## ANNUAL RESEARCH REPORT TO THE ALMOND BOARD 1992

Almond Fruit

Project No. 91-ZG2-

- (a) Relationship of Irrigation Cut-off Date to Occurrence of Hull Rot
  - (b) Etiology of Ceratocystis Limb Canker
  - (c) Bloom Disease Control and Survey of
  - Microflora Inhabiting Almond Flowers (d) Effect of Shot Hole Infection on

Project Leaders:

Dr. Beth L. Teviotdale (209) 891-2500 Dr. Themis J. Michailides Kearney Agricultural Center 9240 South Riverbend Avenue Parlier, CA 93648

Cooperating Personnel:

D. Harper, M. Viveros, and L. Hendricks

A. Relationship of Irrigation Cut-off Date to Occurrence of Hull Rot

Objectives:

1) Determine relationship of irrigation cutoff to moisture content of hulls and to incidence and severity of hull rot.

2) Determine the effect of moisture content of hulls on incidence, and severity of hull rot and search for a moisture status that minimizes hull rot while maintaining tree and crop health.

3) Relate water status of the tree to susceptibility to hull rot fungi.

Hull rot develops in late summer and causes death of fruiting wood and small branches. It is most severe on vigorous, heavily-cropped Nonpareil trees. The disease results from infection of the hull by any of these fungi: Monilinia fructicola, M. laxa (the brown rot fungi), Rhizopus stolonifer, or R. arrhizus (bread mold fungi). R. stolonifer and M. fructicola are the most frequent incitants of hull rot. Hulls are susceptible to infection as soon as they begin to split. The fungi enter through the natural opening, invade the inner hull surface and apparently produce a toxin that is transported into the spur or twig causing death of those tissues.

The incidence of hull rot may be reduced by reducing the interval between hull split and harvest (early harvest). Chemical control of the disease is not available and most likely will not be an acceptable option. Manipulation of cultural practices may prove to be the best chance for management of this disease. In 1989, differences in incidence of hull rot were observed among treatments in Dr. Dave Goldhamer's trial in which he investgated the effect of irrigation cut-off date on almond tree performance (Almond Board Project 89-11). Here, rot was consideralby more severe in trees having the three latest irrigation cut-off dates than in those deprived of water earlier. Our project is designed to examine more closley the relationship of irrigation cut-off date and incidence of hull rot with the hope of finding cultural practices consistent with productive tree culture that also will ameliorate damage caused by hull rot.

#### GENERAL PROCEDURES

#### The irrigation cut-off trial

The experimental orchard was located in Kern County and planted with cultivars Nonpareil and Carmel. The orchard was divided into six replications of eight treatments in a randomized complete block Three of the six replications received a post-harvest design. irrigation in 1989 and 1990, three did not. Our experiments were restricted to cultivar Nonpareil trees in the three replications receiving the post-harvest irrigations. Each replication of cultivar Nonpareil included eight trees, four trees in two adjacent Our experiments were conducted on any of these center eight rows. trees. Each treatment was an irrigation cut-off date, meaning that the trees in that treatment received no further irrigations beyond that date during the summer until the post-harvest irrigation in early fall. All trees received identical and standard irrigation until the experiment began. The irrigation cut-off dates were 25 June, 1, 8, 15, 22, 29 July, 5 and 12 August. These treatments are numbered 1 through 8 and correspond to 52, 46, 39, 32, 25, 18, 8, 11 and 4 days before harvest, respectively. (For further description of the irrigation experimental design and procedure, please refer to the report by Dr. Dave Goldhamer). The trees were harvested on 16 August.

#### Kearney Agricultural Center

Additional experiments were conducted on Nonpareil almond trees grown at the Kearney Agrucultural Center. These trees were 15 years old and uniformly flood-furrow irrigated.

#### Inoculum

The fungi used were isolates of R. stolonifer and Aspergillus niger obtained from almond. They were grown on acidified potato dextrose agar (aPDA) and inoculum was prepared by collecting spores in sterile deionized water containing a wetting agent (Tween 20). Spore concentration was adjusted to  $10^3$ ,  $10^3$ , and  $10^5$  spores/ml (R. stolonifer) and  $10^4$  (A. niger), and an average of .25 to .50 ml inoculum was delivered to each test fruit using an artists air brush. R. stolonifer was the test pathogen unless otherwise indicated. Percent germination was measured by counting germinated conidia in three groups of 100 conidia sown onto three aPDA plates and incubated at room temperature for 8 to 12 hours (R. stolonifer) and 24 hours (A. niger). Percent germination ranged from 92 to 100 percent.

EXPERIMENTS IN THE IRRIGATION CUT-OFF TRIAL

#### Percent hull moisture and percent hull split

On each inoculation date, 20 fruit from each plot were collected at random from each replication in all eight irrigation cut off treatments and examined for opening of the hull suture. Any opening of the suture was designated as split. Hulls were removed and fresh weight measured after which the hulls were air dried at 130F for 72 hr and reweighed. Percent hull moisture content was calculated. Fruit were assigned one of three categories, small, medium, and large, based upon the width of hull split. Hull split sizes were determined by measuring the splits, at the widest point, of 10 fruit representing each catogory. These hulls then were processed as described above for hull moisture content.

The percent hull moisture content and percent hull split of fruit taken as random samples from all plots increased with increasing numbers of irrigations (Table 1). Percent hull moisture declined from early hull split (17 July) through late full hull split (6 Aug) and the percent nuts having split hulls increased during this same time interval.

The percent moisture content of hulls of fruit having small, medium, and large splits exhibited relationships to irrigation cutoff and time that were similar to those described for the random samples (Table 2). Percent dead leaves decreased as hull split size increased.

