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TITLE: HULL ROT OF ALMONDS 78 Q4

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I. OBJECTIVES:

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

II. INTERPRETIVE SUMMARY:

Since control of hull rot through the use of protective fungicide sprays has not resulted in significant disease reduction, the emphasis of this research has turned to epidemiological factors which may point out alternate control measures.

It has been established that almond hulls remain susceptible to infection by Rhizopus stolonifer spores as long as they are moist. Any cultural practice increasing the uniformity of hull split and decreasing the time for hull drying should reduce the incidence of this disease. The use of cultural practices must be weighed against possible detrimental effects on yield. Early harvesting should also reduce the incidence and severity of hull rot. Investigations are underway to establish when almonds can be harvested (UCD Pomology Department) and if early harvesting reduces the damage done by this disease.

The epidemiological factors under study are in two areas: 1) The life cycle of the fungus in the soil and on the tree and conditions which increase the population of Rhizopus. Such studies could be related to control measures if the fungal population can be reduced by cultural programs such as irrigation, cultivation, fertilization, etc. 2) Methods by which the propagules of Rhizopus spread from the source to the splitting hull. If the vector were primarily an insect such as the Nitidulid beetles, the study on their behavior could point to the direction of research which could result in disease control. Such studies have been made on Ceratocystis canker of almonds showing that orchard soil should be dry at the time of harvest.

III. EXPERIMENTAL PROCEDURE

1. Etiology (cause) of hull rot: Samples of Nonpareil almond hulls infected with Rhizopus were collected into individual plastic baggies to avoid cross contamination. Two samples of 40 each were collected at Sugiura in Merced County and McKinley and Academy Ave., Fresno County; a sample of 20 was collected at a third location (Freeman in Fresno County). Species evaluation was carried out as cited in the 1977 annual report except that nutrient agar was used in place of potato dextrose agar (PDA).

Fifty Nonpareil almond hulls infected with brown rot pathogen were collected randomly from the Sugiura orchard and isolated for identification on PDA medium. These were stored at 68°F under continuous light for 5 days. Using this method Monilinia fructicola was discerned from M. laxa by its regular growth and heavy sporulation.

2. Epidemiology of Rhizopus stolonifer: In 1977 it was observed that insects, primarily dried fruit beetles, family Nitidulidae, visit diseased and apparently healthy split almond hulls. Orchard and laboratory experiments in 1978 were designed to establish whether or not these insects can transmit Rhizopus to susceptible almonds.

Cages for orchard transmission studies were constructed from 5/16 x 1-1/8" pine and Dupont Mylar insect netting. At the Sugiura orchard (Merced County) 4 Nonpareil trees with at least 2 small limbs bearing 25 nuts or more each were chosen and the appropriate branches were trimmed to a size that would fit inside the cages. Cages were pulled over the limbs, tied with wire and sealed with florist's clay; 2 cages were placed in each of the 4 trees and 1 uncaged branch in each tree was marked as a control. By August 18 at least 90% of the almonds in each cage had split. Twenty to 50 beetles, family Nitidulidae, Carpophilus freemani, which had been contaminated by feeding on R. stolonifer cultures for 3 hr were released into one cage in each tree. The other 4 cages were retained as controls. The reason for the caged and uncaged controls was to see if exclusion of insects reduced the natural occurrence of hull rot. Disease readings were made on September 1.

A 2'x2'x1' cage of the same materials was constructed for use in laboratory transmission experiments. Three crispers were filled with distilled water and covered with wire hardware mesh through which 8 fruiting shoots each of Nonpareil and NePlus Ultra were placed into each crisper. The crispers were placed into the cage, approximately 100 contaminated beetles (C. freemani) released, and the cage was sealed. After 5 days storage at 68°F, 90% RH, and 12 hours light per day, disease incidence was recorded.

Beetle visitation of split Nonpareil almond hulls was recorded in an orchard at McKinley and Academy avenues in Fresno County; 6 diseased and apparently healthy hulls were observed in each of 5 trees for the presence of beetles. The healthy hulls containing beetles were placed into individual plastic bags and brought to the lab where the beetles were collected and placed into individual petri plates containing PDA + 125 ppm Streptomycin sulfate. In this way the percentage of contaminated beetles in healthy hulls was established.

To establish whether beetles could ingest and excrete viable R. stolonifer spores, 5 individuals (C. freemani) were fed on a 1-wk-old culture for 1 hr, washed 6 times in sterile distilled water, and placed in individual petri plates containing PDA + 125 ppm streptomycin sulfate. Each beetle was observed with the 6.2X objective of a compound microscope until it released excrement onto the medium. This usually occurred within 1 minute and there were between 50 and 200 spores in each of the pelleted excreta observed. The areas on which the droppings were presented were marked off and the plates were incubated for 8 hr at 24 C. The viability was taken as percent germination of 100 spore counts. Spores were considered germinated if the germ tube was at least as long as the spore length.

