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Project No. 96-JA2: Host Resistance and Chemical Management Strategies for Brown Rot Blossom Blight of Almond.

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SUMMARY

In 1996, studies were initiated to evaluate natural host resistance and relative brown rot susceptibility in almond cultivars planted in varietal blocks in Kern and Butte Counties. Additionally, objectives included the development of efficacy data for brown rot control using new fungicides, fungicide-additive combinations, and susceptible-row application of fungicides for brown rot blossom blight control on almonds. These studies were conducted to develop integrated approaches for brown rot blossom blight management in almond. In host resistance studies, comparisons of inoculation methods were made on the equivalent phenological stage of blossom development based on early, mid-, and late blooming varieties. Studies that utilized direct pink bud inoculations gave consistent results for incidence and severity of brown rot between the two plots as compared to studies that utilized inoculation and bagging of blossoms in full bloom or the use of detached blossoms in laboratory inoculations. In the pink bud inoculations, significant differences in brown rot incidence were observed among varieties combined from both plots. Aldrich, Fritz, Price, Sonora, Wood Colony, and 13-1 were significantly higher in disease incidence than Nonpareil, 1-102, and 2-19. Some varieties, however, had more disease in Butte than in Kern Co. and a significant interaction was observed between varieties and plot location. In comparisons of severity or those infections that resulted in a stem lesion, Aldrich, Butte, Fritz, Price, Sonora, Wood Colony, and 13-1 had the largest stem lesions. Again, Nonpareil, 2-19, and 1-102 had the smallest lesions that resulted from pink bud inoculations. Preliminary histological studies showed that blossoms of Nonpareil formed a clear zone and latter an abscission zone in the bud-stem union that inhibited blossom infections from entering the stem. In fungicide studies comparing iprodione and myclobutanil with and without 2% summer oil (Omni Supreme Oil), all treatments significantly reduced blossom blight compared to the check. The oil emulsion significantly improved the efficacy of iprodione but not that of myclobutanil. In evaluations of susceptible-row application of fungicides for brown rot blossom blight control on almonds, no significant difference in disease incidence (less than 0.1% in all treatments) or in crop yield for the cultivar Nonpareil was observed between fungicide treated (12.8 lbs of kernels/tree) and non-treated (13.3 lbs of kernels/tree) trees. For cultivar Carmel, however, yield for the iprodione-oil treatment (13.54 lbs/tree) was significantly higher than non-treated trees. Thus, as expected, susceptible varieties such as Carmel need to be protected with fungicides to prevent yield losses from brown rot, whereas Nonpareil trees may not need fungicide application in years of low disease pressure.

INTRODUCTION

Brown rot blossom blight caused by *Monilinia* species is one of the major diseases of almond in California and potentially can cause extensive crop losses. In the past several years, we have conducted research to develop reduced-spray programs for brown rot control based on improved efficacy of fungicides (e.g., iprodione-oil mixtures), identification of differences in cultivar susceptibility, and development of new application schedules (e.g., susceptible row spray programs). In 1996, we continued to evaluate the effect of oil emulsions in combination with iprodione or other fungicides such as myclobutanil, as well as evaluate new fungicides currently being developed for almond disease management. Additionally, we initiated research to evaluate almond cultivars for their susceptibility to brown rot. Previously, we documented in relative comparisons between selected varieties in field and laboratory studies that Nonpareil was less susceptible, Mission was variable, and NePlus Ultra, Butte, and Carmel were more susceptible to brown rot. In 1996, with the cooperation of Dr. Tom Gradzeil, Extension Specialist Warren Micke, Joe Connell (UCCE-Butte County), and Mario Viveros (UCCE-Kern Co.), we initiated research to evaluate relative brown rot susceptibility in new almond varieties that are also being horticulturally and pomologically evaluated. Information on varietal susceptibility to brown rot and other diseases in these advanced variety trials will be beneficial to growers that are planting new selections and breeders that are planning and developing future varieties.

OBJECTIVES

1. Evaluate natural host resistance and relative brown rot susceptibility in almond cultivars planted in varietal blocks in Kern and Butte Counties.

2. Development of efficacy data for brown rot control using new fungicides, fungicide-additive combinations, and susceptible-row application of fungicides for brown rot blossom blight control on almonds.

MATERIALS AND METHODS

I. *Natural host resistance*

Pink bud inoculations. Thirty-five buds each on individual spurs on three single-tree replications of each cultivar were individually inoculated at pink bud stage (5% open blossoms) with 20 µl of a 50,000 spores/ml suspension of *Monilinia laxa* using a 1.0 ml syringe and 27 gauge needle. Incidence of brown-rotted blossoms was recorded weekly for 3-4 weeks. Severity of brown rotted stems infections was recorded by measuring the length of twig cankers. Treatments were compared using analysis of variance or general linear model and least significant difference (LSD) procedures for multiple comparisons of means using SAS 6.04.

Open bloom inoculations - field. Two branches each with 10 - 20 open blossoms on three trees of each cultivar were manually inoculated at full bloom stage (80%+ open blossoms) by spraying blossoms with a 30,000 spores/ml suspension of *Monilinia laxa* with a hand-held spray bottle. Unopened buds were removed and sprayed branches were then double bagged with a plastic bag with added distilled water and a wet paper towel, and a paper bag to protect the inoculations from radiant heat. Incidence of brown-rotted blossoms was recorded weekly for 3-4 weeks. Severity of brown rotted stems infections was recorded by measuring the length of twig cankers. Treatments were compared using analysis of variance or general linear model and least significant difference (LSD) procedures for multiple comparisons of means using SAS 6.04.

