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TITLE: DEVELOPMENT AND CONTROL OF BENOMYL-TOLERANT BOTRYTIS CINEREA STRAINS ON SNAP BEANS (PHASEOLUS VULGARIS)

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Benomyl-tolerant Botrytis cinerea was found in snap bean fields throughout the Willamette Valley while no tolerant isolates of Whetzelinia sclerotiorum were detected. Thirty-five benomyl-tolerant isolates of B. cinerea had slower radial growth rates than eighteen benomyl-sensitive isolates. Sporulation of an aggressive tolerant isolate was not stimulated when grown on benomyl-treated leaves. The sporulation of benomyl-tolerant strains used to inoculate field plots was less than sensitive isolates. There was a decline in the number of benomyl-tolerant B. cinerea lesions in plots not sprayed with benomyl. Development of a population with a high percentage of benomyl-tolerant B. cinerea occurred within four weeks in benomyl sprayed field plots regardless of the initial level of benomyl-tolerant individuals.

The combination of captan, dichloran, and chlorothalonil with benomyl did not retard the development of a benomyl-

tolerant B. cinerea population. A benomyl-chlorothalonil combination, captan alone, dichloran alone, or chlorothalonil alone controlled gray mold. The incidence of gray mold was greater in plots sprayed only with benomyl than unsprayed plots. White mold was controlled by benomyl alone or in combination with captan, dichloran, or chlorothalonil while captan, dichloran, and chlorothalonil applied alone were not as effective. Benomyl-chlorothalonil combinations offer the most effective control of both diseases.

Thiophanate methyl applied alone controlled white mold but not gray mold. Thiophanate methyl in combination with captan, dichloran, and chlorothalonil controlled gray mold. In a test of experimental fungicides, BAS35204F (structure confidential) provided the best control of white mold on snap beans. Botran applied to the base of bean plants was superior to topical applications applied at the same time and comparable to the best fungicide treatments.

Development and Control of Benomyl-Tolerant *Botrytis cinerea* Strains on Snap Beans (*Phaseolus vulgaris*)

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INTRODUCTION

The development of fungicide tolerance in certain plant pathogens has reduced the efficacy of several fungicides (2, 13, 17, 18, 20). The use of combinations of fungicides having different modes of action has been recommended to delay the appearance of tolerant strains of plant pathogens (4, 7, 8, 10, 18). Once tolerant strains have appeared, discontinuing the use of the fungicide to which tolerance developed has been suggested (18). In the absence of a suitable replacement or where the fungicide continues to provide good control of another disease of the same crop, Dekker (5) suggested that the use of combinations might also be of value in reducing the increase and spread of resistant strains.

In the Willamette Valley of western Oregon, gray mold (*Botrytis cinerea* Pers.) and white mold (*Whetzelinia sclerotiorum* (Lib.) Dork and Dumont = *Sclerotinia sclerotiorum* (Lib.) de Bary) are perennial problems on snap bean. Since 1970, benomyl has provided control of both diseases; however, in 1975, several benomyl-tolerant *B. cinerea* isolates were detected (27). The use of benomyl has continued because of its effectiveness against white mold. This study was initiated to determine (i) the extent of benomyl tolerance in *B. cinerea* populations in Willamette Valley snap bean fields, (ii) the efficacy of various fungicides and fungicide combi-

nations for control of white mold and gray mold, and (iii) the effect of fungicides alone or in combination with benomyl on the increase of benomyl tolerance in the B. cinerea population.

METHODS AND MATERIALS

Field Survey. In 1976 randomly selected gray mold-infected tissue was collected one to seven days before harvest from 37 snap bean fields located throughout the Willamette Valley of Western Oregon. Individual sporulating samples were sealed in plastic bags and stored at 2°C until tested for benomyl tolerance.

Benomyl-tolerance assay. A sterilized inoculating loop containing a drop of sterile distilled water was used to transfer conidia from a B. cinerea lesion to the center of petri dishes containing either benomyl-amended or nonamended potato dextrose agar (PDA). Benomyl-amended PDA (BPDA) contained 10 ppm benomyl (Benlate 50W) for 1976 tests and 5 ppm for 1977 tests. Both media contained 100 ppm streptomycin sulfate. Benomyl and streptomycin were added after autoclaving.

After six to twelve hours incubation at room temperature, conidia were examined for germination with a compound microscope at 100x. Germ tubes of benomyl-tolerant isolates elongated normally. Germ tubes of benomyl-sensitive isolates were stunted and distorted. All plates were observed daily for at least four days. Benomyl-tolerant conidia continued to grow and the resultant colonies sporulated on both PDA and BPDA. Benomyl-sensitive conidia continued to grow and the resultant colonies sporulated only on PDA. Sporulation verified colonies were B. cinerea.

Field plot design. To accomplish objectives (ii) and (iii), high density snap bean plantings were established on May 26, 1976 and May 18, 1977 at the Oregon State University Horticulture Farm. Snap Beans (Phaseolus vulgaris 'Early Gallatin') were seeded in 12-inch rows at 200 lb of seed/A. After the beans had reached the first trifoliate leaf stage, one-half inch of overhead irrigation water was applied twice weekly. High density planting and frequent irrigations created a microclimate conducive to gray mold and white mold.

Treatments were arranged in a randomized complete block design with four replicates. In 1976, each replicate consisted of seven by fifteen foot plots surrounded by a nine foot border of untreated beans. In 1977, replicates contained 12 x 12 foot plots with 12 foot borders.