Infection threshold (number of spores for infection). Laboratory inoculations of R. stolonifer were made onto split Nonpareil (2 trials) and NePlus (1 trial) almond hulls which were attached to shoots. Each treatment consisted of 3 replicates of 8 shoots which were held in crispers with distilled water under the same conditions used for laboratory beetle transmission. Each crisper held 3 replicates of randomly assigned treatments.

The four treatments used were 0, 1, 10, and 100 spores per hull. The first treatment consisted of uninoculated controls. In the second treatment, single spores were transferred from 1.5% water agar to almond hulls with a fine glass needle. After each application the needle was cleaned by stroking through PDA in a petri plate. These plates were incubated at 24 C and checked for R. stolonifer growth after 1 and 2 days to make sure that the spores had come off of the needle in the hulls. For the third and fourth treatments, suspensions containing approximately 10 and 100 spores per µl, respectively, were prepared by using a hemocytometer and making proper dilutions with sterile distilled water. One µl of these solutions was placed into individual hulls with a Drummond Microcapillary pipette. The solutions were shaken lightly between thumb and forefinger after each application to keep them well mixed. Inoculations were read for infection after 5 days.

3. Soil sampling. Three locations were chosen randomly within the Sugiura and JACL orchards in Merced county so that a statistical comparison of the soil populations of R. stolonifer could be made. These two orchards were chosen since they are adjacent and the incidence of hull rot in 1977 was high at Sugiura's and low at JACL. Samples of 40 soil cores were collected at monthly intervals at each of the six locations and evaluated for propagules of R. stolonifer per gram dry soil. The techniques used have been described in previous reports.

4. Control: a) Spray test.--Beginning August 1, 5 weekly applications of Botran 75W, 2,6-dichloro-4-nitroaniline, were made on two single-row plots (Nonpareil and NePlus) at the Sugiura orchard in Merced County. The applications were made with an airblast sprayer at a rate of 4 lb Botran 75W per acre. Each plot consisted of 3 treated (6 trees each) and 3 control (3 trees each) replicates. Hull rot readings were made on September 7 by climbing ladders and evaluating 200 nuts per tree specifically for Rhizopus strikes.

b. Soil treatment.--Puregro contact, 6.25% A.I. 4-6-Dinitro-o-sec-butylphenol per gallon, was applied to the soil at the Boos (Fresno County) and Sugiura orchards at 4.5 and 4 gal/acre, respectively, in an attempt to reduce soil Rhizopus populations and disease incidence. Each plot consisted of 3 large (7-11 rows) sprayed replicates and 3 one-row controls. Since naturally occurring populations of Rhizopus were very low, a spore suspension was sprayed onto a area of each replicate approximately 3' x 20' to help evaluate the efficacy of Dinitro applications. At the Boos orchard this was prepared by mixing diseased Nonpareil hulls with water and at the Sugiura orchard by mixing R. stolonifer cultures with water. Approximately 2 liters of the spore suspension were applied to each replicate with a backpack sprayer. The spore concentrations were 1.8×10^4 and 1.2×10^5 spores per ml at the Boos and Sugiura orchards, respectively. Soil samples were taken immediately after application of Dinitro and after 2 weeks following the normal soil sampling procedures.

RESULTS

Etiology of hull rot: Rhizopus - Isolations from diseased hulls were 100, 100 and 55% R. stolonifer at the Sugiura, McKinley and Academy Ave., and Freeman orchards, respectively, indicating that this organism still predominates. Confidence intervals for the populations are listed in Table 1.

Epidemiology of Rhizopus: Insect transmission. The almonds in orchard cages into which contaminated beetles were released had an average of 87% Rhizopus hull rot. There was only one hull rotted almond in all of the caged controls (1%) and there were no hull rot strikes on the uncaged controls (Table 2). Isolations from diseased hulls onto nutrient agar all yielded R. stolonifer cultures. In the laboratory there was a 50% transmission rate onto Nonpareil and NePlus. This was significantly different from controls (Table 3).

Nitidulid beetles were observed in an average of 54% of the diseased and 33.6% of the healthy Nonpareil almond hulls examined. Seventy-five percent of the beetles collected from healthy hulls were contaminated with R. stolonifer (Table 4).

An average of 56.8% of the R. stolonifer spores ingested and excreted by C. freemani beetles were viable (Table 5).

Although there were no diseased hulls in uncaged controls in the field trial, it is not possible to state conclusively that insects are necessary for the dissemination of the hull rot pathogen. The fact that 75% of the beetles from apparently healthy hulls were contaminated with R. stolonifer indicates that these insects probably do play some role in dispensing the Rhizopus fungus.

Infection threshold: In two of the three laboratory trials conducted there were significant differences in infection between inoculations with 1 and 10 spores. In no case did one spore inoculation differ from controls (Table 6). Field inoculations at the Sugiura orchard were contaminated with brown rot and could not be read. The infection threshold needs to be investigated further since it will help establish which inoculum sources are most important in the hull rot disease.

Soil sampling: Monthly samples from the Sugiura and JACL orchards showed continuously low inoculum levels throughout the growing season (Table 7). There were no significant differences between the soil populations at these locations.