#### Inoculation of fruit

Fruit were inoculated at early (17 July), mid (24 July), and full (30 July and 6 August) hull split. Hulls did not split in most fruit on trees in the earliest three irrigation cut-off treatments thus our inoculation experiments were conducted on trees in the later five cut-off treatments. Twenty-five fruit per replication were inoculated for each treatment. All fruit were removed from the tree during the four days preceding harvest. Each fruit was examined for presence of R. stolonifer or A.niger and leaves associated with each fruit were scored as dead or alive.

The effect of hull split size on infection was examinined by inoculating fruit having average hull splits of small, medium and large (3.3, 8.2, and 20.2 mm wide, respectively) splits with 10<sup>4</sup> conidia per ml. The experiments were conducted on 23, 30 July and 6 Aug in irrigation cut-off treatments six through eight. Percent infected nuts and dead leaves decreased as hull split size increased and increased with later irrigation cut-off dates (Table 2). Percent infected nuts decreased from 24 July through 6 August but there were no significant differences in the percentage dead leaves over this time period.

The effect of inoculum concentration on hull rot severity was examined by introducing conidia, at the three concentrations mentioned above, into the open sutures of nuts having small to medium hull splits. This experiment was conducted on all four inoculation dates in irrigation treatments four through eight. Increase in inoculum concentration was significantly correlated with increase in percent dead leaves but not with percent infected fruit (Table 3).

The influence of number of infected nuts on severity of shoot <u>dieback</u> was examined by inoculating clusters having 1, 5, 10, 15, and 25 nuts. The experiment was conducted on 23 and 30 July on trees in irrigaion cut- off treatments six through eight. There was no increase in the percent dead leaves with increasing numbers of infected fruit per cluster (Table 4).

The pathogenicity of A. niger was tested by inoculating fruits having small to medium sized hull splits with a suspension of 10<sup>4</sup> conidia per ml. The experiment was performed on all four inoculation dates in irrigtion cut-off treatments four, six, and eight. Some inoculated fruit developed lesions on the hulls but did not result in leaf or shoot death or any other apparent damange to the fruit (Table 5).

#### Incidence of natural infection

Clusters of withered or dry leaves on shoots was regarded as the symptom of hull rot. Shoots bearing one or two collapsed leaf clusters were designated as strikes, those having more than two collapsed leaf clusters were regarded as collpased shoots. The number of collapsed shoots, all individual clusters of withered leaves on collapsed shoots, and all independent strikes were counted on all data trees. The length of collapsed shoots was estimated for each tree. Data were collected on 16 August, the day trees were shaken. We collected samples of fruit from the harvested crop on the ground beneath the trees, and evaluated 100 fruit per replication for presence of R. stolonifer and A. niger. The greatest numbers of collapsed shoots, strikes on collapsed shoots, total stikes and inches dead wood were found in the two latest irrigation cut-off treatments (Table 6). The percentage fruit with hulls infected by R. stolonifer increased with increasing numbers of irrigations. Percentage of hulls infected by A.niger was not affected by irrigation cut-off. (Table 7).

#### EXPERIMENTS AT KEARNEY AGRUCIULTURAL CENTER

Two experiments were conducted at Kearney Agricultural Center on 9 August and repeated on 17 August. Fruit infection and leaf death data were collected on 4 September 1991. In one experiment, three methods of inoculation were compared: the suspension of  $10^4$  conidia per ml delivered by 1) artists airbrush or 2) hypodermic syringe (the method used in this work in 1990), and 3) application of dry conidia to the inner surface of the split hull. The third method occurred as follows. A tuft of sporulating mycelia from an aPDA culture plate was grasped with forceps and gently rubbed against the inner surface of the open hull. A small black spore mass could be seen with the naked eye. There were no significant differences among inoculation methods in average percent infected nuts on 9 August (Table 8). Variable significance was found among treatments on the 17 August inoculation date.

The second experiment compared the effect of hull attachment on incidence and severity of hull rot. Fruit having tightly attached or fully loosed hulls were inoculated with 10<sup>4</sup> conidia per ml with the artists airbrush. Noninoculated controls were included in all experiments and percent hull moisture was determined for tight and loose hulls as described above. There were no significant differnces between tightly or loosely attached hulls in percent infected hulls but there was significantly greater death of leaves associated with infected tightly attached hulls (Table 9).

#### DISCUSSION

Natural incidence of hull rot is dramatically influenced by irrigation cut-off date. By every measure, number of strikes, number collapsed shoots, inches wood killed, percentage infected hulls, the most severe hull rot was found in trees in the last two irrigation cut-off dates. This corroborates the data collected in 1990 and strongly suggests that cultural practices may provide control for this disease.

Similar responses were found in our inoculation dates, that is, hull rot increased in the later irrigation cut-off treatments. Two important observations emerged with respect to damage to leaves and They are 1) fruit with firmly attached hulls (those at shoots. early to mid hull split) are more likely to be associated with dead leaves than are those further along in the hull split process and 2) the amount of inoculum affects the amount of leaf damage even though the same percentages of hulls are infected. The attachment of the hull and the amount of fungal growth appeared to have a greater influence on leaf and shoot death than did mere numbers of Hull attachment or, put another way, infected fruit in a group. hull abscission and fungal growth both may be affected by environment and tree water status. Practices which hasten detachment of the hull and retard fungal growth could substantially reduce loss to hull rot.

### B. Etiology of Ceratocystis Limb Canker

Completed in 1990, replaced with Bloom Disease Control

Objectives:

1) Compare several bloom disease control options for efficacy and effect on yield and quality of almonds.

Bloom diseases, brown rot, shot hole and jacket rot, occur in years or areas of high spring rainfall and can cause serious loss in yield. Usually, fungicide efficacy trials examine the effects of individual materials on one disease. Although this provides much needed information about the activity of the fungicides and aids in decisions on which materials are useful in combating which diseases, most growers apply more than one fungicide during a season. There are several registered materials and thus several possible combinations to choose from. Our intent with this experiment was to compare the disease control capabilities and effects on yield of several combinations of treatments that might be used by growers.

