Benomyl-Resistant Strains of *Botrytis cinerea* on Apples, Beans, and Grapes

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ABSTRACT

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Strains of *Botrytis cinerea* resistant to benomyl were isolated from apples, beans, and grapes where the fungicide had been used primarily for control of other disease organisms. The level of resistance for each *Botrytis* isolate from the three hosts could not be related to the history of benomyl usage on those crops. Benomyl-resistant isolates from the three hosts were pathogenic on each of the other two hosts, and more variation in aggressiveness was observed among isolates from the same host than among isolates from different hosts. Since apples, beans, and grapes may be grown in neighboring fields, spread of benomyl-resistant pathogenic isolates from one host to another can be expected to complicate control strategies.

Additional key words: fungicide resistance, *Malus pumila, Phaseolus vulgaris, Vitis* spp.

Strains of *Botrytis cinerea* Pers. ex Fr. resistant to benomyl have been reported from many crops since the first report by Bollen and Scholten in 1971 (3). In most instances where benomyl-resistant *Botrytis* strains arose, *Botrytis* had been the target organism. However, the use of benomyl in New York on apples (*Malus pumila*), beans (*Phaseolus vulgaris*), and grapes (*Vitis labrusca, V. vinifera, V. labrusca* × *V. vinifera*) has usually been aimed at fungal pathogens other than *B. cinerea*. Benomyl is used in apple orchards primarily for *Venturia inaequalis* (Cooke) Wint. and *Podosphaera leucotricha* (Ellis & Everh.) Salm., whereas in packinghouse dip tanks, the primary pathogen is *Penicillium expansum* (Link) Thom. On green beans and grapes the target organisms for benomyl sprays are *Sclerotinia sclerotiorum* (Lib.) de Bary. and *Uncinula necator* (Schw.) Burr., respectively. Benomyl is also favored by some viticulturists because of its benefits in reducing ozone damage (8). Benomyl-resistant strains of *B. cinerea* have not been reported from apple, and the only previously resistant strain from beans was selected under laboratory conditions (11). Benomyl-resistant strains of *B. cinerea* on grape have been reported in France, Germany, and Switzerland (2) and in California (15). This report is of the occurrence of benomyl-resistant strains of *B. cinerea* on these three major crops in New York State.

MATERIALS AND METHODS

Collection of isolates. Pieces of decayed apple fruit collected from seven apple storages in Hudson Valley, one in Champlain Valley, and seven in western New York during the winter of 1978–1979 were plated on Difco potato-dextrose agar (PDA). Snap bean plants with signs of gray mold were collected from 12 bean fields in central and western New York during 1978. Grape clusters with Botrytis bunch rot were collected from various cultivars from the Finger Lakes, Lake Erie grape belt, Niagara peninsula, Hudson Valley, and Long Island grape-growing regions of New York in 1978. *Botrytis* was isolated from both hosts by removing conidia from sporulating lesions and seeding them on PDA.

Tests for benomyl resistance. *Botrytis* isolates from apple were rated resistant if growth was observed on PDA amended with 250 μg/ml of benomyl prepared from Benlate 50W. Isolates from beans and grapes were tentatively identified as resistant to benomyl by placing conidia from diseased tissue or agar culture directly onto plastic quadrant petri dishes of Difco water agar amended with 0, 1, 10, or 100 μg/ml of benomyl (WA-B) prepared from Benlate 50W before autoclaving. Three dishes per isolate were incubated at room temperature (20–25 C); after 24 hr, 50 spores per treatment were examined at × 70. Spores developing long filamentous germ tubes were considered resistant to benomyl, whereas those with short curled germ tubes were considered sensitive. Sporulation was rated after 7 days.

