Influence of Selected Fungicide Regimes on Frequency of Dicarboximide-Resistant and Dicarboximide-Sensitive Strains of *Botrytis cinerea*

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ABSTRACT

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Isolates of *Botrytis cinerea* from the field, sensitive and resistant to the dicarboximide fungicide vinclozolin, were used in evaluating fungicide regimes for managing vinclozolin-resistant populations. On geranium, mixed populations, composed of a strain resistant to and a strain sensitive to dicarboximide, were repeatedly subjected to eight fungicide regimes. Disease control and effect on the frequency of vinclozolin resistance were evaluated using a geranium leaf-disk assay in which disease incidence and the percentage of conidia resistant to vinclozolin were quantified after each exposure to fungicide. The percentage of conidia resistant to vinclozolin increased from an initial frequency of 0.2 to 100% after two applications of vinclozolin alone, with a concomitant increase in disease incidence. The incidence of disease when chlorothalonil and cupric hydroxide were applied alone was approximately 30 and 50%, respectively, and the frequency of vinclozolin resistance remained low. Half-strength mixtures of vinclozolin with either chlorothalonil or cupric hydroxide slightly delayed the increase in frequency of resistance. Full-strength mixtures or alternations of vinclozolin with either chlorothalonil or cupric hydroxide failed to delay the increase in the percentage of conidia resistant to vinclozolin. Disease control under these regimes was characteristic of that obtained with chlorothalonil or cupric hydroxide alone, making the inclusion of vinclozolin superfluous. In the alternating regimes, the level of resistance to vinclozolin did not decrease when chlorothalonil or cupric hydroxide were applied, suggesting that the populations resistant to vinclozolin were stable under the conditions of this study. The stability of the resistant strain in the absence of vinclozolin may partially be due to the similarity in sporulation and germination between the resistant and the sensitive strains.

Populations of *Botrytis cinerea* Pers.:Fr. resistant to benzimidazole and dicarboximide fungicides developed rapidly after the commercial introduction of these chemicals. Resistance to the dicarboximides iprodione, procymidone, and vinclozolin have been reported in European vineyards (13), strawberry fields (9), and vegetable greenhouses (7,10,32), as well as in vineyards and greenhouses in Canada (24). Cross-resistance among dicarboximide fungicides is prevalent in *B. cinerea* (13). Additionally, dicarboximide-resistant strains can be resistant to benzimidazoles (6), which complicates disease management. Most strains of *B. cinerea* obtained from the field or greenhouse have relatively low-level dicarboximide resistance (EC_{50} for mycelial growth of 1–10 μg/ml) compared with resistant strains selected in vitro (EC_{50} > 100 μg/ml) (13,23). Fitness of sensitive and low-level resistant strains have been found to be similar, allowing low-level dicarboximide-resistant subpopulations to persist in the absence of fungicide selection pressure (1).

Low-level dicarboximide-resistant strains are prevalent in many field populations of *B. cinerea*, yet control failures attributable to the predominance of resistant strains have occurred most frequently in greenhouses where crops are grown in an environment favorable for infection by *B. cinerea* and in which fungicides are frequently applied (11,25). In vineyards, the number of dicarboximide applications has been positively correlated with an increase in the frequency of resistant strains and decreased efficacy of dicarboximides (1,13). The detection of dicarboximide-resistant strains in Pennsylvania greenhouses where growers have not used dicarboximides (G. W. Moorman, *unpublished*) suggests that resistant strains may be distributed on plants shipped from propagators using dicarboximides to other growers and eventually to retailers. Although no greenhouse growers in Pennsylvania have reported control failures, disease problems attributable to the buildup of resistance seem imminent, given the history of dicarboximide failures in countries where these fungicides have been in use for many more years.

According to Staub and Sozzi (30), the development of resistance can be delayed by reducing the number of sprays of dicarboximides or by combining or alternating dicarboximides and chemicals with different modes of action. Dekker (4) postulated that alternations, rather than mixtures, should be effective in cases where resistant strains are less fit. Thus, fitness of the resistant and sensitive strains is an important consideration when evaluating the effect of fungicide sprays on population dynamics. For some pathosystems, theoretical models have been developed that predict that mixtures of fungicides from different chemical classes would reduce the selection pressure (14,28). Management of dicarboximide-resistant *B. cinerea* has been investigated in numerous countries on different crops under different fungicide regimes with varying results (9,12,16).