#### PROCEDURES

The experiment is located in a commercial orchard in Kern County. The trees are mature, about 10 years old, and are planted 1 Nonpareil:1 Mission:2 Nonpareil. Merced:2 Six fungicide combinations were selected on the basis that any may be expected to provide some control of the three major bloom diseases and all the The combinations and a nontreated materials are registered. control comprised seven treatments which were replicated four times and arranged in a randomized complete block design. There were approximately 50 Merced and 100 Nonpareil trees pre replication (one and two rows of Merced and Nonpareil, respectively). Trees will be treated with the same materials every year for five consecturive years, beginning in 1989. Disease and yield measurements are taken each year.

#### RESULTS

Brown rot and botrytis jacket rot were not observed in trees of either cultivar. Shot hole was present at higher incidence than in the past. All treatments were significantly better than the control and there was significantly less infection in treatments 1,2 and 6 than in treatments 3,4, and 5 but there were no significant differences in average pounds nutmeats per tree or percent shriveled, laquered or NOW infested nuts among treatments (Table 10).

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#### DISCUSSION

Shot hole incidence appears to be increasing over the years in the untreated control. The very dry weather experienced during this experiment has kept all diseases at bay. To date, disease control programs have not increased yield or quality over untreated controls.

C. Bloom Disease Control and Survey of Microflora Inhabiting Almond Flowers

Objectives:

1) Identify naturally occurring microflora of almond bloom in traditionally and "organically" farmed almonds and screen candidates for biological activity.

Almond culture in California includes one to several annual applications of fungicides during and shortly after bloom. The fungal diseases controlled include brown rot (Monilinia laxa), shot (Wilsonomyces carpophilus), green fruit rot (Botrytis hole (Fusicladium cinerea), scab carpophilus) and leaf blight Recently, much interest has developed in (Hendersonia rubi). organic or sustainable systems which do not include use of disease control materials. Differences in the amount of disease might be expected between organically and conventionally farmed orchards and these farming systems also may affect the population of naturally occuring microorganisms which inhabit almond flowers and fruit. Because pathogens operate within this population of naturally occuring microorganisms, effects on the microflora may influence pathogens and disease development. Furthermore, biological control agents may be present which could aid in disease control.

We monitored populations of fungi and yeasts on flowers and fruit of almond trees grown using organic and conventional farming practices. Our objective was to detect differences in the mycoflora populations on these tissues between the two orchards and to identify any bacterial or fungal antagonists that might be present.

#### PROCEDURES

The observations were made in two commercial almond orchards in Merced County, California in 1990 and 1991. The orchards were adjacent (separated by a county road), planted with alternate rows of cultivars Nonpareil and Carmel, flood irrigated and had cover crops. Irrigation water was drawn from wells and canals and contained about 100 units of nitrogen per four acre-feet of water. The conventionally farmed orchard (conventional orchard) was planted in 1978, regularly pruned, fertilized with composted chicken manure, treated anuually with one zinc nutrient spray and one dormant application of copper, oil and diazinon and one or two bloom sprays of Rovral. The tree rows were strip sprayed with herbicide and the cover crop consisted of annual bluegrass, clover, filaree, and chickweed, and reached a height of 8 to 12 inches before mowing. The organically farmed orchard (organic orchard) was planted in 1980, lightly pruned every few years and was not fertilized or treated with other nutrients or pesticides. The cover crop was primarily vetch and ripgut brome with some chickweed and mustard, and attained a height of 18 to 24 inches prior to mowing.

Each orchard was divided into four quadrants, each quadrant containing five rows each Nonpareil and Carmel, each row was ten trees long. Every other Carmel tree was tagged (twenty five trees per quadrant), and samples were collected from these trees at approximately three-week intervals, beginning at full bloom and ending in mid July. One hundred flowers (four per tree) or 50 young fruit (two per tree) per quadrant were collected in plastic bags and transported to the laboratory. Within two hours of collection, samples were placed in plastic wash bottles, covered with 100 to 500 mls sterilized deionized water to which 0.1ml per 100 ml wash water of 0.1% tween 20 was added, and shaken on a rotary shaker for 30 minutes. Three 10-fold serial dilutions were made of the wash water and 0.1 ml of each dilution spread over each of three acidified potato dextrose agar culture plates. Similar aliquots of the most dilute wash water were seeded onto three nonacidified potato dextrose agar culture plates to detect presence of antagonisitic bacterial growth. Plates were incubated at room temperature (20 to 23C) for 5 to 7 days. Fungal and yeast colonies were counted on plates of the dilution that produced the greatest number of discrete colonies. The average number of colonies from the three plates of that dilution was used to calculate the number of propagules per fruit. Rainfall data were obtained from a weather station operated at Cressey California, approximately ten miles from the orchards.

Disease assessments were made by examining trees for symptoms of brown rot or green fruit rot in April and May during routine sample collections. Sample fruit were inspected each collection date for symptoms of shot hole and scab. In 1991, 100 leaves per quadrant (four per tree) were collected on 9 and 30 May for shot hole and 15 July for scab evaluations, respectively. Severity of shot hole was measured by counting the number of spots per fruit and leaves were rated as healthy, moderately or severely infected. Scab fruit lesions were generally too numerous to count and many had coalesced so fruit were rated on an ascending disease severity scale from one(healthy) to four (severely infected). Scab lesions per leaf were counted.

