Project Number 80-S6
Project Researcher: Ogawa

80-S6

OGAWA

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ALMOND BOARD

Hull Rot and Shot Hole

Annual Report

I. Objective

Shot Hole

1. Establish the best timing and application rates for effective fungicides.

2. Determine the efficiency of dormant copper as an alternative to spring sprays with other fungicides.

Hull Rot

To study the important steps of hull rot epidemiology and identify where effective control measures can be applied.

II. Interpretive summary

Shot Hole

We have completed the second year of a proposed multi-year study on Coryneum blight. Data, thus far, show no advantage of a dormant fixed copper application for Coryneum control. A pink bud application of ziram is as effective as a petal fall application for control of Coryneum on leaves.

Studies to determine if Coryneum is implicated in nut drop are not complete because drop was not a problem in 1979 and 1980.

Hull Rot

Continuing studies indicate that orchards with varying disease levels may have similar amounts of inoculum on leaves, hulls and in the soil. This leads to the conclusion that these inoculum sources are not a limiting factor in disease development.

If these compounds could be identified they could be of use in screening new almond varieties.

III. Expermental Procedure

Shot Hole

Nonpareil and Merced almond cultivars in Turlock, California were airblast sprayed, 100 gallons per acre, at either dormant, pink bud and/or petal fall with either copper oxychloride sulfate (COCS), ziram or captan. Each treatment consisted of approximately 20 trees with 3 replications. Disease evaluations were made on 200 leaves and 100 nuts on each of 3 trees per replication.

Hull Rot

Numbers of propagules on leaves and hulls

Eight random samples of hulls and leaves were collected from five orchards at 3 weekly intervals from 6-26 to 7-11-80. Twenty gram subsamples of leaves and groups of 10 hulls were washed twice with 100 ml sterile distilled water plus 0.25% Tween-20. The wash water was centrifuged at 3000 rpm for 10 minutes and the excess solution was poured off leaving about 10 ml of concentrated wash water. This was distributed 2 ml per plate, on *Rhizopus stolonifer* selective medium and incubated in darkness for 2 days before counting *R. stolonifer* colonies.

Insect transmission

1) Trapping: Insects were collected at 3 locations using traps described in the 1979 annual report. Eight catching and control trap pairs were distributed randomly in each orchard. Sterile mustard seed served as a control to see if airborne contamination occurred. Captured beetles were plated on PDA + 30 ppm each Streptomycin and tetracycline. When colonies began to grow those that were *Rhizopus* like were transferred to nutrient agar slants for identification. Equal numbers of control seed were tested in the same way.

2) Insect release studies: To establish whether nitidulid beetles carry inoculum to susceptible almond hulls it was necessary to contaminate groups of lab reared insects with a "marked" pathogen, release them in orchards and retrieve the pathogen from infected almond hulls. The marker used was tolerance to Botran, a fungicide normally toxic to *R. stolonifer* at 10 ppm. Groups of 300 nitidulid beetles, *Carpophiles freemoni*, were fed on 13 day old single spore cultures of 13dc6, a strain of *R. stolonifer* tolerant to 500 ppm Botran. Three lots of contaminated beetles were released in Fresno and Merced County orchards. Ten days later as many Rhizopus hull rot fruit as possible were collected from each of the areas where beetles were released. Rotting hulls from other areas were collected as controls. Isolations were made onto nutrient agar + 10 ppm Botran and colonies which grew were transferred and identified. All tolerant *R. stolonifer* isolates were compared to the original culture for 1) morphological characteristics and 2) response to different Botran levels.

Effects of hull rot on yield

A three phase program to determine the effects of hull rot on yield was initiated in 1979 (see 1979 annual report for methods). In order to get an estimate of yield loss the yield of each tree or branch was measured again in 1980 and compared individually, to 1979 values. In this way the variation between trees or branches was somewhat limited. The percentage yield loss was established with the equation:

$$\frac{\text{Yield 1979} - \text{yield 1980}}{\text{yield 1979}} \times 100 = \%\ \text{yield loss}$$

Yield loss values were compared to disease levels using regression analysis and best fit curves were established.