Fungicide application. Four fungicides were used in this study: benomyl (Benlate 50 W, E. I. du Pont de Nemours and Co., Wilmington, Delaware), captan (Orthocide 50 W, Chevron Chemical Company, San Francisco, California), chlorothalonil (Bravo 6F, Diamond Shamrock Company, Painesville, Ohio), and dichloran (Botran 75 W, Upjohn Company, Kalamazoo, Michigan). All these fungicides are currently registered for use on snap beans and have known activity against B. cinerea.

A small tractor-drawn plot sprayer with a seven foot boom calibrated to deliver 100 gal/A at 50 psi was used to apply fungicides. Fungicides were applied when beans reached

25% bloom and at one and two week intervals thereafter. Application rates were 1.0, 1.5, 2.25 and 1.2 lb ai/A (1.6 pt 6F/A) for benomyl, captan, dichloran, and chlorothalonil, respectively.

Inoculation of plots. Snap beans had previously been grown in plot areas and ample amounts of W. sclerotiorum were present.

Using the tractor-drawn sprayer, plots were inoculated with a conidial suspension of three benomyl-tolerant and three benomyl-sensitive isolates of B. cinerea. Isolates were grown on PDA for 10 days and conidia were collected by flooding cultures with a 1% Tween 80 solution and sieving the resultant spore suspension through three layers of cheese cloth. Concentration of conidia in each suspension was determined with a haemocytometer and appropriate volumes of each suspension were added to 19 liters of water to produce conidial suspensions containing 50% benomyl-tolerant, 50% benomyl-sensitive (50:50) or 5% benomyl-tolerant, 95% benomyl-sensitive (5:95) mixtures of B. cinerea conidia. Spore concentration in the suspension was 1.5×10^4/ml. In 1976, all plots were inoculated with the 50:50 tolerant to sensitive (T/S) conidia mixture in the evening after the first application of fungicides. In 1977 inoculations were made two days after the first fungicide application. One set of plots was inoculated with the 50:50 (T/S) mixture and the other set with a 5:95 (T/S) mixture. The 5:95 (T/S)

conidial mixture was included to test whether the development of a benomyl-tolerant B. cinerea population depends on the initial number of tolerant individuals present in field plots. Plots received 234 liters of inoculum per hectare (100 gal/A) applied with the boom sprayer. Following incubation, plots received .08 of an inch of water every evening until harvest.

Spray plot sampling procedure. Plots were harvested approximately four weeks after the first fungicide application. Fifty randomly selected plants were pulled from each replicate and examined for the presence of gray mold and white mold. Five to twenty sporulating B. cinerea lesions per replicate were also selected, sealed in plastic bags, and stored at 2°C until tested for benomyl tolerance.

Sporulation of the B. cinerea isolates used in the field study. Sporulation was assessed on detached bean leaf segments. An agar disk containing one of the six isolates was attached with masking tape to the lower surface of each leaf segment. Each isolate was tested on 20 leaf segments. Inoculated leaves were placed on a plastic tray lined with moistened paper towels. The tray was enclosed in a plastic bag and incubated at 20°C with a 12 hour photoperiod (light provided by G.E. warm white flourescent tubes). Seven days after inocultation the leaf segments with sporulating B. cinerea lesions were placed in a test tube. Five milliliters of a 1% Tween 80 solution was added and spore concentration was determined using a haemocytometer.

RESULTS

Field survey. In 29 of 32 bean fields where benomyl had been used, benomyl-tolerant *B. cinerea* isolates were recovered. In 23 of these fields, over 50% of the isolates were tolerant (Table 1). In many of these fields, gray mold was extremely severe in spite of benomyl application. In contrast, only two of thirty-two isolates (6%) obtained from unsprayed fields were benomyl-tolerant. None of the 61 isolates of *W. sclerotiorum*, also collected during this survey, were tolerant to benomyl.

1976 Field plot. In uninoculated border areas 20% of the bean plants were infected with *B. cinerea* versus 52% in the inoculated control, indicating that inoculation of the plots with *B. cinerea* was successful.

White mold incidence was reduced by application of benomyl alone or in combination with captan, dichloran, or chlorothalonil (Table 2). Two applications of benomyl afforded better control than any other treatment. Incidence of white mold in plots treated with captan, dichloran, or chlorothalonil alone did not differ significantly from the control. A captan + benomyl combination had significantly more white mold than benomyl applied alone suggesting captan may reduce the efficacy of benomyl on white mold.

In contrast, benomyl alone or in combination with dichloran or captan did not reduce the incidence of gray mold. In fact, plots treated with two applications of beno-

TABLE 1. SURVEY OF BENOMYL-TOLERANT *B. CINEREA* ON SNAP BEANS GROWN IN THE WILLAMETTE VALLEY OF OREGON

	No. of fields surveyed	No. of isolates collected	% of isolates[1] tolerant to benomyl
Fields sprayed with benomyl:			
Albany	1	10	80
Brooks	4	28	82
Dever-Conner	3	35	71
Greenberry	2	16	12
Harrisburg	2	11	36
Junction City	1	3	33
Mc Dowell Cr.	1	10	70
Monroe	3	10	20
Salem	4	28	89
Scio	1	6	67
Stayton	6	41	49
Woodburn	4	30	83
Total	32	228	64
Fields not sprayed with benomyl:			
Junction City	3	22	4
Harrisburg	1	5	0
Stayton	1	5	20
Total	5	32	6

[1]Isolates tested at 10 ppm benomyl.

