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TITLE: HULL ROT OF ALMONDS 79 05 Correct Project Number 79-T5

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I. OBJECTIVES:

Study the epidemiology of the hull rot pathogens and develop an effective control measure.

II. INTERPRETIVE SUMMARY:

There are still no effective fungicides available for Rhizopus hull rot control and research into epidemiological factors which may point out alternative control measures continues.

The 1979 inoculum studies on leaves and hulls and in soils indicated that orchards with and without hull rot did not differ significantly. Therefore, reducing inoculum, such as propagules in the soil, may be ineffective in disease control. Continuing research on insect transmission has shown that Nitidulid beetles do spread the hull rot pathogen in some orchards. The severity of hull rot in these orchards could be reduced by controlling beetles.

To date basic studies indicate that orchards with hull rot problems are characterized by dense canopies. Trees in these orchards generally have closed centers and vigorous growth. The period from hull split to hull drying is extended in these orchards and hence for disease development. The environment within the dense canopy may also favor disease development. In 1980 experiments will be conducted to change the environment in vigorous orchards to confirm these observations.

Early harvesting is a possibility for controlling hull rot. Studies by Dr. Labavitch, Pomology Department, UC Davis, indicate that this method shows promise but is not yet applicable.

III. EXPERIMENTAL PROCEDURE:

1. Etiology (cause) of hull rot: For procedures consult the 1977 and 1978 annual reports. Rhizopus hull rot samples were collected from three orchards in Merced and Fresno counties in 1979. Each sample consisted of 50 hulls. Brown rot samples were collected in Butte and Merced counties in 1979.

2. Yield loss assessment: In 1979 a three phase program was initiated to evaluate the levels of hull rot necessary to cause significant yield reductions.

Phase 1: Pruning to simulate disease damage. In a Merced County orchard, pole shears were used to prune 0 to 60% of the fruiting wood from 40 trees. The amount of damage was estimated by the following equation:

$$\% \text{ fruit wood pruned} = \frac{\text{weight of nuts from prunings}}{\text{gross yield of tree}} \text{ X100}$$

To calculate the yield per tree the following steps were taken. Each tree was knocked with a commercial shaker and the nuts raked into a pile. The nuts were

run through a deleafer into a large plastic trash barrel and weighed (gross weight). The percentage meat weight of a 1 Kg sample was calculated as follows:

$$\% \text{ meat weight} = \frac{\text{meat weight sample}}{\text{total weight sample}} X100$$

To obtain the actual yield of a tree the gross weight was multiplied by the percent meat weight.

Phase 2: Inoculation to simulate disease damage. Zero to one hundred percent of the hulls on 34 limbs of the Nonpareil variety were inoculated with a spore suspension of R. stolonifer (20,000 spores/ml) at hull split. To estimate the percent fruit wood killed on each limb the following equation was used:

$$\% \text{ fruit wood killed} = \frac{\text{inches fruit wood killed}}{\text{total inches fruit wood}} X100$$

Disease levels ranged from 0 to 85% of the fruit wood killed per limb. The yield per limb was found by removing the nuts, shelling them by hand and weighing the meats on a laboratory scale.

Phase 3: Evaluation of naturally occurring disease. In a severely diseased orchard in Merced County ten Nonpareil trees were evaluated for percentage fruit wood killed. Two hundred feet of fruiting wood was counted per tree. The amount of disease occurring on these trees ranged from 27 to 48% fruit wood killed. The yield per tree was calculated as in phase 1.

In 1980 and 1981 the yield of the trees and limbs used in this study will be compared to the yield and disease levels of 1979. In this way we can establish what levels of disease are necessary for significant yield reductions.

3. Sticktights: Numbers of sticktights were observed on ten trees in two orchards in Merced County. Hull rot was severe in one orchard (average 36% fruit wood killed) but not in the other (average 5% fruit wood killed).

