Correct Project Number: 86-T12

Project No. 86-11 - Almond Diseases
Shot hole and brown rot

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1. Objectives:

A. Shot hole - Continue studies on epidemiology and control with the goal of developing a disease forecasting system.

B. Brown rot - Develop alternative fungicides to control benzimidazole-resistant Monilinia laxa.

2. Interpretive Summary:

Shot hole disease: The goal of this research is to critically study the development and control of the disease and devise a forecasting system based on an infection period index (IPI). The IPI will use paramenters of host susceptibility, inoculum potential, and temperature parameters. Postinfection parameters could be important in the forecasting system. Current studies have shown similarities in the shot hole pathogens isolates collected from the Northern Sacramento Valley to that collected in the Southern San Joaquin Valley as well from the Central Valley. The fungal morphological characters were similar, except that one isolate from the Davis Campus had quite different appearances as well as its ability to grow in culture. This isolate is being studied to compare its pathogenic nature with typical cultures. The cultural growth parameters of the three isolates were similar at various temperature ranges.

Inoculation and incubation tests (in dew chamber) showed that the wetness period required for infection at 20 C on leaves of almonds in potted plants is between 12 to 16hr. Field trials are in progress to determine if a Wetness Simulator and Environmental Monitoring System (WSEMS) is capable of producing conditions necessary for infection. The WSEMS developed is unique in that free moisture without dripping is possible on plant surfaces and that the monitoring system records temperature, humidity, wind spead and diarection, as well as rainfall for later correlation studies with natural conditions of infection and disease development. It was interesting to note that during the first rain in fall 1986 in September triggered infections on inoculated leaves and later environmental conditions were suitable for sporodochial formation. Yet on trees which were severely diseased in previous years and never sprayed for shot hole did not develop disease symptoms indicating insufficant inoculum for infection and disease development.

Brown rot: Preliminary studies on a new compund (SC 0858), introduced by the Stauffer Chemical Company, show high activity against Monilinia. This compound could be equal to or more effective than the benzimidazoles (Benlate and Topsin) in the absence of benzimidazole resistant strains of Monilinia.

Botrytis blossom blight and green fruit rot: In spring 1986, with continuous rains during bloom, prevalence of Botrytis on dead and dying floral parts (shucks) was noted. Also there was many green fruits rotting on the tree as well as rotted fruit on the orchard floor. Isolates from the rotted fruit tissue yielded not only Botrytis but other fungi such as Stigmina, Monilinia, Alternaria, and Geotrichum. Critical studies on the role of these organisms in the blossom blight complex as well as in the fruit rot complex should be investigated

Leaf rust: Until recently leaf rust had been detected only in the northern Sacramento Valley, yet during 1986, leaf rust infections could easily be found in Southern San Joaquin Valley orchards. The importance of leaf rust on almonds has never been assessed nor have studies been done on its disease cycle or control measures.

Captan residue samples. Captan residue samples were collected from the test plot at Tejon (Teviotdale and Viveros), dried, and submitted to the Morris Laboratory in Sacramento.

3. Experimental procedure

Forecasting the shot hole disease: To develop biological data essential to developing a forecasting model, this project is an integral part of the UC IPM project. (A copy of the Annual Report for the UC IPM project is attached). An outline of the procedure is presented here. First in order to more clearly understand the pathogen involved, isolates from the northern, southern, and central almond growing regions were examined for their ability to germinate and grow on a synthetic medium at various temperatures. Secondly, trees grafted by Fowler nursery were placed in pots and pruned for use in the dew chamber. Leaves were inoculated, kept moist without dripping in the dew chamber for 8, 12, and 16 hr, removed and air-dried, and placed in a chamber at 20 C and RH which prevented dew formation. Further, almond cultivars Nonpareil, Carmel, Ne Plus Ultra, and Peerless were tested for their differences in susceptibility by inoculation technique described. With the construction of the wetness simulator and environmental monitoring system, field trials were made on potted trees as well as on young third leaf orchard trees. In addition some data were collected on shot hole disease development under natural conditions following rainfall in September 1986.

Single dormant or pink bud spray on control: One test plot with seven single tree replications was set up in Kern County with dormant sprays applied on September 27, 1985 (50% leaf fall) and on February 7, 1986 (pink bud stage with 1-2% of open blossoms) on 12-year-old almond trees of cultivar Merced. These trees located on the Tejon Ranch (Kern County) had not been sprayed for shot hole control during the last four years. On both dates of treatment, ziram and Kocide were applied at the rates of 2 and 4 pounds proprietary mixture per 100 gallons of spray. Each tree received 5 gallons of spray applied with a handgun sprayer. Disease evaluations were made on March 11.

Brown rot: Stauffer Chemical Company's new experimental fungicide, SC 0858, a water soluble compound was tested for its activity to control brown rot on fruits of plum, peach, sweet cherry in the laboratory and on peaches in the experimental orcahrd. Comparative results with benomyl showed that SC 0858 results show greater systemic action within fruit tissue and better control of brown rot. The compound, according to the manufacturer, is in a class different from benzimidazoles (Benlate and Topsin), erogosterol biossynthesis inhibitors (Funginex and Nustar), or the dicarboximides (Rovral and Ronilan). Plans are to test SC 0858 for control of brown rot blossom blight and Botrytis blossom and fruit rot.

