## Project No. 77-Q3

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Project: Hull rot of almonds

Progress Report: December 6, 1977

Hull rot of almond was limited as compared to the 1976 season; this could be related to lack of summer rains during the hull split period. Severe hull rot resulting in shoot dieback was experienced in orchards with high vigor (excessive shoot growth) and in a test plot with high nitrogen fertilization; this could be related directly to increase in crop and thus the number of infection sites.

The primary cause of hull rot was identified as <u>Rhizopus stolonifer</u>; <u>Monilinia fructicola</u> was found in only a few orchards. Almond hulls of the Nonpareil cultivar were highly susceptible to infection from dry spore inoculation for 8 days after hull split but not susceptible after 14 days at which time the hulls were dry (tests made at UC Davis). A selective medium was developed to determine <u>Rhizopus</u> populations. <u>R. stolonifer</u> was isolated from orchard soils in Turlock from June through August (hull split during middle of July). Air samplings with the Anderson spore trap (one cu ft/min) for 15 min or open petri plates with selective media revealed one or two cultures per sampling. Isolations from leaves and freshly split hulls revealed only one percent of samples with <u>Rhizopus</u>. Examination of hulls with <u>Rhizopus</u> and <u>Monilinia</u> revealed an abundance of dried fruit beetles; whether or not beetles are essential in spread of Rhizopus spores has not been established.

Control of hull rot caused by <u>Rhizopus</u> and <u>Monilinia</u> was shown using a handgun spray treatment with Botran and Botran plus benomyl. Large-scale airblast spray applications with Botran or sulfur in three orchards failed to reduce twig kill or number of hulls infected with Rhizopus, indicating the lack of chemical coverage at the infection sites.

Further studies are necessary to establish the differences in amount of hull rot between vigorous, high-yielding orchards to less vigorous orchards, the role of insects in transmission of spores, and possible control of hull rot by soil chemical treatments or through cultural practices such as time of cultivation. PROJECT NO. 77-Q3 (Continuation of Project No. 75-Q)

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PROJECT: Hull Rot of Almonds - Annual Report

The importance of the hull rot project is related to loss of fruiting wood caused by toxin from diseased hulls and sticktight nuts which harbor the navel orange worm. For two years, study was directed to better understand etiology, epidemiology, and disease control through cultural and chemical means.

Additional information on etiology and control with chemicals was established in 1977. More information is needed on epidemiology.

<u>Etiology (cause) of hull rot</u>: Samples of 40 infected almond hulls were collected at random from three almond orchards (Freeman in Fresno County, Sugiura in Merced County, and Montgomery in Butte County). Isolations to potato-dextrose agar were placed at room temperature (21 C) and at 36 C. Those that grew at 36 C were considered nonsensitive to the fungicide, dicloran (Botran 75W), and could be either <u>Rhizopus</u> <u>arrhizus</u> or other high temperature <u>Rhizopus</u> spp. All cultures which did not grow at 36 C were considered to be either <u>R. stolonifer</u> or <u>R. circinans</u>. Cultures were examined and those with nodding sporangia were identified as <u>R</u>. circinans.

Isolations from diseased hulls were 96, 95, and 90% <u>R</u>. <u>stolonifer</u> at Freeman, Sugiura and Montgomery orchards, respectively. The remaining 4, 5, and 10% were the high temperature Rhizopus spp. resistant to dicloran.

During 1976 and 1977, severe brown rot infections at the Sugiura orchard were caused by <u>Monilinia fructicola</u> and not by <u>M. laxa</u> which was found as part of the hull rot complex organism in Butte County during 1976.

<u>Varietal susceptibility</u>: Disease was most severe on the soft-shelled cultivar, Nonpareil, but was also easily found on the Peerless and other medium-shelled cultivars. Some <u>Rhizopus</u>-infected hulls were found in the Mission cultivar (hardshelled) but no twig dieback was observed.

The Nonpareil hulls were dry inoculated with <u>R</u>. stolonifer to determine how long the hulls remain susceptible. Thirty almond hulls were inoculated each time starting from hull split to 18 days thereafter. Hulls were highly susceptible for the first 8 days and no longer susceptible after 14 days (Fig. 1). Once hulls were dry, infections no longer occurred. It must be noted that all hulls do not open at the same time and, especially in the more vigorous orchards, some hulls were splitting at harvest. Based on observations that vigorous trees appear to have more hull rot, the Department of Pomology nitrogen test plot in Butte County was evaluated for the twig blight phase of hull rot. Levels applied each year ranged from 0 to 8 pounds of actual nitrogen per tree. The number of twig diebacks increased with higher nitrogen levels as shown in Table 1. This increase in twig blight could be related to increase in infection sites as indicated by yield increase or hull split over a longer period of time.

