Frequency of Benzimidazole- and Dicarboximide-Resistant Strains of *Botrytis cinerea* in Western Oregon Small Fruit and Snap Bean Plantings

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ABSTRACT

Johnson, K. B., Sawyer, T. L., and Powelson, M. L. 1994. Frequency of benzimidazole- and dicarboximide-resistant strains of *Botrytis cinerea* in western Oregon small fruit and snap bean plantings. Plant Dis. 78:572-577.

In 1990 and 1991, a total of 3,496 isolates of *Botrytis cinerea* were collected from strawberry, wine grape, raspberry, and snap bean plantings and from nonmanaged wild blackberry hedges located in Oregon's Willamette Valley. The percentage of isolates resistant to benomyl (5 μg/ml) and to vinclozolin (10 μg/ml) averaged 52 and 17%, respectively; 16% were resistant to both fungicides. The frequencies of resistance to benomyl and vinclozolin were significantly higher ($P \leq 0.05$) in *B. cinerea* isolates obtained from strawberry than in isolates from the other crops. Of isolates of *B. cinerea* obtained from nonsprayed wild blackberry hedges, 38 and 6% were resistant to benomyl and vinclozolin, respectively. The frequency of benomyl- and vinclozolin-resistant strains of *B. cinerea* in a planting was significantly correlated ($P < 0.05$) with the number of applications in each respective fungicide class. Vinclozolin resistance was estimated to increase by 20.8% per fungicide application in strawberry compared with 6.4% per application in wine grape, raspberry, and snap beans. In eight strawberry fields, frequency of vinclozolin resistance declined from an average of 74% at harvest in June to 32% the following February or April. Strawberry fruit mummies harbored a higher frequency of dicarboximide-resistant strains than did leaves.

Additional keywords: gray mold

The Willamette Valley of Oregon is a important production area for small fruits and vegetables grown for processing. Major commodities of strawberries, wine grapes, raspberries, and snap beans have a combined annual farm gate value of $75 million (16). These four crops are attacked by the gray mold pathogen, *Botrytis cinerea* Pers.:Fr., which is favored by the cool, maritime climate of the Willamette Valley. Moreover, Himalayan blackberry grows wild throughout the valley along roads and fence lines, and its fruits are commonly infected with *B. cinerea*.

Gray mold of small fruits and snap beans is controlled principally with protectant fungicide sprays applied to blossoms and ripening fruit. Cultural practices, including sanitation, irrigation management, and modification of canopy architecture, are used to improve control. In the 1970s, benzimidazole fungicides (e.g., benomyl) provided outstanding control of gray mold in western Oregon crops until widespread resistance developed in the *B. cinerea* population (26). Subsequently, use of benzimidazoles solely for control of gray mold within this region has declined greatly. In 1988 and 1989, the frequency of benomyl resistance in *B. cinerea* isolated from fruit of wild blackberry in the Willamette Valley averaged 37 and 41%, respectively (9). Several isolates also were resistant to vinclozolin, a dicarboximide fungicide (K. B. Johnson, *unpublished*). The resistance of *B. cinerea* from western Oregon to dicarboximides has not been reported previously.

Oregon Agricultural Experiment Station Technical Paper No. 10,280.

Accepted for publication 22 February 1994.

Dicarboximide-resistant strains of *B. cinerea* have been observed in most areas of the world where these compounds have been used extensively (2,5,8,10–12,15,17,19). Research in New Zealand (3) and Germany (11) indicates that selection for dicarboximide-resistant strains of *B. cinerea* occurs most rapidly during the periods that are most favorable for disease development. Dicarboximide resistance in *B. cinerea*, however, apparently differs from benzimidazole resistance in that the former imposes a metabolic cost that results in reduced pathogenic and reproductive abilities when compared with sensitive strains (2,5,12,13,19,23). These reduced abilities or "fitness" of resistant strains allow for reselection of sensitive strains and a reduction in the resistance frequency within a *B. cinerea* population during periods when fungistatic concentrations of dicarboximides are not present in the environment (3,11).

The climate of western Oregon is similar to that in regions of the world where dicarboximide resistance in *B. cinerea* has been most difficult to manage (3, 10,11). Because alternative fungicides for gray mold control are limited and considered less effective, growers in western Oregon could incur economic losses if dicarboximide resistance became widespread. Characterizing the extent of the fungicide resistance problem within the Willamette Valley and comparing these data to what is known on management of dicarboximide resistance would provide growers with information that could potentially improve their gray mold control programs.

The purpose of this study was to determine the frequency of benzimidazole and dicarboximide resistance in populations of *B. cinerea* in plantings of strawberry, wine grape, raspberry, and snap bean and from hedges of wild blackberry in Oregon's Willamette Valley. The relationship between the frequency of fungicide resistance and the number of fungicide applications also was examined.