In addition, linear growth of mycelia in glass petri dishes of PDA amended with benomyl (PDA-B) was rated. Plugs (5 mm) were cut from the advancing edge of 3-day-old colonies of *B. cinerea* growing in PDA, inverted, and placed in the center of petri dishes of PDA and PDA-B amended with 1, 10, or 100 μg/ml of benomyl, added to the agar before autoclaving. All dishes were incubated at room temperature (20–25 C) under 14 hr of cool-white fluorescent light (2,800 lux) per day, 32 cm above the cultures. Colony diameter was measured daily until the colonies in the control dishes (no benomyl) reached the edge of the dishes (about 4 days). Two diameters were measured from each of four replicates, and the mean colony diameter per dish was calculated. Mycelial growth of each isolate on PDA-B was compared with its growth on PDA as a control and was reported as a percent of the control. Resistant (B-11) and susceptible (B-24) strains of *B. cinerea* were always included as internal standards.

Benomyl-resistant isolates of *B. cinerea* (four from apple, five from bean, and eight from grape) from various geographical locations were compared for growth on PDA and PDA-B at 100, 300, or 600 μg/ml of benomyl. The cultures were incubated in the laboratory, and linear growth was measured as described previously.

Pathogenicity tests. Five benomyl-resistant isolates of *B. cinerea* from apple, three from bean, and three from grape were tested for pathogenicity on apple fruit, snap bean plants, and grape berries. McIntosh apples fresh from controlled-atmosphere storage were punctured 3–4 mm deep by three nails mounted in a cork. For each isolate, the injured surface of four fruits was atomized with a suspension of conidia (about 40,000 conidia/ml) washed from a 13-day-old culture growing on PDA. The fruits were incubated in plastic bags at room temperature. The number of punctures infected and the radius of decayed tissue around each wound were recorded after 5 days. Pathogenicity tests on Early Gallatin snap beans were conducted by inoculating four single-pot replicates of three bean plants per pot with a suspension of conidia (about 40,000 conidia/ml) sprayed onto open blossoms. After inoculation, each pot of beans was misted with distilled water, placed in a large polyethylene bag, incubated under shade in a greenhouse at 20–21 C, and rated for gray mold after 7 days. Grapes used for pathogenicity studies were taken from cold storage (about 1 C) after 2.5 mo, soaked in 5% Clorox for 15 min, and placed on hardware cloth in plastic crispers. The berries were punctured with a sterile needle and inoculated with a drop of conidial suspension (about

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40,000 conidia/ml) applied to the wound with a Pasteur pipet. Two groups of four Concord berries and two groups of five Niagara berries were inoculated with each isolate.

RESULTS

Tests for benomyl resistance. Bean and grape isolates of *B. cinerea* were categorized as sensitive or resistant by first observing germ tube growth habit and sporulation on WA-B and then measuring linear growth on PDA-B. Thirteen of 67 isolates plated on WA-B at 1, 10, and 100 μg/ml of benomyl sporulated at each concentration and were rated resistant. These 13 isolates showed ≤ 1.6% and < 10% curled germ tubes on WA-B at 1 and 100 μg/ml of benomyl, respectively. The linear growth of these 13 isolates on PDA-B ranged from 98.5 to 100% of that of the control at 1 μg/ml of benomyl, from 97.8 to 100% at 10 μg/ml, and from 63.9 to 89.5% at 100 μg/ml. The rest of the 67 isolates showed ≥ 20% curled germ tubes on WA-B at 1 and 10 μg/ml of benomyl and > 10% curled germ tubes at 100 μg/ml; none of the isolates grew on PDA-B at these concentrations of benomyl.

Sensitivity of isolates to benomyl. Twenty-one of 24 *B. cinerea* isolates recovered from seven Hudson Valley apple storages grew on PDA-B amended with 250 μg/ml of benomyl and were rated resistant to benomyl. At least one resistant isolate was found in each storage sampled. Neither of two *B. cinerea* isolates from Champlain Valley and only three of 31 isolates from western New York were resistant to benomyl. Each of the three resistant isolates from western New York was recovered from a different storage, one of which had never dipped fruit in benomyl. Because apple growers may spray various blocks of trees differently and then pool the fruit in storage, the effect of orchard sprays on the occurrence of benomyl-resistant strains in packinghouses could not be determined.

Five of 45 isolates of *B. cinerea* collected from snap bean fields in central and western New York were resistant to benomyl but were limited to two of the 12 fields sampled. Most snap bean growers have used benomyl alone for 6–7 yr to control *S. sclerotiorum,* usually with one application per season. The two fields where benomyl resistance was identified, however, had been sprayed twice each season for two consecutive years in one field and for seven consecutive years in the other field. Crop rotation practiced by bean growers made accurate determination of benomyl usage in the other fields difficult.

