

ANNUAL REPORT - 1990

Commodity Group - Almond Board

Project No. 90-T15 - Epidemiology and Control of Shot Hole, Brown Rot, Leaf Rust, Scab, Green Fruit Rot, and other diseases of almond.

Project Leader: Dr. Joseph M. Ogawa, Department of Plant Pathology, University of California, Davis, CA 95616

Cooperating Personnel: J. E. Adaskaveg, B. T. Manji, J. M. Osorio, A. J. Feliciano, J. Connell, M. Viveros, and L. Hendricks

Introduction:

Shot hole: A forecasting system for shot hole disease was studied for a second year to determine ways of reducing fungicide sprays based on the amount of inoculum and weather conditions during bloom to five weeks after petal fall. Studies included experiments to prevent increases in inoculum (spores of the shot hole fungus, *Wilsonomyces carpophilus* Adaskaveg, Ogawa, and Butler formerly known as *Stigmina carpophila*). Fall application of zinc sulfate was applied to trees to defoliate and remove susceptible host tissue prior to fall rains; thus preventing increases of inoculum for spring infections. If inoculum levels were high in the fall, timing of spring fungicide applications could be based on weather forecasts and time of leaf emergence. If inoculum levels were low in the fall timing of sprays would be based on disease incidence and wetness periods. Using this system, bloom sprays (e.g. ziram) could be delayed until after petal fall and could also benefit in controlling other diseases such as scab and leaf blight that occur in late spring and summer.

Brown Rot: Efficacy and systemicity studies of iprodione (Rovral) and the experimental fungicide E-0858 (ICI Chemical) were completed. One manuscript was submitted for publication; while another is in preparation. Studies were conducted to determine the effectiveness of these fungicides in the control of brown rot blossom blight of almond, the movement of fungicides into blossom parts from green bud or pink bud sprays, and their efficacy in disease suppression or after infection.

Objectives: (1) Shot hole: Develop a minimal but effective protective spray program for shot hole and scab through forecasting and disease monitoring procedures; (2) Brown rot and green fruit rot: Develop efficacy data on alternative, systemic fungicides; (3) Scab: Determine the incidence of scab and the effectiveness of protective sprays; and (4) Wood decay: Publish data.

Materials and Methods:

Shot hole: Field trials were established in an experimental orchard in Solano County (UC Davis) and in commercial orchards in cooperation with Farm Advisors in Kern County (M. Viveros), Merced County (L. Hendricks), and Butte County (J. Connell). Experiments were a split-plot design: fall treatments (late October or early November 1989) consisted of non-treated

trees and trees treated with zinc sulfate (20-30 lbs/A); while in the spring, ziram was applied (8 lbs/A) to trees previously treated with either zinc sulfate or no zinc sulfate and at different stages of bloom (Fig. 1). Blossom stage and date of application of ziram sprays for each plot in the spring of 1990 are shown in Table 1. All treatments had six single tree replications and were surrounded by trees of similar treatment (buffer trees) to prevent spray contamination from adjacent treatments. All spray treatments were applied using an air blast sprayer.

In the first two weeks of December 1989, disease and sporodochia production were evaluated from leaf samples collected from each plot. For this 200 leaves (50/quadrant) were sampled from control trees. In the spring of 1990, 200 leaves (50/quadrant) were sampled from non-treated control trees in each plot in 7-10 day intervals from leaf emergence through 5-wk after petal fall and evaluated for disease and production of sporodochia of the fungus. Percent disease was calculated as the percentage of leaves with 1 or more shot hole lesions divided by the total leaves sampled per tree. All treatments had six single tree replications. Dates of leaf evaluations are indicated in Table 1. Environmental conditions (leaf wetness, temperature, rainfall, relative humidity, and wind speed/direction) were measured using a datalogger in the Merced and Solano Co. field plots and by CIMIS stations closest to each plot location.

To determine the potential of spores of W. carpophilus to survive in the absence of causing leaf infections, spores were produced in vitro, washed onto filter paper, and placed in nylon bags. These bags were then tied to either scaffold branches of almond trees or to wooden stakes under tree canopies with the bags in direct contact with soil. Experiments were done in an experimental orchard in Solano Co. (UCD) and in a commercial orchard in Merced Co. All treatments had 3 replications. Periodically, samples were taken from each bag, spores were plated onto potato dextrose agar, and percent germination was measured after one and two days of incubation at 25 C.

Brown Rot: Studies were done both in vitro, in vivo, and in the field to evaluate the effectiveness of benomyl, iprodione, and E-0858. For in vitro tests, fungicides were incorporated into media with final concentrations of 0, 0.1, 0.3, 0.5, 1.0, 1.5, or 2.0 ppm. Mycelium from cultures was placed onto the media containing a fungicide of specific concentration, incubated in the dark at 25 C, and after 4 days, radial growth was recorded. To determine the potential of the fungicides to suppress disease, closed blossoms were excised from 5 to 6-yr-old almond trees, blossoms were opened in the laboratory after incubation at 25 C, and inoculated with a spore suspension of Monilinia laxa. Fungicides were applied 1, 12, 24, or 36 hr after inoculation at a concentration of 300 ppm. Blossoms were incubated for 3 days at 20 C, >95%RH and evaluated as the percentage of blighted blossoms. For further details see enclosed manuscript.

To determine the systemicity of iprodione and E-0858, radioactive compounds of each fungicide were obtained from their manufacturer. For in vivo studies, blossoms attached to shoots were opened in the laboratory at 25 C and radioactive fungicides were applied specifically to sepals or petals of blossoms using a microsyringe. After 3 days blossoms parts (petals,

sepals, stamens, and pistils) were excised, separated, and frozen in liquid nitrogen. Samples were oxidized by combustion and carbon dioxide was trapped and evaluated for radioactivity. Floral parts also were placed on X-ray film for 4 wks. The film was developed and evaluated for exposure to radioactivity adjacent to blossom parts.

In field studies, fungicides were applied to Drake and Ruby almond trees in two orchards in Solano and Fresno counties, respectively. Fungicides were applied to closed (early pink bud) or fully opened (late full bloom) blossoms at a rate of 1 lb per acre. Disease was evaluated as a percentage of diseased twigs/400 shoots/tree. Treatments were replicated five times (single tree reps) and disease was evaluated 3 weeks after the full bloom spray. For further details see enclosed manuscript.

Results and Discussion:

In the fall 1988, inoculum levels were low in all test plots. In fall 1989, however, several rains in field plots in Solano, Butte, and Merced Counties resulted in numerous leaf infections and high levels of spore production in non-treated trees where leaves remained attached when evaluations were made in the first two weeks of December (Table 2). In trees treated with zinc sulfate, inoculum was low or absent and only a few leaves (<100) remained. Thus, plots with high numbers of spores were considered a high risk for disease in Spring 1990.

In spore survival studies conducted in the field, the shot hole fungus was able to survive in the absence of infection or susceptible host tissue with 50%-55% germination of spores after 6 months and 1-10% after a year (Fig. 2). This indicated that the fungus can survive during hot, dry summers when conditions are unfavorable for growth or during cool, wet winters in the absence of susceptible host tissue (e.g. blossoms, leaves or fruit). Thus, the fungus has the potential to survive as a spore until favorable conditions for at least a year, although the percentage is less than 10%.

