Factors Affecting Control of Blossom Blight in a Peach Orchard with Low Level Benomyl-Resistant *Monilinia fructicola*

R. M. SONODA, Professor, University of Florida, IFAS, Agricultural Research Center, Fort Pierce 33450; J. M. OGAWA, Professor, and B. T. MANJI, Staff Research Associate IV, University of California, Davis 95616; E. SHABI, Division of Plant Pathology, Agricultural Research Organization, Volcani Center, Bet Dagan, Israel; and D. ROUGH, Farm Advisor, San Joaquin County, Cooperative Extension Service, Stockton, CA 95205

ABSTRACT

Sonoda, R. M., Ogawa, J. M., Manji, B. T., Shabi, E., and Rough, D. 1983. Factors affecting control of blossom blight in a peach orchard with low level benomyl-resistant *Monilinia fructicola*. Plant Disease 67:681-684.

Benomyl was applied to peach blossoms in a peach orchard where 36% of the *Monilinia fructicola* on sources of inoculum (mummified fruit) were resistant to benomyl at 0.5–3 μg/ml. Benomyl at 1.1 and 2.2 kg a.i./ha reduced blossom blight to 56 and 43%, respectively, of that on unsprayed trees. Benomyl was highly effective against benomyl-sensitive isolates; 98–99% of isolates from blighted blossoms in benomyl-treated plots were benomyl resistant. Forty-eight percent of blighted blossoms from unsprayed trees harbored only benomyl-sensitive isolates. The difference in blighted blossoms between benomyl-sprayed trees and unsprayed trees could be accounted for by assuming that benomyl controlled blossom blight on the 48% of blighted blossoms attacked by only benomyl-sensitive isolates. Studies showed that there was a mean of almost two distinct *M. fructicola* isolate-types on each blighted blossom. The small number of isolates per blighted blossom, resulting in the probability that only sensitive isolates would attack some blossoms in the peach orchard, helps explain the reduction in blossom blight in the orchard. Results also indicate that benomyl treatments had little effect on *M. fructicola* resistant to benomyl.

Resistance of *Monilinia fructicola* (Wint.) Honey to benomyl has been reported from the United States (1,5) and Australia (2). In California, resistant isolates were resistant to a maximum of 5 μg benomyl per milliliter (5), which is much lower than the benomyl concentration to which isolates from Michigan (400 μg/ml) (1) and Australia (20 μg/ml) (2) are resistant. In California, benomyl appeared effective in controlling *M. fructicola* blossom blight in some peach and nectarine orchards but not in other orchards infested with isolates resistant to 5 μg benomyl per milliliter. Because the level of resistance was low, important questions were what degree of control of resistant isolates were the recommended benomyl sprays providing and how much control of *M. fructicola* could be expected where mixtures of benomyl-resistant and benomyl-sensitive isolates were present. Other important questions were what effect there was on subsequent proportions of benomyl-resistant isolates in the populations when benomyl was applied in an orchard where benomyl-resistant isolates occurred and at what point a fungicide with moderate effectiveness against the disease would be as effective as benomyl. Studies were conducted to address these questions.

Accepted for publication 2 December 1982.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Blossom blight control in peach orchard. The orchard in which the first benomyl-resistant *M. fructicola* in California was found (5) was used for field studies. The orchard was in Lockeford, San Joaquin County, CA, in a low-lying pocket of land adjacent to the Mokelumne River. The orchard consisted of 15-yr-old Loadel peach trees planted on corners of squares measuring 6.1 × 6.1 m. Benomyl (Benlate 50W) was applied by handgun (16.3 L/tree) in suspensions of 298 and 596 mg a.i./L of spray (equivalent to 1.1 and 2.2 kg/ha, respectively) to drip stage when about 5% of the peach blossoms were fully open on 27 February 1980. Captan (Orthocide 50W) was applied at 1,192 mg a.i./L (4.4 kg/ha) in the same manner at the same time and again 6 days later when 75% of the peach blossoms were fully open. One set of trees was left unsprayed. Unsprayed trees bordered each treatment tree (including unsprayed treatment trees) on all four sides. There were six single-tree replicates per treatment in a randomized complete block design.