Twenty-nine isolates of *B. cinerea* were collected from 16 vineyards in various parts of New York. Ten of the 29 isolates were resistant to benomyl and were collected from nine vineyards representing the five major grape-growing regions in

Table 1. Linear growth of benomyl-resistant isolates of *Botrytis cinerea* from apple, bean, and grape hosts on PDA amended with various concentrations of benomyl

Isolate	Host	Years of benomyl usage	No. of field applications per year	Linear growth on benomyl-amended PDA[u] as percent of control		
				100 μg/ml	300 μg/ml	600 μg/ml
3-4	Apple	4	...[v]	80.5 a[w]	76.9 a	41.8 bc
3-3	Apple	4	...	75.7 bc	62.4 abc	40.4 bcd
1-5	Apple	4	...	77.0 b	72.7 ab	43.3 ab
5-3	Apple	6	...	73.0 cd	71.9 abc	47.1 a
29	Bean	2	2	79.9 a	72.6 ab	41.6 bc
26	Bean	7	1–2	71.9 de	67.4 abc	39.3 bcde
1	Bean	7	1–2	70.3 def	66.2 abc	38.4 cdef
38	Bean	7	1–2	68.9 efg	62.5 abc	34.5 f
11	Bean	...[x]	...	68.6 fg	62.1 abc	39.9 bcde
205	Grape	5	5	61.1 ij	56.9 c	39.8 bcde
218	Grape	...[y]	...	64.3 hi	57.9 bc	40.9 bc
233	Grape	4	2–3	76.5 b	70.5 abc	41.8 bc
230	Grape	2	3	60.1 j	58.3 bc	30.2 g
215	Grape	4	4–5	64.7 h	57.5 bc	37.8 cdef
225	Grape	2	3	67.1 gh	61.1 bc	36.3 def
0102-1	Grape	4	3–4	78.0 ab	71.7 abc	36.1 ef
0102-6	Grape	4	3–4	72.2 d	60.1 abc	40.6 bc
24 (sensitive ck)	Bean	6	1	0[z]	...	...

[u] Each treatment was replicated four times. Control plates consisted of PDA without benomyl. After four days, colony diameter was measured in two directions and averaged.
[v] Not possible to determine.
[w] Letters indicate groupings of treatments that do not differ significantly ($P \leq 0.001$) according to Waller-Duncan's exact Bayesian K-ratio (LSD) rule.
[x] Isolate 11 was selected in the laboratory from a sensitive strain by F. J. Polach.
[y] Isolate 218 was collected from an experimental vineyard with a discontinuous history of benomyl usage since 1968.
[z] Omitted from statistical analysis.

Table 2. Pathogenicity of benomyl-resistant *Botrytis cinerea* on apple, bean, and grape

Isolate	Host	McIntosh apple fruit		Early Gallatin snap bean	Grape berries			
		Percent puncture sites infected[v]	Mean radius (mm) decay at infected puncture sites	Mean infection rating[w]	Percent berries infected[x]		Mean percent surface area[y] with mycelium	
					Niagara	Concord	Niagara	Concord
2-5	Apple	0 c[z]	...	2.5 a	10 c	0 c	0.2 d	0 f
3-4	Apple	50.0 abc	15.0	2.7 a	100 a	100 a	45.1 ab	92.1 a
3-3	Apple	58.6 abc	13.2	2.0 a	100 a	100 a	22.4 abc	74.1 bc
1-5	Apple	2.3 c	3.0	1.6 ab	100 a	100 a	63.4 a	78.1 ab
5-3	Apple	50.0 abc	8.8	2.5 a	70 b	63 b	8.8 bcd	5.0 ef
26	Bean	14.6 bc	5.9	1.8 a	100 a	100 a	15.6 bcd	52.5 c
1	Bean	41.4 abc	7.2	2.1 a	50 b	63 b	3.7 cd	6.2 def
38	Bean	0 c	...	0.5 b	100 a	100 a	27.7 abc	86.3 ab
205	Grape	41.4 abc	4.3	2.1 a	70 b	75 a	3.3 cd	3.5 ef
233	Grape	85.3 ab	6.5	2.4 a	70 b	38 b	4.5 cd	14.8 de
225	Grape	94.3 a	13.6	2.2 a	100 a	100 a	19.5 bcd	21.9 d