TABLE 2. EFFECT OF FUNGICIDES ON WHITE MOLD AND GRAY MOLD INCIDENCE ON SNAP BEANS IN 1976 AND THE INCIDENCE OF BENOMYL TOLERANCE IN THE *B. CINEREA* POPULATION[1]

Fungicides and time of application:			% Plants infected		% of *B. cinerea* population
25% Bloom	1 Week later	2 Weeks later	White mold	Gray mold	tolerant to benomyl
Benomyl			28 b[2]	62 de	100 e
Benomyl	Benomyl		8 a	74 e	100 e
Benomyl + chlorothalonil			40 bc	26 ab	94 e
Benomyl + dichloran			40 bc	47 cd	98 e
Benomyl + captan			46 c	50 cd	95 e
Benomyl + chlorothalonil	Chlorothalonil	Chlorothalonil	47 bc	16 a	100 e
Benomyl + dichloran	Dichloran	Dichloran	24 b	33 bc	100 e
Benomyl + captan	Captan	Captan	40 bc	38 bc	100 e
Chlorothalonil	Chlorothalonil	Chlorothalonil	89 d	26 ab	34 cd
Dichloran	Dichloran	Dichloran	90 d	24 ab	15 ab
Captan	Captan	Captan	95 d	25 ab	3 a
Inoculated control	(no fungicide)		90 d	52 cd	26 bc

[1]All treatments, including the control, inoculated with a 50:50 (benomyl-tolerant/benomyl-sensitive conidial suspension.

[2]Mean of four 50-plant replicates; means followed by the same letter are not significantly different according to Duncan's Multiple Range Test (P=0.05) based on arc-sin transformed data.

myl had more gray mold than the inoculated control. Captan, dichloran and chlorothalonil applied alone and benomyl-chlorothalonil combinations resulted in a significant reduction of gray mold. A benomyl-chlorothalonil application plus two additional chlorothalonil applications was the most effective treatment for gray mold.

Application of benomyl to field plots, alone or in combination, resulted in the development of nearly 100% tolerance to benomyl in the B. cinerea population. The frequency of benomyl-tolerant isolates in inoculated (50:50 T/S mixture) plots not sprayed with benomyl declined. Why captan reduced the number of benomyl-tolerant isolates below that of the control (Table 2) cannot be explained and may be due to experimental error. Two of the three benomyl-tolerant isolates used in the 1976 and 1977 experiment produced significantly fewer spores than two of the three benomyl-sensitive isolates (significance determined using Duncan's Multiple Range Test).

1977 Field plot. A record drought during the summer of 1977 in western Oregon reduced the incidence of both diseases. However, border areas had approximately 10% of the plants infected with gray mold versus 20% in the inoculated control, again indicating a successful inoculation. In spite of the lower disease incidence, many of the trends in the 1976 data were repeated. Benomyl alone and combined with chlorothalonil or captan controlled white mold while chlorothalonil and

and captan applied alone did not.

None of the fungicide treatments significantly reduced gray mold incidence below that of the control (Table 3). However, plots sprayed with chlorothalonil or a benomyl-chlorothalonil combination had significantly less gray mold than plots sprayed with only benomyl or a benomyl-captan combination. Benomyl alone and benomyl plus captan tended to increase gray mold incidence as compared to the inoculated control.

There was no significant difference in the final incidence of gray mold (or white mold) in plots inoculated with a 50:50 or 5:95 (T/S) mixture of B. cinerea. Frequency of benomyl-tolerant isolates in the B. cinerea population increased in all treatments receiving applications of benomyl. In the absence of benomyl sprays, the number of tolerant isolates declined in plots initially sprayed with a 50:50 (T/S) conidia mixture. In plots inoculated with a 5:95 (T/S) spore mixture, benomyl-tolerant isolates of B. cinerea appeared to increase. However, 33% of the B. cinerea collected from border areas assayed as benomyl-tolerant: a tolerant population sufficiently large to mask any decline in benomyl-tolerant individuals in field plots.

TABLE 3. EFFECT OF FUNGICIDES ON WHITE MOLD AND GRAY MOLD INCIDENCE ON SNAP BEANS IN 1977 AND LEVEL OF BENOMYL TOLERANCE IN THE B. CINEREA POPULATION

Fungicides and time of application:		% Plants infected						% of B. cinerea population tolerant to benomyl	
		White mold			Gray mold				
25% Bloom	1 Week later	5:95[1]	50:50	Avg	5:95	50:50	Avg	5:95	50:50
Benomyl	Benomyl	9	0	4 a[2]	33	30	32 b	100 b[3]	100 b
Benomyl + chlorothalonil	Benomyl + chlorothalonil	2	6	4 a	11	16	14 a	97 b	97 a
Benomyl + captan	Benomyl + captan	8	3	6 a	32	38	34 b	100 b	100 b
Chlorothalonil	Chlorothalonil	50	56	53 b	16	9	12 a	12 a	37 a
Captan	Captan	62	62	62 b	22	16	19 ab	17 a	36 a
Inoculated control (no fungicide)		52	42	47 b	23	16	20 ab	17 a	46 a

[1]Plots inoculated with either a 5:95 (T/S) or a 50:50 (T/S) conidial suspension.

[2]Means of four 50-plant replicates; means followed by the same letter are not significantly different according to Duncan's Multiple Range Test (P=0.05) based on arc-sin transformed data. Data analyzed using a three-factor analysis of variance. No significant interaction was detected between fungicide control in 5:95 (T/S) and 50:50 (T/S) inoculated plots.

[3]Data analyzed using two-factor analysis of variance.