#### RESULTS

The most common fungal species were Cladosporium, Penicillium, and Aspergillus (Table 11). Other fungi included the genera Alternaria, Eppicoccum, Mucor, Fusarium, Botrytis, Paecilomyces, Coniothyrium, Botryosphaeria and several unidentified Phoma, species. The plant pathogens M. laxa and W. carpophilus were not found in either year. Yeasts also were present and in far greater numbers than the fungi. The dominant yeasts were Aureobasidium and two other species, one which formed a small, opaque creamy white colony, the other a small bright orange colony. The data can not be statistically analysed but generally, there were no blatant differences in the number or composition of the mycoflora between the two orchards. The populations in both orchards increased from bloom through mid summer and there were higher counts of propagules in 1991 than in 1990. Rainfall from 1 March through 31 July was 3.21 inches in 1990, half of which (1.67 inches) fell during the last week of May. In 1991, 6.50 inches rain were recorded for the same period and 84% (5.46 inches) fell in March. The average rainfall for these months is 3.5 inches. The exceptionally wet weather during bloom and early fruit growth may have promoted rapid increase in populations that resulted in higher numbers of microorganisms for that season. Bacteria were present at each collection date but no antagonism was observed.

Brown rot and green fruit rot were not observed in either orchard in either year. (Leaf blight has not been reported from this area of the state.) The incidence of shot hole infections on fruit in both orchards in 1990 was low and similar and the disease was not detected on leaves (Table 12). Scab was not found on leaves or fruit. In 1991, shot hole infection of fruit and leaves was much greater in the organic than in the conventional orchard but the average disease severity rating of scab on fruit was similar, 2.5 and 2.6 from the organic and conventional orchards, respectively. Although scab lesions could be found infrequently on leaves in the orchards, none were present on those picked for evaluation from either orchard. Shot hole caused some defoliation in the organic orchard, none in the conventional.

#### DISCUSSION

The mycoflora inhabiting the surfaces of flowers and fruit from these two orchards were remarkably similar. There were greater differences between years than between orchards. The most striking contrast was the much higher population of yeasts in 1991 than in 1990. To a lesser extent, Cladosporium and the group designated 'other fungi' also were favored by the increased rainfall.

The climate within these two orchards seemed quite different in many respects. Leaves were larger and darker green, the tree structure more open and shoot growth more vigorous in the conventional than in the organic orchard. The organic orchard was darker, more humid and generally cooler than was the traditional orchard. We often had to collect from the traditional orchard first because the fruit and leaves in the organic orchard remained wet until later in the morning. The minimal pruning in the organic orchard allowed less light into the trees and the lush cover crop grown there may have contributed to this lingering dampness. These apparent dissimilarities in orchard climate, along with the use of fungicide during bloom, did not appreciably alter the composition of the mycoflora in the conventional orchard in contrast to that in the organic orchard.

The higher incidence in shot hole and scab found in 1991 than in 1990 can be attributed to the above average rainfall in 1991. The conventional orchard was treated with the fungicide Rovral which is effecitve against the shot hole fungus but not the scab organism. Hence the reduction in shot hole infection in the conventional orchard but comparable levels of scab infections present in both orchards. There appeared to be no connection between mycflora populations and disease incidence.

These data were not collected from an experiment and thus cannot be analysed statistically. Valid experiments that can be analysed would greatly benefit the study of nonconventional farming systems. The practical obstacles to this are many, including the cost of orchards large enough to include replicated plots of sufficient size and number to be useful. Reliable information on comparative farming methods awaits such experimentation.

## D. Effect of Shot Hole Infection on Almond Fruit

Objectives:

1) Determine the stage of fruit development susceptible to susceptible to damage by the shot hole fungus.

2) Determine the importance on inoculum density on fruit infection and damage.

Shot hole, caused by the fungus <u>Wilsonomyces carpophilus</u> (aka <u>Stigmina carpophila</u>), produces lesions on leaves, fruit and occasionally young shoots of almond trees. When severe, leaf drop ensues, which may lead to fruit drop and chronic weakness. Fruit may bear many lesions on the hull without sustaining any apparent damage. Gumming is associated with fruit infection, but often the nutmeats of fruit with gumming hulls are healthy. The direct effect of shot hole infection on fruit has not been investigated.

#### PROCEDURES

The experiments were conducted on cultivar Mission almond trees at the Kearney Agricultural Center, Parlier, California. An environment of free moisture on fruit surfaces, a condition favorable to infection, was provided using an automatic misting system. Mist was produced for 1 to 5 minutes (longer periods were needed in late spring) at 10 to 15 minute intervals for 48 hours unless otherwise specified. Although some run-off from leaves was observed, in most instances the fruit surfaces were wet but water did not drip.

The fungus was grown on acidified potato dextrose agar for four to six weeks. On the day of inoculation, conidia were collected in sterile deionized water and concentrations adjusted to  $10^3$ ,  $10^4$ ,  $10^5$ , and  $10^6$  conidia/ml. The  $10^6$  conidia per ml concentration was included on the last four inoculation dates only. Two 1.5 percent water agar plates were seeded with 0.1 ml of the  $10^4$  conidial concentration and incubated for 24 to 48 hours at room temperature. The number of germinated conidia in three groups of 100 were counted. Germination ranged from 82 to 96 percent except on 2 and 9 April. No germination accurred in the 2 April inoculum thus this experiment was reinoculated 5 April with a suspension of conidia that had an 89 percent germination. Poor germination (27 percent) also occurred during the 9 April experiment.

Approximately 0.25, 0.50 and 1.00 ml of inoculum was applied to flowers, young fruit and expanded fruit, respectively, using a hand-held hand pump spray bottle. These inoculum concentrations and a noninoculated control comprised the treatments in each experiment. There were four replications, one in each of the four quadrants of a tree. During and shorly after bloom, 75 to 100 blossoms per replication, usually each replication located on one branch, were inoculated because the number of blossoms that would eventually become fruit could not be determined at the time of inoculation. As the season progressed and viable fruit could be identified, 25 to 35 fruit were inoculated per replication. These were located on one to three branches depending upon availability of fruit. Misting began immediately after inoculation. The length of 20 fruit taken randomly from the experimental tree was measured at each inoculation date. This procedure was repeated at weekly intervals beginning at full bloom (1 March) and ending when fruit were full sized (16 May). Usually, test trees were located in one row, and one tree, randomly assigned, was used on each inoculation Three trees were used on the 23 and 30 April inoculation date. Inoculated fruit were observed weekly for evidence of dates. infection, and on 14 June, all fruit were harvested, counted, and shot hole lesions counted.