According to a survey of growers in Pennsylvania (G. W. Moorman, *unpublished*), weekly or biweekly applications of benomyl or chlorothalonil are the mainstays of most *B. cinerea* control programs, even though benomyl resistance is widespread (20). A leaf-disk assay has shown that vinclozolin provides excellent control of benomyl-resistant strains, and chlorothalonil is effective against strains that have double resistance to benzimidazoles and dicarboximides (21). Because *B. cinerea* is not known to develop resistance to the ethylenebisdithiocarbamate fungicides (EBDCs), these compounds have been used in controlling *B. cinerea*. However, pending registration changes may limit the use of the EBDCs, leaving chlorothalonil, cupric hydroxide, and dicloran as alternatives to benzimidazoles and dicarboximides. Dicloran did not provide adequate control in leaf-disk assays (21), and cross-resistance to this fungicide has been reported in dicarboximide-resistant strains (10,25). Cupric hydroxide leaves a bluish residue on foliage, has a short residual time, and is not registered on many important ornamentals.

The studies reported here were undertaken to determine the efficacy of mixtures and alternations of vinclozolin with nonsystemic fungicides in controlling *B. cinerea* and to determine the influence of these strategies on the frequency of a sensitive and a resistant strain in an experimentally mixed population. An assay was used in which experimental populations of resistant and sensitive conidia were cycled on leaf disks from fungicide-treated plants. This allowed evaluation of eight fungicide regimes in

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a relatively short time and avoided cross-contamination between treatments, a common problem in greenhouse studies (29). Several fitness parameters of the resistant and the sensitive strain were evaluated, as well as competition between these strains in vivo. These are discussed in relation to competitiveness and population dynamics.

MATERIALS AND METHODS

Strains. Geranium tissue infected with *B. cinerea*, collected from a commercial geranium propagator in Pennsylvania, was incubated in plastic bags with damp paper towels at 21 C, with an 18-hr photoperiod to promote sporulation. Conidia were aseptically removed with a sterile needle and placed on half-strength potato-dextrose agar (PDA) (19.5 g of Difco PDA and 7.5 g of agar per liter of deionized water). Strains used in cycling experiments were selected based on EC_{50} of vinclozolin, similarity in virulence on geranium, and germination or lack of germination on PDA amended with vinclozolin at 20 μg a.i./ml (VPDA). The strain of *B. cinerea* sensitive to vinclozolin (BVS) was unable to grow on geranium leaf disks sprayed with the manufacturer-recommended rate of 0.6 g (a.i.) of vinclozolin per liter and had an EC_{50} for mycelial growth on amended PDA of 0.084 μg of vinclozolin per milliliter. The vinclozolin-resistant strain (BVR) colonized and sporulated on vinclozolin-treated leaf disks and had an EC_{50} of 1.18 μg of vinclozolin per milliliter in vitro (31). Conidia of BVR germinated in the presence of 20 μg of vinclozolin per milliliter of PDA, whereas germination of BVS conidia was completely inhibited at this concentration.

Plant material. Geraniums (*Pelargonium* × *hortorum* L. H. Bailey 'Red Elite') were grown from seed in 8-cm plastic pots filled with Metro-Mix 350 (W. R. Grace Co., Cambridge, MA). Plants were grown at 21 ± 5 C in a walk-in growth chamber. A 16-hr photoperiod was maintained with 40W Grolux and cool-white fluorescent lights. Average irradiance was 0.0045 W/m^2, as determined with a Radiometer 550 (EG & G Inc., Salem, MA). Plants were subirrigated when the potting medium became dry and fertilized every 3 wk with 200 μg/ml nitrogen (15:16:17 N-P-K) soluble fertilizer (Peters Fertilizer Products, W. R. Grace Co., Fogelsville, PA). No pesticides were applied. All experiments were conducted with 12-wk-old plants.

Fungicides. The following formulated fungicides were used: vinclozolin (Ornalin 50WP), cupric hydroxide (Kocide 101, 77WP), and chlorothalonil (Daconil 2787, 4.17F). Vinclozolin was diluted in sterile distilled water before addition to cooled (50 C) molten PDA. Fungicides were applied to plants until runoff with a CO_2-powered sprayer (89–104 kg/cm^2) fitted with a flat-fan nozzle. The full rates applied were as follows: vinclozolin, 0.6 g a.i./L; cupric hydroxide, 0.92 g a.i./L; chlorothalonil, 1.01 ml a.i./L; full-rate mixtures of vinclozolin and cupric hydroxide; or full-rate vinclozolin and chlorothalonil. Half-rate mixtures included vinclozolin and cupric hydroxide, 0.3 g a.i./L + 0.46 g a.i./L, respectively, and vinclozolin and chlorothalonil, 0.3 g a.i./L + 0.5 ml a.i./L, respectively. Regimes of vinclozolin, cupric hydroxide, or chlorothalonil consisted of sprays every 10 days (one cycle) for 40 days. These regimes were each tested five times. Regimes of full- and half-rate mixtures were sprayed in the same manner (i.e., every 10 days) and tested twice each. Alternating regimes consisted of vinclozolin sprayed at days 1 and 30 (i.e., cycle 1 and 3) and the nonsystemics (cupric hydroxide and chlorothalonil) sprayed at days 20 and 40 (cycle 2 and 4). Alternating regimes were evaluated four times.