DISCUSSION

Factors affecting the increase of fungicide resistance of fungal pathogens include: the fitness of tolerant strains to survive or reproduce, the level of genetic selection pressure exerted by the fungicide, and the life cycle of the particular fungus being considered (5). The reduced fitness of benomyl-tolerant B. cinerea was indicated by the reduced numbers of benomyl-tolerant isolates recovered from field plots not treated with benomyl. Lack of fitness is supproted by the sporulation study where two of the three benomyl-tolerant isolates produced fewer conidia than two of the three benomyl-sensitive isolates. In two additional tests, 18 benomyl-sensitive isolates collected from the Oregon State University Horticulture Farm had radial growth rates on PDA that were significantly greater than that of 35 benomyl-tolerant isolates collected near Stayton, Oregon (Johnson, M. S. Thesis). Thus, it is unlikely that the increase of benomyl-tolerant individuals in the B. cinerea population is due to greater fitness of tolerant strains. Bollen and Scholten (1) also found that a benomyl-tolerant strain of B. cinerea on cyclamen was less aggressive than sensitive strains.

The selection pressure exerted on B. cinerea by benomyl was extremely high. After four weeks, all bean plants sprayed with benomyl produced almost 100% benomyl-tolerant isolates. Other researchers have also found that after

continued use of benomyl, the B. cinerea population consisted mainly of benomyl-tolerant individuals (9, 14, 15, 19, 24). Dekker (9) states that pathogens which sporulate abundantly on aerial parts of a crop may spread rapidly after the elimination of the sensitive fungal population by the fungicide. B. cinerea sporulates abundantly on infected tissue and can complete several infection cycles in one season. One application of benomyl at 25% bloom has been the recommended control for both white mold and gray mold since 1970. Thus the continual use of benomyl for mold control and the abundant sporulation of B. cinerea have resulted in the selection and development of a benomyl-tolerant B. cinerea population in Willamette Valley snap bean fields.

W. sclerotiorum has only one infection cycle per season; primary infections arise from ascospores and no secondary spores are produced on infected tissue. This limits the potential for current season increase of benomyl-tolerant strains and would delay the development of a tolerant population. Before the development of tolerance in the B. cinerea population, a single application of benomyl controlled both gray mold and white mold (26).

The majority of fungi for which fungicide tolerance has been reported are heavily sporulating fungi with multiple disease cycles (4, 18). The capacity to produce large quantities of spores several times each season is apparently

important in the development of a fungicide-tolerant fungal population. Dekker and Ogawa *et al.* (5, 18) have suggested that once tolerance to a fungicide develops, use of combinations containing the fungicide may suppress an increase in tolerant strains. Littrell (16) reported the use of chlorothalonil-benomyl combinations reduced the benomyl-tolerant population of *Cercospora arachidicola*; however, none of the combinations used in our studies prevented the increase of tolerant strains in the *B. cinerea* population (Tables 2 and 3). Thus, it appears that at least for *B. cinerea* on snap beans, combinations of conventional fungicides with benomyl do not delay an increase in the number of tolerant isolates already present in the field.

There was some concern that the T/S ratio in the inoculum might influence the final incidence of gray mold. However, after four weeks, there was no significant difference in gray mold incidence in plots inoculated with either a 50:50 or 5:95 (T/S) mixture of conidia (Table 3). Apparently *B. cinerea* reproduces at a rate that enables benomyl-tolerant isolates to dominate the population in four weeks no matter whether the initial level of tolerant individuals was 5% or 50% of the *B. cinerea* population. Thus benomyl used alone or in combination with captan, dichloran, or chlorothalonil had no effect on the level of tolerance in the *B. cinerea* population four weeks after application of these fungicides.

In 1977, captan, chlorothalonil, and the inoculated control plots exhibited an apparent increase in the benomyl-tolerant B. cinerea population over the 5% benomyl-tolerant population of conidia used to inoculate the plots. However, 33% of the B. cinerea population in border areas was benomyl-tolerant and could probably account for the increase.

There was a statistically significant increase of gray mold in all plots sprayed with two applications of benomyl. Although not statistically significant in the 1977 experiment, this increased disease incidence was still apparent. In 1976 a set of thiophanate methyl treated plots showed the same trends (Johnson, M. S. Thesis).

Laboratory studies failed to demonstrate increased pathogenicity or direct stimulation of tolerant strains of B. cinerea by sub-lethal concentrations of benomyl (Johnson, M. S. Thesis). An increase in disease incidence of B. cinerea observed on cyclamen was attributed to elimination of antagonistic fungi (1, 14). Perhaps the gray mold increase in our study can be explained by competition between gray mold and white mold for the same infection court, i.e. senescing petals. Thus, effective control of white mold by benomyl may leave more bean tissue available for B. cinerea colonization, sporulation, and infection.

In western Oregon, benomyl-chlorothalonil combinations seem to provide the best solution to controlling both gray mold and white mold in snap beans. However, chlorothalonil

is not registered for gray mold control. Use of benomyl alone for white mold control aggrevates the gray mold problem. Use of the other combinations tested would provide little control of gray mold and might even reduce the level of white mold control (Table 2). Until effective combinations are registered, snap bean growers should be advised to discontinue benomyl use and to apply two conventional fungicides, such as captan and ziram, for control of gray mold and white mold, respectively.

BIBLIOGRAPHY

1. BOLLEN, G. J. and G. SCHOLTEN. 1971. Acquired resistance to benomyl in a strain of B. cinerea in cyclamen. Neth. J. Plant Pathol. 77:83-90.

2. BOLTEN, A. T. 1976. Fungicide resistance in Botrytis cinerea, the result of selective pressure on resistant strains already present in nature. Can. J. Plant Sci. 56:861-864.