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<u>Epidemiology of the Rhizopus</u>: A study was made on the location of <u>R</u>. <u>stolonifer</u> propagule within the orchard. The soil as a medium for <u>Rhizopus</u> survival has been reported. A selective medium was developed for assessing the number of Rhizopus propagules in soil. Each sample of 6-10 soil cores was collected with a soil auger to the depth of one inch and placed in a plastic bag. The auger was surfacesterilized with 10% sodium hypochlorite between sampling in orchards. From June 23 to August 20, eight samples were taken from the Sugiura orchard in Merced County. Individual samples varied from 0 to 144 propagules per gram of soil. The averages for the 6-core samples ranged from 13 to 69 propagules per gram of soil. This study shows the abundance of Rhizopus propagules in the soil (Table 2).

After harvest soil samples were taken again from various almond orchards throughout the state. The number of <u>Rhizopus</u> propagules in the Sugiura orchard increased to 600 per gram of soil. The numbers of propagules in almond orchards were different and appeared to be related to the amount of hull rot within the orchard. In Kern County very little, if any, hull rot was observed. Propagule numbers were higher in the Sugiura orchard with high hull rot incidence compared to the adjacent JACL orchard with little hull rot (Table 3).

Air at the Sugiura orchard was also sampled from June 23 through August 20. Two techniques were used. In one test, 20 open petri dishes with the selective medium were placed on the orchard floor for one hour. After incubation for 2 to 3 days at room temperature the number of <u>Rhizopus</u> colonies which grew on the medium were counted. The second method involved use of the Anderson Spore Trap with an air sampling rate of one cubic foot per minute. The spore trap was run for 15 minutes. Results given in Table 4 show the presence of spores in the orchard air throughout the sampling period. This would indicate that split hulls could become contaminated quite easily. Yet when 1-cm diameter agar discs were placed on each of 90 leaves at random during hull split, only one sample with <u>Rhizopus</u> was detected. Also at the same time, a portion of the endocarp under the newly split hull was isolated for <u>Rhizopus</u>. Only one out of 90 samples taken developed cultures of <u>Rhizopus</u>. Thus there is some indication that split hulls do not get contaminated easily nor are spores on leaf surfaces easily transferred to split hulls.

The possibility of insect transmission of <u>Rhizopus</u> spores was examined by observing orchards with severe hull rot infections. Studies showed that 72% of diseased green hulls had dried fruit beetles in hulls, just split 14%, while in dry hulls with or without hull rot no beetles were found. This suggests that beetles fed on diseased hulls and left when the hulls dried. There is a possibility the beetles were initially contaminated in the soil and moved into the split hulls or the beetles were attracted to diseased hulls.

<u>Control</u>: Botran 75W, 2-6-dichloro-4-nitroaniline, is effective in control of diseases caused by <u>Rhizopus stolonifer</u>. The fungicide is registered for use on many crops including postharvest applications on cherries, apricots, peaches, plums, and nectarines. The residue tolerance for postharvest use on these crops is 20 ppm.

Preliminary studies in 1975 and 1976 using branch tests and single tree replications with hand-gun sprays significantly reduced hull rot and twig kill. During 1977 the hand-gun spray test was repeated on Peerless almond trees at Sugiura. One spray was applied at hull split. The treatments were Botran 75W (1-1/2 lb. per 100 gal. spray) and the combination of Botran 75W plus Benlate 50W (1-1/2 lb. and 0.5 lb., respectively, in 100 gal spray). At harvest the untreated hulls had 45% <u>Rhizopus</u> while the Botran and the Botran plus Benlate treatment reduced hull rot to 31 and 36%, respectively. For brown rot control in the same plot, the untreated hulls had 64% while the Botran had 71% and with Botran plus benomyl the hull rot was reduced to 28%. This experiment again provided evidence that chemicals applied with a hand gun at hull split can reduce hull rot.

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During 1977, three large test plots were sprayed twice with chemicals using an airblast rig to simulate commercial application, once at hull split and the second 2 weeks later. Hull rot counts made on two, 20-lb. samples collected from each of the three replications showed that neither the two applications of sulfur, nor the one and two applications of dicloran reduced hull rot by <u>Rhizopus</u> or <u>Monilinia</u> (Table 5, 6). The Montgomery plot is located near Chico (Butte County) and this year the <u>Rhizopus</u> incidence was unusually low in the area. The Sugiura orchard located near Turlock (Merced County) had high incidences of both <u>Rhizopus</u> and <u>Monilinia</u>. <u>Rhizopus</u> incidence was also high at Freeman's orchard (Fresno County). Identification of <u>Rhizopus</u> species showed that over 90% was <u>R</u>. <u>stolonifer</u>, the species sensitive to dicloran (Botran 75W).