MATERIALS AND METHODS

Resistance survey. Commercial plantings of strawberry (*Fragaria* × *ananassa* Duchesne), grape (*Vitis vinifera* L.), raspberry (*Rubus idaeus* L.), and snap bean (*Phaseolus vulgaris* L.) and nonmanaged hedges of Himalayan blackberry (*R. discolor* Weihe & Nees) located in the Willamette Valley of Oregon were sampled for *B. cinerea* in the spring, summer, and/or fall of 1990 and 1991. In each season, samples of *B. cinerea* were taken from 19–20 plantings each of strawberry, raspberry, and wine grape as fruit matured in June, July, and October, respectively. Ten fields of snap bean were sampled near harvest during July–September in 1990 and 20 fields were sampled in 1991. Eighteen hedges of Himalayan blackberry were sampled during September–October in 1990 and 38 in October 1991. Plantings were selected to provide a representative distribution of field size and farming intensity throughout the valley (Fig. 1). All plantings were selected without knowledge of previous fungicide use.

Field sampling. Cotton swabs were placed individually into screw-cap test tubes and autoclaved. With small fruit crops, conidia of *B. cinerea* were collected by touching a cotton swab to a sporulating lesion on a ripe fruit. Snap bean isolates were collected similarly from diseased pod, stem, and blossom tissues. After sampling a lesion, each cotton swab was put back in the test tube and transported to the laboratory. Twenty samples were made in each planting or hedge. A zigzag sampling pattern was used within commercial plantings, and blackberry hedges were sampled along their entire length.

Individual isolates of *B. cinerea* were recovered by streaking the conidia-bearing cotton swabs onto Difco malt agar amended with 200 μg/ml of streptomycin sulfate (MAS). After streaking, the isolates were incubated at room temperature (20–26 C) for 7–10 days on a laboratory bench near large east-facing windows and allowed to sporulate. Several thousand conidia from each sporulating culture were then transferred with a flamed culture loop onto MAS amended with 10 μg/ml of vinclozolin (13) or 5 μg/ml of benomyl (25). After 24 hr of incubation at 20–26 C, 100 conidia were evaluated for germination, defined as a straight germ tube at least three times as long as the diameter of the spore (about 40 μg). Culture dishes were examined again for sporulation of *B. cinerea* after incubation for 6–10 days. Isolates were considered resistant only if more than 90% of transferred conidia germinated after 24 hr and if new conidia were subsequently produced (13,25).

Osmotic sensitivity dicarboximide-resistant isolates. A subset of 20 dicarboximide-sensitive and 27 dicarboximide-resistant isolates of *B. cinerea* was further characterized for their sensitivity to osmotic stress (1) and for relative sensitivity to two concentrations of vinclozolin. Sensitivity to high osmotic pressure has been shown to be a pleiomorphic effect of a single gene mutation that confers resistance to dicarboximide fungicides (6,7), and the degree of osmotic sensitivity has been shown to be dependent on the level of dicarboximide resistance within a strain (2,7). Original cultures that had not been previously grown on fungicide-amended medium were used in these assays. Plugs 6 mm in diameter from 4-day-old cultures were transferred to 9-cm-diameter petri dishes containing MAS, MAS amended with 0.68 M NaCl (40 g/L), or MAS amended with 2 or 5 μg/ml of vinclozolin. Each isolate was replicated twice. Colony diameter was measured after 1 and 4 days of incubation at 20–24 C, and an average daily growth rate was calculated. The average daily growth rate on MAS amended with vinclozolin or NaCl was used to classify isolates into categories of sensitive, ultra-low-level-resistant, and low-level-resistant isolates, as defined by Beever et al (2).

Fungicide-use survey. After sampling of *B. cinerea* was completed each season, questionnaires were mailed to individuals who could be identified as managers of sampled plantings. Managers were requested to list the fungicides applied and the date of applications during the same season that isolates of *B. cinerea* were collected.

Overwintering of resistant strains in strawberry. On 4 April 1991 and 27 February 1992, 30 mummified fruit, young