Further analysis of sticktights was made by inoculating 50 freshly split Nonpareil almond hulls with a 20,000 spore/ml suspension of R. stolonifer. At maturity nuts were removed with a pressure testing device. In addition to inoculated nuts, actual sticktights, healthy nuts and nuts on blighted shoots adjacent to inoculated nuts were removed also with a pressure testing device.

4. Insect transmission of Rhizopus hull rot: Investigations into insect transmission of hull rot continued in 1979. To determine if dried fruit beetles, family Nitidulidae, were carrying the primary inoculum for disease development eight traps were deployed at each of three locations in Merced and Fresno counties from mid-June to mid-August. Each trap consisted of a one pint plastic container placed within a one quart container. The bottom of the smaller vessel was replaced by 30 mesh stainless steel screening. In this way the insects caught were kept separated from the bait. Bait consisted of dried cull figs which were autoclaved for 15 minutes at 121° C, soaked overnight in sterile distilled water and inoculated with dry active yeast. Four figs were placed in the bottom of each quart container and covered with sterile distilled water. A one

inch square hole was cut in the lid of each trap to allow insect entry. A one half inch slice of Shell No Pest Strip was stapled to the inside of each lid to kill entering insects. Traps were suspended by coat hangers at eye level. At weekly intervals, beetles were collected and a maximum of ten per trap were transferred to potato glucose agar (PGA) plus 75 ppm each of streptomycin sulfate and chlorotetracycline HCl with sterile forceps. Controls consisted of sterile wheat seed in baitless traps to establish the extent of aerial contamination. Developing Rhizopus colonies were transferred to nutrient agar slants and speciated by methods described previously (1977 report).

Beetle visitation of split Nonpareil almond hulls was observed in an orchard in Fresno County. On 7/26, 7/31 and 8/6, before the onset of severe hull rot, 2500 randomly selected healthy hulls (split) were examined for the presence of insects. On these occasions a few diseased hulls (8m 45 and 60, respectively) were found and also examined. On 8/22, near the end of the hull split period, 150 each of healthy hulls and dried or green diseased hulls were examined for beetles.

On 8/22 three observations were made on each of 75 diseased hulls to further support the hypothesis of beetle transmission. First, the direction that the suture was facing was rated on a scale of one to five, one being up, three sideways and five down. Second, the hull split stage of infected hulls was rated on a one to five scale, one representing hulls that were beginning to split (but without separation) and five representing fully opened hulls. Third, the location of infections on hulls was rated by the distance, in centimeters, from the sutures.

Orchard transmission-exclusion experiments were conducted in Fresno County by the methods listed in the 1978 annual report.

5. Soil sampling: For methods refer to 1977 and 1978 reports. In 1979, 25 locations were sampled within each orchard.

6. Sampling of plant surfaces: One hundred, 5 leaf samples and single hull samples were collected into individual baggies from two Merced County orchards. Samples were placed into sterile plastic containers and 10 ml of PGA + 75 ppm each streptomycin and tetracycline was added to each baggie to cover the sample. Empty baggies were filled with 10 ml of this medium as controls.

7. Liberation of inoculum from soils: A cyclone spore collector was run during orchard floating to test the hypothesis that this operation liberates R. stolonifer propagules. Eight, two minute samples were taken into collecting vials containing 10 ml of sterile distilled water while the tractor was passing on the west side of the rows. After each sample the spore trap was cleaned thoroughly with 95% ETOH and dried. Control samples were taken when the tractor was not moving. The sample liquid was plated onto the selective medium used in soil sampling and analyzed for the number of R. stolonifer propagules.

8. Influence of environment on disease development: In Merced County, disease severity, general canopy condition and time from hull split to hull drying were observed in adjacent orchards. At a Fresno County location, the percent fruit wood killed was evaluated at the east and west edges as well as inside the orchard. One hundred feet of fruit wood were evaluated on 5 trees at two places on the east and one on the west side of the orchard.