3. CHO, J. J. 1977. Shoot and flower blight of Leucospermum cordifolium incited by a benomyl-tolerant strain of Botrytis cinerea. Phytopathology 67:124-127.

4. DEKKER, J. 1976. Acquired resistance to fungicides. Ann. Rev. Phytopathol. 14:405-423.

5. DEKKER, J. 1976. Prospects for the use of systemic fungicides in view of the resistance problem. Proc. Amer. Phytopathol. Soc. 3:60-66.

6. ERWIN, D. C. 1973. Systemic fungicides, disease, control, translocation and mode of action. Ann. Rev. Phytopathol. 11:389-422.

7. EVANS, E. 1971. Problems and progress in the use of systemic fungicides. Proc. 6th Brit. Insectic. Fungic. Conf. 3:758-763.

8. EVANS, E. 1971. Systemic fungicides in practice. Pestic. Sci. 2:192-196.

9. FLETCHER, J. T. and S. M. SCHOLEFIELD. 1976. Benomyl-tolerance in isolates of Botrytis cinerea from tomato plants. Ann. Appl. Biol. 82:529-536.

10. FLETCHER, J. T. 1975. Tolerance of fungal pathogens to systemic fungicides in relation to disease control. Proc. 8th Brit. Insectic. Fungic. Conf. 3:751-762.

11. GEESON, J. D. 1976. Comparative studies of methyl-benzimidazole-2-ylcarbamate-tolerant and sensitive isolates of Botrytis cinerea and other fungi. Trans. Brit. Mycol. Soc. 66:123-129.

12. HARTMANN, H. T. and D. E. KESTER. 1975. Plant Propagation Principles and Practices. Prentice-Hall, Inc., New Jersey. 30 p.

13. JARVIS, W. R. and K. SLINGSBY. 1975. Tolerance of B. cinerea and rose powdery mildew to benomyl. Can. Plant Dis. Surv. 55:44.

14. JORDAN, V. W. L. and D. V. RICHMOND. 1974. Diseases of Strawberries. Long Ashton Res. Sta. Rep. p. 125-126.

15. JORDAN, V. W. L. and D. V. RICHMOND. 1975. Perrenation and control of benomyl-insensitive Botrytis affecting strawberries. Proc. 8th Brit. Insectic. Fungic. Conf. 3:758-763.

16. LITTRELL, R. H. 1974. Tolerance of Cercospora arachidicola to benomyl and related fungicides. Phytopathology 64:377-378.

17. MILLER, M. W. and J. T. FLETCHER. 1974. Tolerance in B. cinerea isolates from glasshouse crops. Trans. Brit. Mycol. Soc. 62:99-103.

18. OGAWA, J. M., B. T. MANJI and A. H. BEHADI. 1975. Tolerance of plant pathogens to fungicides and bactericides. Fungicide and Nematicide Tests 31:3-8.

19. OGAWA, J. M., B. T. MANJI and G. A. CHASTAGNER. 1976. Field problems due to chemical tolerance to plant pathogens. Proc. Amer. Phytopathol. Soc. 3:47-52.

20. POLACH, F. J. and W. T. MOLIN. 1975. Benzimidazole-resistant mutant derived from a single ascospore culture of Botryotinia fuckeliana. Phytopathology 65:902-904.

21. RICHMOND, D. V., R. J. PRING and H. TARR. 1973. Resistance to benomyl. Rep. Long Ashton Res. Sta., p. 126.

22. SHAY, J. R. 1975. Seedling stands of root crops as influenced by chloropicrin applications and status of benlate for white mold control in beans. 89th Proc. Ore. Hort. Soc. 66:43-47.

23. SIJPESTEIJN, A. KAARS. 1972. Effects on fungal pathogens. Pages 132-155 In R. W. Marsh, ed. Systemic Fungicides. Halsted Press, New York. 135 p.

24. STAUNTON, W. P. and T. KAVANAGH. 1975. Resistance of fungal pathogens to benomyl in Ireland and results of alternate spray programmes for disease control. Proc. 8th Brit. Insectic. Fungic. Conf. 1:1-4.

25. VAN DOMMELEN, L. and G. J. BOLLEN. 1973. Antagonism between benomyl-resistant fungi on cyclamen sprayed with benomyl. Acta Bot. Neerl. 22:169-170.

26. VAUGHAN, E. K. and R. D. IRWIN. 1973. Fungicide control of gray and white mold with benomyl Botrytis cinerea and Sclerotinia sclerotiorum on Phaseolus vulgaris. Fungicide and Nematicide Tests 29:53.

27. ZALEWSKI, J. C. 1975. Bush bean mold control. 90th Proc. Ore. Hort. Soc. 67:34-35.

APPENDICES

APPENDIX A

SURVEY FOR BENOMYL-TOLERANT STRAINS OF W. SCLEROTIORUM

The appearance of benomyl-tolerant gray mold in commercial snap bean fields has diminished the value of benomyl as a chemical control. Although benomyl still controls white mold, the possibility of benomyl-tolerant strains in the W. sclerotiorum population cannot be overlooked.

Sclerotia of W. sclerotiorum collected from Willamette Valley snap bean fields in 1976 were tested for benomyl tolerance. One half of each sclerotia was placed on PDA amended with 10 ppm benomyl while the other half was placed on nonamended PDA. Of the 61 isolates tested on benomyl amended PDA, 75% showed no measureable mycelial growth after 12 days incubation at room temperature (20°C). Sclerotia of the remaining 15 isolates (25%) germinated, but were unable to colonize benomyl-amended PDA. Shay (22), in a previous survey, also found no tolerance to benomyl by W. sclerotiorum. The abscence of benomyl-tolerant W. sclerotiorum probably explains the continued effectiveness of benomyl in controlling white mold in the Willamette Valley.