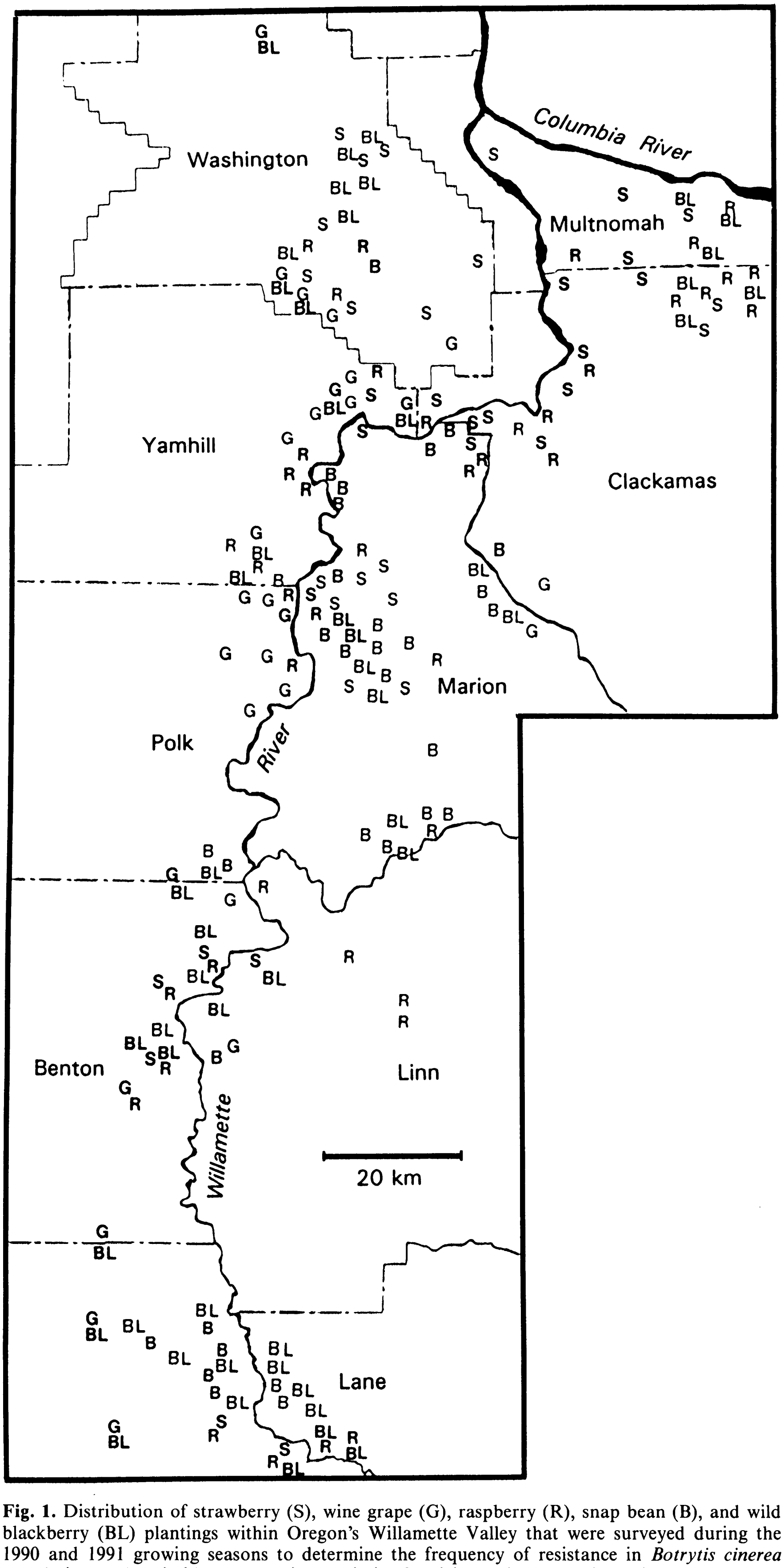

Fig. 1. Distribution of strawberry (S), wine grape (G), raspberry (R), snap bean (B), and wild blackberry (BL) plantings within Oregon's Willamette Valley that were surveyed during the 1990 and 1991 growing seasons to determine the frequency of resistance in *Botrytis cinerea* populations to benzimidazole and dicarboximide fungicides. Lines and tributaries denote county boundaries.

leaves, and old leaves were sampled from each of four strawberry plantings from which isolates of *B. cinerea* had been collected the previous June. Mummified fruit were surfaced-sterilized with 0.5% NaOCl for 2 min, rinsed in sterile distilled water amended with 200 μg/ml of streptomycin sulfate, and then incubated on wire-screen supports inside plastic crisper boxes maintained at room temperature (20–24 C) and 100% relative humidity. Leaves were similarly disinfested, cut into 1 cm^2 pieces, and placed into petri dishes containing water agar (1%) amended with 20 μg/ml of paraquat (21). Conidia of *B. cinerea* were produced on fruit and leaf pieces after 4–10 days of incubation at 20–24 C. Conidia were transferred with a sterile dissecting needle to MAS and then screened for resistance to benomyl and vinclozolin as described above.

Data analysis. For each planting, the frequency of resistance in *B. cinerea* to benomyl, to vinclozolin, and to both was calculated by dividing the number of resistant isolates by the total number of isolates recovered on MAS expressed as a percentage. The effect of host crop on frequency of resistance was analyzed with the general linear models procedure of SAS (22). Resistance frequency histograms also were developed for each crop by categorizing sample sites into the following classes for mean frequency of resistance to benomyl or vinclozolin: 0%, 1–20%, 21–40%, 41–60%, 61–80%, and 81–100%.