9. Varietal susceptibility: Varietal susceptibility was evaluated in a Fresno County orchard with severe hull rot. The percentage twigs blighted and hulls infected were counted on ten trees each of the Nonpareil, Merced and Mission varieties.

IV. RESULTS:

Etiology of hull rot: In 1979 all of the Rhizopus hull rot samples were found to be R. stolonifer. The brown rot samples were all Monilinia fructicola.

Sticktights: Trees in the severely diseased orchard had about five times as many sticktights as those in nondiseased (Table 1). The average yield loss in the diseased orchard was calculated to be 20 lbs. per acre.

In the inoculation study actual sticktights were hardest to remove (Table 2) but were closely followed by nuts on blighted shoots caused by adjacent inoculated nuts. This occurs when shoots are blighted before dehiscence and abscission occur and seems to be the main source of hull rot related sticktights.

Insect transmission: Before the onset of hull split none of the beetle catch was significantly more contaminated with R. stolonifer than the sterile controls. By the middle of August, when Rhizopus hull rot had begun to develop, 83% of the Nitidulid beetles and 12% of the controls were contaminated (significant at P = 0.01 level) indicating that these insects are picking up the fungus from infected hulls and carrying it around.

Beetle visitation of healthy hulls was found to be rare with only three of the 7500 healthy hulls examined containing Nitidulids. Infected hulls were found to contain large numbers of Nitidulids especially Carpophilus freemani and C. lugubris while dried diseased hulls contained few (Table 3) indicating that the beetles do move out of infected hulls possibly to healthy ones. Supporting evidence for the vectoring of hull rot by beetles was gained by three observations made on 8/22. The direction of sutures of infected nuts was random (average 3.04) with downward facing hulls infected as frequently as upward facing ones. Thirteen of 75 hulls were infected at stage 1 (suture not yet separated) and on most of these a hole could be seen along the suture where at least one beetle had burrowed into the hull. These hulls, when opened, were found to contain at least one C. freemani beetle each. On the 75 hulls infections had been initiated, on the average, 1.5 cm from the suture. None of these three conditions should have existed if insect transmission of hull rot was not involved.

In orchard transmission-exclusion experiments, it was found that natural levels of disease developed in cages when 50 contaminated beetles, C. freemani, were released into each cage. In control-exclusion cages (no beetles released) the level of disease was significantly lower (Table 4).

The data presented support the hypothesis that Rhizopus hull rot is spread by Nitidulid beetles. The evidence is not yet clear whether these insects are the carriers of primary inoculum.

Sampling of soil and plant surfaces: Table 5 compares the levels of inoculum in the soil and on plant surfaces with the disease level in two adjacent orchards in Merced County. This study indicates no positive association between inoculum and disease levels and it can be concluded that reducing inoculum levels may not be the easiest way to reduce disease levels.

Liberation of soil-borne inoculum: Although there was a trend toward more inoculum in spore trap samples during floating, no statistical differences were observed. This study will be continued in 1980 with the additional use open petri plates and plant parts as assay methods to help increase precision.

Environmental influences: Observations in adjacent orchards in Merced County show an association between a dense canopy, longer hull split period and increased disease levels (Table 6). Trees on the open east and west edges of a Fresno County orchard had significantly less disease than trees in the interior (Table 7). The edge trees are both more open to air movement and exposure to sunlight. In 1980 we will attempt to elucidate the specific factors that contribute to the reduction in disease and relate these to possible control measures.

Varietal susceptibility: Observations in the past have indicated that hard-shelled varieties of almonds are most resistant to hull rot. Table 8 illustrates that even with high inoculum the Mission variety is nearly immune to the disease. This is possibly due to the fast separation of the hull from the peduncle and low susceptibility of the shell to infections.

Monilinia (brown rot) hull rot: New fungicides effective against brown rot blossom and shoot blight are becoming available (Table 9). Some of these compounds have systemic activity and will be tested for efficacy in controlling hull rot in 1980.