APPENDIX B

GROWTH OF B. CINEREA ISOLATES TAKEN FROM WILLAMETTE VALLEY SNAP BEAN FIELDS

Previous studies on growth and sporulation of benomyl-tolerant versus benomyl-sensitive isolates are contradictory. Bollen and Scholten (1) reported the reduced radial growth of a benomyl-tolerant isolate while other researchers have noted no differences (3, 11, 21). The radial growth of 35 benomyl-tolerant and 18 benomyl-sensitive isolates of B. cinerea were compared in this study.

Benomyl-tolerant isolates were collected near Stayton, Oregon and benomyl-sensitive isolates at the OSU Horticulture Farm. Four-mm diameter agar plugs cut from the edge of expanding colonies were placed on the center of petri plates containing PDA. Isolates were incubated in the dark at room temperature. The diameter of each colony was measured 24 and 48 hours after inoculation. This experiment was repeated twice. Discrepancies in sample size between experiments resulted from contamination losses (Table 1B).

Benomyl-tolerant strains of B. cinerea grew slower than benomyl-sensitive strains. This would suggest the number of benomyl-tolerant individuals in the B. cinerea population will decline in the absence of benomyl applications if there is a relationship between growth rate and sporulation.

TABLE 1B. THE GROWTH RATE OF 53 B. CINEREA ISOLATES COLLECTED FROM WILLAMETTE VALLEY SNAP BEAN FIELDS

Isolates	Experiment 1		Experiment 2	
	No. of Isolates	Mycelial growth rate (cm/day)	No. of Isolates	Mycelial growth rate (cm/day)
Benomyl-sensitive	14	2.8	18	3
Benomyl-tolerant	35	2.3	31	2.7
Significance of T test[1]		**		*

[1]At P=0.05, * = significant; at P=0.01, ** = significant.

APPENDIX C

CHARACTERIZATION OF THE ISOLATES USED IN FUNGICIDE SPRAY TRIALS

Reduced competitive ability may result if benomyl-tolerant isolates have slower growth rates. In addition, the ability to sporulate should be assessed when comparing the aggressiveness of isolates.

Three benomyl-tolerant and three benomyl-sensitive isolates were tested to determine growth rate and sporulation *in vitro* and under greenhouse conditions. Inoculum consisted of six-mm plugs cut from the edge of the cultures incubated in the dark at 20°C for three days.

The laboratory experiment was arranged in a completely randomized design. A one factor analysis of variance was used to test differences in sporulation and growth of the isolates. Eight petri plates of each isolate were grown on PDA and maintained in a growth chamber at 22°C with a 12 hour photoperiod (G.E. warm white flourescent lights provided 2.4 x 10^3 lux).

Cultures were observed daily to detect the onset of sporulation. Radial growth was measured on the second day of the experiment and the amount of sporulation was assessed after seven days. To determine the amount of sporulation of each isolate, the entire contents of each culture dish was transferred to a large test tube containing a 1% Tween 80 solution. After the final volume of the solution was ad-

justed to 50 ml, the concentration of conidia was ascertained using a haemocytometer.

The greenhouse experiment was arranged in a completely randomized design. Differences in treatments were tested using a one factor analysis of variance. Early Gallatin snap beans were planted in 20 pots containing UC soil mix (12), three plants per pot. Each pot represented a replication of the experiment. Agar plugs containing one of the six isolates were attached with masking tape to the lower surface of leaves arising from the third node of each plant. After inoculation, plants received two and one half seconds of mist every five minutes. Greenhouse temperatures ranged from 35.5^{o}C during the day to 22^{o}C at night.

Lesion diameters were measured on the second and third days to determine the growth rate (cm/day). On the fourth day, lesions were cut from the leaves, washed with distilled water, and placed on a moistened tray. The tray was enclosed in a plastic bag and incubated in the growth chamber at 22^{o}C with a 12 hour photoperiod. Three days later sporulating lesions were placed in test tubes with five ml of 1% Tween 80 to remove conidia for counting with a haemocytometer.

In the laboratory study of the six isolates no differences were detected in radial growth (Table 1C). Sporulation began on the second day for all isolates except T45 and T47. Two of the three tolerant isolates produced significantly

TABLE 1C. GROWTH CHARACTERISTICS OF SENSITIVE (S) AND TOLERANT (T) *B. CINEREA* ISOLATES ON PDA

Isolate	Diameter of colony after 3 days (cm)	No. of days until sporulation	Conidia/ml ($1x10^6$) after 7 days
S11	5.5 a[1]	2 b	2.22 c
S16	5.4 a	2 b	2.35 c
S18	5.6 a	2 b	2.31 c
T33	5.6 a	2 b	1.94 bc
T45	5.6 a	2.9 c	1.22 ab
T47	5.0 a	1.6 a	1.82 b

[1]Means average of eight observations. Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test (P=0.05).

fewer conidia than all three benomyl-sensitive isolates. In the greenhouse study isolate S16 had a significantly greater radial growth than the other five isolates (Table 2C). Two of the three tolerant isolates produced significantly fewer conidia than two of the three benomyl-sensitive isolates.

The composition of two populations competing for the same ecological niche is in part a function of the ability of each population to reproduce. The results of this study indicate benomyl-tolerant isolates produce fewer conidia (Tables 1C and 2C). Thus, in plots not sprayed with benomyl, the benomyl-tolerant population may not produce as many conidia, resulting in fewer chances for infection. This may explain the observed decline in frequency of benomyl-tolerant isolates in field plots not receiving benomyl. Growth and generation time are also important factors in determining the fitness of isolates or populations. However, neither of these factors varied sufficiently between tolerant and sensitive isolates to explain the decline in tolerance noted in the field experiments.