Population dynamics and evaluation of fungicide regimes. The cycling technique (19) is summarized in Figure 1. An experimental population of 1:500 resistant/sensitive conidia (0.2% resistant) from 5- to 7-day-old half-strength PDA cultures was mixed in sterile 0.1 M dextrose in deionized water and used to initiate all cycling experiments. Whole geranium plants were sprayed until runoff with fungicides or water (control). The next day, the fifth leaf counting from the apical meristem was removed. Disks were cut from the periphery of the leaf with a 10-mm-diameter cork borer and placed onto moist filter paper in petri dishes (10 leaf disks per dish, five dishes per treatment). Each leaf disk was inoculated with 25 μl, containing approximately 100 conidia of the experimental population. Dishes were sealed with Parafilm and incubated at 21 C under a 16-hr photoperiod. After 10 days, the number of disks showing water-soaked, dark lesions or sporulation of *B. cinerea* was determined. Conidia were collected from leaf disks by vacuum removal as follows. A 1-cm^2 piece of 1-μm-mesh nylon filter fabric (Spectrum Medical Industries, Inc., Los Angeles, CA) was held in place between tubing connected to a vacuum pump and a truncated dis-

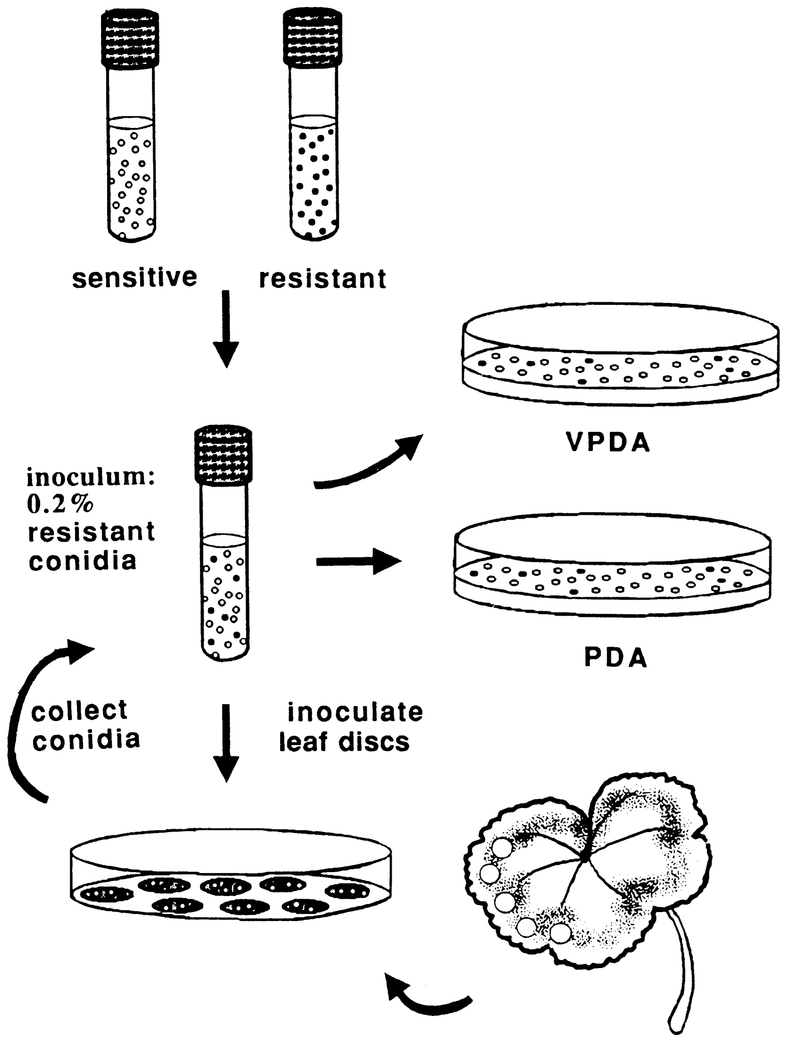

Fig. 1. Technique for cycling a mixed population of vinclozolin-resistant and vinclozolin-sensitive conidia of *Botrytis cinerea* on geranium leaf disks. The resistant and sensitive subpopulations are partitioned on PDA and PDA + vinclozolin at 20 μg/ml (VPDA).

posable pipet tip. The filter fabric with the conidia and the pipet tip were inserted in a test tube containing sterile 0.1 M dextrose and triturated. To determine the number of resistant conidia, aliquots were plated onto PDA and VPDA. Germinated conidia on unamended agar were counted after 1 day, and those on VPDA were counted after 3 days. Conidia were scored as germinated if the germ tube length exceeded the width of the conidium. These conidial suspensions also were used to inoculate new leaf disks from sprayed plants, thus completing one cycle and initiating the subsequent cycle. Mixed populations of BVS and BVR for each fungicide treatment were cycled in this manner for four consecutive 10-day periods.