Open bloom inoculations - laboratory. Several branches with pink buds, but with open blooms removed, from three trees of each cultivar were returned to the laboratory in a cold box. Branches were cut at the base, placed in fresh water, and forced open at room temperature. Eighteen to twenty-four open blooms from each cultivar were then removed, placed on moist vermiculite, and sprayed to run off with an aqueous suspension of *M. laxa* conidia (15,000 spores/ml). Treatments were compared using analysis of variance or general linear model and least significant difference (LSD) procedures for multiple comparisons of means using SAS 6.04.

Natural incidence of brown rot caused by M. laxa - field All cultivars were evaluated for natural incidence of blossom blight disease on March 31, 1996. Two hundred blossoms on each of three trees(reps)/treatment were rated for incidence of brown rot. Treatments were compared using analysis of variance or general linear model and least significant difference (LSD) procedures for multiple comparisons of means using SAS 6.04.

Histology. Almond stems, including axillary regions with attached inoculated and non-inoculated blossoms were excised in the field and immersed in either 2.5% glutaraldehyde in phosphate buffer or in Carnoy-Lebrun fixative. Specimens initially fixed in glutaraldehyde were later transferred to Carnoy-Lebrun. Following 2-3 days in Carnoy-Lebrun fixative, specimens received three, one hour rinses in 95% ethanol, were trimmed to smaller sizes, and immersed for 3-4 days in Sinha's modified version of Mukerji's solution for softening. After softening, specimens were rinsed in 3 changes of 95% ethanol, and transferred in sequence through 100% ethanol, 100% ethanol + TBA (1:1), three rinses of 100% TBA. Infiltration was initiated with TBA + paraffin oil (1:1) in the vacuum oven. Three to four changes in melted paraffin in the vacuum oven preceded final embedding in paraffin. Sections, 10 µm in thickness, were made using an American Optical Spencer 820 microtome. Ribbons were mounted on glass slides with Mayer's egg albumin adhesive. Sections were deparaffinized with xylene, rehydrated through ethanol to 70%, and stained 36-48 hours in safranin, followed by counter staining in fast green (Johansen, 1940). Final dehydration was with ethanol and final clearing with xylene. Cover glasses were mounted with Permount. Photographs were taken using Zeiss Axioscope and Zeiss camera at 400x magnification on Kodak Ektachrome film, digitized using Photoshop and a slide scanner and printed on a Sony D8800 dye-sublimation printer.

II. *Fungicide Evaluations*

Evaluation of fungicides for brown rot management. Drake almond trees planted at the Department of Plant Pathology Research Field at the University of California, Davis were used for these studies. A randomized plot of four, single-tree replications for each treatment was used. Fungicides were applied using a 3.5 gal capacity, back-pack sprayer calibrated for 100 gal/A. The following fungicide products were evaluated: Rally 40W, RH141647-8 30EC, Rovral 50WP (iprodione), Captan 50WP (captan), Abound 80WDG, Benlate 50WP (benomyl). Additives used with designated treatments were Omni Supreme Spray Oil, Kinetic, or Latron CS-7. In a second study, selected rates of Rally and Rovral were evaluated alone or in combination with 2% Omni Supreme Spray Oil. In both studies, treatments were applied at pink bud and full bloom and there were four (study 1) and three (study 2), single-tree replications per treatment. For both studies, brown rot twig and spur blight caused by *Monilinia laxa* were evaluated based on 200 spurs/tree in early April. Treatments were compared using analysis of variance or general linear model and least significant difference (LSD) procedures for multiple comparisons of means using SAS 6.04. Flowering stages were recorded by date for each *Prunus* species studied.

Evaluation of a susceptible-row spray program for brown rot management. A Nonpareil-Carmel-Monterey (50-25-25%) almond orchard in Bakersfield, CA was used for this study. Rows of Nonpareil (30 trees/row) were flanked by rows of Carmel and Monterey (both 29 trees/row) cultivars. In half of the plot, alternate rows of susceptible cultivars, Carmel and Monterey were sprayed, whereas Nonpareil rows were not sprayed. In the second half of the plot, all rows were sprayed. Fungicide treatments were iprodione (Rovral 50WP; 1 lb/acre) and myclobutanil (Rally 40WP; 6 oz/acre) each with and without 1.5% summer oil. Treatments were applied on February 26, 1996. Control rows were not sprayed. Each treatments had 4 single row replications. Commercial harvesters were used to harvest the almonds from each row (rep) into a gondola; four reps/treatment. Each gondola used were tared and gross weight of each gondola used was recorded. Net weight of row yield was calculated by subtracting the tare weight from the gross weight. Four pound sub-samples were taken from each row harvested. Total nuts were counted from each four-pound sample, and from these, 100 nut meats were weighed for final yield calculations per tree.