Inoculum of *M. fructicola* was present in the form of conidia on mummies on treatment and border trees and apothecia on mummies on the orchard floor. Mummies were collected from trees before spraying.

Isolations were made and isolates were grown on PDA amended with 1 μg benomyl per milliliter to determine the percentage of inoculum resistant to benomyl. Six weeks after spraying, the incidence of blighted blossoms per tree was determined by observing 75 blossoms at 1.5–2.5 m and 50 blossoms at 2.5–4.0 m above ground level.

Blossom assay for isolate-types. Thirty-one blighted blossoms with visible evidence of *M. fructicola* sporulation, each blossom at least 30 cm from another blighted blossom, were collected at random from unsprayed trees within the

experimental plot. The blossoms were picked using small plastic bags as gloves to minimize cross-contamination with conidia from other blossoms. The blossoms were kept separate and transported to the laboratory. Within 4 hr after collection, 17 blossoms were suspended in 2 ml streptomycin (500 μg/ml)-neomycin (100 μg/ml) solution in a test tube. The test tube was shaken on a Vortex mixer for 20 sec and the suspension was spread on water agar for single sporing. The other 14 blossoms were dipped in 200 μg/ml NaOCl for 1 min, rinsed twice in sterile distilled water (SDW), and incubated separately in sterilized test tubes containing a piece of sterile moistened cotton for 48 hr to induce fresh sporulation of the fungus. These blossoms were then placed in 2 ml streptomycin-neomycin solution and prepared for single sporing as described before. The conidial concentration in the streptomycin-neomycin solution was usually between 0.5 and 5.0 × 10^4/ml. Twenty germinating conidia from each blighted blossom were transferred onto potato-dextrose agar acidified with lactic acid (LA-PDA).

The presence or absence of barrages (mycelial interactions) (3) between the 20 single-conidial isolates from each blossom, their colony characteristics (6), and their resistance or sensitivity to benomyl were used to differentiate between isolate-types of *M. fructicola*. Four-millimeter disks of 4- to 7-day-old LA-PDA cultures of the 20 isolates from the same blossom were placed 2.5 cm apart in various random combinations on the corners of equilateral triangles on oatmeal agar (OMA) in petri dishes (150 × 15 mm) (4). There were three replicates of each combination. The cultures were incubated for 10–14 days on the laboratory bench and the margins of the colonies were inspected for presence or absence of barrages. Plating on OMA was repeated until all isolates from the same blossom were separated into groups of non-barrage-forming isolates. Non-barrage-forming isolates were checked for differences in cultural characteristics on OMA. The isolates were grown on 1 μg benomyl per milliliter in PDA to determine their reaction to the chemical. Radial growth was recorded 48 hr after transfer and incubation at room temperature (21 C). Isolates from any one blossom were not compared with isolates from another blossom. Non-barrage-forming isolates from the same blossom that had similar cultural characteristics and similar response to benomyl were considered to belong to the same isolate-type.

Blossom assay to monitor benomyl resistance. Ten blighted blossoms with conidia of the fungus sporulating on their surface were collected from each replicate of the benomyl-treated trees and from unsprayed trees. The ten blossoms from each replicate were suspended together in 10 ml SDW. The suspensions were shaken for 20 sec with a Vortex mixer and drops of the resulting conidial suspension spread on water agar for single sporing. Germinated conidia, 20/replicate, were transferred to LA-PDA. In 4–5 days, 4-mm disks of mycelium were transferred to PDA amended with 0.5 and 1 μg benomyl per milliliter. Radial growth on the media was determined 48 hr after transfer and incubation on the laboratory bench (21 C). Isolates were designated sensitive if they were reduced 90% or more in radial growth. Resistant isolates were transferred to 2 and 3 μg benomyl per milliliter in PDA and the growth criteria described previously were used to characterize their response to those levels of benomyl.