Fitness parameters. Parameters evaluated included sclerotia production, sporulation, virulence, and germination. Experiments were conducted in a randomized block design with sampling in which replicates were treated as blocks. Sclerotia production was determined from five center-inoculated half-strength PDA dishes. After 30 days of incubation, sclerotia were harvested by homogenizing the contents of each petri dish in 500 ml of water in a blender for 10 sec, decanting the agar, and collecting the sclerotia after they settled. Sclerotia were air-dried and weighed. Disease data for the area under disease progress curves (AUDPC) and time for 50% of leaf disks to display symptoms (T_{50}) were obtained by monitoring daily, for 10 days, the number of diseased leaf disks inoculated with either BVS or BVR (10 disks per dish, five dishes). AUDPCs were calculated according to Berger (2) using the average of the five dishes. Sporulation (conidia per square centimeter) was determined by vacuum removal of conidia from the leaf disks after 10 days, as described above, and counting with a hemacytometer. Average percent germination of conidia was determined from total counts of germlings for each of five PDA dishes onto which 0.5 ml of sterile distilled water containing 200 conidia per milliliter had been plated. Evaluations of all fitness parameters described above were independently replicated three to six times. Data for each fitness parameter were subjected to the GLM procedure of SAS (27).

RESULTS

Population dynamics. In the experimental population of BVR and BVS, the percentage of BVR did not increase significantly above the initial 0.2% resistant level when carried for four cycles on leaf disks from plants sprayed with water (Fig. 2A). The BVR portion of the population remained between 0.2 and 1.8%. The percentage of leaf disks infected with *B. cinerea* from water-sprayed plants ranged from 80 to 100% (Fig. 2A). On leaf disks sprayed with vinclozolin, BVR increased from 0.2 to 100% after two cycles, with a concomitant increase in the percentage of diseased leaf disks (Fig. 2B). After two cycles, disease incidence was comparable to leaf disks from water-sprayed plants (Fig. 2B).

On leaf disks sprayed with cupric hydroxide, the level of BVR in the experimental population remained less than 5% (Fig. 2C). Protection by this chemical was poor, with approximately 50% of the leaf disks infected throughout each cycling experiment (Fig. 2C). Chlorothalonil was more effective than cupric hydroxide in controlling disease caused by *B. cinerea*. The level of disease ranged between 20 and 40% (Fig. 2D), whereas the level of BVR in a mixed population remained less than 0.8% throughout each experiment. (Fig. 2D).

On leaf disks sprayed with a full-strength mixture of vinclozolin and cupric hydroxide, 100% of the conidia were resistant after two cycles (Fig. 3A). The increase of BVR when this mixture was used was similar to results obtained when vinclozolin was used alone. Disease incidence under this regime showed a trend similar to that when cupric hydroxide was used exclusively, except that after the first spray, disease incidence was approximately 10% (Fig. 3A). When the half-rate mixture of vinclozolin and cupric hydroxide was used, disease incidence was higher than when the full-rate mixture of these chemicals was used, but the development of resistance was slightly delayed (Fig. 3B). After the first cycle, the level of BVR was approximately 45%, compared to 90% when full-strength mixtures were used (Fig. 3A and B). Full-strength mixtures of vinclozolin and chlorothalonil prevented sporulation on leaf disks in both experiments, and the conidial populations were insufficient for cycling. One experiment with half-rate mixtures of these chemicals was terminated after two cycles because no sporulation