RESULTS AND DISCUSSION

Evaluations of natural host resistance and relative brown rot susceptibility in almond cultivars planted in varietal blocks in Kern and Butte counties. Because of the enormous potential for variation in environmental conditions and bloom dates between varieties during the spring from year to year and between research sites, our research approach was to evaluate almond varieties in the regional varietal trials using several different methods of evaluation. These methods included: natural incidence of brown rot, pink bud and blossom inoculations in the field, and laboratory inoculations using detached blossoms. Varieties evaluated for natural incidence of brown rot were Aldrich, Butte, Carmel, Fritz, Mission, Monterey, Nonpareil, Padre, Price, Rosetta, Sonora, Wood Colony, 1-87, 1-102, 2-19, 2-43, 13-1, and 25-75. In late-March, evaluations for natural incidence of brown rot, average disease incidence was less than 7% among the 16-18 varieties evaluated in each of the Kern and Butte Co. plots. Because trees in both plots are young, natural incidence of brown rot was low. During bloom, however, approximately 1.86 inches of rain occurred from Feb. 19 - March 15 in Kern Co. In Butte Co., approximately 2.6 inches of rain occurred from Feb. 23 - March 12. Early to mid-blooming varieties Sonora, Price and 2-43 had significantly higher incidence of disease than the mid- to late blooming varieties Nonpareil, 2-19, and 1-102. Other varieties were intermediate in disease incidence.

In comparisons of inoculation methods, inoculations were made on the equivalent phenological stage of blossom development based on early, mid-, and late blooming varieties. Evaluations were made 1-4 weeks after inoculation. Studies that utilized direct pink bud inoculations gave consistent results for incidence and severity of brown rot between the two plots (Figs. 1,2). In studies that included inoculation and bagging of blossoms in full bloom for 24-hrs, incidence and severity of brown rot were more variable (Figs. 3,4). **In the pink bud inoculations, significant differences in brown rot incidence were observed among varieties combined from both plots. Aldrich, Fritz, Price, Sonora, Wood Colony, and 13-1 were significantly higher in disease incidence than Nonpareil, 1-102, and 2-19 (Figs. 1,2).**

Some varieties, however, had more disease in Butte than in Kern Co. Thus, a significant interaction was observed between varieties and plot location. Interestingly, Aldrich and Rosetta were the most variable between the two locations. In comparisons of severity or those infections that resulted in a stem lesion, Aldrich, Butte, Fritz, Price, Sonora, Wood Colony, and 13-1 had the largest stem lesions (Fig. 6). Again, Nonpareil, 2-19, and 1-102 had the smallest lesions that resulted from pink bud inoculations (Fig. 6).

In full bloom inoculations studies, branches with 6-10 blossoms that were in full bloom were selected at random and the exact age of each blossom was not known. Blossoms in earlier stages of development were removed from the branch. Therefore, variation was expected due to blossom age or from natural inoculum contaminating selected blossoms. In the Bakersfield plot, all varieties had relatively high levels of disease in these tests (Figs. 3,4). Butte, Carmel, Wood Colony, and 25-75 had significantly higher levels of disease than the other varieties evaluated. In the Butte plot, disease levels were lower probably due to the lower temperatures. Severity of disease (canker length) resulting from full bloom inoculations was significantly higher in Wood Colony and Sonora varieties than other varieties evaluated (Fig. 7). In laboratory studies with detached blossoms that were forced open, incidence of anther infections was less variable as compared to field opened blossoms (Fig. 5). All varieties had relatively high levels of disease in these tests with over 50% anther infection occurring in all varieties. Combining laboratory opened blossoms for 2 experiments using blossoms from Butte Co. and 2 experiments using blossoms from Kern Co., Mission, Aldrich, Butte, Sonora, Monterey, and 1-102 had the highest anther infection and were significantly different from the other varieties evaluated. Interestingly, Nonpareil and other varieties that had low incidence and severity of disease in pink bud inoculations had a high incidence of anther infection in these laboratory studies.

Histological evidence for natural host resistance in almond to brown rot. Histological studies were done on selected varieties showing significant differences in susceptibility to brown rot in several methods of evaluation. Based on laboratory inoculations, all of the varieties have susceptible blossom tissues (e.g., anthers, stigma, petals), however, several varieties evaluated showed significantly fewer infections that resulted in stem lesions. Upon examination of pink bud and full bloom inoculations in the field, varieties that were more resistant to stem lesions had fewer blossoms that were inoculated attached to the stem at the time of evaluation. **Preliminary histological studies showed that blossoms of Nonpareil formed a clear zone and latter an abscission zone in the bud-stem union that inhibited blossom infections from entering the stem (Fig. 8).** This was not observed in Butte or Drake varieties. Additionally, varieties will be evaluated to determine if this mechanism can be correlated with the reduced incidence of brown rot infections in selected almond varieties evaluated.

Efficacy of fungicides for management of blossom blight and shot hole of almond. Incidence of brown rot infected spurs was extremely high in non-treated trees with 72.6% spur infection (Fig. 9, 10; Table 1). All fungicide treatments evaluated significantly reduced brown rot compared to the non-treated check trees. Abound, Abound-Benlate/Abound, Abound/Rovral, Rovral/Captan, and Rally (with or without Latron CS-7) had the lowest incidence of brown rot spur infections with less than 16.8% incidence (Fig. 9). Rovral-oil/Captan, Rovral-Kinetic, and RH141647-8 treatments had slightly higher levels of disease. In these treatments, Rovral was used at the low rate of 0.5 lb product/A. Slight phytotoxicity was observed in the Rovral-oil/Captan and the Rally-oil treatments. The Rally-oil treatment had the highest level of disease compared to the other fungicide treatments. In the second almond study, Rally and Rovral significantly reduced the incidence of disease to less than 24% as compared to the 77.3% incidence of the check (Table 2-I). The efficacy of Rovral at 1.0 lb ai/A in 2% oil (Omni Supreme Spray) was significantly improved compared to Rovral alone (Fig. 10A). The Rovral-oil treatment (1 lb-2%) had the lowest disease incidence in comparisons of all treatments (Fig. 10B, Table 1). The efficacy of Rally at 6 oz/A (19.7%) was statistically similar to Rovral at 1 lb/A (24%). The efficacy of Rally, however, was not significantly improved with the addition of oil alone (Fig. 10A,B). No phytotoxicity was observed in the oil treatments of the second plot. In comparisons of fungicides regardless of rates, only Rovral-oil had significantly lower brown rot blossom blight than Rovral alone (Fig. 10B, Table 1). Similarly, Rovral-oil had the lowest incidence of disease; whereas the Rovral, Rally, and Rally-oil treatments formed statistically intermediate groups as compared to the check (Fig. 10B, Table 1).