#### RESULTS

No significant differences among inoculum concentrations were found

in percent fruit dropped, except on 16 May (Table 13). Significant differences were found among treatments in percent fruit infected (23, 30 April and 7 May) and in average number lesions per fruit (9, 23, 30 April and 7 May). There was no correlation (P=0.05) with average percent fruit dropped and inoculum concentration for any inoculation date. Significant correlation with average percent infected fruit or average number lesions per fruit and inoculum concentration date. Significant correlation dates 23, 30 April and 7 May.

Significant differences among misting time treatments were found for average percent fruit dropped and average number lesions per fruit, but not for average percent infected fruit (Table 14). Correlations calculated with and without noninoculated control data were not significant for all three parameters measured on both dates.

#### DISCUSSION

We did not observe the increased drop of young inoculated fruit that occurred in 1989 and 1990. According to past experience, the stage at which fruit drop, if inoculated, is an approximately two week period during and shortly after emergence from the shuck (fruit length 14 to 26 mm). This occured during the two weeks of 2 and 9 April in 1991 when we had problems with germination of the inoculum. Also, bloom was early, rapid, short and accompanied by cold wet weather that inhibited bee activity and was inimical to pollination. Consequently, natural fruit drop was particularly severe and more than 80 % of the experimental fruits were lost to drop for the 5 April inoculation date. Later inoculations (23 April through 16 May) were accomplished with no technical difficulties. However, the window of susceptibility had passed. Fruit drop was not affectd by inoculation or inoculum concentration and disease incidence (percent infected fruit) and severity (number lesions per fruit) increased with increasing inoculum. Susceptibility to infection decreased rapidly when fruit reached full size as can be seen by the dramatically lower disease incidence and severity found between 7 and 16 May.

The experiment on misting duration had to be conducted after the drop susceptible stage of fruit development, for reasons mentioned earlier, thus very little can be derrived from this experience. Even though significant differences were not found, there was a trend toward increased disease incidence and severity with increased wetness period on both dates.

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	Irrigation date	cut-off duration (days)	X Percent hull moisture <sup>a)</sup>	X Percent hull <sup>a)</sup>
1	25 June	52	71.4	5.4
2	1 Jul	46	74.5	25.6
3	8 Jul	39	73.2	21.6
4	15 Jul	32	76.0	38.8
5	22 Jul	25	75.2	60.4
6	29 Jul	18	75.9	69.4
7	5 Aug	11	79.0	70.4
8	12 Aug	4	79.9	61.4
Anov r (F	P = 0.05 = 0.05		.000	.000
Inocula	tion date			
1 2 3 4	17 July 23 July 30 July 6 August		80.1 77.6 75.3 69.6	16.9 45.8 54.7 59.0
AN	IOVA, P =		.000	.000
r (	P=0.05)=		767	.503

Table 1. Effect of irrigation cut off on percent hull moisture and split of almond. 1991. Kern County.

<sup>a)</sup> Twenty fruit per replication, three replications per treatment.

	$\overline{X}$ Percent infected nuts <sup>1</sup>	X Percent dead leaves	X Percent <sup>a)</sup> hull moisture
Hull split size			
Small	56.3	62.3	78.5
Medium	50.4	41.6	78.1
Large	34.4	19.4	76.9
P =	.000	.000	.005
Irrigation cut-off		10 v. 147 100	
29 Jul	31.2	32.4	75.7
5 Aug	42.6	42.7	78.5
12 Aug	67.3	48.3	79.2
P =	.000	.085	.001
Date inoculated			
24 July	55.6	42.2	79.7
30 July	43.5	43.9	79.5
6 Aug	41.9	37.3	74.3
P =	.016	N.S.	.000
		· · · · · · · · · · · · · · · · · · ·	
Interactions $(P =)$			
Irrigation x Siz	e N.S.	N.S.	N.S.
Irrigation x Dat	e .002	.016	.000
Size x Date	.411	.318	.002
Trigation x SIZ	215	NC	272
x Date	. 515	N.J.	. 212

Table 2. Effect of hull split size on incidence and severity of hull rot of almond and percent moisture content of hulls. 1991. Kern County.

<sup>a)</sup> Arcsine analyzed, actual presented.

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Table 3. Effect of inoculum concentration on incidence and severity of hull rot of almond. 1991. Kern County.

Inoculum concentration (conidia/ml)	$\overline{X}$ Percent infected nuts	$\overline{X}$ Percent dead leaves
$10^{5}$ $10^{4}$ $10^{3}$ Control	44.6 35.6 39.6 13.7	48.5 46.2 39.9 14.5
ANOVA, $P =$ r (P=0.05) = r 10 <sup>5</sup> -10 <sup>3</sup> , no check (P=0.	.000 813 .05)=422	.000 904 918
Irrigation cut-off		
15 Jul 22 Jul 29 Jul 5 Aug 12 Aug	16.1 21.6 34.8 42.5 51.9	16.3 40.8 48.8 41.0 39.4
ANOVA, $P = r (P=0.05) =$	.000 .994	.033 .602
Inoculation date		
17 Jul 24 Jul 30 Jul 6 Aug	35.6 28.8 36.0 33.2	46.5 32.1 48.3 22.2
ANOVA, $P = r$ (P=0.05)=	.279 234	.000 653

9 • 12<sup>2</sup>

·		24 J	uly	30 J	uly
			Average	e percent	
Irrigation cut-off date	# Nuts/ cluster	Infected nuts	Dead leaves	Infected nuts	Dead leaves
29 Jul	1	46.6	50.0	66.7	91.0
	5	55.0	83.3	63.3	100.0
	10	70.0	55.6	38.3	60.0
	15	41.4	63.6	34.3	90.3
	25	49.9	62.4	43.8	61.5
5 Aug	1	64.2	9.6	33.3	0.0
	5	66.7	66.7	57.8	83.3
	10	70.4	51.2	46.2	33.3
	15	41.3	31.0	55.3	70.4
	25	46.7	20.1	33.8	36.7
12 Aug	1	50.0	18.7	1.5	12.7
	5	90.0	50.0	73.3	66.7
	10	53.6	37.5	80.0	61.1
	15	53.4	-	69.8	80.0
	25	76.1	35.8	44.9	65.4

Table 4. Effect of number of infected fruit on severity of hull rot. 1991. Kern County.