Because the amount of conidia produced in seven days may also be a function of the amount of mycelium produced in this time interval, this study did not measure the inherent genetic potential of each isolate to produce different yields of conidia. Different environmental conditions could also have changed the conidia production of isolates.

TABLE 2C. GROWTH CHARACTERISTICS OF TOLERANT (T) AND SENSITIVE (S) *B. CINEREA* ISOLATES ON BEAN LEAVES

Isolate	Radial growth Rate (cm/day)	Conidia Produced/Lesion ($1x10^6$) after 7 days
S11	.3 ab	1.33 c
S16	.4 b	.95 c
S18	.2 a	1.20 bc
T33	.3 ab	.79 b
T45	.2 a	.28 a
T47	.2 a	.82 bc

[1]All means average of 20 observations. Means followed by the same letter are not significantly different (P=0.05) according to Duncan's Multiple Range Test.

Since all isolates were grown at the same temperature and photoperiod, each isolate may not have been growing in its optimum environment.

APPENDIX D

EXPLANATIONS FOR INCREASED GRAY MOLD IN BENOMYL-TREATED SNAP BEAN PLOTS

Gray mold incidence increased following applications of benomyl to field plots of snap beans. To determine if increase in gray mold resulted from benomyl stimulating growth and sporulation of B. cinerea, the following laboratory study was conducted.

Leaf samples were collected from a fungicide spray trial at the OSU Horticulture Farm three days after an application of benomyl. Sixty Early Gallatin snap bean leaves were collected from plots sprayed with benomyl, from control plots in the field, and from border areas. Leaves were taken to the laboratory and each leaf was trimmed to a 3 cm^2 segment. Six-mm diameter agar plugs of tolerant isolate T47 were attached with masking tape to the lower surface of 45 leaf segments. Plugs of the sensitive isolate (S18) were attached to the lower surface of 15 leaf segments. The leaf segments were then incubated in the growth chamber at 22^0C and a 12 hour photoperiod. Growth rate and the amount of sporulation on leaf tissue were determined as described previously. Only the growth rate of isolate S18 was determined. Differences in growth and sporulation were tested using a one factor analysis of variance.

The sensitive isolate had a significantly reduced growth rate on benomyl-treated leaves (Table 1D). Thus a

TABLE 1D. EFFECTS OF BENOMYL ON GROWTH AND SPORULATION OF TOLERANT AND SENSITIVE ISOLATES OF B. CINEREA

Source of test leaves[3]	Tolerant isolate (T47)[1]		Sensitive isolate (S18)[2]
	Growth rate (cm/day)[4]	Conidia/ml ($1x10^5$) after 4 days	Growth rate (cm/day)
Benomyl plots	1 a[5]	1.44 a	.2 a
Border areas	.9 a	1.79 b	.9 b
Control plots	1 a	1.88 b	.9 b

[1]Means of 45 observations.

[2]Means of 15 observations.

[3]Substrate was 3 cm^2 bean leaf segments.

[4]Growth rate calculated for the 24 hour interval between days two and three.

[5]Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test (P=0.05).

sufficient quantity of benomyl was present in leaves picked from benomyl field plots to inhibit growth.

The growth rate of the tolerant isolate did not differ on benomyl-treated and non-treated leaves. Sporulation was significantly reduced on treated leaves. Thus, the increased incidence of gray mold in field plots cannot be explained by benomyl directly stimulating the benomyl-tolerant isolate.

Four other explanations are possible for the increased incidence of gray mold in field plots: benomyl-tolerant strains are more pathogenic, host resistance is reduced with an application of benomyl, organisms antagonistic to B. cinerea are eliminated by benomyl, and B. cinerea competes with other organisms (particularly W. sclerotiorum) for the same infection court. Greenhouse studies demonstrated isolate T47 was less pathogenic than sensitive isolates. Reduced host resistance is unlikely since isolates on benomyl-treated leaves did not have increased growth or sporulation. Any organism antagonistic to B. cinerea that would be eliminated by applications of benomyl would probably be absent on benomyl-treated leaves used in this experiment. Since no increase in growth or sporulation of B. cinerea was detected on benomyl-treated leaves, elimination of antagonists is also unlikely.

Competition, like antagonism, is not substantiated by this experiment. However, this author believes competition between W. sclerotiorum and B. cinerea is the best explanation

for increased gray mold incidence in benomyl-treated plots. Both fungi usually colonize senescing petals or other senescing flower parts before infecting healthy tissue. Both diseases are prevalent in snap beans grown in the Willamette Valley. Benomyl controls white mold in field plots resulting in more tissue available for colonization by benomyl-tolerant B. cinerea. Thus increased gray mold in field plots treated with benomyl may be due to reduced competition for the infection court.

APPENDIX E

THIOPHANATE METHYL FUNGICIDE FIELD TEST

In a 1976 field experiment, thiophanate methyl was tested alone and in combination with other fungicides for control of gray mold and white mold. Since this systemic fungicide and benomyl have similar chemical activities against fungi (6, 23), both should have the same effect against gray mold and white mold.

Thiophanate methyl (Topsin M 70 WP, Agchem-Decco Division of Pennwalt Corp., Tacoma, Washington) was applied alone or in combination at 1 lb ai/A. All other fungicide rates and experimental procedures are outlined in the benomyl field study.