Effect of hull split period on disease

Five orchards in Merced and Fresno Counties were used to compare the length of hull split period with disease level. The locations chosen represented a range of hull rot severities in past years. The trees, variety Nonpareil, were chosen randomly at each site and after the onset of hull split records of % hull split and disease levels were collected every week to ten days. Hull split percentage was determined by counting 50 nuts per guadrant (200/tree) and disease levels were established by evaluating 50 ft of fruiting wood per guadrant for twig blight (200 ft fruit wood/tree). Values of total hull split days and disease were compared to see if there was an association between these factors.

Early harvesting to control hull rot

Ten pairs of trees were chosen randomly at the Freeman orchard in Fresno County. On 8-27-80 all 20 trees were evaluated for percent hull split and disease. These data were compared to make sure there were no differences between the trees at this time. One tree from each pair was shalsen and eight days later (8-4-80) the trees were re-evaluated for disease. The early and mature harvest trees were compared by analysis of variance to see if there was a significant reduction in disease.

Resistance of Mission variety

After preliminary inoculations showed that hulls of the Mission variety were not easily infected with R. stolonifer, water extracts of fresh hulls were made to determine if this was due to compound(s) in the hulls. 800 g of hulls (varieties Nonpareil and Mission) were surface sterilized for 4 min in 400 ppm NaOCl. Each sample was then blended for 1 min with 1L sterile distilled water. The mixtures were then filtered through sterile cheesecloth and Whatman No. 1 filter paper. 50 ml of filtrate from each variety was distributed to each of 8 sterile 125 ml flasks. Four flasks of each variety were inoculated with 20,000 R. stolonifer spores and all 16 flasks were incubated for

48 h on a rotary shaker at room temperature (N23°C). The non-inoculated flasks served as controls. The mycelial mats were collected and washed on pre-weighed filter papers. These were dried to constant weight at 90°C and weighed to establish mg dry weight of mycelial growth.

IV. Results

Shot Hole (see table 1)

A dormant fixed copper spray did not reduce Coryneum on hulls. A single captan or ziram sprays at either pink bud or petal fall were equally effective and both treatments were significantly better than the unsprayed check for control of Coryneum on hulls. A two-spray program, pink bud and petal fall, was significantly better than a single spray. All disease comparisons on the leaves were not significant at the 5% level.

Numbers of propagules on leaf and hull surfaces

None of the sample groups collected on any of the dates showed a greater amount of inoculum on leaves on hulls in orchards which went on to have severe hull rot. Table 2 illustrates the average number of *R. stolonifer* propagules on leaves and hulls on one of the dates as compared to the final average disease levels.

Insect transmission

1) Trapping: On the two sampling dates before the onset of hull rot, nitidulid beetles contaminated with *R. stolonifer* were collected at all three locations. In no cases were the sterile seeds used as controls contaminated (Table 3). This indicates that these insects are present in almond orchards and may be sources of primary inoculum.

2) Insect Release Study: At least one hull from each of the replications at the Merced County orchard (Sugiura) was contaminated with the Botran tolerant *R. stolonifer* strain. In Fresno County one hull out of all three replicates was contaminated. No control hulls were found to contain Botran tolerant *R. stolonifer* (Table 4). The low number of tolerant isolates was expected and since no controls were contaminated we conclude that contaminated beetles do visit and transmit the hull rot pathogen to healthy almonds in the field.

Effects of hull rot on yield

The experiments comparing natural hull rot levels and simulated disease by inoculation with yield loss showed significant yield reductions with increasing disease (figures 1 & 2). These curves though dissimilar in intercept are similar in slope. The inoculation experiment started with a higher yield loss level because individual branches were used. A whole tree has more chance for recovery since some wood without fruit one year (and therefore without hull rot) will become fruiting wood the next year. The figures show that low levels of hull rot (<20% FWK) probably do not cause economic yield losses.

Effect of hull split period on disease

Table 5 shows a trend of increasing hull rot with increasing hull split period. This means that any practice to reduce the amount of time that susceptible fruit is on the tree should reduce the level of hull rot damage. One possible way of achieving this was tested.

Effect of early harvesting on hull rot

Table 6 illustrates a reduction in disease from 47.5% to 13.7% by harvesting 10 days early. This shows that this practice may be effective in controlling this disease. The timing of this procedure needs to be further worked out to reduce the amount of green fruit left on the tree at harvest.