Pounds of nitrogen	Average number of
applied per tree	twig dieback per tree
8	23.1 x <sup>a</sup>
4	21.5 x
3	17.6 xy
2	14.7 yz
0	9.3 z

Table 1. Effects of nitrogen fertilizer application on twig dieback from hull rot.

<sup>a</sup>Statistical significant values having a letter in common do not differ significantly at the 5% level. Single mature Nonpareil trees replicated 5 times.

Time soil Average number of propagules sampled per gm of soil	
June 2369July 148July 833July 1544July 2233July 3051August 6Not sampledAugust 1313August 2030	

Table 2. Populations of <u>Rhizopus</u> propagules in soil at Sugiura in Merced County.

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<sup>a</sup>Average of six soil samples in one location of orchard for each sampling time.

County and Orchard or location	Propagules per gm of soil <sup>a</sup>
Butte County	
Birdseye orchard	100
Durham Road Fimple Road	< 50 660
Goodspeed-Watt Road	200
Montgomery orchard Nottelmann orchard	430 180
Merced County JACL orchard	< 50
Sugiura orchard	600
Fresno County Cunha orchard	120
Freeman orchard	260
Kern County Arvin area Highway 65	< <b>5</b> 0 < 50

Table 3. Comparison of <u>Rhizopus</u> populations in orchard soils

<sup>a</sup>Soil samples collected in October, 1977.

Date air sampled	Ander	of Rhizopu son Sample in 10 min	r	ules collected by <sup>a</sup> Open petri dish one hr
June 23 July 1 July 8 July 15 July 22 July 30 August 6 August 13 August 20	0 0 0 0 0 0 0 0 0		0 2 1 3 1 2 2 1	1 2 0 1 0 1 2 1

Table 4. Number of <u>Rhizopus</u> propagules collected by air sampling inside Sugiura orchard in Merced County.

<sup>a</sup>Selective medium for <u>Rhizopus</u> used.

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Table 5. Percent <u>Rhizopus</u> infected hulls and twig blight in Nonpareil almond orchards

	Percent hull rot				Avg. No. twig blight/ 200 fruiting twig			
a		Replication			Replication			
<u>Orchard &amp; treatment<sup>a</sup></u>	I	II	III	Avg.	I	II	III	Avg.
Montgomery orchard Control	2.1	2.6	3.4	2.8	22.7	20.4	12.4	18.5
Sulfur 2x	2.2	4.0	4.3	3.8	21.6	19.3	18.5	19.8
Botran lx	0.75	1.1	2.8	1.6	11.4	13.6	9.6	11.5
Botran 2x	2.8	2.8	2.0	2.5	16.5	20.5	17.7	18.2
Sugiura orchard Control Sulfur 2x Botran 1x Botran 2x	15.2 23.4 21.6 17.4	15.7 17.5 16.4 21.1	14.4 14.3 22.5 13.4	15.4 19.0 19.8 17.1	148.8 132.3 127.4 133.1	141.6 144.5 129.8 140.2	142.4 137.6 123.4 136.5	144.3 138.1 126.9 136.6
Freeman orchard Control Sulfur 2x Botran 1x Botran 2x	18.2 6.7 18.4 13.6	11.8 16.2 9.9 11.4	7.6 31.0 5.9 7.9	12.6 18.4 11.4 9.3	14.0 22.3 21.9 25.7	16.9 46.5 15.8 17.8	14.6 50.8 31.9 17.2	15.2 39.9 23.2 20.2

<sup>a</sup>All treatments were made with airblast equipment using 100-250 gallons of spray per acre. Wettable sulfur was used at 20 pounds/acre while Botran 75W was used at 4 pounds/acre. The Montgomery orchard is located in Butte County, Sugiura orchard in Merced County, and Freeman orchard in Fresno County. The sprays were applied at hull split and 2 weeks thereafter for the 2x application and only the hull split application for the 1x application.

Orchard & treatment <sup>a</sup>	Repl.	I	II	III	Average
Montgomery orchard Control			None		
Sugiura orchard Control Sulfur 2x Botran 1x Botran 2x		1.95 2.50 2.40 1.20	2.75 2.90 2.15 5.35	3.20 2.15 2.05 1.45	2.63 2.52 2.20 2.67
Freeman orchard Control			None		

## Table 6. Percent Monilinia fructicola infected Nonpareil hulls.

<sup>a</sup>Treatments same as in Table 5.

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Fig. 1. Susceptible period of split Nonpareil hulls to infection caused by inoculation with dry spores of <u>Rhizopus</u> stolonifer.

