Bioassay for Dicarboximide Resistance in *Botrytis cinerea*

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ABSTRACT

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A simple, inexpensive bioassay successfully differentiated dicarboximide-resistant and dicarboximide-sensitive isolates of *Botrytis cinerea*. Inoculum was placed directly into vinclozolin-containing droplets on the growing tips of seedlings of the sunflower cultivar Sunspot. Resistant isolates killed fungicide-treated and fungicide-free seedlings in 3–7 days, whereas sensitive isolates killed only fungicide-free seedlings.

Botrytis cinerea Pers.:Fr., the causal agent of gray mold, is a major pathogen of greenhouse crops in the northeastern United States, posing a threat to production at all stages of plant growth throughout most of the growing season. The few classes of fungicides effective against gray mold in greenhouses are used repeatedly to protect crops, particularly when conditions are highly favorable for disease development. When populations of *B. cinerea* are repeatedly exposed to dicarboximide fungicides, selection for resistance can be rapid (1, 2,5,7). *B. cinerea* populations from approximately one-half of the greenhouse operations surveyed in Pennsylvania were found be resistant to dicarboximides (6). Although the presence of resistance in a *B. cinerea* population can render dicarboximides ineffective for controlling gray mold, it is difficult for a grower to detect and verify reduced fungicide efficacy. For these reasons, a simple, reliable bioassay was developed for growers.

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MATERIALS AND METHODS

Ten seeds of the sunflower (*Helianthus annuus* L.) cultivar Sunspot were planted in 250-ml Tri-Pour single-use beakers (Oxford Labware, St. Louis, MO) containing Metro-Mix 350 (W. R. Grace & Co., Foglesville, PA) potting medium and grown until the cotyledons were fully expanded and in the horizontal position. Then, 10 ml of 18 g/L of glucose in tap water was placed into each of two 15-ml disposable dropper bottles, and 0.012 g of the dicarboximide fungicide vinclozolin (Ornalin 50WP) was added to one of the bottles. The resulting concentration of fungicide was equal to the label-recommended rate of 600 μg a.i./ml (1 lb of product/100 gal). Bottles were shaken vigorously, and one drop (0.05–0.1 ml) of the fungicide/glucose suspension was placed onto the apical meristem of each seedling in a beaker. As a check, seedlings in other beakers were treated with glucose solution without fungicide.

For inoculation of the seedlings, 18 previously described (6) dicarboximide-

resistant and -sensitive *B. cinerea* isolates were grown on potato-dextrose agar. Small (8 mm^3) blocks of mycelium-containing agar were cut from the culture dishes and placed directly into the droplet on each seedling. Alternatively, geranium leaf disk tissue infected with *Botrytis*, as previously described (6), was cut into small (10–20 mm^2) pieces, which then were used as inoculum. Each beaker was placed into a clear plastic bag with 20–30 ml of tap water in the bottom to maintain high relative humidity. The bags were sealed and left in a room (20–25 C) receiving bright, indirect sunlight. Three to 7 days later, the number of fungicide/glucose-treated and glucose-treated seedlings killed by each strain of *Botrytis* was recorded. Each isolate was tested at least twice.

Table 1. Comparison of the sunflower seedling (*Helianthus annuus* cv. Sunspot) bioassay with the previously reported[a] leaf disk technique for dicarboximide resistance in strains of *Botrytis cinerea*

Isolate no.	Infected vinclozolin-treated leaf disks[b]	Infected glucose-treated sunflower seedlings[c]	Infected vinclozolin-treated sunflower seedlings[d]
1	+[e]	+	+
2	+	+	+
3	+	+	+,−
4	+	+	+
5	−	+	−
6	−	+	−
7	−	+	−
8	+	+	+
9	−	+	−
10	+	+	+
11	+	+	+
12	−	+	−
13	−	+	−
14	−	+	−
15	−	+	−
16	−	+	−
17	+	+	+
18	+	+	+

[a]Moorman and Lease (6).
[b]Vinclozolin (Ornalin 50WP), 600 μg a.i./ml, was sprayed on 12-wk-old Red Elite geraniums the day before tissue was excised and inoculated (6).
[c]Glucose, 0.05–0.1 ml of 18 g/L, was applied to the apical meristem of each of 10 sunflower seedlings planted in beakers, and inoculum was placed directly in each drop.
[d]Vinclozolin (Ornalin 50WP), 0.05–0.1 ml of 600 μg a.i./ml in 18 g/L of glucose, was applied to the apical meristem of each of 10 sunflower seedlings planted in beakers, and inoculum was placed directly in each drop.
[e]+ = Infected, − = not infected.

RESULTS AND DISCUSSION

Preliminary experiments using coreopsis, zinnia, muskmelon, sweet pepper, lettuce, green bean, and cucumber seedlings as assay plants yielded erratic results. However, seedlings of the sunflower cultivar Sunspot were infected consistently by *B. cinerea*, especially when the inoculum droplet contained 18 g/L of glucose. All *Botrytis* isolates tested killed the glucose-treated sunflower seedlings (Table 1). Except for isolate 3, *B. cinerea* isolates previously identified as resistant to dicarboximides by the geranium leaf disk technique (6) also killed the vinclozolin-treated sunflower seedlings (Table 1). None of the isolates found in previous research (6) to be sensitive to dicarboximides killed vinclozolin-treated sunflower seedlings. Isolate 3 killed fungicide-treated seedlings in some tests but not in others. Isolate 3 has an EC_{50} value of 0.1 ± 0.01 μg/ml of vinclozolin, approximately equal to that of the dicarboximide-sensitive isolates used here. The reason for its inconsistent performance is not known.

A similar fungicide-resistance test (8) required spraying plants, a practice that may deter growers from using the test. The test described here makes it economically feasible to assemble kits containing sunflower seeds and disposable dropper bottles with measured amounts of fungicide plus glucose and of glucose alone. Bottles can be marked with the level to which tap water should be added to obtain the desired concentration of chemicals. Since the percentage of spores carrying dicarboximide resistance can vary greatly in a greenhouse (3,4), depending on the time of year, it is important to test several isolates collected from different locations in each greenhouse. We believe that by testing 10 isolates, a resistant strain of *Botrytis* will be readily detected if it is present in a commercial greenhouse. Therefore, at least 200 seeds should be provided to the user so that fungicide-treated and check plants can be inoculated with each isolate tested. The greenhouse operator can supply 20 pots and potting medium for growing the plants and plastic bags for incubating the inoculated plants.

When given clear directions, including a description of *Botrytis* or a fact sheet on gray mold, growers can use *Botrytis*-infected tissue collected in their facility as inoculum without isolation onto agar. When this was done in Pennsylvania, we were able to confirm, by using the geranium leaf disk technique and by determining the EC_{50} of the isolate on fungicide-amended agar, that the kit users had successfully determined whether the *Botrytis* isolate tested was or was not resistant to dicarboximides. However, the loss of viability of the fungus in infected sunflower tissue during shipment of most samples to our laboratory has hampered our full assessment of the use of test kits by growers. Using the sunflower bioassay method described here, students in introductory plant pathology courses over a 3-yr period have consistently differentiated dicarboximide-resistant and -sensitive isolates in blind tests.

The test described here provides a simple, inexpensive, reliable method for growers to determine whether fungicide-resistant *Botrytis* is present in their production facilities. Growers can then make an informed decision on the disease management strategy to employ and possibly avoid the application of and exposure to a fungicide that would not be effective.

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