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	Average	percent <sup>a)</sup>	
Irrigation	Infected	Dead	ç
cut-off date	hulls	leaves <sup>b)</sup>	
15 Jul	79.3	13.8	
29 Jul	61.3	24.5	
12 Aug	73.0	21.8	
ANOVA, P=	.219	.161	
Inoculation	Infected	Dead	
date	hulls	leaves	
17 Jul	82.0	22.2	
23 Jul	72.7	22.2	
30 Jul	67.3	22.2	
6 Aug	62.9	13.5	
ANOVA, P=	.168	N.S.	
Irrigation x date, P=	.040	.345	

Table 5. Infection of almond hulls inoculated with <u>Aspergillus</u> <u>niger</u> and association with dead leaves. 1991. Kern County.

<sup>a)</sup> Arcsine transformed data analysed; actual values presented.

<sup>b)</sup> <u>Rhizopus</u> stolonifer also present in associated fruit.

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Table 6. Effect of irrigation cut-off date on amount of shoot death of almond trees naturally infected with <u>Rhizopus stolonifer</u>, causal agent of hull rot disease. 1991. Kern County.

				X Number			
<u>Irrigatior</u> date	<u>n cut-off</u> duration	Inches dead wood	Collapsed shoots <sup>a)</sup>	Strikes on collapsed shoots <sup>b)</sup>	Strikes alone <sup>c)</sup>	Total strikes	
25 Jun 1 Jul 8 Jul 15 Jul 22 Jul 29 Jul 5 Aug 12 Aug	52 46 39 32 25 18 11 4	8.0 18.0 24.3 28.0 63.0 83.0 671.3 662.0	0.3 1.3 1.7 4.7 4.0 7.3 42.3 37.3	4.7 4.3 11.3 24.7 20.0 44.7 314.3 259.3	8.3 30.7 11.0 20.0 36.3 71.0 284.7 362.7	13.3 36.3 24.0 49.3 61.0 123.0 641.3 659.3	
ANOVA, P= r (P=0.05)=	:	.000 .80	.000 .86	.002	.000 .81	.001 .81	

<sup>a)</sup> Shoots or spur short, less than three inches long, with a maximum of two collapsed leaf clusters.

<sup>b)</sup> Individual leaf clusters present on collapsed shoot.

 $(\Box)$ 

<sup>c)</sup> More than two collapsed leaf clusters, short greater than three inches long.

Table 7. Effect of irrigation cut off date on percentage of almond hulls infected by <u>Rhizopus</u> <u>stolonifer</u> and <u>Aspergillus</u> <u>niger</u>. 1991. Kern County.

		X Percent frui	t infected with <sup>b)</sup>
Irrigation date	n cut-off duration (days)	% Rhizopus	% Aspergillus
25 June	52	0.0	17.7
1 Jul	46	7.0	32.3
8 Jul	39	0.3	28.0
15 Jul	32	7.7	34.0
22 Jul	25	6.7	32.3
29 Jul	18	9.0	31.0
5 Aug	11	16.3	30.7
12 Aug	4	31.7	32.3
Anov	va, P =	.000	N.S.
r (P=0	0.05) =	.760	.190

<sup>a)</sup> Irrigation ceased at weekly intervals beginning 25 June. Trees shaken for harvest 16 August.

<sup>b)</sup> Data collected immediately after fruit shaken from trees for harvest. All clusters of dead leaves ("strikes") counted on eight trees per plot. Three replications per treatment. Nonpareil almond trees grown in Kern County, California. One hundred fruit collected at random from beneath trees in each plot immediately after fruit were shaken from trees for harvest. Each fruit examined for presence of <u>Rhizopus stolonifer</u> and <u>Aspergillus niger</u>. Table 8. Comparison of three inoculation methods on infection of almond hulls with <u>Rhizopus</u> <u>stolonifer</u>. 1991. Kearney Agricultural Center.

	$\overline{X}$ Perce	nt infected nuts	
Inoculation methods	Inocula 9 Aug	ition date 17 Aug	
Air brush	65.2	95.3 ab	
Syringe	73.0	82.8 bc	
Dry spores	44.0	100.0 a	
Control	58.1	50.7 c	(8)
ANOVA, P=	N.S.	.017	

Table 9. Effect of almond hull attachment on infection by <u>Rhizopus stolonifer</u> and severity of leaf death. 1991. Kearney Agricultural Center.

x Percent	X	Percent
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Hulls	Dead leaves	Infected nuts	
firmly attached loosely attached	82.6 20.7	80.2 58.2	
ANOVA, P=	.007	N.S	
<u>Date</u> 9 Aug 17 Aug	50.9 52.3	60.6 77.8	
ANOVA, P=	N.S.	.022	

Table 10. Effect of several disease control funicide programs on shot hole disease, yield and quality of almond trees. 1991. Kern County.