Fungicide application data were summarized by crop. Mean frequency of benomyl or vinclozolin resistance within a planting was regressed on the number of respective benzimidazole or dicarboximide applications made within the same season. Indicator variables (18), representing the crops from which the isolates were obtained, also were added to the regression model with a stepwise multiple regression procedure (22).

RESULTS

Resistance survey. A total of 3,496 isolates of *B. cinerea* were obtained from samples collected in 145 strawberry, wine grape, raspberry, and snap bean plantings and from 56 wild blackberry hedges, with an average of 17 isolates per sample site. Overall, the frequency of resistance to benomyl and vinclozolin averaged 52 and 17%, respectively; 16% of the isolates were resistant to both fungicides. During both 1990 and 1991, isolates of *B. cinerea* obtained from strawberry had the highest frequency of resistance to benomyl (average 79%) and vinclozolin (average 41%) (Table 1). Over both years, resistance to benomyl and vinclozolin in isolates of *B. cinerea* obtained from wine grape, raspberry, snap bean, and wild blackberry averaged 57 and 15%, 46 and 11%, 31 and 15%, and 38 and 6%, respectively (Table 1). With the exception of wild blackberry, each crop had at least one planting where resistance to benomyl and vinclozolin exceeded 80 and 60%, respectively (Fig. 2).

Osmotic sensitivity dicarboximide-resistant isolates. Vinclozolin-resistant isolates of *B. cinerea* cultured on MAS amended with NaCl or vinclozolin separated into the two classes ultra-low-level and low-level resistance, as defined previously (2). Compared with the ultra-low-level-resistant isolates, the low-level-resistant isolates showed reduced growth on MAS amended with 0.68 M NaCl with denser than normal colony margins and a higher growth rate on the vinclozolin-amended medium (Table 2). Isolates in the low-level resistance class were obtained from plantings with an average frequency of resistance to vinclozolin of 91%. In contrast, eight of 12 isolates in the ultra-low-level resistance class were obtained from plantings that averaged 33% resistant isolates.

Fungicide-use survey. Records of fungicide applications were obtained for 74 (51%) of the 145 commercial plantings surveyed over the 2-yr period. The proportion of plantings from which application records were obtained ranged from 40% for raspberry to 60% for strawberry.

Strawberry crops received an average of 2.7 and 3.7 fungicide applications for gray mold control in 1990 and 1991, respectively (Table 1). Over both years, wine grape, raspberry, and snap bean plantings were sprayed with fungicides an average of 1.9, 1.2, and 0.9 times, respectively, for gray mold control. Of fungicides applied to control gray mold, dicarboximides (vinclozolin or iprodione) were used in 55% of applications made in strawberry and 86% of applications made in the other crops (Table 1). The benzimidazole fungicide benomyl or thiophanate-methyl was included in 14% of applications, usually in a mixture with captan. In only one of the 74 plantings for which fungicide application records were obtained was a benzimidazole fungicide applied more than once in the same season.

For both the benzimidazole and dicarboximide fungicides, the percentage of *B. cinerea* isolates resistant to benomyl or vinclozolin was significantly correlated ($P \leq 0.05$) with the number of applications within each respective fungicide class. The *y* intercept for frequency of resistance to benomyl was 47.3% ± 29.5 (SE), and the slope of the regression model was estimated to be 30.4% ± 7.4 ($R^2 = 0.17$) (Fig. 3). Regression of the frequency of vinclozolin resistance on number of dicarboximide applications

Table 1. Mean frequency of resistance to the fungicides benomyl and vinclozolin in isolates of *Botrytis cinerea* collected near harvest in 1990 and 1991 from strawberry, wine grape, raspberry, and snap bean plantings and wild blackberry hedges in Oregon's Willamette Valley, and class of fungicide and mean number of applications made during the season in which isolates of *B. cinerea* were collected

Year / Crop	No. of plantings	Mean no. of isolates per planting	Resistance frequency[v] (%) Benomyl	Vinclozolin	Benomyl and vinclozolin	Mean no. of fungicide applications Benzimidazole	Dicarboximide	Total[w]
1990								
Strawberry	20	17.5	77 a[x]	32 a	31 a	0.4 (0.5)[y]	1.4 (1.7)	2.7 (3.0)
Wine grape	19	18.4	64 ab	14 ab	11 ab	0.3 (0.7)	1.3 (0.9)	1.5 (1.0)
Raspberry	20	14.8	46 b	12 b	9 b	0.4 (0.5)	0.7 (1.0)	1.1 (1.4)
Snap bean	8	15.9	52 b	31 a	25 ab	0.0 (0.0)	0.5 (0.6)	0.5 (0.6)
Wild blackberry	18	18.4	58 ab	10 b	9 b	...[z]	...	...
1991								
Strawberry	20	17.3	81 a	50 a	48 a	0.5 (0.5)	2.1 (1.1)	3.7 (1.0)
Wine grape	18	18.2	50 b	16 b	16 b	0.1 (0.3)	2.0 (1.2)	2.2 (1.1)
Raspberry	20	18.1	47 b	12 bc	10 bc	0.3 (0.5)	1.0 (1.0)	1.3 (1.1)
Snap bean	20	16.6	22 c	8 bc	7 bc	0.0 (0.0)	1.0 (0.0)	1.0 (0.0)
Wild blackberry	38	17.7	27 c	4 c	3 c	...	...	...