Resistance of Mission variety

Table 7 illustrates the difference in growth of *R. stolonifer* in water extracts of Mission and Nonpareil hulls. This difference is probably due to inhibitory compounds present in the Mission variety. Further work is needed to identify compounds that could be used in a breeding program or as biological control.

V. Discussion

Since the inoculum densities in orchards with and without hull rot are relatively equal attacking the initial inoculum level is not a good method of controlling hull rot.

The data on insect transmission indicate that protecting split hulls from visitation by nitidulid beetles should reduce hull rot levels. There is no known control for these insects at present.

It has now been established that hull rot can reduce yields. The yield data when considered with early harvest results would indicate that early harvesting could reduce hull rot to non-economic levels if properly applied. Other methods of reducing the hull split period could also be helpful in controlling hull rot.

If compounds responsible for resistance in the Mission variety can be identified they could be useful in screening new almond varieties and possibly biological control.

FIGURE 1

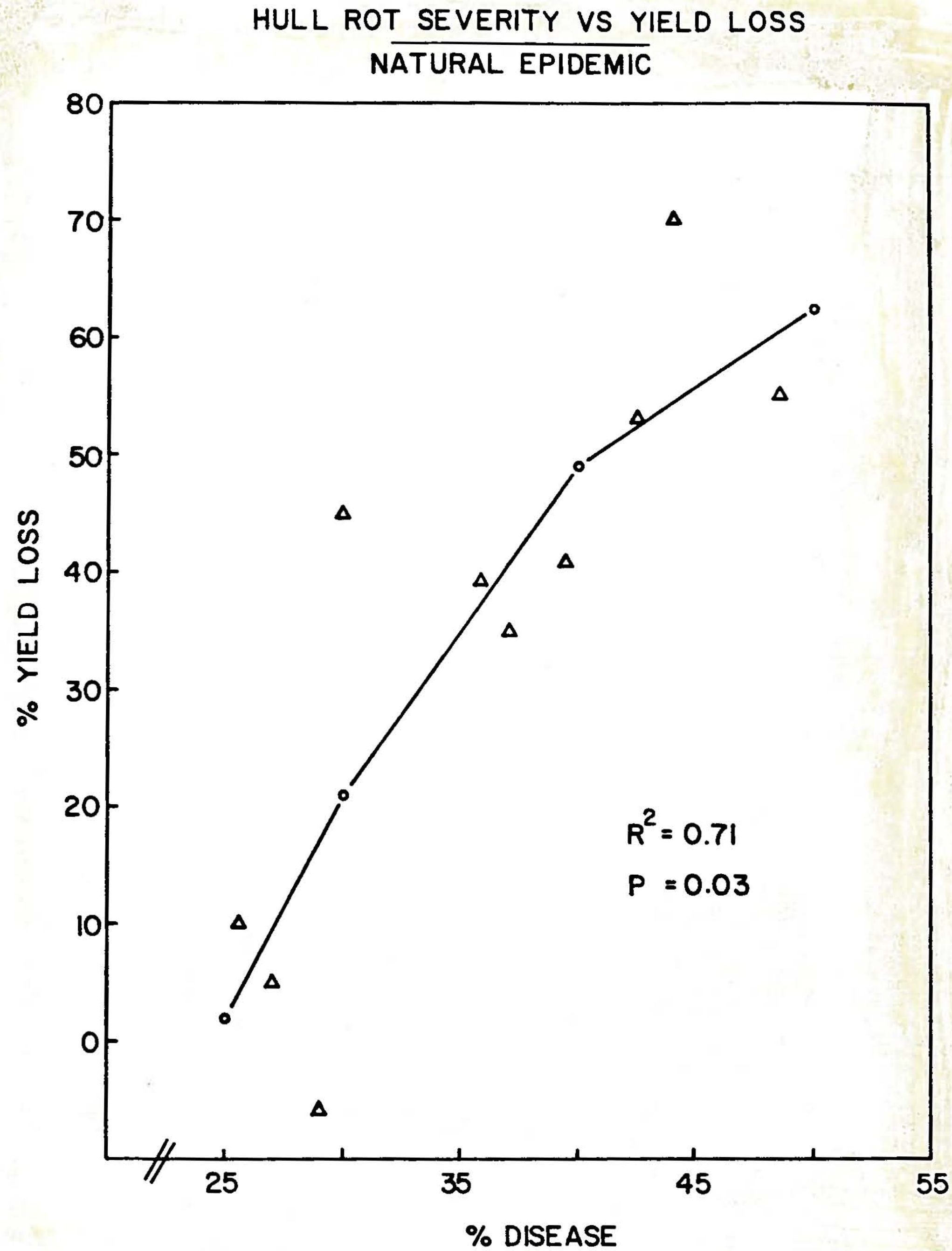
HULL ROT SEVERITY VS YIELD LOSS
NATURAL EPIDEMIC
% YIELD LOSS
80
70
60
50
40
30
20
10
0
25
35
45
55
% DISEASE
R² = 0.71
P = 0.03