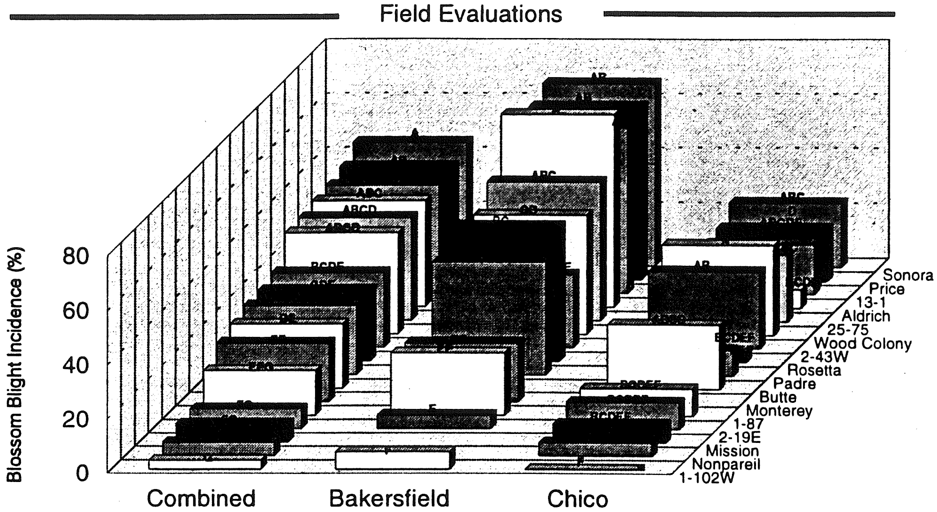
Efficacy of susceptible-row application of fungicides for brown rot blossom blight control and yield benefits on almonds. In Kern Co., studies were also continued to determine the benefits of a susceptible-row spray program. In one 30 acre orchard planted in 1993, cultivars and planting design were: Nonpareil, Monterey, Nonpareil, and Carmel. All varieties were on Nemaguard rootstock. In half of the block all varieties were treated with one pink bud fungicide application, whereas the other half of the block only the Carmel and Monterey cultivars were treated. **No significant difference in disease incidence (less than 0.1% in all treatments) or in crop yield was observed between fungicide treated (13.1 lbs of kernels/tree) and non-treated (12.5 lbs of kernels/tree) Nonpareil (Table 2).** Crop yield was based on 8 rows per treatment and 30 trees per row. This was consistent with two previous years of research in Merced Co. where disease incidence and crop yield were also evaluated in trials comparing treated and non-treated, 12 year-old, Nonpareil trees (Almond Reports 1993, 1994).

In the same Kern Co. orchard, fungicide treated and nontreated, susceptible varieties Carmel and Monterey were also compared for disease incidence and yield. In these studies, the incidence of brown rot infected spurs was determined 4 weeks after bloom in a sample of 50 spurs/tree on 10 different trees/rep and 4 replications per treatment. The average incidence of brown rot for both varieties in the check (3 infected spurs) was significantly higher than iprodione (1), iprodione-oil (0.25), myclobutanil (0.5) and myclobutanil-oil (0.25). When all varieties were compared, no differences in yield were observed. The following yields for each treatment were: iprodione-oil (13.3 lbs/tree), iprodione (12.76), and myclobutanil (12.34) followed by the check (12) and myclobutanil-oil (11.5) treatments. For cultivar Monterey, no significant differences were observed in yield between fungicide (13.38 lbs of kernels/tree) and no fungicide (12.25) treatment (Table 2). For cultivar Carmel, however, yield of the iprodione-oil treatment (13.54 lbs/tree) was significantly higher than non-treated trees (Table 2). As expected, susceptible varieties such as Carmel need to be protected with fungicides to prevent yield losses from brown rot.

LITERATURE CITED

1. Adaskaveg, J. E. and J. M. Ogawa. 1993. *Almond Board Annual Report-1993.*
2. Adaskaveg, J. E. and J. M. Ogawa. 1994. *Almond Board Annual Report-1994.*
3. Johansen, 1940. *Plant Histology.*