[v] Mean percentage of isolates that exhibited at least 90% conidial germination and subsequent sporulation on MAS amended with benomyl (5 μg/ml) or vinclozolin (10 μg/ml), or on both benomyl- and vinclozolin-amended MAS.
[w] Captan and thiram were the other fungicides applied for gray mold control.
[x] Within a column and year, means followed by the same letter are not significantly different according to Fisher's least significant difference test at $P = 0.05$.
[y] Numbers in parenthesis are the standard deviation of the mean.
[z] Nonmanaged plantings.

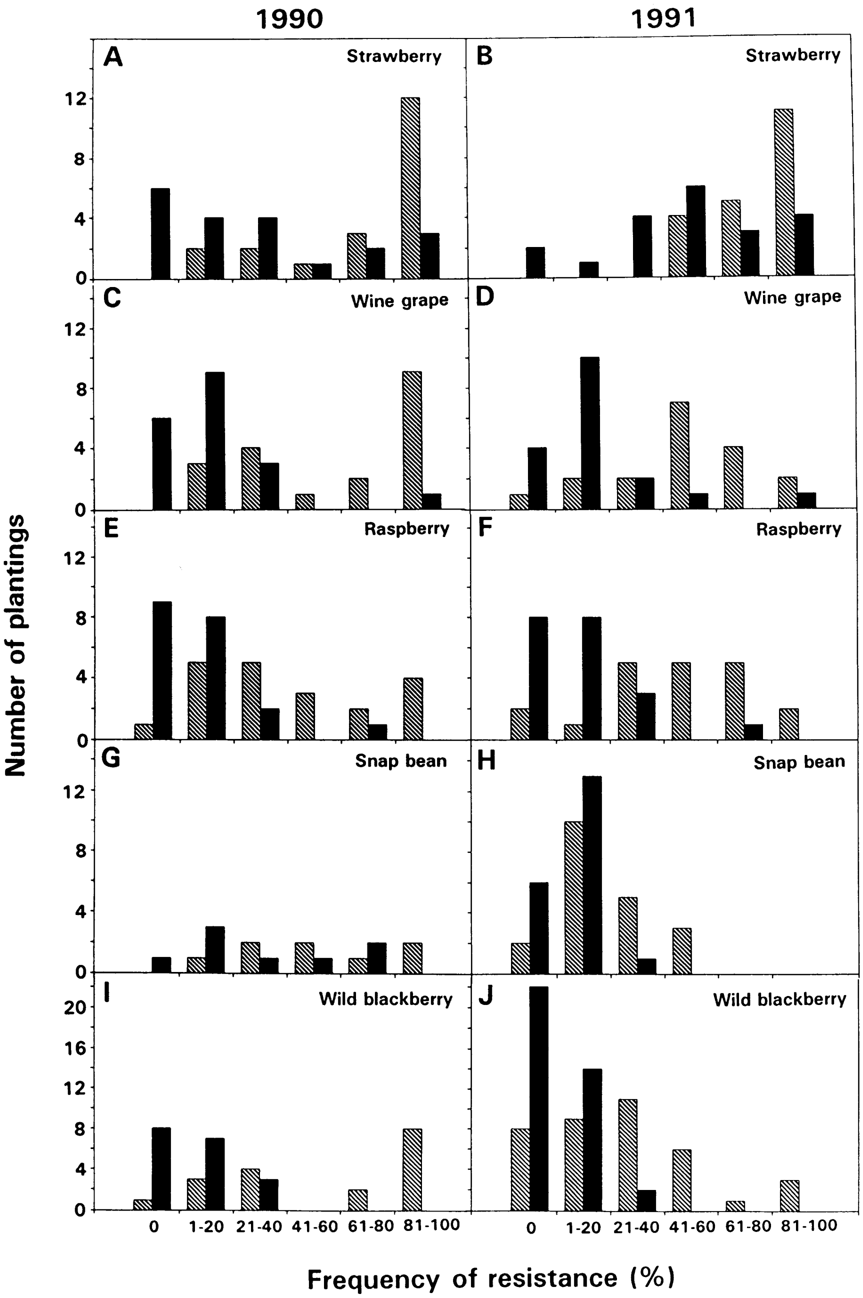

Fig. 2. Histograms depicting estimated frequency classes of benzimidazole resistance (hatched bars) and dicarboximide resistance (solid bars) in *Botrytis cinerea* populations sampled near harvest during 1990 and 1991 from commercial plantings of **(A and B)** strawberry, **(C and D)** wine grape, **(E and F)** raspberry, and **(G and H)** snap bean and from **(I and J)** wild blackberry hedges in Oregon's Willamette Valley. An average of 17 isolates per planting were assayed on malt agar amended with benomyl (5 μg/ml) or vinclozolin (10 μg/ml).