In the spring of 1990, most of the plots were relatively dry except for Merced County where high-angle sprinkler irrigations supplemented leaf wetness. In the Butte, Kern and Solano plots, low-angle or furrow irrigation practices did not increase leaf wetness as detected by leaf wetness sensors or visual evaluation. Leaf wetness or rainfall monitored in or near test plots throughout the spring are shown for the Butte and Kern plots in Figs. 3 and 4; while total water measured for each test plot location is summarized in Table 2. Leaf samples for the Butte and Kern plots showed only a few new leaf infections (Figs. 3, 4) with a minimal production of sporodochia (0-2%). After monitoring wetness periods, leaf infections, and sporodochial formation, one timing spray for each plot was applied near the 5-wk after petal fall date; while grower sprays were applied in each plot based on blossom stage (pink bud and petal fall) and leaf emergence. No significant differences were observed between nonsprayed treatments, grower sprayed treatments (two ziram sprays), or our forecasted single-spray treatments (ziram). In these tests, scab, green fruit rot, and leaf rust symptoms were so scarce, data were not recorded. These results were similar to those obtained in the spring of 1989.

In the Merced plot, leaf wetness periods from rain were supplemented by the growers irrigation schedule and high-angle sprinklers that supplied water into the canopy of the tree (Fig. 5). Five weeks after petal fall, percent disease in control (non-treated) trees was 21.8% (Fig. 6). As in the other plots, grower sprays were at pink bud and at petal fall; while a timing spray was applied based on the observation of sporodochia in shot hole lesions on leaves from control trees (Fig. 6). Based on percent disease in the spring, no significant differences were observed between zinc sulfate (fall defoliation) and check treatments; although a trend for lower disease was observed non-treated (spring sprays), zinc sulfate sprays than in non-treated checks (Fig. 7). Significant differences were observed, however, between non-treated and ziram treatments (spring sprays) regardless of the non-treated and zinc sulfate fall treatments. Furthermore, no significant difference was observed in percent disease between the one timing spray (based on wetness periods and sporodochia production) and the two currently recommended blossom sprays (Fig. 8).

Benefits of this forecasting program could forewarn growers of high risk orchards based on the amount of inoculum formed during the previous fall season. Timing of sprays for the following spring would be based on the disease incidence during the previous fall season. Preliminary and on-going studies on spore survival indicate that spores can survive several months in an orchard during unfavorable environmental conditions for infection. Spores from fall infections serve as the primary inoculum. In the spring, our current understanding indicates that the primary inoculum has to be redistributed (e.g. by rain) to susceptible tissue. If disease was limited in the fall and spring, based on disease monitoring techniques, spray treatments may not be required until after the first infection and sporodochial development period (Fig. 6). The newly formed spores are considered secondary inoculum and are formed directly on susceptible tissue. At this time, preventative spray treatments are most effective in preventing disease. Thus, spray treatments (e.g. ziram or captan) could be delayed until after the petal fall stage of bloom. At this time, spray treatments would benefit in controlling shot hole, scab, and leaf blight. When severe outbreaks of shot hole occur in the fall, control practices could begin as soon as leaves emerge in the spring and additional spray treatments could be governed by wetness period and disease outbreak for individual orchards being monitored.

Brown rot blossom blight: Laboratory studies with radioactive labelled iprodione (Rovral) and an experimental fungicide E-0858 were conducted on Drake and Ne Plus Ultra almond blossoms. Labelled compounds placed on the sepal or petal at the pink bud stage of bloom were shown to move into the anthers and pistils. The amount of radioactive compound translocated for E-0858 was higher than that of iprodione. Bioassay tests made on detached blossoms, using benomyl-sensitive isolate of Monilinia laxa showed equivalent disease control between benomyl and iprodione. In almond orchards (Mission and Ruby cultivars) with 100 percent benomyl-resistant strains (1 µg a.i./ml benomyl) both iprodione and E-0858 provided effective control of brown rot blossom blight but not benomyl. In an orchard (Drake cultivar) with only benomyl-sensitive strains of M. laxa and high disease pressure based on inoculum level, benomyl provided better disease control than iprodione in comparisons of a single spray treatment at pink bud or two spray treatments (pink bud and full bloom). Evidence

of systemicity of iprodione when applied at pink bud stage of bloom supports data on benefits derived in blossom blight control from a single spray treatment. While application of a systemic fungicide at full bloom (80% of blossoms open) provides good coverage of the most susceptible blossom parts (anthers and stigma) and further offers protection of all blossom parts (sepals, petals, anthers, and stigma) of those bloom showing pink bud. This is the first report showing movement of the fungicide iprodione from sepals and petals into anthers and pistil of almond blossoms. For specific information see enclosed manuscript.

Note: One manuscript submitted to Plant Disease (enclosed without figures) and one currently in preparation.

Survey of wood decay organisms. A manuscript of wood decay fungi on fruit and nut trees of California was published summarizing the species and the amount of decay found in a three year study (Adaskaveg and Ogawa 1990).

Publications

1. Adaskaveg, J.E., and Ogawa, J.M. 1990. Wood decay pathology of fruit and nut trees in California. Plant Disease 74: 341-352.
2. Adaskaveg, J.E., Ogawa, J.M., and Butler, E.E. 1990. Morphology and ontogeny of conidia in Wilsonomyces carpophilus, gen. nov. comb. nov., causal pathogen of shot hole disease in Prunus species. Mycotaxon 37: 275-290.
3. Shaw, D.A., Adaskaveg, J.E., and Ogawa, J.M. 1990. Influence of moisture and temperature on infection and development of shot hole disease of almond caused by Wilsonomyces carpophilus. Phytopathology 80: 749-756.
4. Adaskaveg, J.E., Shaw, D.A., and Ogawa, J.M. 1990 A moisture generator and environmental monitoring system for field studies on shot hole disease of almond. Plant Disease 74: 558-562.
5. Osorio, J.M., and Ogawa, J.M. 1991. Comparison of systemic fungicides in the control of almond brown rot blossom and twig blight. Plant Disease, submitted.
6. Osorio, J.M., Ogawa, J.M., Bostock, R., and Ryugo, K. 1991. Systemicity in almond blossoms and efficacy of E-0858 and iprodione for control of almond blossom blight. Phytopathology, in preparation.

Table 1. Location, stage of growth, date of spray application, treatment, and date of disease evaluation for shot hole test plots in almond orchards during spring 1990.

<u>PLOT¹</u> <u>LOCATION</u>	<u>GROWTH²</u> <u>STAGE</u>	<u>DATE OF SPRAY³</u> <u>APPLICATION</u>	<u>TREATMENT⁴</u>	<u>DATE OF¹</u> <u>DISEASE</u> <u>EVALUATION</u>
SOLANO	PINK	2/27	R	3/20
	PETAL FALL	3/8	R	3/29
	FIVE WEEK	4/10	T	4/5
	----	---	---	5/4
BUTTE	PINK	2/28	R, T2	3/24
	PETAL FALL	3/22	R	4/10
	FIVE WEEK	4/13	T1	5/7
MERCED	PINK	2/28	R	3/19
	PETAL FALL	3/14	R	3/25
	FIVE WEEK	4/19	T	4/4
	----	---	---	5/2
KERN	PINK	3/1	R	3/19
	FULL BLOOM	3/9	R	3/26
	PETAL FALL	3/19	R	4/3
	FIVE WEEK	4/27	T	4/9

¹ Plot location by county.