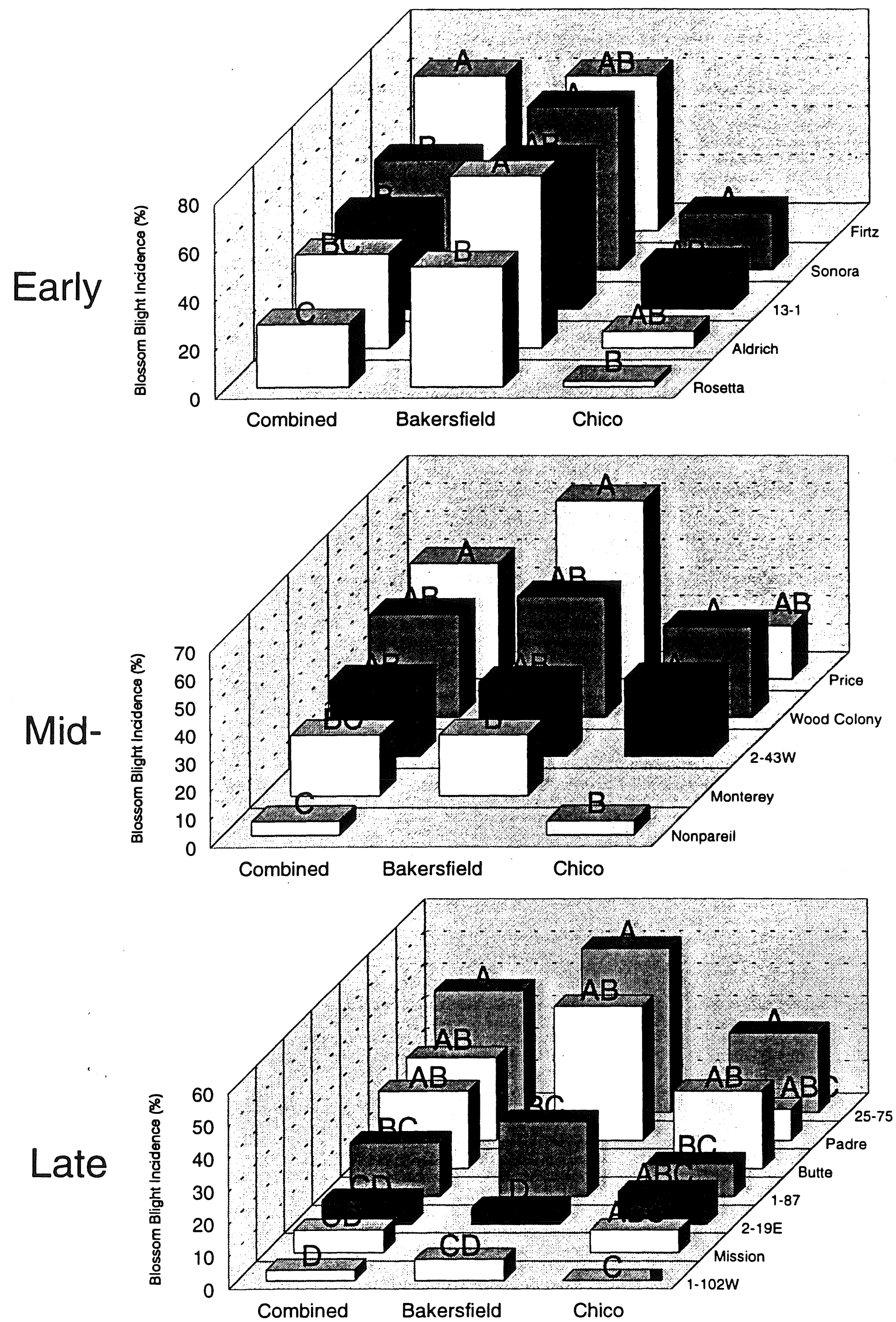
Fig. 1. Incidence of Brown Rot Blossom Blight
Caused by *M. laxa* in Pink Bud Inoculated Blossoms
of Selected Almond Varieties



Blossoms in pink bud stage of development were inoculated with conidia (30K conidia/ml) in the field using syringe, and evaluated for incidence of blossom blight after 3 weeks.

Fig. 2 Incidence of Brown Rot Blossom Blight Caused by *M. laxa* in Pink Bud Inoculated Blossoms of Selected Almond Varieties

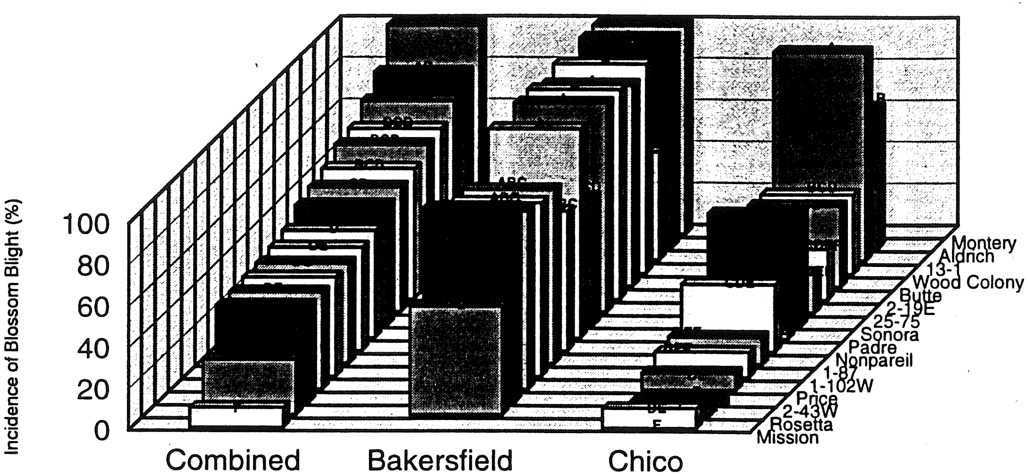
Field Evaluations



Blossoms in pink bud stage of development were inoculated with conidia (30K conidia/ml) in the field using syringe, and evaluated for incidence of blossom blight after 3 weeks.