FIGURE 2

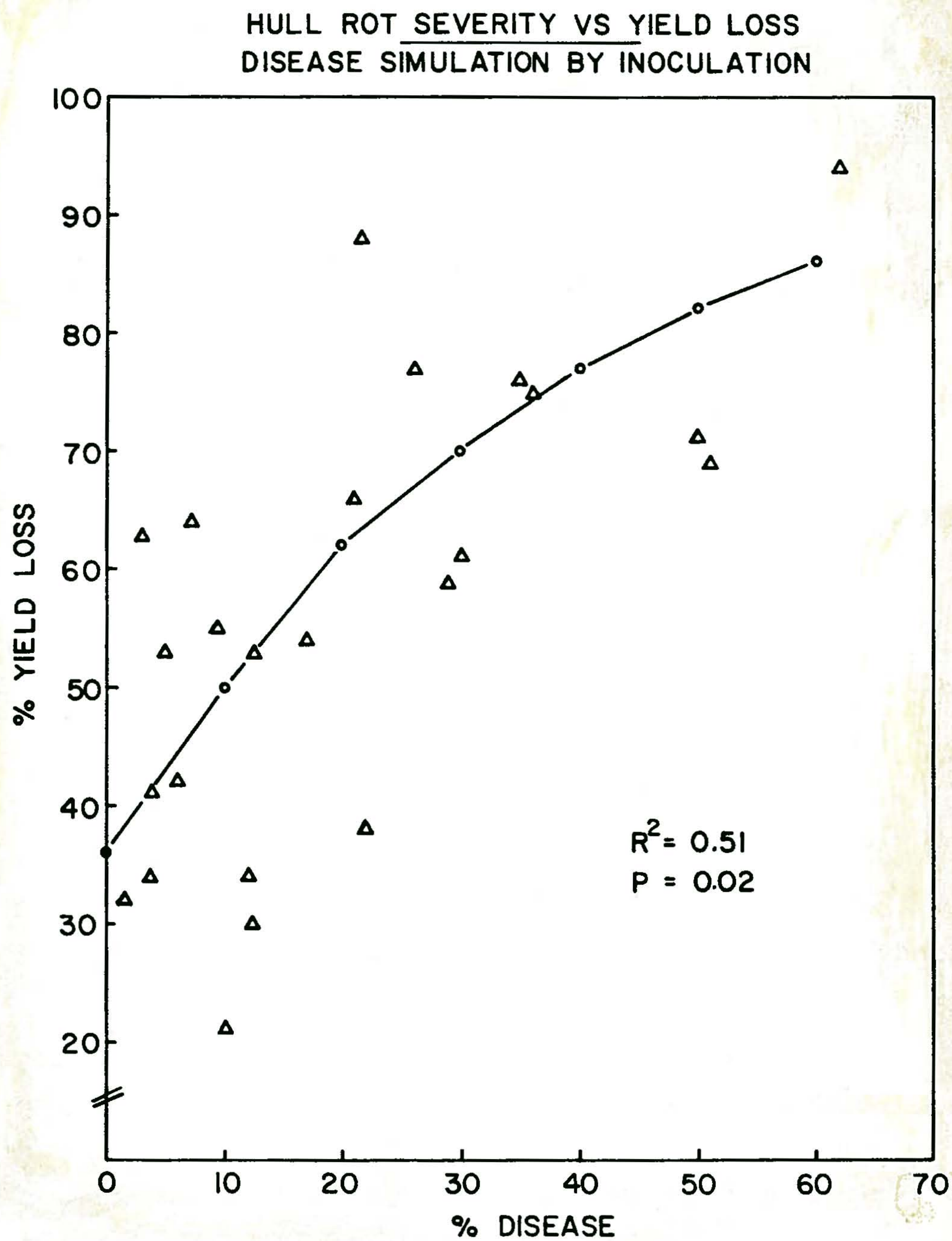
HULL ROT SEVERITY VS YIELD LOSS
DISEASE SIMULATION BY INOCULATION
% YIELD LOSS
100
90
80
70
60
50
40
30
20
0
10
20
30
40
50
60
70
% DISEASE
R2= 0.51
P = 0.02