² Growth stage of almond blossoms.

³ Date of ziram application.

⁴ Treatment code: R = recommended spray; T = timing spray based on presence of disease and sporodochia; as well as weather forecasts.

Table 2. Percentage of leaves with sporodochia from defoliated and non-defoliated plots from each of four test plots during December 1989.

PLOT LOCATION BY COUNTY	PERCENT SPOROCHIA ¹	
	DEFOLIATED	NON-DEFOLIATED
KERN	0	0.0
BUTTE	< 2	28.2
SOLANO	< 2	42.5
MERCED	< 2	30.1

¹ Percentage of leaves with sporodochia from trees defoliated with zinc sulfate or from non-treated trees.

Table 3. Comparison of rainfall and percent disease from non-treated trees in each of four test plots in the spring 1990.

PLOT LOCATION BY COUNTY	SPRING ¹ PRECIPITATION	PERCENT ² DISEASE
KERN	1.2	2.8
DURHAM	2.5	1.0
SOLANO	1.1	2.5
MERCED	3.4	21.8

¹ Spring precipitation in inches for the months of March and April 1990.

² Percent disease is the number of leaves with shot hole from 200 leaves collected from non-treated trees.

CHECK

ZINC SULFATE



Fig. 1. Split-plot design in test plots for comparing timing and recommended spray treatments for control of shot hole of almond.

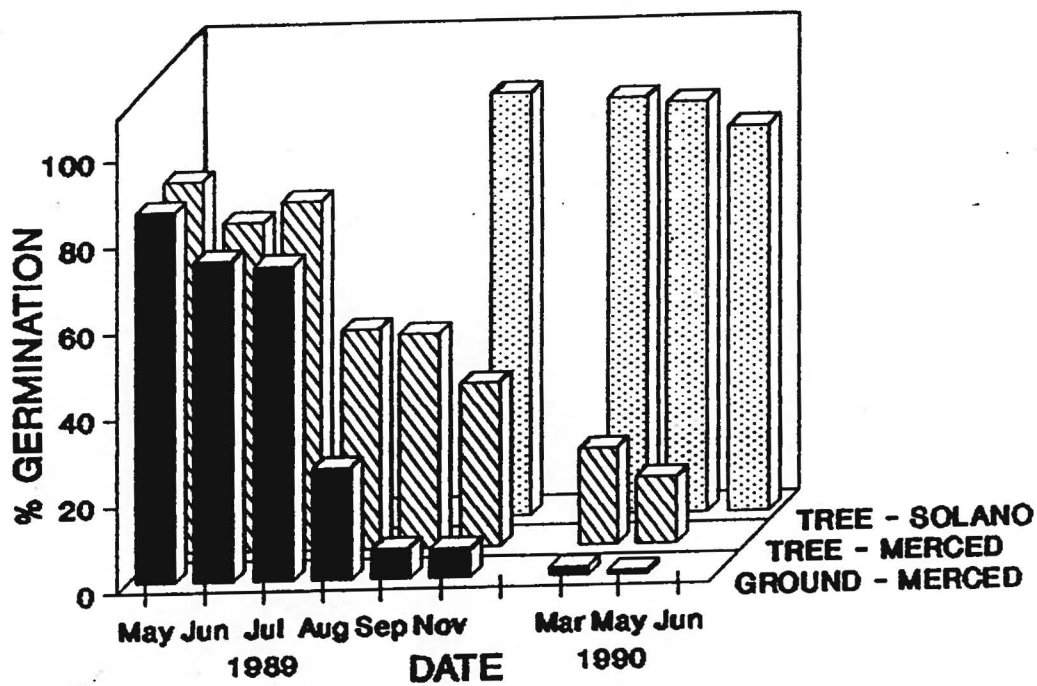


Fig. 2. Spore survival of *Wilsonomyces carpophilus* in almond orchards in Merced and Solano counties during the 1989-90 season.

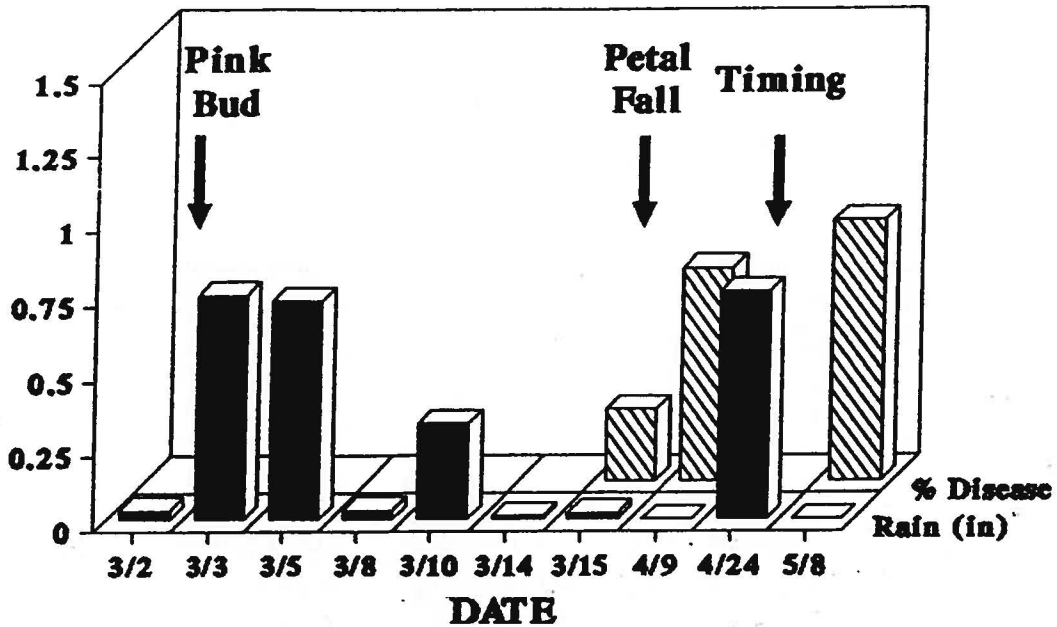


Fig. 3. Percentage of diseased leaves from non-treated trees and total rainfall at the field plot in Butte county in the spring (March and April) 1990. Pink bud and petal fall indicate blossom stage when recommended sprays were applied; while timing indicates when the a ziram spray was applied based on disease and weather forecasts. Precipitation was measured by CIMIS stations.