Botrytis blossom and fruit rot. Based on information from Peter Yamamoto that the almond fruits show brown lesions, on March 10 and 11, 1986, an orchard in Cortez and the test plot in Bakersfield was examined. Abundance of "Botrytis" sporulation was found on almond blossom shucks and fruit lesions on the upper furface of fruit showed irregular brownish rust lesions on many of the fruits on the lower branches of trees of Nonpareil (30.8% with jacket) Mission (45.2% with jacket) and Merced (33.6% with jacket) cultivars. From the Tejon test plot, 100 fruit and leaves were collected at random for evaluation from each of the five treatments (Fall ziram, fall copper, spring ziram, spring copper, and control).

Leaf rust. Control of leaf rust was attempted in Kern County during the middle of summer before almond harvest on trees where over 50% of the leaves showed rust symptoms. Single spray application of wettable sulfur and mancozeb were applied.

Captan residue on almonds. Within the test plot at Tejon, Farm Advisor Mario Viveros applied captan fungicides after the 5 week after jacket fall to provide residue samples. The samples were harvested through the help of Tejon Farming personnel as well as the Farm Advisor. The samples were dried in Davis and the Morris laboratory personnel picked up the samples for analyses.

4. Results

Shot hole. Data related to forecasting are provided in the attached UC IPM annual report. Results provided here reflect control measures using a single dormant or pink bud spay on control of shot hole: Evaluations made on March 18, 1986 showed the following:

Treatments		% Fruit with lesions	% Fruit with rot	% leaves with spots
9/27/85	Ziram	99	11	50
	Fixed copper	100	9	54
2/7/86	Ziram	98	9	55
	Fixed copper	98	10	53
Nontreated		99	11	53

Fruit evaluation: Average of 7 replications of 50 fruit
Leaf evaluation: Average of 7 replications of 100 leaves

Isolations from the above samples which had been surface sterilized for 3 minutes in 1:9 dilution of 5.25% sodium hypochlorite and distilled water showed from the fruit rots on medium containing lactic acid PDA, 46% Stigmina, 40 % Alternaria, 8% Botrytis and on 523 bacteria detecting medium 22% Stigmina, 48% Alternaria, 4% Botrytis, and 26% bacteria. While on lesions which appeared like shot hole lesions on lactic acid PDA 54% Stigmina and 28% Alternaria and on 523 medium 585% Stigmina, 8% Alternaria, and 6% bacteria. Similar data on isolation was obtained by Dr. Hassan Zehsazian and Dr. Themis Michailides.

Pathogenicity tests were made on detached Merced almond cultivar. Inoculation were make on 20 puncture wounds and fruit incubated at 18 C and examined for percent fruits with depression (lesions or rot) and average diameter of depression recorded in mm. Isolation data from diseased fruit from each experiment are indicated below each table with an asterisk (*).

Exp. No. 1. Percent water soaked fruit with depression.

	Incubation period (hr)				
Inoculum	24	48	72	96	144
Botrytis	0	25 (5mm)	40 (6mm)	100 (8mm)	
Botrytis & Stigmina	0	20 (3mm)	20 (4mm)	20 4mm)	35 (5mm)
Botrytis & Alternaria	0	0	0	5 (4mm)	35 (6mm)
Stigmina	0	0	15 (4mm)	25 (6mm)	75 (7mm)
Alternaria	0	0	0	0	80 (6mm)
Stigmina & Alternaria	0	25 (3mm)	25 (3mm)	25 (5mm)	85 (--)
Control	0	0	0	0	0

*Reisolation from following inoculations on 10 diseased fruits:

Inoculation	Isolation: Botrytis	Stigmina	Alternaria
Botrytis	8		
Botrytis & Stigmina	10		
Botrytis & Alternaria	10		
Stigmina		7	3
Alternaria			10
Stigmina & Alternaria			10
Control			2

Exp. No. 2. Percent non-watersoaked fruit with depression

Inoculum	Incubation period (hr) 24	48	72	96	144
Botrytis	45	70	85	85	85
Botrytis & Stigmina	0	35	50	80	--
Botrytis & Alternaria	30	70	70	100	--
Stigmina	0	0	0	100	--
Alternaria	0	0	15	15	--
Stigmina & Alternaria	0	10	10	100	
Control	0	0	0	0	0

*Reisolation from inoculations on 10 diseased fruit.