Blossom blight control in nectarine orchard. Information obtained from a fungicide spray trial in a nectarine orchard in Parlier, CA, in 1979 is presented to support some of the findings made in the peach orchard in Lockeford. In 1978, the nectarine orchard had a low population of benomyl-resistant *M. fructicola* inoculum (5.8% on mummified fruit), with resistance to up to 5 μg benomyl per milliliter. Benomyl (0.8 kg/ha), captan (4.5 kg/ha), and benomyl (0.8 kg/ha) plus captan (4.5 kg/ha) were applied by airblast (935 L/ha) at late pink bud on 2 March 1979, before the anthers were showing. Captan was reapplied on 7 March 1980, when 75% of the blossoms were fully open. The number of blighted blossoms per tree was counted 1 mo after treatment.

RESULTS

Blossom blight control in peach orchard. Thirty-six percent of the isolates on mummified fruit collected from the peach orchard before spraying were resistant to benomyl. The disease pressure in the orchard was judged moderately heavy because 56.7% of the blossoms on unsprayed trees were blighted. Blossom blight in plots sprayed with benomyl was significantly lower than in unsprayed plots (Table 1).

Number of isolate-types per blighted blossom. There were 2.2 distinguishable isolate-types per blossom on the 17 unsprayed blighted blossoms processed immediately after collecting blighted blossoms from the peach orchard. The 14 surface-sterilized unsprayed blossoms incubated for 48 hr yielded 1.9 distinguishable isolate-types per blossom. Because the data for the two procedures were similar, results from all 31 blossoms are combined in Table 2. The mixed population on individual blossoms was probably not due to contamination from conidia produced on other blossoms because the number of isolate-types per blossom was similar for surface-sterilized and non-surface-sterilized blossoms. Also in most cases, a substantial portion of the conidia on a blighted blossom was made up of each isolate-type.

Estimates of the number of isolate-types per blossom were made using the following relationship:

$$n = \frac{\log (1 - E)}{\log (1 - P)},$$

where n = number of isolate-types per blossom, E = proportion of blighted blossoms with at least one benomyl-resistant (or benomyl-sensitive) isolate-type, and P = proportion of benomyl-resistant (or benomyl-sensitive) isolates in inoculum ($P \neq 0$). (Assumption:

Table 1. Effect of fungicide sprays on incidence of *Monilinia fructicola* blossom blight and effect of benomyl in vitro on single-spore isolates from benomyl-sprayed and unsprayed blighted peach blossoms

Fungicide (kg/ha)	Blossom blight incidence[x] (%)	Percent *M. fructicola* isolates growing (benomyl conc., μg/ml) 0.5	1.0	2.0	3.0
None	56.7 a	43	35	6	0
Benomyl (1.1)[y]	25.0 b	98	78	44	0
Benomyl (2.2)[y]	32.5 b	99	91	25	1
Captan (4.5 + 4.5)[z]	35.5 b	...	...	...	...

[x] Means in a column followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.
[y] Applied when 5% of blossoms were fully open.
[z] Applied twice, when 5% of blossoms were fully open and when 75% of blossoms were fully open.

Table 2. Incidence of benomyl-sensitive and benomyl-resistant isolate-types of *Monilinia fructicola* on 31 separate unsprayed blighted peach blossoms from an orchard at Lockeford, CA

No. blossoms tested[y]	*M. fructicola* isolate-types	Composition[z]
11	1	1S
2	1	1R
1	2	2S
4	2	1S, 1R
3	3	3S
1	3	3R
5	3	2S, 1R
2	3	1S, 2R
1	4	2S, 2R
1	4	1S, 3R

[y] Twenty single-spore isolates from each blossom.
[z] S = lines sensitive to 5 μg benomyl per milliliter PDA; R = lines resistant to 5 μg benomyl per milliliter PDA.

benomyl-resistant isolates are as fit as benomyl-sensitive isolates.)