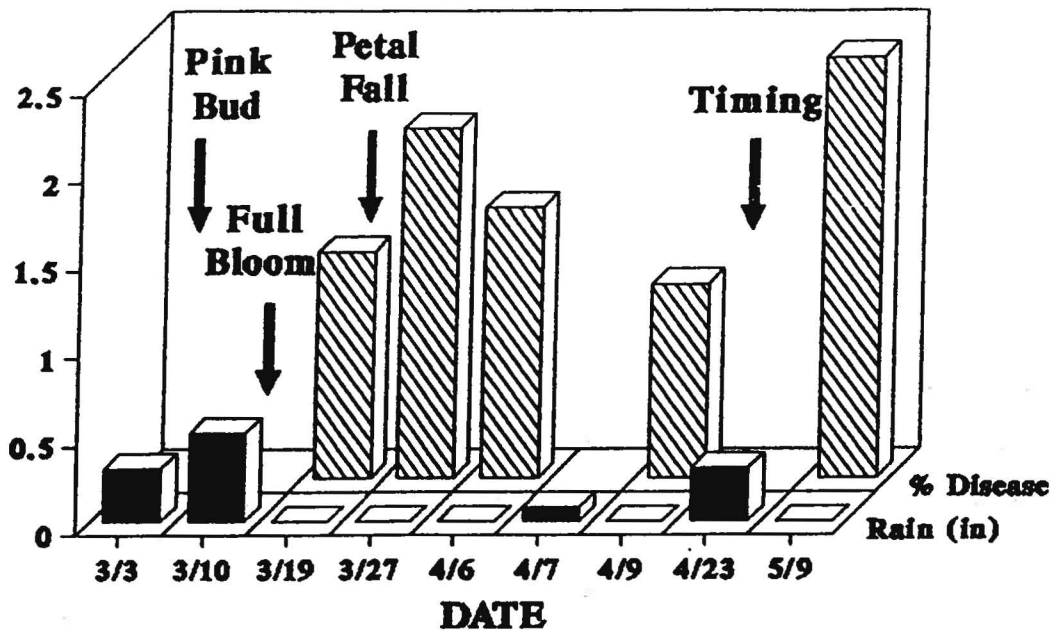


Fig. 4. Percentage of diseased leaves from non-treated trees and total rainfall at the field plot in Kern county in the spring (March and April) 1990. Pink bud and petal fall indicate blossom stage when recommended sprays were applied; while timing indicates when the a ziram spray was applied based on disease and weather forecasts. Precipitation was measured by CIMIS stations.

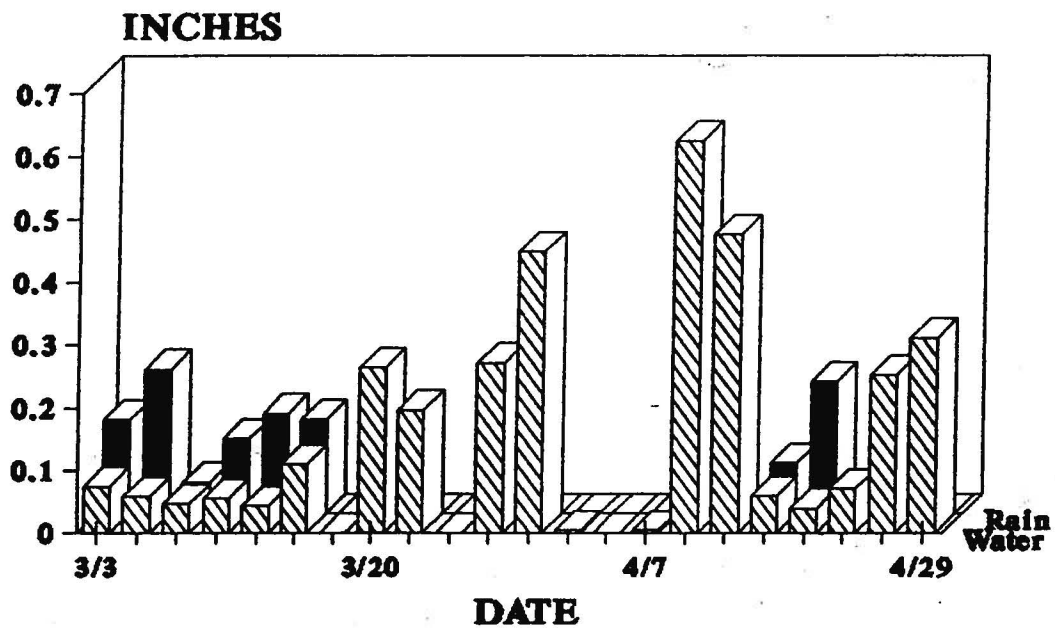


Fig. 5. Total rainfall and water applied by high-angle sprinkler irrigation at the field plot in Merced county in the spring (March and April) 1990. Precipitation was measured by a datalogger in the field plot and by CIMIS stations.

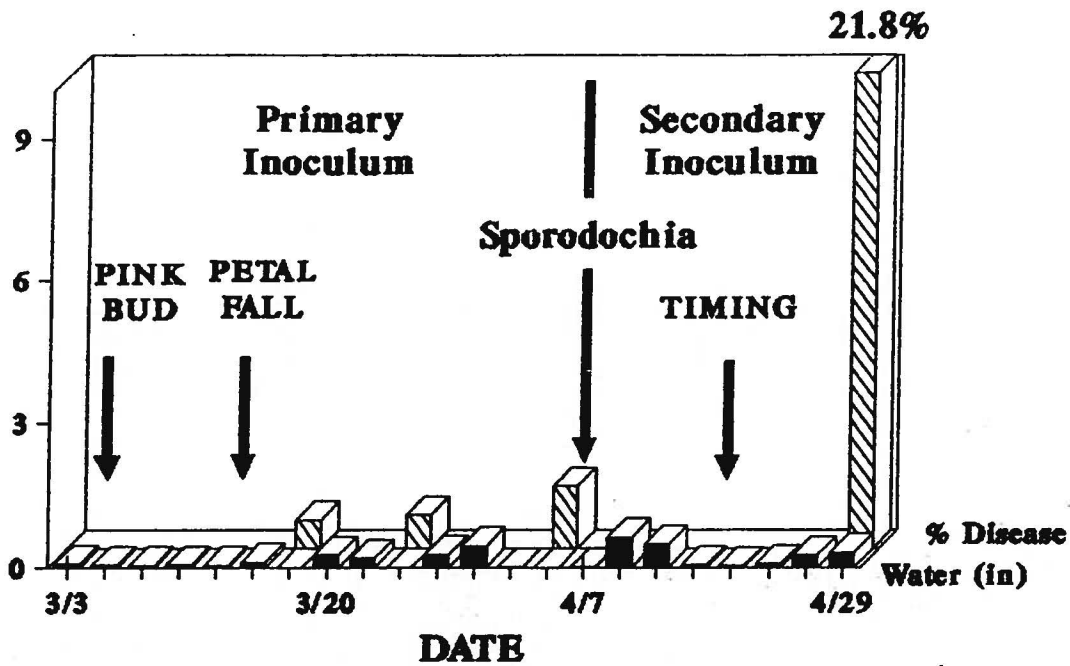


Fig. 6. Percentage of diseased leaves from non-treated trees and total rainfall at the field plot in Merced county in the spring (March and April) 1990. Pink bud and petal fall indicate blossom stage when recommended sprays were applied; while timing indicates when the a ziram spray was applied based on disease and weather forecasts. Sporodochia indicates the production of new (secondary) inoculum first detected on diseased leaves. Prior to this event, spores produced in the previous fall are considered primary inoculum. Precipitation was measured by a datalogger in the field plot and by CIMIS stations.