Cultivar Merced

Shot Hole<sup>a)</sup>

Yield Quality

Treatment <sup>b)</sup>	X Percent infected nuts	X No. spots/nut	$\overline{X}$ Lbs. nutmeat per tree	$\overline{X}$ P shrivel	ercent nu laquer	its NOW	
1	31.7 a	0.1 a	25.1	2.5	0.2	9.2	
2	30.0 a	0.5 a	24.0	1.7	1.0	7.0	
3	51.0 b	2.4 b	27.1	1.7	1.0	5.5	
4	49.7 b	1.8 b	24.8	1.0	0.5	8.0	
5	53.8 b	2.6 b	24.9	1.2	1.0	4.8	
6	30.7 a	3.6 a	24.8	2.0	0.5	6.5	
7	73.2 c	6.4 C	25.6	2.0	1.0	8.2	
ANOVA, P = 0.05	+++	+++	N.S.	N.S.	N.S.	N.S.	
		Cultiv	ar Nonpareil				
1	4.0 a	0.1 a	22.3	1.6	1.3	2.1	
2	3.5 a	0.1 a	18.6	2.7	0.0	2.9	
3	37.2 b	1.8 b	31.3	1.8	0.0	3.4	
4	34.5 b	2.0 b	22.5	2.7	0.0	3.1	
5	48.0 b	3.1 b	22.4	1.9	0.0	1.9	
6	4.0 a	0.1 a	23.9	0.9	0.0	2.5	
7	67.5 c	5.1 c	25.1	1.2	0.0	2.7	
P = 0.05	+	+	N.S.	N.S.	N.S.	N.S.	

a) One hundred fruit per replication.

b) (see next page)

## Table 10. Continued.

b) Treatment code.

Treatment no.	Dormant	5-10% Bloom	FB - PP	≈ 5 weeks later	*
1		Captan	Captan	Captan	
2	Kocide	Captan	Captan	Captan	
3		Rovral	Rovral	Rovral	
4		Rovral	Rovral	Ziram	
5	Kocide	Rovral	Rovral		
6		Topsin/ Captan	Rovral	Ziram	
7	,	Cont	rol		

Four replications

Approximately 50 trees/plot (Merceds) and 100 trees/plot (Nonpareil)

Materials applied by grower, airblast sprayer

Shot hole evaluation: 100 fruit/rep (10 fruit from 10 trees) examined 23 June 1991

Brown rot: None observed

Green fruit rot: None observed

Table 11. Survey of mycoflora inhabiting flowers and fruit of Carmel almond trees in conventionally and organically farmed orchards. 1990-1991. Merced County.

		Cla	dosporium	n	Penicillium					
 Data		1990	1991		19	90	19	91		
collected <sup>b)</sup>	org <sup>c)</sup>	conv <sup>c)</sup>	org	conv	org	conv	org	conv		
1	0.422	0.438	0.490	0.168	0.000	0.002	4.300	11.825		
2	0.152	0.105	0.000	0.000	0.000	0.000	125.000	39.575		
3	1.020	1.158	25.450	21.000	0.000	0.005	0.000	0.000		
4	32.850	12.370	652.000	111.400	12.175	0.995	2.800	5.200		
5	88.075	21.121	145.950	107.200	0.000	1.350	116.900	8.475		
6	174.800	59.850	775.500	1545.250	0.000	84.000	40.250	18.250		

 $\overline{x}$  Number propagules/fruit  $(X10^4)^{a}$ 

Aspergillus

Other Fungi

Date	1990		1991		19	90	1991	
collected	org	conv	org	conv	org	conv	org	conv
1	0.000	0.000	1.268	4.975	0.047	0.282	0.015	0.000
2	0.000	0.000	0.075	0.000	0.052	0.015	1.000	0.650
3	0.000	0.000	0.000	0.000	0.098	0.022	0.675	3.000
4	0.313	0.000	0.000	0.000	0.773	0.537	12.400	12.800
5	0.000	0.150	6.175	0.150	1.550	0.375	3.700	1.425
6	0.200	0.225	0.000	0.000	1.625	0.175	47.250	35.000

Total Fungi

Yeast

Date		1990		1991		1990	1991		
collected	org	conv	org	conv	org	conv	org	conv	
1	0.470	0.497	6.075	16.950	1.595	3.280	5.150	7.700	
2	0.170	0.120	125.825	40.225	0.673	0.792	3315.000	5337.500	
3	1.118	1.185	26.125	26.700	0.160	0.740	1167.750	8778.000	
4	46.025	13.902	667.200	124.000	22.375	53.575	11968.000	9874.000	
5	89.600	22.996	429.200	117.150	181.000	80.300	10064.250	9138.000	
6	176.625	115.575	915.250	1580.250	200.600	130.400	10230.500	12082.000	

## Table 11. continued.

1.0

	Total Fungi and Yeast								
Data	199	90	1991						
collected	org	conv	org	conv					
1	2.065	3.778	11.225	24.650					
2	0.843	0.912	3440.000	5377.825					
3	1.278	1.677	1193.875	8804.750					
4	70.900	67.477	12635.250	8774.000					
5	270.350	103.296	10493.450	9255.150					
6	377.225	245.975	11145.25	13662.225					

# $\overline{x}$ Number propagules/fruit (x10<sup>4</sup>)

<sup>a)</sup> 400 flowers (first collection date only), 200 fruit per orchard.

<sup>b)</sup> Date collected	1990	1991	
1	8 Mar	8 Mar	
2	19 Mar	28 Mar	
3	9 Apr	19 Apr	
4	3 May	9 May	
5	24 May	30 May	
6	3 July	15 July	

c) Org = organically-farmed orchard, conv = conventionally-farmed orchard.

Table 12. Incidence of shot hole disease on Carmel almond trees in conventionally and organically farmed orchards. 1990-1991. Merced County.