Table 2. Average growth rates of *Botrytis cinerea* isolates in three dicarboximide resistance classes on malt agar plus streptomycin (MAS) and on MAS amended with sodium chloride or vinclozolin

		Radial growth rate[x] (cm/day)			
				MAS + vinclozolin	
Resistance class[w]	No. of isolates	MAS	MAS + NaCl (40 mg/ml)	2 μg/ml	5 μg/ml
Sensitive	20	1.9 ± 0.5[y]	1.3 ± 0.3 (68%)[z]	0.1 ± 0.1 (5%)	0.0 ± 0.0 (0%)
Ultra-low-level	12	2.0 ± 0.2	1.5 ± 0.3 (75%)	1.1 ± 0.4 (55%)	0.2 ± 0.1 (10%)
Low-level	15	2.0 ± 0.3	1.1 ± 0.3 (55%)	1.5 ± 0.5 (75%)	0.5 ± 0.2 (25%)

[w] Low-level-resistant isolates had denser colony margins on MAS amended with sodium chloride than ultra-low-level-resistant isolates, as has been observed previously (2).
[x] Average daily increase in colony diameter 1–4 days after 0.6-mm plugs of the isolates were transferred to the surface of the growth medium.
[y] Standard deviation of the mean.
[z] Percentage of the value obtained on MAS within each class.

resulted in the equation: $y = 11.7 + 6.4 \cdot x_{bgrs} + 14.4 \cdot x_s$ ($R^2 = 0.46$), where y is the frequency of vinclozolin resistance within a planting, x_{bgrs} is the number of dicarboximide applications made in any crop, and x_s is the number of dicarboximide applications made if the crop was strawberry (zero otherwise). Consequently, the first regression coefficient ($\beta_{bgrs} = 6.4\% \pm 3.3\%$) represents the rate of increase in the frequency of resistance to vinclozolin owing to each dicarboximide application in wine grape, raspberry, or snap bean (Fig. 4). The second regression coefficient ($\beta_s = 14.4\% \pm 3.2$) represents an additional, significant ($P < 0.05$) increase in the frequency of vinclozolin resistance owing to each dicarboximide application made in strawberry; i.e., for strawberry, the

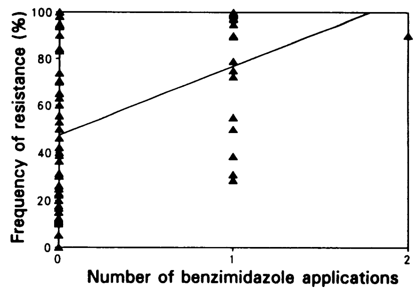

Fig. 3. Regression of the estimated frequency of benzimidazole-resistant strains of *Botrytis cinerea* on number of benzimidazole applications made in strawberry, wine grape, raspberry, and snap bean plantings in Oregon's Willamette Valley. Data from 74 plantings are plotted; $y = 47.3 + 30.4 \cdot x$, where y is the frequency of benomyl resistance within a planting and x is the number of benzimidazole applications.

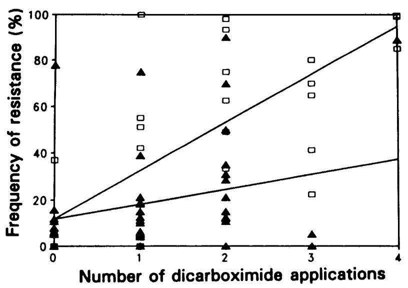

Fig. 4. Regression of the estimated frequency of dicarboximide-resistant strains of *Botrytis cinerea* on number of dicarboximide applications made in strawberry, wine grape, raspberry, and snap bean plantings in Oregon's Willamette Valley. The upper line and open boxes represent data obtained from strawberry; $y = 11.7 + 20.8 \cdot x$, where y is the frequency of vinclozolin resistance within a strawberry planting and x is the number of dicarboximide applications. The lower line and solid triangles represent data obtained from wine grape, raspberry, and snap bean; $y = 11.7 + 6.4 \cdot x$, where y is the frequency of vinclozolin resistance within a wine grape, raspberry, or snap bean planting and x is the number of dicarboximide applications. Data from 74 plantings, 24 of which were strawberry, are plotted.