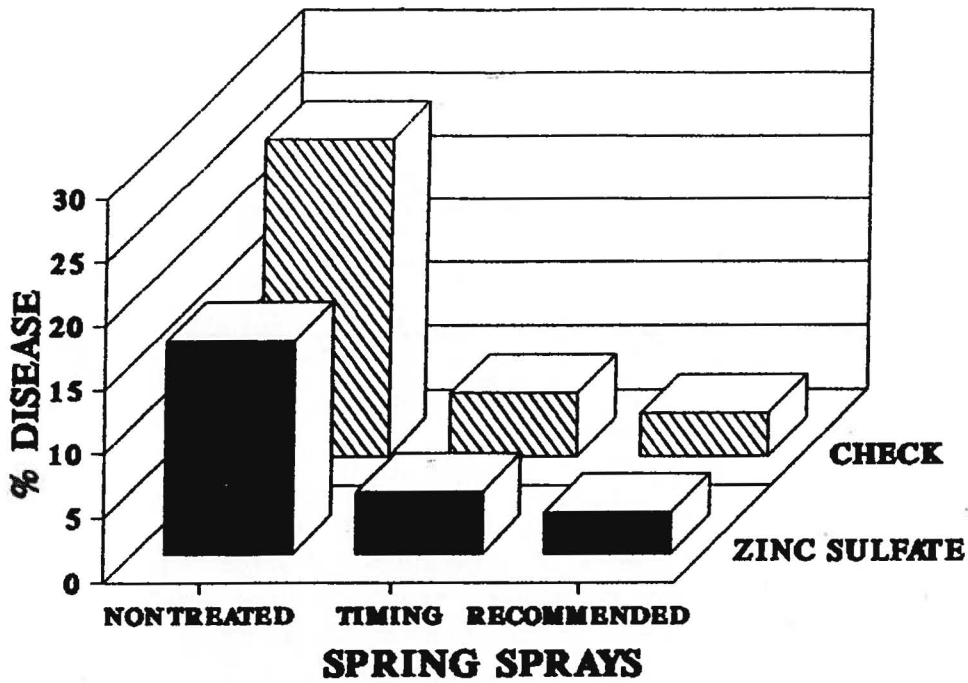


Fig. 7. Comparison of percentage of leaves with shot hole in timing (1 ziram spray), recommended (2 ziram sprays), and non-treated trees in zinc sulfate (defoliated) and check (non-defoliated) plots from the Merced Co. test plot in the spring 1990.

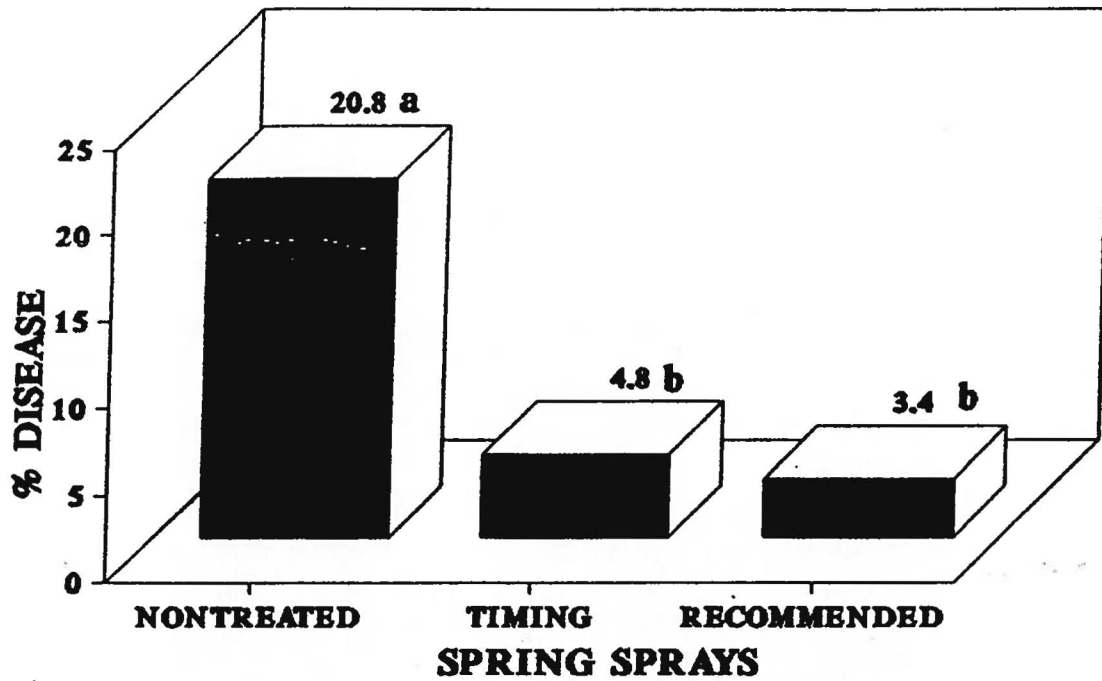


Fig. 8. Comparison of percentage of leaves with shot hole in timing (1 ziram spray), recommended (2 ziram sprays), and non-treated trees regardless of fall defoliation treatments in the Merced Co. test plot in the spring 1990.

1 Comparison of Systemic Fungicides for Control of Almond Brown Rot Blossom and
2 Twig Blight#

3 J. M. OSORIO and J. M. OGAWA. Graduate student and Professor, respectively.
4 Department of Plant Pathology, University of California, Davis, CA 95616.#

5
6 ABSTRACT#

7 Osorio, J. M. and Ogawa, J. M. 1990. Comparison of systemic fungicides for
8 control of almond brown rot blossom and twig blight. Plant Dis. 74:#

9
10 The fungicides E-0858, iprodione, and benomyl were compared for their efficacy
11 as in vitro inhibitors of mycelial growth of species of Monilinia and in vivo
12 control of brown rot blossom and twig blight. The effective dosage (ED₅₀) of
13 E-0858 in Czapek's medium required for inhibition of mycelial growth of M. laxa
14 was 0.30 for benomyl-sensitive and 0.31 µg a.i./ml for benomyl-resistant
15 isolates, whereas the ED₅₀ of mycelial growth of M. fructicola was 0.74 and 0.80
16 µg a.i./ml for benomyl-sensitive and -resistant isolates, respectively. Unlike
17 benomyl and iprodione, E-0858 when incorporated in potato-dextrose agar medium
18 did not inhibit mycelial growth of M. laxa or M. fructicola isolates. Iprodione
19 incorporated into Czapek's medium at 0.5 µg a.i./ml, however, was equally
20 inhibitory to both benomyl-sensitive and -resistant isolates of M. laxa and M.
21 fructicola. In laboratory studies, disease symptoms were suppressed when open
22 blossoms were sprayed with E-0858 or iprodione within 24 hr after inoculation.
23 In almond orchards with and without benomyl-resistant populations of M. laxa,
24 pre-bloom sprays of E-0858 and iprodione effectively controlled post-bloom
25 blossom and twig blight, which suggests movement of these fungicides from the