Thiophanate methyl (TM) controlled white mold (Table 1E). In fact, two applications provided better control of white mold than all other treatments. Dichloran-TM combinations or a captan-TM combination supplemented with two additional applications of captan also provided significant control of white mold. However, both TM-chlorothalonil combinations and the captan-TM combination did not control white mold. No TM combination tested in this experiment was superior to all other TM combinations tested.

This systemic fungicide did not control gray mold. In fact, two applications provided less control than one application. Like benomyl, TM in combination with chlorothalonil

TABLE 1E. EFFECTS OF THIOPHANATE METHYL ALONE OR IN COMBINATION WITH OTHER FUNGICIDES ON WHITE MOLD AND GRAY MOLD CONTROL OF SNAP BEANS IN 1976[1]

Fungicides and time of application:			% Plants infected	
25% Bloom	1 Week later	2 Weeks later	White mold	Gray mold
Thiophanate methyl			56 b[2]	56 de
Thiophanate methyl	Thiophanate methyl		18 a	67 e
Thiophanate methyl + chlorothalonil			78 cde	24 ab
Thiophanate methyl + dichloran			58 bc	31 ab
Thiophanate methyl + captan			84 def	28 ab
Thiophanate methyl + chlorothalonil	Chlorothalonil	Chlorothalonil	78 de	14 a
Thiophanate methyl + dichloran	Dichloran	Dichloran	68 bcd	34 abc
Thiophanate methyl + captan	Captan	Captan	74 bcd	40 bcd
Chlorothalonil			89 ef	26 ab
Dichloran			90 ef	24 ab
Captan			95 f	26 ab
Control-inoculated (no fungicide)			90 ef	52 cd

[1]All treatments, including the control, inoculated with a 50:50 (benomyl-tolerant/benomyl-sensitive) conidial suspension.

[2]Mean of four 50-plant replicates; means followed by the same letter are not significantly different according to Duncan's Multiple Range Test (P=0.05) based on arc-sin transformed data.

supplemented with two additional applications of chlorothalonil provided the best control for B. cinerea. Gray mold was also controlled by applications of dichloran, captan, or chlorothalonil applied alone or in combination with TM. No significant differences in gray mold control were detected between captan, dichloran, or chlorothalonil applied alone or in combination with TM.

Benomyl and thiophanate methyl have similar modes of action against gray mold and white mold. This fungicide experiment demonstrates that thiophanate methyl, like benomyl, controls white mold, but not gray mold. In addition, the use of thiophanate methyl also resulted in selection for benomyl-tolerant strains of B. cinerea in field plots. Two applications of thiophanate methyl were less effective against gray mold than one application. Thiophanate methyl, like benomyl, appears to increase the incidence of B. cinerea in field plots.

APPENDIX F

EFFECTIVENESS OF EXPERIMENTAL CHEMICALS FOR WHITE MOLD CONTROL

Experiments were conducted to test the activity of experimental and recommended fungicides against W. sclerotiorum. Several new fungicides effectively controlled white mold. Results of this test have been submitted to "Fungicide-Nematicide Tests", published by the American Phytopathological Society.

Tests were conducted on the Oregon State University Horticulture Farm. Dyfonate and Treflan were applied pre-plant and Dinitro amine post-plant. Beans were seeded on June 9, 1977 in 12 inch rows at a rate of 200 lb/A. One half inch of irrigation water was provided by overhead sprinkler two times per week throughout the growing season. A ten minute irrigation in the evening was started on July 30 and continued until August 22, 1977. Treatments consisted of seven by twelve foot plots replicated four times in a randomized block design. Foliar applications of the test fungicides were applied July 28 (25% bloom = B) and August 8 (full bloom = FB) in 100 gal water/A at 50 psi using a tractor-drawn boom sprayer equipped with cone nozzles. Percent of plants with white mold was recorded at harvest (August 22) by examining a random sample of 50 plants from each replicate. Moderate

disease incidence and variability between replicates was attributed to hot dry weather during the infection period.

Significant control of white mold was provided by BAS35204F, Benlate, and RP26019. Botran applied at the base of plants or Botran applied in a tank-mix with Benlate also provided a significant control.

TABLE 1F. EFFECTS OF FUNGICIDES ON WHITE MOLD CONTROL OF SNAP BEANS IN 1977.

Treatment, rate (ai/A) and time of applications:	% White mold[1]
BAS35204F 1.0 lb B FB	7.0 a
Botran 75 W 2.25 lb + Benlate 1.0 lb B; Botran 75 W 2.25 lb FB[2]	10.0 a
Benlate 50 W 1.0 lb B	16.0 ab
Benlate 50 W 1.0 lb B FB	21.0 ab
RP26019 1.0 lb (2) B FB	22.0 ab
Botran 75 W 2.25 lb[3] B FB	23.0 ab
Topsin M 70 W 1.0 lb B	28.0 abc
DPX4424 0.25 lb B FB	28.0 abc
DPX4424 0.5 lb B FB	32.0 abc
BAS35204F 0.5 lb B FB	35.0 abc
Topsin M 70 W 1.0 lb B FB	48.0 abc
Bravo 6F 1.2 pt B FB	48.0 abc
Bravo 6F 1.6 pt B FB	56.0 bc
Botran 75 W 2.25 lb[4] B FB	59.0 bc
Check - no fungicide	66.0 c

[1]Mean of four replicates; means followed by the same letter do not differ significantly at .05 level using Duncan's Multiple Range Test.

[2](+) indicates tank-mix; Botran alone was applied 1 week later.

[3]Applied at the base of plants.

[4]Applied over row.