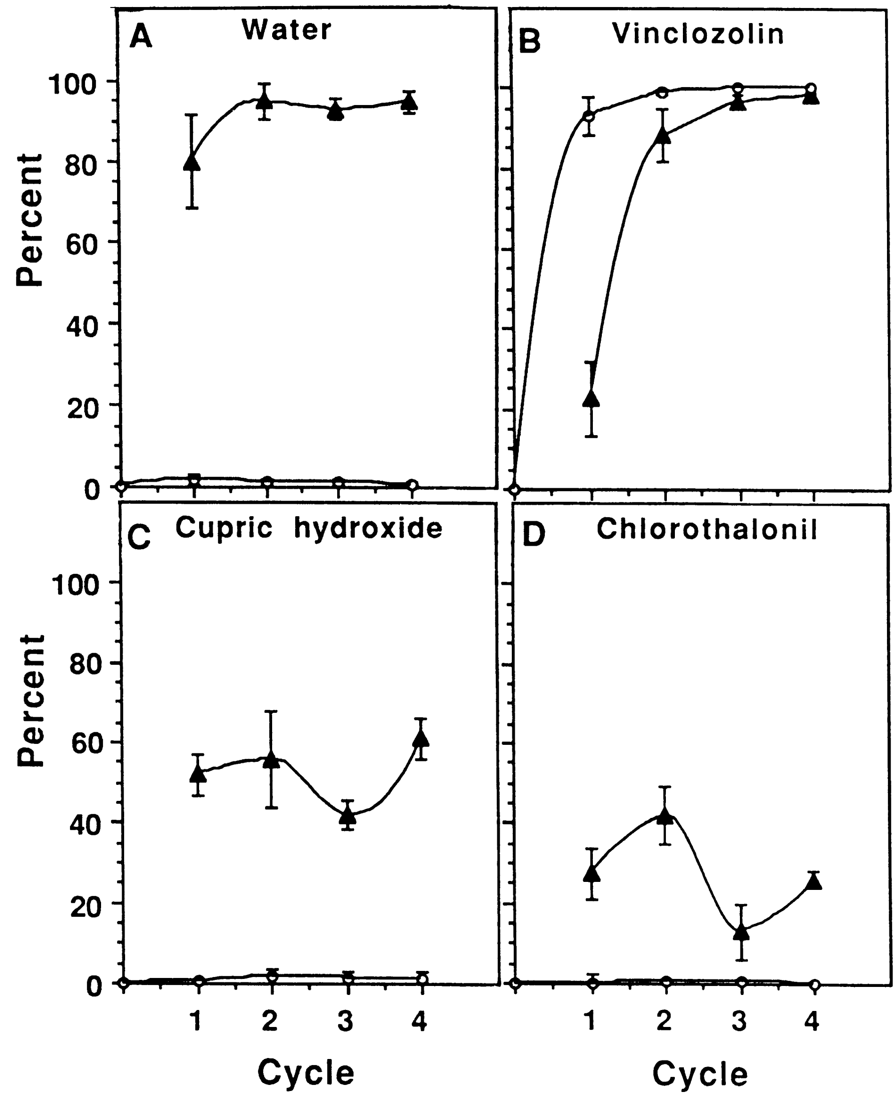

Fig 2. Changes in the proportion of vinclozolin-resistant conidia of *Botrytis cinerea* (BVR) (○) and percent infected leaf disks (▲) during four cycles on leaf disks from geranium plants sprayed every cycle with **(A)** water, **(B)** vinclozolin, **(C)** cupric hydroxide, and **(D)** chlorothalonil. Averages from five experiments are shown.

Table 1. Comparison of fitness parameters[t] for strains of *Botrytis cinerea* resistant (BVR) and sensitive (BVS) to vinclozolin

Strain	Sclerotia[u] (mg/dish)	Sporulation[v] (× 10^5 conidia/cm^2)	T_{50}[w] (days)	AUDPC[x]	Percent germinated conidia[y]
BVS	74 a ± 5.4[z]	68.3 a ± 6.7	6.2 a ± 0.44	322 a	60.7 a ± 3.4
BVR	62 b ± 4.6	87.5 a ± 14.9	8.0 b ± 0.58	229 b	62.8 a ± 3.9

[t] Means ± standard error.
[u] Dry weight of sclerotia harvested from half-strength potato-dextrose agar dishes 30 days after inoculation.
[v] Conidial counts obtained from geranium leaf disks 10 days after inoculation.
[w] Time in days for 50% of leaf disks to show symptoms.
[x] Area under the disease progress curve values.
[y] Conidia assessed for germination after 24 hr of incubation on half-strength PDA using ×12.5 magnification.
[z] Numbers within columns followed by same letter are not different according to *t* test ($P \leq 0.05$).

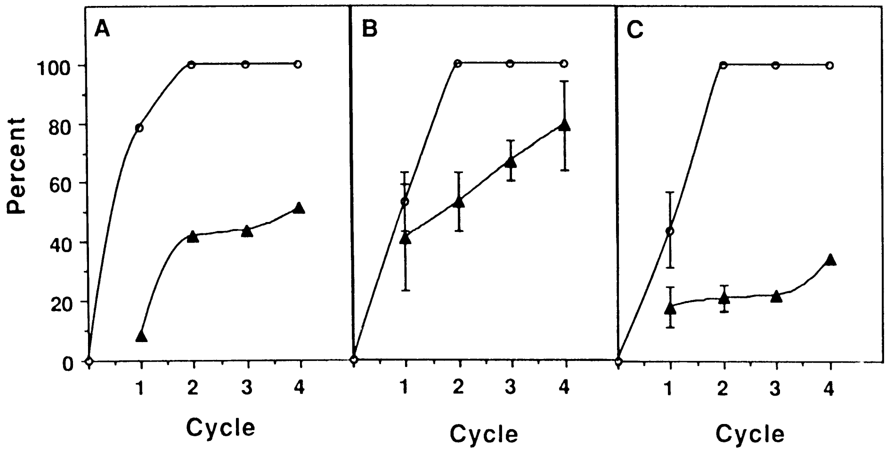

Fig. 3. Changes in the proportion of vinclozolin-resistant conidia of *Botrytis cinerea* (BVR) (○) and percent infected leaf disks (▲) during four cycles on leaf disks from geranium plants sprayed with mixtures of fungicides. **(A)** Full-rate cupric hydroxide (0.92 g a.i./L) + vinclozolin (0.6 g a.i./L); **(B)** half-rate mixture cupric hydroxide (0.46 g a.i./L) + vinclozolin (0.3 g a.i./L); and **(C)** half-rate mixture chlorothalonil (0.5 g a.i./L) + vinclozolin (0.3 g a.i./L). Averages from two experiments are shown.