1 exposed petals and sepals to the unexposed pistils and stamens. #

2 Additional keywords: Monilinia laxa, Prunus dulcis, iprodione, E-0858. #

3
4 **INTRODUCTION#**

5 Brown rot blossom and twig blight, caused by Monilinia laxa (Aderh. &
6 Ruhl.) Honey and M. fructicola (Wint.) Honey, is a devastating disease causing
7 severe crop losses and control costs of almond and other stone fruit crops (16).
8 In California, the disease is controlled by one to two fungicide applications
9 during bloom so that blossom infections are prevented. In almond blossoms, the
10 stigma, anthers, and petals are the most susceptible tissues (6); therefore,
11 timing of fungicide applications in relation to blossom development is
12 critical. #

13 When non-systemic fungicides such as captan are used, at least two
14 applications are necessary to provide continued coverage of susceptible tissues.
15 With the introduction of benomyl, however, Ramsdell and Ogawa (12) reported that
16 a surface application of the chemical (applied at the pink bud stage of
17 blossoms) was translocated systemically to the pistils and stamens reducing
18 almond blossom blight by 92 percent. Ogawa et al. (9) demonstrated that a
19 single spray application of benomyl showed localized systemic activity and was
20 equivalent to two spray applications with protectant fungicides. Because of its
21 high efficacy in controlling brown rot blossom blight, benomyl was used
22 exclusively at first and later in combination with some protectant fungicides.
23 Benomyl-resistant populations of M. laxa and M. fructicola, however, have become
24 widespread throughout California (5,8,13) and other stone fruit production areas
25 (3,14,15,17,18), thus reducing the efficacy of benomyl in the control of brown
26 rot. Furthermore, populations of resistant strains have remained stable for

1 several years after benomyl had been withdrawn from the disease control program
2 (1,2). Therefore, with the establishment of benomyl-resistant populations,
3 there is need for comparative studies in the timing of applications of
4 alternative fungicides in orchards. The objectives of this study were to
5 determine the effect of the experimental compound E-0858 (SC-0858) and iprodione
6 on mycelial growth, disease suppression in infected blossoms, and control of
7 brown rot blossom and twig blight in almond orchards with and without benomyl-
8 resistant populations M. laxa.#

9 MATERIALS AND METHODS#

10 Fungal isolates. One hundred brown rot blighted twigs with sporodochia
11 were collected in 1987 from Drake almond trees in Solano County and in 1988 from
12 Ruby almond trees in Fresno-County. The samples were individually collected in
13 plastic bags, plated on lactic acid potato-dextrose agar (LPDA) and identified
14 as isolates of M. laxa. Sensitivity of these isolates to benomyl was assessed
15 by transferring 4-mm-diameter agar disks, taken from the edge of 3-day-old
16 colonies grown on LPDA, onto petri plates with Difco potato-dextrose agar (PDA)
17 amended with benomyl at 1 µg a.i./ml. Two representative isolates, one from the
18 Solano orchard (S1-87) and the other from the Fresno orchard (F1-88), were
19 selected and used in this study. The isolates were stored on potato-dextrose
20 agar (PDA) slants at 5 C, and grown on PDA at 23 C in the dark for production
21 of mycelium and on oatmeal agar at 23 C under fluorescent light for conidial
22 production. Benomyl-sensitive (MUK-1) and benomyl-resistant (489-81) isolates
23 of M. fructicola were obtained from the University of California, Davis,
24 collection for comparative studies, since both M. fructicola and M. laxa have
25 been reported in some orchards (7).#

26 Mycelial growth inhibition. In vitro inhibition of mycelial growth was

1 determined by placing 4-mm-diameter agar disks, taken from the edge of actively
2 growing 3-day-old colonies on PDA, onto petri plates with Difco Czapek agar
3 medium amended with 0, 0.1, 0.3, 0.5, 1.0, 1.5 or 2.0 µg a.i./ml of E-0858
4 (Experimental Fungicide E-0858 50WP, ICI Americans Inc., Sunnyvale, CA 94087),
5 or iprodione (Rovral 50WP, Rhone-Poulenc, Inc., Williamston, NC 27892), or 0,
6 0.05, 0.08, 0.10, 0.30, 0.50, 1.00 or 1.50 µg a.i./ml of benomyl (Benlate 50WP,
7 E. I. duPont de Nemours & Co. (Inc.), Wilmington, DE 19898). The Czapek agar
8 was amended by adding sterile water suspensions of the fungicides to the
9 autoclaved agar medium cooled to 50 C. Radial growth was assessed after 4-day
10 incubation in the dark at 23 C by measuring the distance from the edge of the
11 inoculum plug to the advancing margin of the colony. The percent inhibition of
12 mycelial growth was determined by comparing radial growth measurements of
13 treated cultures with the fungicide-free control. Linear regression analysis
14 was performed and the percent inhibition on a probit scale was plotted against
15 the log of the fungicide concentration to establish ED₅₀ values for each
16 compound. The experiment was duplicated and each treatment was replicated five
17 times. #

18 Disease suppression. Drake almond shoots with closed blossoms were
19 excised from 5- and 6-year-old trees. The cut ends were placed in water and
20 held at 25 C until the blossoms were open. Fully open blossoms were detached
21 from the shoots, placed in plastic food containers (100 X 235 X 315 mm)
22 containing sterile moist sand, and inoculated by spraying with a spore
23 suspension (1.2 X 10⁴ conidia/ml) of a benomyl-sensitive isolate of M. laxa (S1-
24 87). Blossoms were sprayed with E-0858, iprodione, or benomyl at 300 µg a.i./ml
25 1, 12, 24 or 36 hr after inoculation with M. laxa. Untreated blossoms, sprayed

1 with sterile water, served as controls. After inoculation blossoms were
2 incubated at 20 C, at RH \geq 95%, and evaluated 3 days later using a scale of 0-4
3 (0 = no visible symptoms; 1 = 1-25% of the blossom blighted; 2 = 26-50%; 3 = 51-
4 75%; and 4 = 76-100% of the blossom blighted). The experiment was duplicated
5 and each treatment was replicated 4 times (10 blossoms/replication).#

6 Field tests. Field experiments were conducted during 1988 and 1989 in a
7 5-year-old, Drake almond orchard in Solano County and in a 12-year-old Ruby
8 almond orchard in Fresno County. Treatments of E-0858 (Experimental Fungicide
9 E-0858 50WP), iprodione (Rovral 50WP) or benomyl (Benlate 50WP), each at the
10 concentration of 1.12 kg a.i./ha., were applied to either closed blossoms, fully
11 opened blossoms, or at both stages. Non-sprayed trees served as controls. The
12 fungicides were mixed in water and applied with a handgun sprayer (Rear's
13 Manufacturing CO., Eugene, OR) at a pressure of 1694 kpa to runoff. Each
14 treatment was replicated five times (one-tree/replication) in a randomized
15 complete block design. Disease severity was based on the percentage of blighted
16 twigs three weeks after the full bloom application. For this evaluation, 100
17 randomly selected twigs from each quadrant of the tree were examined for disease
18 symptoms. Values from each quadrant were averaged to obtain the percentage of
19 diseased twigs per tree. Meteorological data during the test period in each
20 plot were recorded using a Campbell 21X micrologger (Campbell Scientific Inc.,
21 Logan, UT), except for the 1988 in the Fresno field plot. Data for 1988 were
22 obtained from the Fresno airport weather station located about 10 km northwest
23 of the plot.#