Table 3. Frequency of benzimidazole and dicarboximide resistance in isolates of *Botrytis cinerea* obtained from strawberry fruit at harvest and in mummified fruit and young and old leaves sampled from the same fields the following spring[x]

Year Field	Resistance frequency in fruit sampled in June of preceding season[y]		Frequency of resistance in spring[y]								
			Fruit mummies			Old leaves			Young leaves		
	b	v	n	b	v	n	b	v	n	b	v
1991											
1	100	80	10	100	40	9	100	0	22	91	5
2	100	100	28	89	64	22	100	86	26	100	73
3	100	70	—[z]	—	—	19	100	0	11	100	0
4	100	100	25	96	28	12	100	0	10	100	0
Av.	100	88	21	95	44	16	100	22	17	98	20
1992											
5	53	47	25	68	40	2	50	0	22	68	9
6	94	56	2	50	100	—	—	—	7	57	29
7	94	63	25	96	60	6	100	100	15	67	27
8	67	56	—	—	—	3	33	0	29	69	7
Av.	77	56	17	71	67	4	61	33	18	65	18

[x] Fields were sampled on 4 April 1991 and 27 February 1992 before the fungicide spray program for the upcoming season had begun.
[y] Percentage of isolates exhibiting at least 90% conidial germination and subsequent sporulation on MAS amended with 5 μg/ml of benomyl (b) or 10 μg/ml of vinclozolin (v). n = Number of isolates obtained.
[z] Field not sampled.

model estimates that vinclozolin resistance increased 20.8% ($\beta_{bgrs} + \beta_s$) with each dicarboximide application (Fig. 4).

Overwintering of resistant strains in strawberry. Isolates of *B. cinerea* were recovered from fruit mummies and from young and old leaves sampled from strawberry fields in early spring (Table 3). Overall, the percentage of isolates resistant to vinclozolin declined from 74% in the initial June survey to 32% in the spring. In contrast, the frequency of resistance to benomyl did not decline over the winter (Table 3). Fruit mummies harbored a higher frequency of vinclozolin-resistant strains than did leaves (Table 3). The decline in the frequency of vinclozolin resistance was most variable for isolates of *B. cinerea* obtained from leaves; two fields showed relatively small changes in the frequency of vinclozolin resistance from summer to spring, but in the other two fields, no vinclozolin-resistant strains were recovered from leaves sampled in the spring (Table 3).

DISCUSSION

The approach used in this study to survey isolates of *B. cinerea* associated with several crop hosts and with a wild host provided useful information on the distribution and frequency of benzimidazole- and dicarboximide-resistant strains of the fungus within a defined agricultural production area. As a general conclusion, the frequencies of benzimidazole and dicarboximide resistance within Oregon's Willamette Valley are similar to those reported from small fruit and vegetable production areas in northern Europe (8,10–13,15,23), New Zealand (2,3,20), and Canada (19). Like these other production areas, the environment of Willamette Valley is conducive to the development of gray mold, and the benzimidazole and dicarboximide classes of fungicides have been registered for gray mold control for an extended period of time (15–20 and 8–10 yr, respectively).

The data obtained from wild Himalayan blackberry hedges are particularly useful for understanding the significance of the fungicide resistance problem. Wild blackberries grow commonly along roadways and fence lines throughout the Willamette Valley, fruit diseased with gray mold are present in most seasons, and the hedges are not sprayed with fungicides for gray mold control. When we include findings from surveys conducted in 1988 and 1989 (9), an average of 40% of the isolates obtained from wild blackberry in the Willamette Valley were resistant to benomyl (Table 1). This high frequency of resistance to benomyl has been maintained despite relatively limited use of benzimidazole fungicides for gray mold control (Table 1). In 1991, the frequency of benzimidazole resistance in isolates obtained from wild blackberry averaged 27%, compared with 58% the previous season (Table 1). We attribute this difference to having chosen a higher proportion of hedges from less intensively farmed areas of the valley in 1991 and more hedges from the intensively farmed north central area of the valley in 1990. Lower frequencies of benomyl-resistant isolates nearer to the edges of the valley may indicate that the frequency of benzimidazole resistance within an area could decline over time in areas where there is potential for immigration of sensitive strains from nonfarmed (forested) areas.

Isolates of *B. cinerea* resistant to the dicarboximide fungicide vinclozolin also were obtained from wild blackberry during both 1990 and 1991 but at about one-sixth the frequency at which benomyl-resistant isolates were found. This lower frequency of vinclozolin-resistant strains on wild blackberry may reflect, in part, their reduced reproductive fitness relative to sensitive strains (2,3,5,14,19). Beever et al (3) proposed that the frequency of dicarboximide resistance in a *B. cinerea* population will tend to an equilibrium value that is governed by selection for resistant strains as a result of fungicide use and by selection against resistant strains because of their lower reproductive fitness. In New Zealand, this equilibrium value was estimated to be about 15% in grapes not treated with a dicarboximide (3); our overall estimate of the frequency of vinclozolin-resistant strains on nontreated wild blackberry was 6% (Table 1).