Using the proportion of benomyl-resistant isolates in the peach orchard (0.36) and the proportion of blighted blossoms with at least one benomyl-resistant isolate (0.52) (Table 2), the estimated number of isolates per blossom (n) was 1.65. Using the proportion of benomyl-sensitive isolates in the peach orchard (0.64) and the proportion of blighted blossoms with at least one benomyl-sensitive isolate (0.91) (Table 2), the estimated number of isolates per blossom (n) was 2.35. An estimate of 1.5 isolate-types per blighted blossom was obtained using Vanderplank's (7) equation for multiple infection of plants: $m = -\log_e (1 - y)$, where m = mean number of infections per blighted blossom and y = proportion of blossoms blighted. In our case, $y = 0.56$.

Proportion of benomyl-resistant isolates on blighted blossoms. Forty-three percent of the isolates obtained from suspensions made with 10 blossoms from each unsprayed replicate tree were sensitive to 0.5 μg benomyl per milliliter. Nearly all *M. fructicola* single-conidial isolates from benomyl-treated trees were resistant to 0.5 μg benomyl per milliliter. On 3 μg benomyl per milliliter, most of these resistant isolates were reduced 90% in radial growth. After seven days of incubation, no growth occurred on 0.5 μg benomyl per milliliter for isolates considered sensitive to 0.5 μg benomyl per milliliter; however, most of those considered resistant at 2 μg/ml but sensitive at 3 μg/ml had grown out on 3 μg benomyl per milliliter.

Blossom blight control in nectarine orchard. Benomyl significantly reduced blossom blight in the nectarine orchard with a low proportion of benomyl-resistant *M. fructicola* (Table 3). The combination of benomyl plus captan provided similar control. Most of the *M. fructicola* isolates from blighted blossoms from these two treatments were resistant to benomyl. Captan alone applied at pink bud and full bloom (75% of blossoms fully open) failed to provide disease control (Table 3).

Table 3. Effect of benomyl sprays on control of blossom blight and proportion of benomyl-resistant *Monilinia fructicola* isolates on blighted blossoms in a nectarine orchard in Parlier, CA

Treatment[w]	Time of application[x]	Rate (kg a.i./ha)	Mean blossoms blighted	Percent isolates resistant to 1 μg benomyl/ml PDA[y]
None	...	...	26.2 a[z]	20
Benomyl	Pink bud	0.8	2.7 b	87
Captan	Pink bud and full bloom	4.5	20.2 a	9
Benomyl + captan	Pink bud	0.8 4.5	3.0 b	86

[w] Trade formulations were Benlate 50W (benomyl) and Orthocide 50W (captan). Sprayed with airblast (935 L/ha) to drip stage during the 1979 season.
[x] Pink bud is before anthers are showing and full bloom is when about 75% of blossoms are open.
[y] Frequency of resistant *M. fructicola* strains in the orchard during the 1978 season was 5.8% on the mummies collected throughout the orchard.
[z] Means of 10 replicates of two trees per plot. Means in a column followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

DISCUSSION

Benomyl sprays provided a significant reduction of *M. fructicola* blossom blight in the peach orchard with 36% of inocula resistant to benomyl. The reduction in incidence of blossom blight was 56 and 43% (calculated from data in Table 1) for 1.1 and 2.2 kg a.i. benomyl per hectare, respectively, compared with unsprayed plots. The following factors contributed to the reduction: 1) benomyl sprays were highly effective against benomyl-sensitive isolates as shown by assay of blighted blossoms obtained from benomyl-treated plots (Table 3), and 2) in unsprayed plots, 48% of the blighted blossoms were infected by only benomyl-sensitive isolates (Table 3). The difference between benomyl-treated plots and unsprayed plots can be attributed to control by benomyl of blossom blight on the 48% of blossoms that were attacked by only benomyl-sensitive isolates.

The fact that there was not a greater reduction in blossom blight in plots sprayed with benomyl indicates that the benomyl sprays were not effective in controlling blossom blight caused by benomyl-resistant *M. fructicola* isolates. A reduction in blossom blight greater than 48% would be expected especially for the higher rate of 2.2 kg a.i. benomyl per hectare, which reduced blossom blight by 43%.