24 RESULTS#

25 Mycelial growth inhibition. All isolates of M. laxa obtained from the
26 Solano orchard were sensitive to benomyl, whereas in the Fresno orchard all were

1 benomyl-resistant (1 μg a.i./ml). Using benomyl, ED50 values for S1-87
2 (benomyl-sensitive) and F1-88 (benomyl-resistant) isolates of M. laxa were 0.08
3 and 0.70 μg a.i./ml, respectively (Table 1). The benomyl-sensitive (MUK-1) and
4 the benomyl-resistant (249-81) isolates of M. fructicola followed the same trend
5 as isolates of M. laxa. Benomyl-sensitive and -resistant isolates of M. laxa
6 and M. fructicola were similar in sensitivity to E-0858 and iprodione. The ED₅₀
7 values of M. laxa isolates S1-87 and F1-88 were 0.30 and 0.31 μg a.i./ml for E-
8 0858 and 0.60 and 0.44 μg a.i./ml for iprodione, respectively (Table 1).
9 Whereas, M. fructicola isolates MUK-1 and 249-81 required 0.80 and 0.74 μg
10 a.i./ml of E-0858, respectively, to attain ED₅₀, their growth was suppressed
11 with iprodione at lower ED₅₀ values (0.52 and 0.58 μg a.i./ml, respectively).#

12 Disease suppression. Symptoms of brown rot blossom blight, under
13 laboratory conditions, were observed after a 24 to 28 hr incubation period. E-
14 0858, iprodione, and benomyl at 300 μg a.i./ml suppressed almond blossom blight
15 development when applied after infection had occurred. Blossoms treated with
16 fungicides after inoculation had significantly lower disease indexes than non-
17 fungicide treated blossoms. Significant differences were not observed ($P \geq$
18 0.05) between the fungicide treatments (Fig. 1). Comparisons of time of
19 fungicide application within any fungicide treatment indicated no significant
20 differences ($P \geq 0.05$) between the 1, 12, and 24 hr after inoculation
21 treatment.#

22 Field tests. In 1988, low rainfall and an average temperature of 15 C
23 during bloom in the Solano plot (Fig. 2) resulted in only 4.3% of the twigs
24 blighted in non-sprayed trees. There were no differences in disease severity
25 as a function of fungicide type and the time or numbers of fungicide

1 applications (Table 2). In contrast, the Fresno plot (Fig. 3) had 22 mm of
2 precipitation over 3 days, and a daily average temperature of 14 C during bloom.
3 An average of 12% blighted twigs was found on non-sprayed trees, whereas, a
4 single application of E-0858 or iprodione at full bloom reduced disease
5 incidence to 0.9 and 1.7% blighted twigs, respectively. Two applications of E-
6 0858 or iprodione did not further reduce disease incidence (Table 2).#

7 In 1989, weather conditions were conducive to high disease incidence in
8 both plots. In the Solano plot (Fig. 2) rains fell for 10 days during bloom,
9 totalling 67 mm of precipitation, with a daily average temperature of 12 C
10 during this period. On the non-sprayed trees, 43.7% of the twigs were blighted;
11 in contrast, on trees receiving a single application of E-0858 and benomyl at
12 pink bud stage, 3.8 and 4.9% of the twigs were blighted, respectively. On trees
13 sprayed at pink bud and full bloom, a significant difference in disease
14 incidence was not observed for E-0858 and benomyl but was observed for iprodione
15 (Table 2).#

16 The Fresno plot had 5 days of rain totalling 7 mm of precipitation and a
17 daily average temperature of 15 C during bloom (Fig. 3). These conditions led
18 to 21.7% blighted twigs on non-sprayed trees. The fungicide E-0858 provided
19 similar disease control in both the Fresno and Solano plots (Table 2).#

20 Iprodione applied to closed blossoms significantly reduced disease
21 incidence ($P \leq 0.05$) as compared to non-sprayed trees; however, disease control
22 was better ($P \leq 0.05$) when iprodione was applied twice during bloom. Overall,
23 benomyl provided less disease control than E-0858 and iprodione in the Fresno
24 plot which had benomyl-resistant isolates of M. laxa, but was significantly
25 better than iprodione in the Solano plot which did not have benomyl-resistant
26 isolates of M. laxa. Monilinia fructicola was not isolated from either plot.#

1 **DISCUSSION#**

2 Benomyl-resistant populations of M. laxa and M. fructicola have been
3 reported in California and other areas of North America (1,3,5,8,13,18). The
4 levels of resistance (1 to 4 µg a.i./ml) in isolates from California are
5 relatively low compared to the levels (300 to 1000 µg a.i./ml) reported in
6 isolates from South Carolina (18) and Michigan (3). In orchards with high
7 populations of isolates resistant at 1 to 4 µg a.i./ml, disease control with
8 benomyl has been ineffective (1,13). Our previous and current studies indicate
9 that E-0858 and iprodione inhibited mycelial growth on Czapek medium of both
10 benomyl-sensitive and resistant isolates of M. laxa and M. fructicola at similar
11 concentrations (10). E-0858 did not show any fungitoxicity at the concentration
12 evaluated on potato-dextrose agar medium. Matheron and Matejka (4) also found
13 that E-0858 (former code SC-0858) provided control of Sclerotinia sclerotiorum
14 comparable to that of iprodione under field conditions. In their in vitro tests
15 with PDA, however, ED₅₀ values were 158 and 0.42 µg a.i./ml for E-0858 and
16 iprodione, respectively. These data indicate that E-0858 is stable in Czapek
17 but not in PDA medium.#

18 In vivo laboratory tests revealed that, E-0858, iprodione, and benomyl
19 were capable of reducing the rate of almond blossom blight development after
20 infection. The suppression of disease on blossoms, treated with fungicides 24
21 hr after inoculation, were similar to results obtained when fungicides were
22 applied 1 hr after inoculation. These results suggest that benefits could be
23 obtained from applications of these fungicides after an infection period and
24 initial symptoms of the disease appear. Low temperature during bloom delays
25

1 disease development (6) which could possibly extend the effective period for
2 spray applications. #

3 Field experiments showed that E-0858 and iprodione applied to closed
4 blossoms (unexposed pistil and stamens) significantly reduced disease incidence.
5 This information supports the hypothesis that these compounds are translocated
6 from the point of contact to other blossom parts to inhibit growth of the
7 pathogen as have been demonstrated with benomyl (12). Additionally, these field
8 data support in vivo laboratory results that ¹⁴C-labelled E-0858 and iprodione
9 applied to either the sepals or petals are translocated to the stamen and pistil
10 (11). #