Fig 3. Incidence of Brown Rot Blight Caused by *M. laxa* in Full Bloom Spray Inoculated Blossoms of Selected Almond Varieties

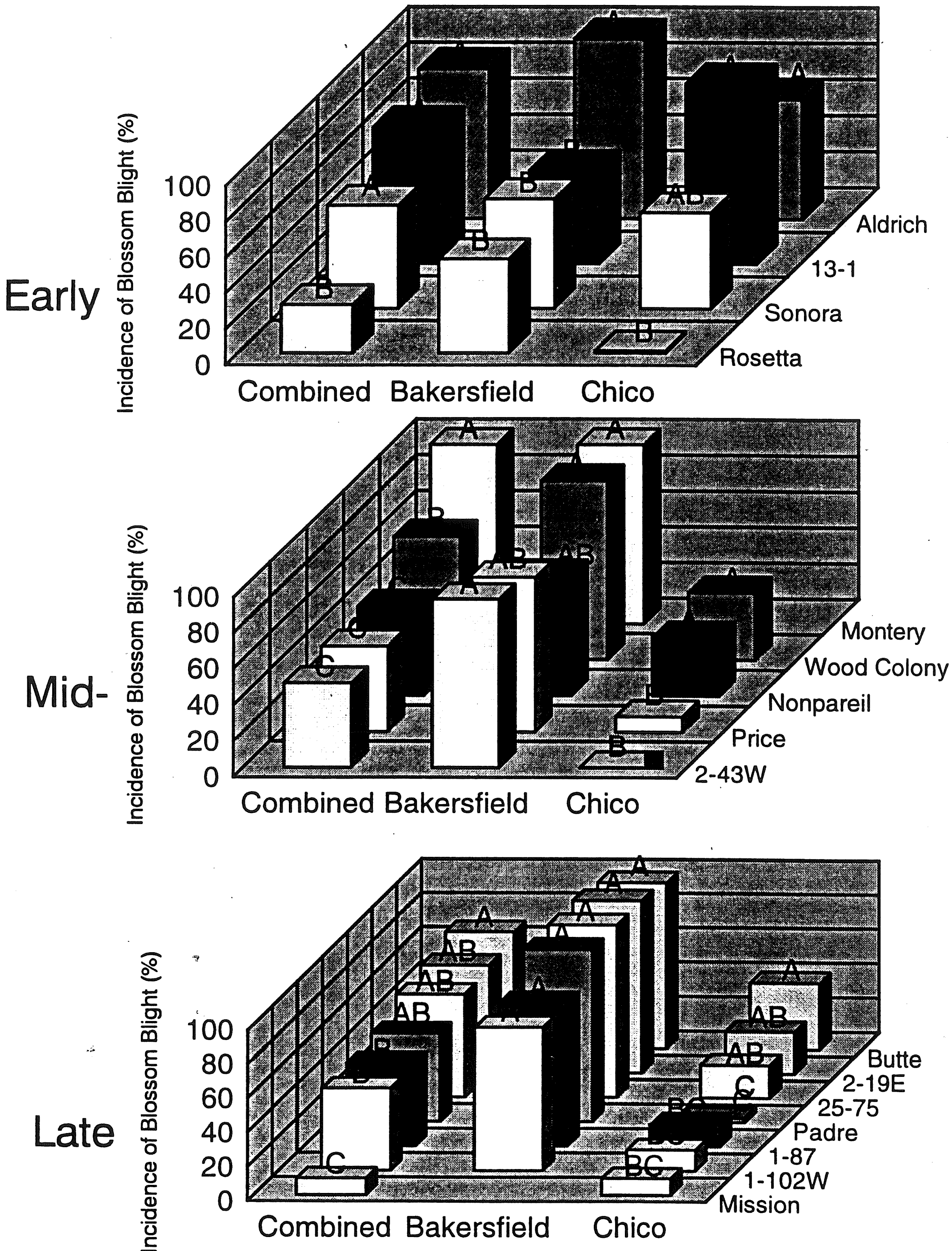
Field Evaluation



Blossoms were inoculated with conidia (30K spores/ml) in the field using a atomizer, bagged for 12 hr, and evaluated for incidence of blossom blight after 3 weeks.

Fig. 4. Incidence of Brown Rot Blight Caused by *M. laxa* in Full Bloom Spray Inoculated Blossoms of Selected Almond Varieties

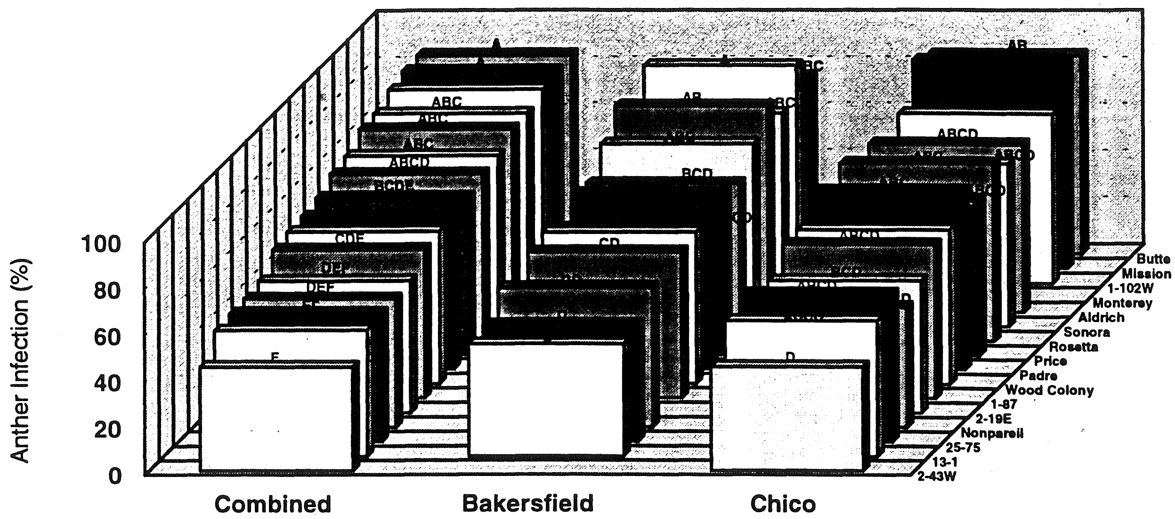
Field Evaluations



Blossoms were inoculated with conidia (30K spores/ml) in the field using an atomizer, bagged for 12 h, and evaluated for incidence of blossom blight after 3 weeks.

