothalonil + mancozeb—1×	0.0 Ad	0.0 Ag	0.0 Aj	0.4 Ag-j
Vinclozolin + chlorothalonil + mancozeb—0.33×	0.0 Bd	0.3 ABg	0.3 ABij	1.1 Ad-h
Vinclozolin + chlorothalonil + cupric hydroxide—1×	0.0 Ad	0.0 Ag	0.0 Aj	0.0 Aj
Vinclozolin + chlorothalonil + cupric hydroxide—0.33×	0.1 Ad	0.2 Ag	0.4 Ah-j	0.1 Aij
Vinclozolin + cupric hydroxide + mancozeb—1×	0.0 Ad	0.0 Ag	0.0 Aj	0.0 Aj
Vinclozolin + cupric hydroxide + mancozeb—0.33×	0.0 Ad	0.0 Ag	0.0 Aj	0.1 Aij
Vinclozolin + chlorothalonil + cupric hydroxide + mancozeb—1×	0.0 Ad	0.0 Ag	0.0 Aj	0.0 Aj
Vinclozolin + chlorothalonil + cupric hydroxide + mancozeb—0.25×	0.0 Ad	0.0 Ag	0.5 Ag-i	0.4 Ah-j

[y] Plants were watered directly in the pots.
[z] The arcsine transformation of the number of infected leaf disks was subjected to analysis of variance and the Waller-Duncan k-ratio t test was applied. Numbers within a row followed by the same uppercase letter or within a column followed by the same lowercase letter are not significantly different ($P = 0.05$).

other components provided excellent disease control. Because vinclozolin is at risk to the development of resistance in *B. cinerea* populations, it should not be used alone. To obtain the least exposure of *B. cinerea* populations to vinclozolin, the quarter-strength four-component mixture provides good protection for 21 days. As an alternative to vinclozolin, various combinations of chlorothalonil, cupric hydroxide, and mancozeb appear to be choices superior to any of these chemicals used singly based on the initial protection and the residual efficacy that mixtures provided.

Systemic chemicals are particularly at risk to the development of fungicide resistance in *B. cinerea* populations because of their specific, single-site modes of action. Systemics are generally thought to have greater residual efficacy than protectants because of the uptake by the plant. In theory, the uptake and longer residual efficacy of systemic fungicides could result in a partitioning of mixtures of systemics and protectants on the plant. If this occurs, the fungus could, over time, be exposed to the systemic chemical alone. In this research, vinclozolin, which is considered to have some systemic properties, had less residual efficacy than either chlorothalonil or mancozeb. The residual efficacy was extended far more in the mixtures tested than in the single materials. Partitioning of chemicals may not have occurred. It is not known whether the residual activity of vinclozolin as one component of the mixtures was exended or if the components interacted equally in the extended activity. Although it was demonstrated here that the net result is enhanced disease control, information is needed on what effects these mixtures have on the population when some of the *B. cinerea* spores used as inoculum are resistant to vinclozolin. Research has been conducted that indicates that full-strength fungicide mixtures that include vinclozolin as a component favor the buildup of vinclozolin-resistant strains in a population of *B. cinerea* when inoculum is applied immediately after treatment (13). Future research will focus on the effects that fungicide mixtures have on a population of *B. cinerea* containing resistance when the spores are used to inoculate tissue 7, 14, and 21 days after fungicide application.

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