	A Human		.0110/1	LULU	A 1010	Chie Int			
Collection	1990		1991		1990		1991		 
date	org <sup>b)</sup>	conv <sup>b)</sup>	org	conv	org	conv	org	conv	 
9 Apr 1990, 9 May 1991	0.0	0.0	1.3	0.2	0.0	0.0	26.2	2.0	
24 May 1990, 30 May 1991	0.5	0.3	4.6	0.4	13.0	11.0	67.5	5.6	
	x Leaf infection severity rating <sup>c)</sup>				x Percent infected leaves			cted	
9 Apr 1990, 9 May 1991	N.A. <sup>d)</sup>	N.A.	2.1	1.0	N.A.	N.A.	81.3	5.0	
24 May 1990, 30 May 1991	N.A.	N.A.	1.9	-	N.A.	N.A.	75.3	_e)	

 $\overline{x}$  Number lesions/fruit<sup>a)</sup>  $\overline{x}$  Percent infected fruit

<sup>a)</sup> Two hundred fruit per orchard.

<sup>b)</sup> Org = organically farmed orchard, conv = conventionally farmed orchard.

c) Four hundred leaves per orchard, rating system: 1= healthy, 2= moderate infections, 3= severe infection.

<sup>d)</sup> Leaf samples not collected; shot hole lesions not observed on leaves in orchard.

e) Herbicide-induced lesions on leaves obscured shot hole lesions, no counts made. Table 13. Effect of inoculum concentration of *Wilsonomyces carpophilus* on infection and drop of almond fruit, 1991.

				Ave	rage % F	ruit Dro	pped			
Inoculation concentration (conidia/ml)	1	March 7	18	2	Apr 9	ril 23	30	May 7	16	
10 <sup>5</sup> 10 <sup>5</sup> 10 <sup>4</sup> 10 <sup>3</sup> Control	90.8 93.8 92.5 90.6	94.2 89.2 94.7 91.6	76.5 79.1 81.4 82.3	85.6 85.8 89.1 89.2	18.3 19.6 11.4 12.6	5.4 10.4 5.3 8.3 4.5	7.7 14.7 7.9 20.8 13.9	3.2 4.6 1.4 8.1 1.2	12.5 7.8 4.4 0.0 2.1	
ANOVA, P=0.05 <sup>2)</sup> r =	N.S. -	N.S. -	N.S.	N.S. -	N.S. -0.19	N.S. -0.08	N.S. -0.41	N.S. -0.02	+ -0.56	
				Aver	age % Fr	uit Infe	ected			
10 <sup>6</sup> 10 <sup>5</sup> 10 <sup>4</sup> 10 <sup>3</sup> Control	7.8 8.5 4.2 15.8	8.9 21.2 0.0 11.5	14.8 18.2 10.7 17.4	8.6 11.5 18.0 13.9	28.0 4.8 10.5 7.1	48.1 45.2 21.2 16.5	66.9 23.0 10.5 10.9 8.3	35.5 61.4 46.5 24.0 9.6	79.71 12.9 7.8 8.0 2.4	
ANOVA, P=0.05 r =	N.S. -	N.S. -	N.S.	N.S. -	N.S. -0.05	N.S. -0.68	++ -0.79	++ .93	N.S. -0.30	
				Averag	je Number	Lesions	:/Fruit			
10 <sup>6</sup> 10 <sup>5</sup> 10 <sup>4</sup> 10 <sup>3</sup> Control	. 10 . 33 . 17 . 94	.10 .32 .01 .20	.58 .54 .32 1.18	.41 .28 .60 .58	1.10 .06 .22 .18	8.23 2.49 1.42 0.48 0.43	1.01 0.79 0.29 0.31 0.13	3.09 1.73 1.32 0.84 0.79	.52 .35 .18 .18 .04	
ANOVA, P=0.05 r =	N.S. -	N.S.	N.S. -	N.S.	+ N.S.	+ .73	++ .80	+++ .75	N.S. N.S.	

<sup>a)</sup> Cultivar Mission almond trees planted at Kearney Agricultural Center were used. One hundred flowers, then 25 to 35 fruit, per replication inoculated each date with conidial suspensions containing  $10^2$ ,  $10^3$ ,  $10^4$ ,  $10^5$ ,  $10^6$  conida per ml, approximately 0.25, 0.35, and 1.0 ml per flower, young fruit, or expanded fruit, respectively. All treatments, four replications per treatment, for each date located on a single tree date. Experimental sections of the tree misted for 1 to 3 minute periods at 10 to 15 minute intervals for 48 hours immediately following inoculation. Data collected 14 June 1991.

<sup>b)</sup> Where required, arcsine or square root transformations were performed on data for before nalysis; actual data are presented.

Misting duration	Av Dropp	verage per ed	cent fruit <sup>®</sup> Infe	Average Lesions per			
(hours)	23 Apr	30 Apr	23 Apr	30 Apr	23 Apr	30 Apr	
16	23.7	21.5	22.0	9.7	.76	.33	
24	34.0	0.0	23.1	16.7	.81	1.00	
48	10.4	4.7	48.1	23.0	2.49	.79	
48 (noninoculat	4.5 .ed	13.9	16.5	8.3	.43	.13	
		in					
P=0.05 <sup>b)</sup>	+	++	N.S.	N.S.	N.S.	++	
r =	.542	.212	.050	.420	.062	.296	
r = (without con	.322 trol)	.177	. 527	.085	.516	.030	

Table 14. Effect of misting duration on infection and drop of almond fruit inoculated with *Wilsonomyces carpophilus*, 1991.

<sup>a)</sup> Cultivar Mission almond trees planted at Kearney Agricultural Center were used. Twenty-five to 35 fruit per replication inoculated each date with a suspension of 10/5 conida per ml, approximately 0.5 ml per fruit. Four replications per treatment, each misting duration treatment located on a separate tree. Experimental sections of the tree misted for 1 to 3 minute periods at 10 to 15 minute intervals for 16, 24, and 48 hours.

<sup>b)</sup> Where required, arcsine or quare root transformations were performed on data for before analysis; actual data are presented.