Fig. 5. Incidence of Anther Infection Caused by *M. laxa* in Detached Inoculated Blossoms of Selected Almond Varieties

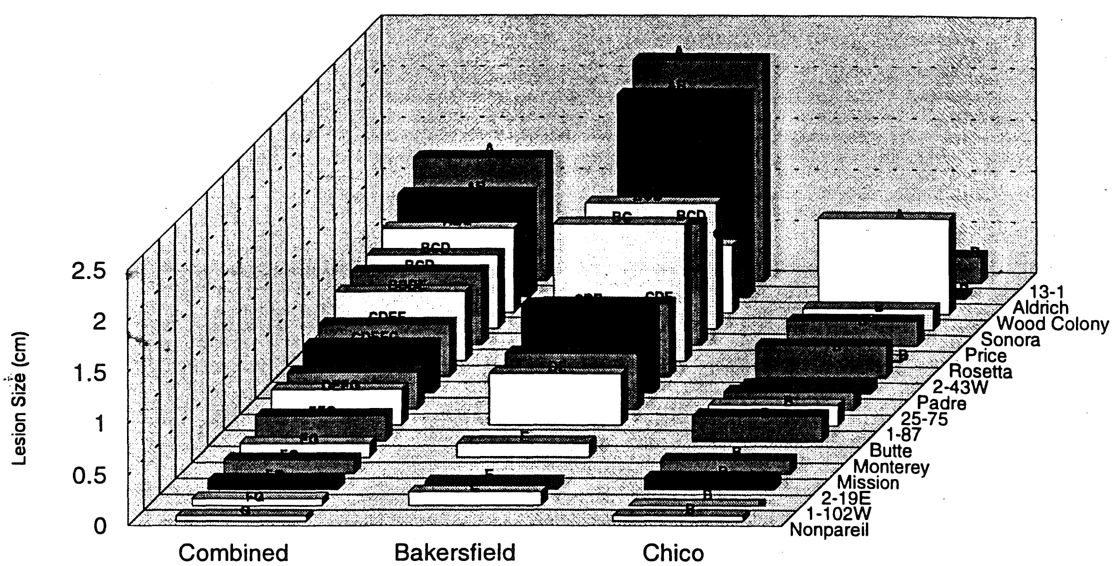
Laboratory Evaluation



Blossoms were detached, placed on wet vermiculite, and inoculated with conidia (30K conidia/ml) using an atomizer, and incubated for 3-5 days at 20 C. Incidence of anther infection = Infected anthers / Total anthers per blossom.

Fig. 6. Severity of Brown Rot Stem Infections Caused by *M. laxa* for Selected Almond Varieties

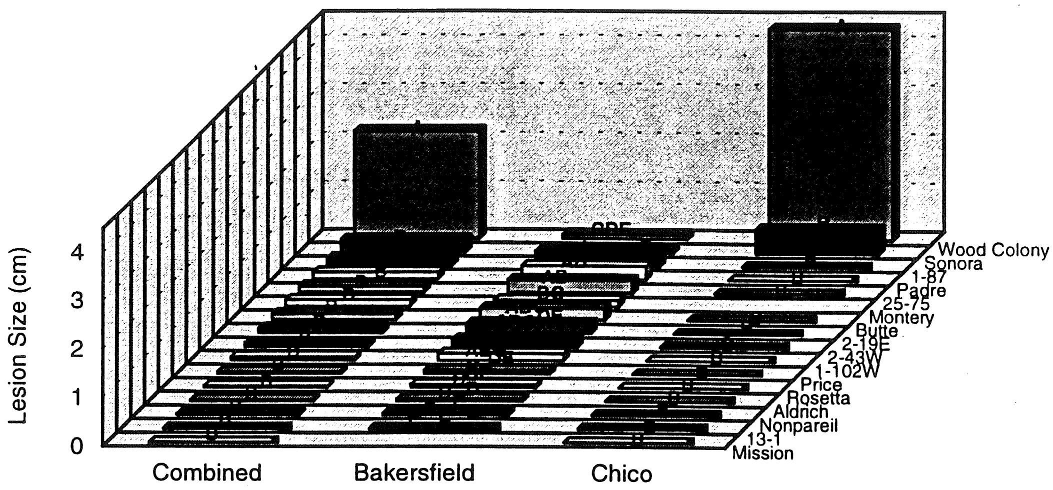
Field Evaluations of Pink Bud Inoculated Blossoms



Blossoms in pink bud stage of development were inoculated with conidia (30K conidia/ml) in the field using a syringe, twigs were cut 3 weeks after, and measured for twig canker size in the laboratory.