Control: Significant differences in Rhizopus hull rot were not observed in the Botran 75W spray plot (Table 8). This could have been due to the very low incidence of this disease at the location tested and this test should be repeated in 1979.

There were no significant differences observed in soil populations of R. stolonifer between control plots and plots sprayed with Dinitro (Table 9). Laboratory experiments are in progress to establish the effects of Dinitro and other chemicals on soil populations of R. stolonifer.

Discussion.--Further studies are needed to evaluate the relative importance of various inoculum sources. The parameters to examine are: survival in and dispersal from soils, survival of inoculum on the trees, insect transmission, and long distance dispersal. To complement these, susceptibility of the host is being studied in detail from the standpoint of infection thresholds and requirements.

Once the inoculum sources and methods of spread for this disease are evaluated we can better decide the types and timing of various control measures to be complemented.

Research related to reducing the population of the causal agents, Rhizopus stolonifer and Monilinia fructicola, should be continued to develop control measures. Such studies include dormant treatment of the almond orchard with an eradicant type fungicide such as sodium pentachlorophenate to determine its value in reducing the Monilinia fructicola inoculum, Rhizopus stolonifer inoculum on the tree and in the soil, and possibly the chemical's role in killing the navel orangeworm. Such experiments were conducted in a year with little rain and no effects were shown; during 1978-79 year we have had both rain and fog which could help penetration of the chemical. Treatments have been applied during December 1978. The other aspect of control is the treatment of soil with fungicides or herbicides; although this has not provided significant results during 1978, the principle of reducing population of pathogen in the soil appears to be worth continuing. Along with this line of control measures, the exact role of the nitidulid beetles should be examined. This has been discussed with the entomologists and research is progressing.

Table 1. 95% confidence intervals[a] for Rhizopus hull rot populations in 3 almond orchards in 1978

Organism			95% CI
Rhizopus stolonifer			
	Total		85.3 - 96.7%
Orchard 1		Sugiura	100%
	2	McKinley & Academy	100%
	3	Freeman	31.1 - 78.9%

[a]95% probability that populations fall within the ranges listed.

Table 2. Orchard transmission of Rhizopus hull rot by Carpophilus freemani

	Control		Contaminated beetles
	Caged	Uncaged	Caged
% Hull rot[a]	1	0	87**

** significant difference at P < 0.01

[a]Each treatment consisted of 4 replications of 25 hulls

Table 3. Laboratory transmission of Rhizopus hull rot by Carpophilus freemani

	Nonpareil		NePlus	
	Control	Transmission	Control	Transmission
% hull rot[a]	12.5	50*	4.1	50**

* significant difference at P = 0.025
** significant difference at P < 0.01

[a] each treatment consisted of 3 replications of 8 hulls.

Table 4. Percent beetle visitation - Nonpareil

	Diseased hulls	Healthy hulls
Avg.	54	33.6*+
95% C.I.	37.8 - 70.2	20.2 - 47.0

* significant difference between means at P = 0.05.

+ 75% of beetles from healthy hulls were contaminated with R. stolonifer.

Table 5. Percent viability[a] of Rhizopus spores passing through Nitidulid beetles

Replication					Avg.	95% C.I.
1	2	3	4	5		
95	34	30	68	57	56.8	23.8 - 89.8

[a] germination of 100 spores counted after 8-hr incubation at 24 C.

Table 6. Number of spores required for infection of susceptible almond hulls by R. stolonifer

	% Hull rot[y]		
No. spores	Trial 1 Nonpareil	Trial 2 Nonpareil	Trial 3 NePlus Ultra
0	8.4[NS]	12.5a[*]	4.1a
1	20.8	8.4a	0.0a
10	25.0	41.6 b	25.0 b
100	41.6	41.6 b	33.4 b

[*] P = 0.05. Letters indicate probability groups.
Y = Each treatment consisted of 3 replicates of 8 hulls/replicate.

Table 7. Populations of R. stolonifer in orchard soils 1978

	Propagules 1 g dry soil[*]	
	Sugiura	JACL
May	14	< 5[NS]
June	5	< 5
July	< 5	< 5
August	< 5	< 5
September	< 5	< 5

[*] each value is the average of 3 replications.
NS = no significant differences between locations.

Table 8. Hull rot control plot - Botran[Y]

	% Rhizopus Hull Rot[*]
Sprayed	2.26 NS
Non sprayed	0.05 NS

[*]Each value represents the average of 3 replications. 200 hulls were evaluated per tree.

[Y]Botran 75W applied by airblast sprayer at 4 lb/acre at 5 weekly intervals beginning August 1.

Table 9. Effect of dinitro on soil populations of R. stolonifer[Y]

Propagules/g dry soil[*]			
Sugiura		Boos	
Treated	Control	Treated	Control
27	31[NS]	24	30[NS]

[Y]Spores of R. stolonifer sprayed on soil. Dinitro applied at 4 gal/A (Sugiura) and 4.5 gal/A (Boos).

[*]Values represent average of 3 replications.