Table 1

CHEMICALS AND APPLICATION TIMING ON INCIDENCE OF CORYNEUM BLIGHT OF ALMOND - 1980

Treatment[a]	Conc/acre	Application: Dormant 1/5	Application: Pink bud 2/12	Application: Petal fall 3/4	% leaves with Coryneum[b]: Merced	% leaves with Coryneum[b]: Nonpareil	% Hulls with Coryneum[c]: Merced
COCS 50%,	16 lb.	+	-	-	8.1	10.0	7.1 x[d]
Ziram 76%	8 lb.	-	+	+			
Ziram 76%	8 lb.	-	+	+	14.2	10.5	10.9 x
Captan 50W	8 lb.	-	-	+	15.1	10.8	34.2 y
Ziram 76%	8 lb.	-	-	+	12.6	10.9	35.7 y
Ziram 76%	8 lb.	-	+	-	10.2	10.3	37.8 y
Captan 50W	8 lb.	-	+	-	11.8	9.0	53.4 y
COCS 50%	16 lb.	+	-	-	10.8	8.7	87.4 z
Check	--	-	-	-	17.2	15.0	84.3 z

[a]Chemicals were applied with airblast sprayer, 100 gallons/acre. Each treatment had approximately 20 trees with 3 replications.

[b]Two hundred leaves were evaluated on each of 3 trees per replication on 3/25/80. Data not significant at the 5% level.

[c]One hundred nuts were evaluated on each of 3 trees per replication on 5/15/80.

[d]Numbers in vertical columns followed by the same letter are not significantly different, P = 0.05.

TABLE 2

NUMBERS OF *R. STOLONIFER* PROPAGULES ON LEAF AND HULL SURFACES COMPARED TO FINAL HULL ROT LEVELS

	original no. of propagules on:		
orchard no.	hulls	leaves	% FWK
1	0.2	0	48 a
2	0.2	4.2	43 a
3	1.2	5.0	28 b
4	0.2	5.4	6 c
5	1.0	3.0	1 c

TABLE 3

NITIDULID BEETLES CONTAMINATED WITH *RHIZOPUS STOLONIFER*

BEFORE THE ONSET OF HULL ROT

Location	Date	No. of Beetles Examined	No. Contaminated	No. of Controls Contaminated
Fresno Co. I				
	7-16	35	11	0
	7-23	16	6	0
Fresno Co. II				
	7-16	135	30	0
	7-23	136	28	0
Merced Co.				
	7-16	23	4	0
	7-23	2	1	0

TABLE 4

RETRIEVAL OF BOTRAN TOLERANT *R. STOLONIFER*

FROM NONPAREIL ALMOND HULLS

	No. Botran tolerant from:	
Location	release areas	control
Fresno County	1	0
Merced County	5	0

TABLE 5

HULL SPLIT DURATION VS. HULL ROT SEVERITY

Location	Hull split[+] period (days)	% Fruit wood killed[z]
Merced Co. I	50	43
Fresno Co. I	42	48
Fresno Co. II	41	28
Merced Co. II	30	6
Fresno Co. III	27	>1

[+]Mean of 10 replicates.

[z]200 feet of fruit wood evaluated per tree.

TABLE 6

EFFECT OF EARLY HARVESTING ON HULL ROT SEVERITY

	% Hull Split[+]	% Disease[+]
8-27-80		
Early harvest - shaken[z]	97[NS]	7[NS]
Late harvest	93[NS]	9[NS]
9-4-80		
Early harvest		13.7**
Late harvest	100	47.5

[+]Mean of 10 trees.