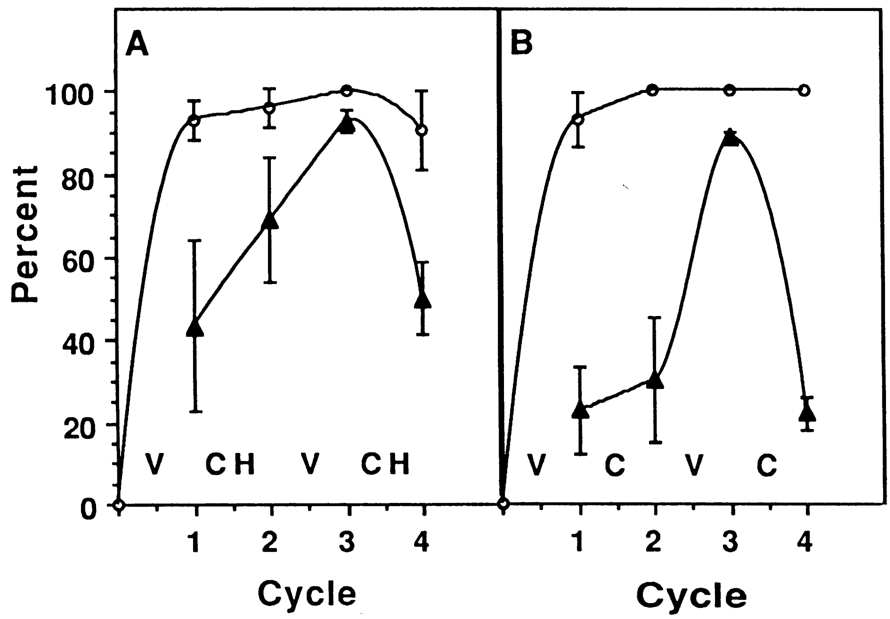

Fig. 4. Changes in the proportion of vinclozolin-resistant conidia of *Botrytis cinerea* (BVR) (○) and percent infected leaf disks (▲) during four cycles on leaf disks from geranium plants sprayed with alternations of fungicides. **(A)** Vinclozolin (V) sprayed during cycles 1 and 3 and cupric hydroxide (CH) sprayed during cycles 2 and 4; or **(B)** chlorothalonil (C) sprayed during cycles 2 and 4. Averages from four experiments are shown.

occurred. In the second experiment with a half-rate mixture, the percentages of infected leaf disks ranged to levels similar to those on leaf disks from plants sprayed with chlorothalonil at the full rate (Figs. 2D and 3C). The level of resistance was slightly less after the first cycle, but 100% BVR was detected after the second cycle (Fig. 3C).

Alternations of vinclozolin with either cupric hydroxide or chlorothalonil did not slow the increase in the vinclozolin-resistant component (Fig. 4A and B). Disease control was provided by the first vinclozolin spray, but BVR neared 100% after the second cycle. After the first vinclozolin spray, the percentage of infected leaf disks was lower when the alternate chemicals were used in the second and forth cycles than when vinclozolin was applied (Figs. 2B and 4A and B). Disease incidence when chlorothalonil and cupric hydroxide were used was about 30 and 60%, respectively.

Sporulation was highly variable within any given treatment over the replicated experiments (*data not shown*). Sporulation on leaf disks sprayed with vinclozolin was comparable to that on disks sprayed with water. Sporulation on disks treated with chlorothalonil was consistently low. Full-strength mixtures of vinclozolin and chlorothalonil prevented sporulation. In the alternating regimes, sporulation was lower during the second and forth cycles when the partner chemical was used.

Comparison of fitness parameters. Sporulation and germination of conidia did not differ between BVS and BVR (Table 1). However, virulence, described by AUDPC and T_{50}, differed significantly. Disease progress curves (*data not shown*) for inoculated leaf disks showed that the lag period after inoculation was 1–2 days longer for BVR, resulting in significantly lower AUDPC value at day 10 and T_{50} (Table 1). Sclerotia production also differed significantly. In addition, BVR retained resistance to vinclozolin after 30 weekly transfers on unamended half-strength PDA.