V. DISCUSSION:

Through studies on the life cycle of Rhizopus stolonifer and the effects of environment on disease development it is becoming clear where weak links in the disease chain are occurring. Although not finalized, studies on various inoculum sources indicate that inoculum may not be limiting. This means that reducing the initial inoculum may be a difficult and unfruitful path to control. Since dried fruit beetles are spreading the hull rot pathogen in some orchards their control should greatly reduce disease incidence. There is no effective control measure for these insects at this time.

The resistance of hard-shell varieties to infection and twig dieback offers a possible way of controlling hull rot. These varieties are also more resistant to insect damage (such as by NOW and peach twig borer) of kernals.

Environment appears to have a greater impact on the development of severe hull rot. Experiments are being planned to find ways to alter orchard environment and tree vigor to reduce hull rot without yield reduction.

For brown rot hull rot, fungicide sprays during bloom and at hull split will establish if M. fructicola overwinters on blighted blossoms and if systemic fungicides can prevent or suppress hull infections.

Registration of Topsin M for M. laxa control on almond blossoms is suggested.

Table 1. Evaluation of sticktight numbers in diseased and healthy trees.

	Mean disease level (% fruit wood killed)[a]	Mean number sticktights/tree[a]
Orchard 1	36**	94**
Orchard 2	5	19

[a]10 trees evaluated for disease and sticktights at each location.

**Significant difference at P = 0.01 level.

Table 2. Pressure necessary for removal of sticktights, healthy nuts and sticktights simulated by inoculation.

	Mean pressure for removal of nuts (grams)[a]
Actual sticktights	875 a*
On blighted shoots caused by inoculations	639 b
Inoculated	339 c
Healthy	214 c

[a]50 replicates.

*Different letters indicate significant differences at P = 0.01 level.

Table 3. Beetle visitation of healthy and green or dried diseased hulls of the Nonpareil variety.

	% hulls observed with Nitidulid beetles[a]
Green diseased hulls	37
Dried diseased hulls	2
Healthy hulls	2

[a]Sample size = 150.

Table 4. Insect vectoring of R. stolonifer exclusion and controlled transmission.

	% hulls infected[a]
Transmission[b]	86 a*
Control[c]	83 a
Exclusion[d]	16 b

[a]Average of 3 replicates.

[b]50 contaminated beetles (Carpophilus freemani) released into each cage at 50% hull split.

[c]Samples stripped from branches adjacent to exclusion cages.

[d]Exclusion cages on branches before hull split.

*P = 0.01 letters indicate probability groups.

Table 5. Comparisons of inoculum and disease levels in almond orchards.

	Orchard 1	Orchard 2
Propagules/100 g soil (R. stolonifer)	64.7	26.7**
# leaf samples/100[a] with R. stolonifer	14	16 ns
# hull samples/100[b] with R. stolonifer	9	8ns
Disease severity (% fruiting wood killed)	5	36**

[a]Each sample consisted of five leaves in an individual baggie.

[b]Each sample consisted of one closed hull in a plastic bag.

**Significant difference at P = 0.01 level.

Table 6. Environmental effects. Some characteristics of adjacent orchards.

	Orchard 1	Orchard 2
Disease severity[a]	5	36**
General canopy condition	Open	Dense
Time-hull split to hull drying (days)	33	50[b]

[a]Expressed as percentage fruit wood killed--average of 10 trees at each location.

[b]Orchard shaken at this time--green fruit were still present on some trees.

**Significant difference at P = 0.01 level.

Table 7. Environmental effects. Disease severity. East and west edges vs. interior of orchard.

Location		% fruit wood killed[a]
East 1	Edge	6.3**
	Interior	55.2
East 2	Edge	7.9**
	Interior	54.5
West	Edge	5.7**
	Interior	50.5
Overall average		
	Edge	6.6**
	Interior	53.4

[a]Five trees at each location--evaluated 100 ft. of fruiting wood per tree.