Fig. 7. Severity of Brown Rot Stem Infections Caused by *M. laxa* for Selected Almond Varieties

Field Evaluations of Full Blossom Inoculated Blossoms



Blossoms were inoculated with conidia (30K spores/ml) in the field using a atomizer, bagged for 12 hr, and measured for twig blight after 3 weeks.

A.

B.

C.

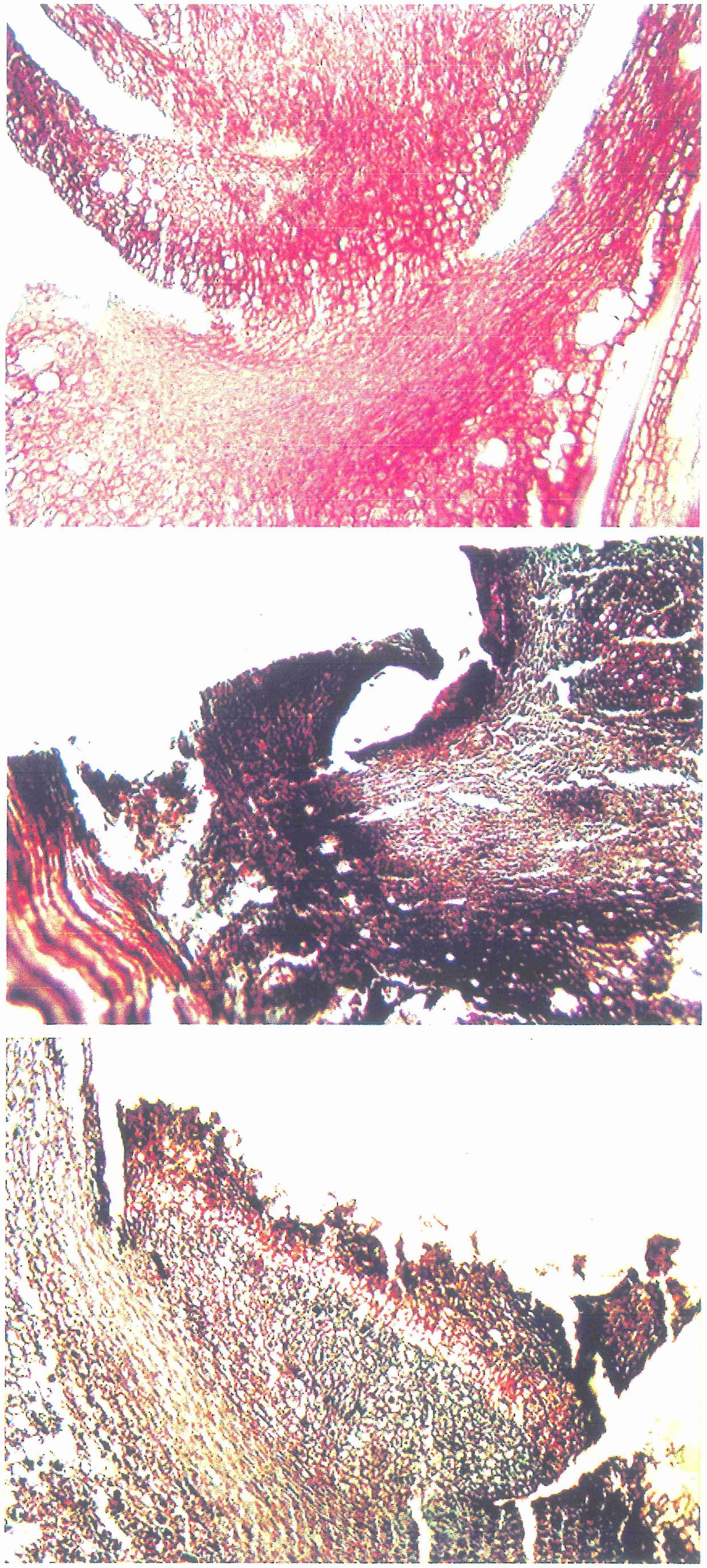


Fig. 8. Histological sections of almond blossoms and peduncles: A. Healthy Nonpareil peduncle and basal section of blossom; B. Brown-rotted Butte blossom with dead colonized tissue and no abscission or clear zone; and C. Abcisson and clear zone of non-infected Nonpareil peduncle. Brown rotted blossom has been abcissed.

Table 1. Efficacy of iprdione and myclobutanil treatments with and without the addition of an oil emulsion for control of brown rot blossom blight of Drake almonds - 1996.

Treatment*	Product Rates (/A)	Brown Rot Incidence (%)	LSD Grouping
Check	—	77.3	a
Rally	6 oz	19.7	b
Rally -Oil	6 oz / 2%	19.3	bc
Rovral	1 lb	22	bc
Rovral - Oil	1 lb / 2%	9.7	c

* - Treatment were applied using an air-blast sprayer callibrated for 100 gal/A. Fungicides were applied at pink bud and full bloom.

Table 2. Yield Comparison Between Fungicide Treated and Non-treated Almond Trees by Variety

Variety	Treatment**	Number of Rows	Yield (lb/tree)*	LSD
Nonpareil	Sprayed (Rovral 1lb + Oil 2%)	4	13.14	a
	Non-sprayed	4	12.55	a
Carmel	Sprayed (Rovral 1lb + Oil 2%)	4	13.54	b
	Non-sprayed	4	11.42	a
Monterey	Sprayed (Rovral 1lb + Oil 2%)	4	13.38	a
	Non-sprayed	4	12.25	a

* Average kernel weight was based on 19 to 20 trees from 4 rows
** Treatments were applied using an air-blast sprayer calibrated for 100 gal/A