DISCUSSION

Strains of *B. cinerea* with low-level resistance to dicarboximide fungicides (EC_{50} = approximately 1.2 μg/ml vinclozolin) have been detected in Pennsylvania greenhouses where ornamentals and bedding plants are grown (20). Spraying dicarboximides at the recommended rates has given protection against these low-level resistant strains. Similar results have been reported in some protected crops (8) but not others (25). Because field populations with high proportions of resistant strains often can be correlated with frequent dicarboximide use (23), the goal of management practices has been to reduce use of these fungicides by using fewer sprays per season or by mixing or alternating fungicides with different modes of action. In strawberry fields and vineyards, timing dicarboximide sprays with flowering and fruiting periods has provided disease control with fewer sprays (22). In greenhouse production however, protection is required throughout the season, and timing sprays with phenology is not as feasible.

Experimental populations initially composed of 0.2% BVR stabilized between 0.2 and 2% during cycling on water-sprayed leaf disks for four cycles. The fluctuations in percent BVR may be due to experimental error, because the detection limit of the leaf-disk assay was approached at such low percent BVR. The stability of this population in the absence of fungicide selection pressure suggests that the resistant and sensitive strains used were of comparable fitness in vivo. This is in agreement with greenhouse studies by Katan (11), although some field studies indicated a gradual decline of the resistant portion of the population in the absence of dicarboximide selection pressure (9,16,26).

Of the two strains used, BVR appeared to exhibit diminished fitness for two of the five parameters evaluated. Sclerotia production in vitro and virulence on leaf disks were significantly greater for BVS than for BVR. Of the fitness parameters monitored, AUDPC and sporulation probably had the most influence on the population dynamics in the experimental system used. Likely, other fitness parameters are involved in population dynamics in vivo. Other studies with low-level resistant strains, such as BVR, have shown fitness similar to sensitive strains (1,24). Although BVS had a higher AUDPC value than BVR, the rate (slope) of disease development appeared similar for BVS and BVR; a 1- to 2-day lag for BVR was responsible for the lower AUDPC value at day 10. Published results concerning fitness are conflicting, in part because *B. cinerea* is genetically heterogeneous and because fitness differences also can be attributable to factors independent of fungicide resistance.

Resistance to vinclozolin in BVR appeared to be stable in vitro, based on results of 30 weekly transfers on half-strength PDA (*data not shown*). Numerous authors also have shown that resistance is stable after repeated transfers on fungicide-free media (3,10,32). Because the experimental populations appeared stable in the absence of fungicide in the cycling experiments, any changes in the ratio of resistant to sensitive would, theoretically, be due to selection pressure imposed by the fungicide treatment used, not differences in fitness.

Neither chlorothalonil nor cupric hydroxide selected for dicarboximide resistance. The slightly higher percent resistant conidia when cupric hydroxide was used may be due to the difficulty of counting colonies on VPDA, as contamination by *Penicillium* or *Trichoderma* spp. was a problem when plants had been sprayed with cupric hydroxide. The high variability in percent diseased leaf disks when cupric hydroxide was applied may have been due to growth of contaminants on the leaf disks, which affected assessment. However, poor disease control with cupric hydroxide may be partially attributable to the relatively short residual efficacy of this fungicide (21). The incidence of disease was lower when chlorothalonil was used. The reduction in disease incidence during the third cycle, and the increase during the fourth, when both chlorothalonil and cupric hydroxide were applied, was attributed primarily to experimental error. Considering the level of disease control and the low risk for the development of fungicide resistance, chlorothalonil appears to be an effective alternative to vinclozolin.

The rapid shift to almost 100% resistant conidia after two cycles with vinclozolin in the current study is similar to the results of Löcher et al (16), who reported rapid shifts to 95–100% resistance after one spray of vinclozolin in vineyards. Katan and Ovadia (12) reported shifts to 100% resistant populations after four sprays in greenhouses. The number of vinclozolin sprays required for 100% resistance is a function of the initial population size and frequency of resistant strains, as shown by models for the development of fungicide resistance in plant pathogen populations (18).

Although disease control was improved when full-strength mixtures of vinclozolin and cupric hydroxide were applied, this strategy did not delay the increase in the frequency of resistance. Mixtures of vinclozolin or procymidone with other nonsystemics such as captan or thiram have yielded similar results (5,24). Because resistance approaches 100% so rapidly, full-rate mixtures seem to be of little practical use because efficacy obtained is attributable solely to the partner fungicide. Half-rate mixtures of vinclozolin and cupric hydroxide were only slightly effective in delaying resistance buildup after the first spray, and disease control was little improved over that obtained with vinclozolin alone. Cupric hydroxide alone provided similar disease control, making the addition of vinclozolin superfluous. Northover and Matteoni (24) and Hunter et al (9) tested half-rate mixtures of a dicarboximide with companion fungicides and reported no delay in resistance increase. Vinclozolin in a half-rate mixture with chlorothalonil was more effective than mixtures with cupric hydroxide in controlling disease incidence and delaying resistance increase, however the delay was apparent for only one cycle. This finding is in agreement with Löcher et al (16), who reported that mixtures of vinclozolin and chlorothalonil failed to delay resistance increase, although disease control was improved.