With regard to the criteria we used to judge an isolate resistant or sensitive, the sporulation test was more important for screening isolates on benomyl. The germination test also was useful, but most benomyl-sensitive *B. cinerea* isolates produced short, curved germ tubes on the benomyl-amended medium (19), and after 24 hr, distinguishing resistant and sensitive isolates was not always straightforward. For evaluating vinclozolin resistance, we considered the germination test a more reliable criterion because sensitive isolates did not germinate within 24 hr on the vinclozolin-amended medium. About 4% of isolates we classified as sensitive to vinclozolin on the basis of low germination subsequently sporulated on the medium. These isolates may have been mixtures of strains or, perhaps, heterokaryons with a low number of nuclei with the dicarboximide resistance allele (6,7).

More than one-half of the subset of vinclozolin-resistant isolates grown on a medium amended with 0.68 M NaCl had reduced growth rates indicative of abnormal sensitivity to high osmotic pressure (1,2,7). The osmotically sensitive isolates fit the description of "low-level resistant isolates" used by Beever et al (2), whereas resistant isolates that were not affected by the addition of NaCl have been described as "ultra-low-level resistant isolates" (2). For the 27 isolates we evaluated, the degree of resistance (or osmotic sensitivity) appeared to be positively correlated with the frequency of dicarboximide resistance in the planting from which they were obtained, suggesting that the intensity of the selection pressure (fungicide use) in a planting will influence the relative proportions of low-level and ultra-low-level resistant strains. Low-level and ultra-low-level isolates of *B. cinerea* made up 82 and 18%, respectively, of dicarboximide-resistant strains recovered in a New Zealand survey of grape production areas (2).

The incidence amount of gray mold on fruit in fields or hedges surveyed in this study was variable and usually less than 10%. Thus, no conclusions could be drawn from the survey data regarding loss of control from the presence of fungicide-resistant strains of *B. cinerea*. In discussions with growers, most were aware that benzimidazole resistance was widespread and most were satisfied with the level of control they were obtaining from use of dicarboximide fungicides. Several strawberry fields and one raspberry field, however, had significant amounts of disease in spite of three or more applications of vinclozolin or iprodione. Isolates of *B. cinerea* obtained from these fields had high frequencies of resistance to vinclozolin (>80%). Survey data from New Zealand (2) indicate that dicarboximide fungicides begin to lose efficacy for control of gray mold in grape when the percentage of resistance in a *Botrytis* population at harvest exceeds 40%. Thus, for the crops we surveyed, the measured frequencies of dicarboximide resistance (Table 1), the number of times that fungicides are applied (Table 1), and our observations of disease development in surveyed fields indicate that strawberry is most at risk of control failures from use of dicarboximide fungicides.

Regression of the frequency of vinclozolin-resistant strains on the number of dicarboximide applications (Fig. 4) also indicated that use of dicarboximide fungicides has a higher potential risk of control failure in strawberry than in the other crops we surveyed. In the Willamette Valley, strawberries have a long bloom period and are usually subjected to cool, wet weather during the bloom and fruit maturation periods (April–May) when fungicides to control gray mold are applied. In contrast, for wine grape, raspberry, and snap bean, the bloom periods are shorter and the weather during bloom and/or fruit maturation (late June through September) is typically warm and dry. Consequently, the more favorable conditions for disease development in strawberry probably account for the higher rate of selection of vinclozolin-resistant strains than in the other crops (Fig. 4). In other areas with climates conducive to gray mold, e.g., Germany (11) and New Zealand (3), a limit of two dicarboximide applications has been recommended in order to maintain resistance frequencies below levels at which control failure is likely to occur. Our results suggest that the same recommendation is appropriate for Willamette Valley strawberry producers.

Several strawberry fields were sampled in the spring following the initial survey in June to determine if the frequency of dicarboximide resistance in *B. cinerea* populations declined after a winter under Willamette Valley conditions. The decline in resistance frequency that we measured, from 74% at harvest to 32% in the spring, was similar to other reports where the frequency of dicarboximide resistance was monitored throughout the year (11,13,19,20). We were surprised, however, with the variability in the recovery of vinclozolin-resistant isolates from field to field and among strawberry tissues that were sampled. Strawberry plantings in the Willamette Valley are typically grown for 3–4 yr and renovated between seasons by mowing the crop canopy during the summer. Research is needed to determine if the timing and method of field renovation influence the persistence of fungicide-resistant strains of *B. cinerea* in strawberry leaves, which are a significant source of inoculum during bloom the following season (4,24). In addition, alternative renovation practices such as flaming leaves with a propane burner could potentially destroy a large proportion of fungicide-resistant strains within a field.

ACKNOWLEDGMENTS

The research was supported by the Oregon Caneberry, Strawberry, and Vegetable commissions, the Wine Advisory Board, and the Oregon Agricultural Experiment Station.

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