11 Weather conditions during bloom are important factors in disease
12 development. In 1988, between pink bud and full bloom, more rain and higher
13 disease levels occurred in non-treated blossoms in the Fresno plot than in the
14 Solano plot. In 1989, however, more rain and disease occurred in the Solano
15 plot (Figs. 2, 3; Table 2). In both years for plots with higher rainfall,
16 prolonged wetness periods during bloom were favorable for twig blight caused by
17 benomyl-sensitive and benomyl-resistance populations of *M. laxa*. Regardless of
18 rainfall and temperature, one or two applications of the experimental fungicide
19 E-0858 provided equal or better disease control than iprodione in both orchards
20 (one of which had benomyl-sensitive and the other benomyl-resistant populations
21 of *M. laxa*). The experimental fungicide also provided control equivalent to
22 that of benomyl in the orchard with benomyl-sensitive populations. #

23 This study indicates that under laboratory conditions E-0858 and iprodione
24 have a suppressive effect on infected blossoms similar to that of benomyl. Both
25 are highly effective in controlling almond blossom and twig blight in orchards

1 with and without benomyl-resistant populations of M. laxa. They also provided
2 protection to inner blossom parts when applied to closed blossoms. #

3 ACKNOWLEDGMENTS#

4 This investigation was supported in part by a grant from the Almond Board of
5 California. We thank R. M. Bostock, R. Ryugo, J. E. Adaskaveg, B. T. Manji, and
6 H. English for the technical support provided in this research. #

7 LITERATURE CITED#

8 1. Adaskaveg, J. E., Manji, B. T., and Ogawa, J. M. 1987. Stability and control
9 of benomyl-resistant populations of Monilinia species in the absence or presence
10 of benzimidazoles. (Abstr.) *Phytopathology* 77:1749. #

11 2. Delp. J. C., Ed. 1988. Resistance management strategies for benzimidazole.
12 pp 41-43 In: *Fungicide resistance in North America*. APS Press 133 p. #

13 3. Jones, A. L. and Ehret, G. R. 1976 Isolation and characterization of benomyl-
14 tolerant strains of Monilinia fructicola. *Plant Dis. Rep.* 60:765-769. #

15 4. Matheron, M. E., and Matejka, J. C. 1989. In vitro and field comparison of
16 six new fungicides with iprodione and vinclozolin for control of leaf drop of
17 lettuce caused by Sclerotinia sclerotiorum. *Plant Disease* 73:727-730. #

18 5. Michailides, T. J., Ogawa, J. M. and Opgenorth, D. C. 1987. Shift of
19 Monilinia spp. and distribution of isolates sensitive and resistant to benomyl
20 in California prune and apricot orchard. *Plant Dis.* 71:893-896. #

21 6. Ogawa, J. M., and English, H. 1960. Relative pathogenicity of two brown rot
22 fungi, Sclerotinia laxa and Sclerotinia fructicola, on twigs and blossoms.
23 *Phytopathology* 50:550-558. #

24 7. Ogawa, J. M., English, H., and Wilson, E. E. 1954. Survey for brown rot of
25 stone fruits in California. *Plant Dis. Rep.* 38:254-257. #

26 8. Ogawa, J. M., Manji, B. T., Bostock, R. M., Cañez, V. M., and Bose, E. A.

- 1 1984. Detection and characterization of benomyl-resistant Monilinia laxa on
2 apricots. Plant Dis. 68:29-31. #
- 3 9. Ogawa, J. M., Manji, B. T., and Sonoda R. M. 1985. Management of the brown
4 rot disease on stone fruits and almond in California. pp 8-15. In: Proceedings
5 brown rot of stone fruit workshop. N. Y. State Agric. Exp. Sta., Geneva Report
6 No. 55. #
- 7 10. Osorio, J. M., Ogawa, J. M., Feliciano, A. J., and Manji, B. T. 1987.
8 Efficacy of experimental fungicide SC-0858 in control of brown rot diseases of
9 stone fruits (Abstr.). Phytopathology 77:1241. #
- 10 11. Osorio, J. M., Bostock, R. M., and Ogawa, J. M. 1989. Systemicity in almond
11 blossoms and efficacy of E-0858 and iprodione for control of brown rot blossom
12 blight (Abstr.). Phytopathology 79:911. #
- 13 12. Ramsdell, D. C. and Ogawa, J. M. 1973. Systemic activity of methyl-2-
14 benzimidazolecarbamate (MBC) in almond blossoms following prebloom spray of
15 benomyl + MBC. Phytopathology 63:959-964. #
- 16 13. Sonoda, R. M., Ogawa, J. M., Manji, B. T., Shabi, E., and Rough, D. 1983.
17 Factors affecting control of blossom blight in a peach orchard with low level
18 benomyl-resistant Monilinia fructicola. Plant Dis. 67:681-684. #
- 19 14. Szkolnik, M., and Gilpatrick, J. D. 1977. Tolerance of Monilinia fructicola
20 to benomyl in western New York state orchards. Plant Dis. Rep. 61:654-657. #
- 21 15. Trivellas A. E. 1988. Benzimidazole resistance monitoring techniques and the
22 use of monitoring studies to guide benomyl marketing. pp 28-30. In: Fungicide
23 resistance in North America. APS Press 133 p. #
- 24 16. University of California. 1965. Estimates of Crop Losses and Disease-Control
25 Costs in California, 1963. Univ. of Calif. Agric. Exp. Sta. and Agric. Exten.
26 Serv. 102 pp.

1 17. Whan, J. H. 1976. Tolerance of Sclerotinia fructicola to benomyl. Plant Dis.
2 Rep. 60:200-201. #

3 18. Zehr, E. I. 1982. Control of brown rot in peach orchards. Plant Dis.
4 66:1101-1105. #

5 **FOOTNOTES**

6 Senior author's current address: Facultad de Agronomia, David, Chiriqui,
7 Panama. #

8 Accepted for publication _____ (submitted for electronic processing). #

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

LEGENDS#

Fig. 1. Indices of blossom blight comparing fungicide treatments to non-treated blossoms at selected time limits after inoculation with M. laxa. Blossoms were inoculated with a conidial suspension (1×10^4 conidia/ml) of a benomyl-sensitive isolate of M. laxa (S1-87), incubated for 3 days (20 C, > 95%RH), and evaluated for disease on a scale of 0-4: 0 = no visible symptom; 1 = 1-25% of blossoms blighted; 2 = 26-50%; 3 = 51-75%; and 4 = 76-100%. Values are the means of four replications (10 blossoms/replication) and were analyzed using a square root transformation. Values within a time treatment followed by the same letter were not significantly different according to Duncan's multiple range test for mean separation ($P \leq 0.05$).#

Fig. 2. Rainfall, average daily temperatures, and dates of flowering when fungicides were applied to Drake almond trees in the Solano orchard during the 1988 and 1989 seasons.#

Fig. 3. Rainfall, average daily temperatures, and dates of flowering when fungicides were applied to Ruby almond trees in the Fresno orchard during the 1988 and 1989 seasons.#