Results from greenhouse studies on tomato and strawberry, where procymidone and chlorothalonil were sprayed, have shown that this mixture failed to control *B. cinerea* or delay resistance buildup (6). Because mixtures failed to significantly delay resistance increase in the present work, vinclozolin in mixture with chlorothalonil does not seem to be a feasible regime if resistance is present in the population. Although full-rate mixtures of vinclozolin and chlorothalonil were effective enough to prevent sporulation in our experiments, the situation in a greenhouse would be different. Because spray coverage is never complete, the likelihood of escapes is high. This, coupled with the very rapid reproduction rate of *B. cinerea*, makes the risk of using this regime high. Three-component, reduced-rate mixtures of vinclozolin, chlorothalonil, and an EBDC fungicide, such as mancozeb, may be effective in improving control, as has been shown for *B. squamosa* J. C. Walker on onion (17). Whether a three-component mixture significantly delays resistance increase remains to be tested.

Dekker (4) suggested that alternations of dissimilar fungicides would be more effective than mixtures in delaying resistance if the resistant strains were less fit. In this study, it is not clear if BVR was less fit than BVS. However, alternating regimes were not effective in delaying resistance. Resistance neared 100% after one cycle with vinclozolin and remained high during the alternate cycles with chlorothalonil or cupric hydroxide. Possibly, the comparable fitness of BVR and BVS allowed BVR to maintain itself in the absence of dicarboximide selection pressure. On greenhouse-grown cucumbers, Katan and Ovadia (12) found that alternations of chlorothalonil and iprodione slightly delayed resistance increase, whereas several sprays of chlorothalonil between iprodione sprays were most effective. Although a delay was achieved, neither of these regimes resulted in a decrease in percent resistance after the application of iprodione. Alternations of captan with mixtures of captan and dicarboximide or several captan sprays between dicarboximide sprays resulted in reduced frequency of iprodione-resistant isolates on grape (23). This regime could easily be tested using the leaf-disk assay described here. On greenhouse-grown tomatoes, Löcher (15) showed that using a nonsystemic (dichlofluanid, chlorothalonil, or folpet) early in the season and application of dicarboximides later in the season improved disease control. Although control is improved, this regime carries great risk since large numbers of conidia would be exposed to the dicarboximide, making the selection of resistant strains more probable.

Diethofencarb, a compound with negative cross-resistance to benzimidazoles, controls dicarboximide-resistant strains that are also benzimidazole resistant. Gullino et al (5) reported a decrease in percent resistance when procymidone was alternated with a mixture of benzimidazole and diethofencarb. However, diethofencarb is not registered for use in the United States, and *Botrytis* has been reported to develop double resistance to this fungicide and dicarboximides (5). Regardless of the regime, alternations can only be effective when the resistant subpopulation is less fit than the sensitive or when the resistant strains are more sensitive to the alternate fungicide.

Because the leaf-disk assay provided information consistent with field and greenhouse studies, it offers a relatively rapid evaluation of the relative resistance selectivity among various fungicide regimes. Many regimes can be assessed in a limited space. Cross-contamination between plots has been a problem in several studies (12,23) but can be avoided with this technique. However, important factors such as infection pressure, environmental conditions, plot size, and overlapping generations cannot be taken into consideration with this technique. Selection pressure due to fungicide coverage and exposure also may be higher in this technique than in a greenhouse. Mixed experimental populations of conidia from several randomly sampled resistant and sensitive strains, as opposed to a mixture of one resistant and one sensitive strain as used in this study, may comprise a more realistic population. However, the resistant and sensitive strains used in these studies, compared to more than 25 isolates in our collection, are representative based on evaluation of fitness parameters and sensitivities to fungicides. In the field and greenhouse, it is not known how many different strains occur in a limited, fungicide-treated area. Future studies should include cycling experiments with mixed populations of more than one sensitive and one resistant strain and evaluation under greenhouse situations.

None of the regimes that included vinclozolin delayed the development of resistance to dicarboximides significantly. Although resistant strains of *B. cinerea* have been isolated from many greenhouses in Pennsylvania, control failures have not been reported. This may be a result of either a lack of careful evaluation by growers of fungicide efficacy in commercial greenhouses or the use of reduced humidity to assist in controlling *B. cinerea*. Dicarboximide rates commercially used may provide control of low-level resistant strains. However, the threat of increased incidence of resistance and concomitant control failures or selection of resistant strains with improved fitness cannot be ignored. Based on the present study, chlorothalonil appears to be a suitable alternative to benzimidazoles and dicarboximides.

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