[z]Average of 8.5% of crop remained on trees.

**Significant difference at P=0.01.

TABLE 7

GROWTH OF RHIZOPUS STOLONIFER IN WATER

EXTRACTS OF ALMOND HULLS

Treatment	mg dry mycelium+
Mission	
inoculated	16.4**
control	0
Nonpariel	
inoculated	155.1
control	0

**Significant difference between Nonpareil and Mission at P=0.01 level

+Mean of 4 replicates

Project No. 80-S6
(Continuation of Project No. 79-T5)

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Project: Almond Diseases
Hull Rot
Shot Hole (Coryneum Blight)

Objectives: To develop control measures for hull rot by obtaining required critical information on the epidemiology of the pathogen.

To continue study of shot hole and determine role of fungicide sprays on control during the winter, early bloom and petal fall.

Progress: Studies on hull rot of almond since 1975 have indicated that hull rot can affect crop production through losses in fruiting wood, unharvested nuts and overwintering of the navel orangeworm (n.o.w.). The main hull rot pathogen has varied in the almond production regions but *Rhizopus stolonifer* continues to be one of the primary pathogens in all regions except during 1979 when it was absent in Butte County. *Monilinia fructicola* occurrence has been erratic. During 1979 *M. fructicola* was the only pathogen causing hull rot in Butte County while both *R. stolonifer* and *M. fructicola* occurred in Merced County. In Fresno County, *M. fructicola* occurred in other years but not during 1979. *M. laxa* continues to play a minor role in hull rot. Brown rot blossom blight, caused by *M. fructicola*, was verified in both Butte and Fresno Counties (Kearney Horticultural Field Station). Of the almond cultivars, Mission continues to escape damage from hull rot.

Extensive studies have shown that *Rhizopus* spores are commonly found on almond trees and in the soil and insects and environment play a role in transmission and disease development.

Coryneum blight (shot hole disease) was controlled with a single spray of ziram or captan during bloom in 1979. Blossom blight was not observed and leaf infections were minimal. A dormant sodium pentachlorophenate application appeared to have no benefits on fruit infections. Application of copper at delayed dormant needs further study. Two to three additional years of fungicide testing with applications during dormant, early bloom, and petal fall are necessary to establish the best timing for shot hole control. The chemicals to be tested are fixed copper, ziram and captan.

Plans: For 1980, critical studies are planned to delineate more precisely the role of insects and environment for infection and disease. It is felt that these two areas of study would best establish a foundation for disease control. For studies on insects, more surveys are planned to correlate the movement of beetles from the soil to newly cracked fruit and from diseased to healthy fruit. Further, it will be necessary to establish the source of *Rhizopus* spores for the initial contamination of insects, whether it is from the soil or from the almond tree. Insecticide sprays, such as malathion, will be used to determine if beetle control during the initial stages of hull crack would delay hull rot development. For the environmental studies, it is believed that relative humidity in the orchard affects not only the infection period but possibly insect movement. Both questions will be answered by selecting orchard sites with different relative humidities or using wind machines to change the relative humidity.

Further studies will be made to determine why the Mission cultivar escapes the disease. The main question to be answered is whether Mission hull is resistant to fungal infection or that toxins are not translocated into the shoots to cause sticktights and dieback.

Crop losses will be determined by pruning out certain percentages of fruiting wood to simulate losses from hull rot. Initial and continued studies on yield from these trees should provide evidence on possible crop losses resulting from shoot kill.

Tests will be continued with alternate fungicides to control blossom blight caused by _Monilinia laxa_ and _M. fructicola_. The purpose is to register new fungicides in the event of benomyl-resistant strains or the loss of captan through RPAR.

A survey is planned to evaluate the diseases in almond orchards. It is essential for each grower to identify his disease problems so he can properly manage the control program. As examples: Leaf blight appears to be moving farther south. Scab was serious in certain orchards in Butte County. Brown rot blossom blight and hull rot caused by _M. fructicola_ killed considerable amount of fruiting wood in Butte County.

Almond Industry Participation $14,450