Thirty-six percent of the inocula were benomyl resistant. Thirty-five percent of the isolate-types obtained by assaying individual blighted blossoms were benomyl resistant (Table 2). Forty-three percent of the isolates from the six suspensions made with 10 blighted blossoms from each unsprayed plot were benomyl resistant. These results indicate that benomyl-resistant isolates are as competitive as benomyl-sensitive isolates as incitants of blossom blight on peach.

The fact that there was a small number (1.9 and 2.2) of isolate-types per blighted blossom as determined by in vitro methods, ie, barrage formation, cultural characteristics, and differences in sensitivity to benomyl, was supported by the estimates based on the percentage of unsprayed blighted blossoms affected by at least one benomyl-resistant or at least one benomyl-sensitive isolate and by the estimate using Vanderplank's equation for multiple infection of plants. Hypothetically, if less than 10% of the *M. fructicola* inoculum in an orchard is resistant to benomyl and if conditions are such that fewer than two isolate-types infect each unsprayed blossom, plots sprayed with benomyl would have about 80% fewer blighted blossoms than unsprayed plots. Although no assay to determine the number of isolate-types per blossom was made in the nectarine orchard, the effectiveness of benomyl in reducing blossom blight in that orchard indicates that a small number of isolates infected each blossom.

In the peach orchard, benomyl sprays were as effective as captan. In the nectarine orchard, captan was ineffective. If there were only a choice between the two compounds, if the disease pressure were expected to be high, and if the proportion of *M. fructicola* resistant to benomyl in the orchard were low, the more effective treatment would be benomyl. The recent registration of alternative compounds for blossom blight control in peach and nectarine orchards, however, removes the need for making a choice between these two compounds.

Use of benomyl for blossom blight control in an orchard with benomyl-resistant isolates increased the proportion of benomyl-resistant *M. fructicola* on blighted blossoms. Whether this would result in the total inoculum in the orchard being shifted as drastically as that on the blossoms could not be determined in the peach orchard because unsprayed border trees supported benomyl-sensitive isolates. Further work is needed to determine whether blighted blossoms are a significant source of inoculum for infection of fruit. Other sources of inoculum for fruit infection include mummies from the preceding year and stem cankers.

ACKNOWLEDGMENTS

Research was conducted at the University of California, Davis, with partial support from a United States-Israel Binational Agricultural Research and Development Fund-BARD grant and Federal Pesticide Impact Assessment Program grant. The first author was also partially supported by a University of Florida Faculty Development grant. We thank Dr. Mary Ann Sall, E. A. Bose, and T. E. Esser for technical advice and help on this project.

LITERATURE CITED

1. Jones, A. L., and Ehret, G. R. 1976. Isolation and characterization of benomyl tolerant strains of *Monilinia fructicola*. Plant Dis. Rep. 60:765-769.
2. Penrose, L. J., Davis, K. C., and Koffmann, W. 1979. The distribution of benomyl-tolerant *Sclerotinia fructicola* (Wint.) Rehm. in stone fruit

orchards in New South Wales and comparative studies with susceptible isolates. Aust. J. Agric. Res. 30:307-319.
3. Sonoda, R. M., Ogawa, J. M., Esser, T. E., and Manji, B. T. 1982. Mycelial interaction zones among single ascospore isolates of *Monilinia fructicola*. Mycologia 74:681-683.
4. Sonoda, R. M., Ogawa, J. M., and Manji, B. T. 1982. Use of interactions of cultures to distinguish *Monilinia laxa* from *M. fructicola*. Plant Dis. 66:325-326.
5. Szkolnik, M., Ogawa, J. M., Manji, B. T., Frate, C. A., and Bose, E. A. 1978. Impact of benomyl treatments on populations of benomyl-tolerant *Monilinia fructicola*. (Abstr.) Phytopathol. News 12:239.
6. Thind, D. S., and Keitt, G. W. 1949. Studies on variability of *Sclerotinia fructicola* (Wint.) Rehm. Phytopathology 39:621-636.
7. Vanderplank, J. E. 1975. Principles of Plant Infection. Academic Press, New York. 216 pp.