**Significant differences at P = 0.01 level.

Table 8. Evaluation of varietal susceptibility in an orchard with severe hull rot.

Variety	% twigs blighted[a]	% hulls infected[b]
Merced	100 a[c]	100 a
Nonpareil	55 b	--
Mission	<1 c	7 b

[a]Mean of 10 trees, 100 fruiting shoots per tree.

[b]Mean of three samples, 100 hulls each. Evaluated for infection by R. stolonifer.

[c]P = 0.01. Letters indicate probability groups.

Table 9. Evaluation of fungicides for brown rot (Monilinia laxa) blossom and shoot blight control of Drake almond.

Treatment[a]	Conc/100 gal	% shoot strike[b]
Ronilan 50W	6 oz.	4.3 x[c]
Bayleton 50W	4 oz.	7.8 x
Baycor 25W	16 oz.	11.0 x
Bravo 500 flowable	4 pt.	19.5 y
Check	---	53.5 z

[a]One blossom spray applied with hand-gun sprayer, 7 gal/tree at 10% bloom.

[b]Average of 100 spurs on each of four trees. Disease read on 3/22/79.

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

Shot Hole Disease of Almond

1. OBJECTIVES: Timing of fungicide sprays for control of shot hole disease on almond caused by Coryneum beijerinckii.

2. INTERPRETIVE SUMMARY: Leaf infections caused by Coryneum result in premature defoliation. Infections occur most frequently at initial leafing because of rains during this period. The best control has been the application of ziram or captan around shuck fall stage, followed by ziram when required. More recently, blossom infections by Coryneum have been observed in a few orchards. Blossom infections were important in the late 1930s and early 1940s. Dormant copper sprays have been applied by growers (late December or early January) to control shot hole, brown rot, and blast. Limited scientific data support its use for blast control. No data are available for shot hole. During 1979, test plots which included both dormant and blossom sprays showed that leaf infections can be effectively controlled with a single blossom spray of ziram. Shot hole blossom blight and blast, caused by Pseudomonas syringae, did not develop. Tests conducted in 1979 will be repeated in 1980 in an attempt to establish the benefits of dormant copper sprays to control plant diseases.

3. EXPERIMENTAL PROCEDURE: Same as in 1979.

4. RESULTS: Table 10 shows the data for the 1979 experiment where one ziram spray during bloom effectively controlled leaf infections caused by C. beijerinckii.

5. DISCUSSION: Leaf infection was adequately controlled with ziram sprays applied after leafing. The benefits of dormant copper sprays to control Coryneum and Pseudomonas needs to be established. Captan, the alternate fungicide for control of Coryneum, is under pre-RPAR and should receive support for re-registration.

Table 10. Chemicals and application timing on incidence of Coryneum blight of Nonpareil almond - 1979.

Treatment and timing[a]					
Dormant 12/8	Delayed dormant 1/25	Pink bud 2/27	Petal fall 3/14	% Leaves[b] with shot hole	% Fruit[c] with shot hole
	Cu	Captan	Captan	17.1 xy[d]	0.7 x
	Cu	Ziram	Ziram	8.9 x	1.0 x
	Cu		Ziram	22.6 y	3.3 x
	Cu	Ziram		16.1 xy	7.7 x
SPCP	Cu	Ziram		16.5 xy	14.7 x
Cu	Cu			32.7 y	42.7 y
SPCP	Cu			22.8 y	55.3 yz
	Cu			49.5 z	65.7 z

[a]Airblast sprayer, 100 gal/acre.

SPCP = sodium pentachlorophenate 76%, 16 lb/acre.
Cu = COCS, 16 lb/acre.
Captan 50W, 8 lb/acre.
Ziram 76%, 8 lb/acre.

[b]Percentage based on 12-8" shoots with three replications/treatment.

[c]Percentage based on 300 fruit with three replications/treatment.

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