[v] Mean of four apples per isolate, each inoculated at three puncture sites per apple.
[w] Rating system on a scale of 0 to 3 where 0 = healthy, 1 = slight lesion development on leaf or blossom, 2 = moderate lesion development on both leaf and blossom or stem or pod, and 3 = severe lesion development on both stem and pod.
[x] Data from five Niagara and four Concord berries, each replicated twice, 9 days after wound inoculation.
[y] Data from five Niagara and four Concord berries, each replicated twice, as determined by Barratt-Horsfall ratings of hemispheric area contered on inoculation site.
[z] Letters indicate groupings of treatments that do not differ significantly ($P \leq 0.05$) according to Waller-Duncan's exact Bayesian K-ratio (LSD) rule.

the state. In eight of these vineyards, benomyl had always been used in combination with another fungicide (usually captan) for control of downy mildew; in the other one, however, benomyl had been used alone two or three times per season for four consecutive years.

The host from which benomyl-resistant isolates were collected and the history of benomyl usage did not influence the growth of isolates at different concentrations of benomyl (Table 1). Within the range of benomyl concentrations tested (0, 100, 300, and 600 μg/ml), as much variation occurred among isolates from the same host as among those from differing hosts.

Pathogenicity tests. Pathogenicity tests of benomyl-resistant *B. cinerea* isolates from apples, beans, and grapes gave variable results when inoculated back into their respective hosts (Table 2). Some isolates (apple 3-4 and 3-3 and grape 225) were equally pathogenic on all three hosts, whereas many isolates (apple 1-5 and 5-3, bean 26 and 1, and grape 205 and 233) were more aggressive on two of the three hosts. In some instances, isolates appeared more pathogenic on hosts other than the host of origin (apple 1-5, bean 38, and grape 233). Variations in pathogenicity among isolates from the same host were generally greater than variations among isolates from different hosts. With only two exceptions (apple 2-5 and bean 38), the isolates were pathogenic to some extent on all hosts.

DISCUSSION

The results of our study illustrate several interesting phenomena regarding the development of benomyl resistance. First, the use of benomyl on apples, beans, and grapes in New York has generally not been aimed at *Botrytis* so the development of benomyl-resistance in *Botrytis* may be classified as development in a nontarget organism, a situation predicted by Ogawa et al (10) in 1976. Second, the use of tank mixes of unrelated fungicides to avoid the buildup of resistant strains has been suggested by several researchers (2,5,15), but benomyl-resistant strains of *B. cinerea* exist in vineyards in New York despite the consistent use of tank mixes of benomyl and another fungicide, usually captan.

Our results suggest, especially in those instances where benomyl was used only two seasons on beans and grapes, that the presence of benomyl-resistant strains of *B. cinerea* cannot be explained by the history of benomyl usage. This conclusion is supported by several examples in the literature (4,11,13) and by the growth of benomyl-resistant isolates at relatively high concentrations of benomyl (600 μg/ml) regardless of the history of benomyl usage (Table 1).

Reciprocal pathogenicity tests were conducted with benomyl-resistant isolates collected from apples, beans, and grapes for several reasons: 1) these crops are frequently grown in proximity to one another in New York, 2) reports in the literature indicate a wide host range for *B. cinerea* (1) and the lack of host specificity among isolates (4,7,12), and 3) widespread dispersal of conidia in *B. cinerea* has been well established (6) and spread of resistant strains by airborne conidia has been theorized (9,14). Our results indicate that isolates developing benomyl resistance on one host may be pathogenic on another. Therefore, spread of resistant isolates from field to field and from one host to another (assuming fitness is equal to sensitive strains) may be important in rapid buildup of benomyl resistance on crops without a long history of benomyl usage or on crops never sprayed with benomyl (4).

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