Inoculation	Isolation: Botrytis	Stigmina	Alternaria
Botrytis	8		
Botrytis & Stigmina	10		
Botrytis & Alernaria	10		
Stigmina		7	3
Alternaria			10
Stigmina & Alternaria			10
Control			2

Exp. No. 3. Percent water soaked and dried fruit with depression

Inoculation	Incubation period (hr) 24	48	72	96	144
Botrytis	40	60	95	100	--
Botrytis & Stigmina	0	60	60	100	--
Botrytis & Alternaria	55	80	85	85	--
Stigmina	0	20	50	100	--
Alternaria	0	25	25	25	--
Stigmina & Alternaria	0	0	0	100	100
Control	0	0	0	0	0

*Reisolations from inoculations on 10 diseased fruit.

Inoculation	Isolation: Botrytis	Stigmina	Alternaria
Botrytis	9		
Botrytis & Stigmina	9		
Botrytis & Alternaria	9		1
Stigmina		9	1
Alternaria			10
Stigmina & Alternaria			10
Control			1

Brown Rot. Data collected for SC0858 were on other stone fruits and not on almonds. Results show the activity of chemical in controlling the brown rot organism and further the systemic activity of the chemical within fruits. Fungicide will be applied to almond blossoms during spring 1987.

Botrytis blossom and fruit rot. Obervations in orchards clearly indicate the abundance of Botrytis infections on dead and dying floral parts. Although the symptoms on the fruit resembled decay previously experienced from Botrytis, based on data presented on inoculations of detached fruit rot could have been caused by other pathogens in the orchard such as Stigmina and Monilinia.

Leaf rust. Data obtained from spray trees indicate that neither wettable sulfur nor mancozeb were effective in preventing further spread of leaf rust in an orchard with over 50% of leaves already infected.

Captan residue samples. Information on the residues obtained on the dried almond fruits have not been provided by Morris laboratory at this time.

5. Discussion

Shot hole. Critical biological data required for developing a forecasting model will be conducted during spring 1987. As least one or two infection periods would benefit in developing a model. Both laboratory, dew chamber, and field data simulating infection periods will be obtained as supplement to support field information. Data on spore survival and chemical control will be obtained on trees sprayed with spores during winter 1986 and fungicides will be applied during dormancy and again during the leafing process to determine the effect of the fungicide on the dormant spores as well as in control of the disease.. The possible decay of fruit in the field with Stigmina infections will be tested in the laboratory on detached fruit as well as on the tree.

Brown rot. Evidence for systemic activity of SC 0858 in blossom parts and in the fruit is important to determing the future plans for research and registration processes.

Botrytis blossom and fruit rot. Evaluation of fruit rot symptoms developing on almonds is required with reference to their cause. Plans are to make inoculations in the field with the various pathogens and provide infection periods with the wetness simulator.

Leaf rust. Survey of leaf rust in the major almond producing region is required as well as plans for registration of fungicides other than sulfur if needed.

6. Publications

Highberg, L. M., and Ogawa, J. M. 1986. Yield reduction in almond related to incidence of shot hole disease. Plant Disease 70:825-828. (Attached)

Highberg, L. M., and Ogawa, J. M. 1986. Survival of shot hole inoculum in association with dormant buds. Plant Disease 70:828-831. (Attached)

Copies provided to: Bob Curtis, Almond Board
Investigators & cooperators

December 31, 1986

UNIVERSITY OF CALIFORNIA, DAVIS

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COLLEGE OF AGRICULTURAL AND
ENVIRONMENTAL SCIENCES
AGRICULTURAL EXPERIMENT STATION
DEPARTMENT OF PLANT PATHOLOGY

DAVIS, CALIFORNIA 95616

December 31, 1986

Mr. Bob Curtis
Research Director
Almond Board of California
P.O. Box 15920
Sacramento, CA 95852

Dear Bob:

Please find enclosed annual reports for my project with the Almond Board and the UC IPM project. In order not be be repetitive, I have included the details of the forecasting information in UC IPM report into the Almond Board report.

Thanks for you wise counsel on our projects for the past years. Best to you for the coming year.

Sincerely,

J. M. Ogawa
Professor

JMO/vjs

cc: Manji, B. T.
Shaw, Dave
Feliciano, A.
Osorio, Juan
Viveros, FA Kern County

Enclosures: Annual reports: Almond Board and UC IPM
Manuscripts on shot hole by Highberg and Ogawa

ANNUAL REPORT

UC IPM PROJECT REPORT FOR 1986

Title: Develop Program for Forecasting Shot Hole on Almonds

Investigators: J. M. Ogawa, J. J. Marois, and B. T. Manji
Department of Plant Pathology University of California, Davis, CA 95616

Cooperators: D. A. Shaw, A. Feliciano, and J. Adaskaveg, Department of Plant Pathology, University of California, Davis, CA 95616. J. H. Connell, L. C. Hendricks, and M. Viveros, Farm Advisors: Butte, Merced, and Kern counties.

I. OBJECTIVES AND TIMETABLE

A. Develop a biological basis for forecasting the shot hole disease (spore source, method of overwintering, infection sites, and infection periods). (UC IPM and Almond Board Funds)

Time table: 1985-1988. Delayed set up of dew chamber but major portion should be completed during spring 1987 with final data during spring 1988.

B. Monitor and compare infections in fall before leaf drop to disease incidence in the following spring in orchards with and without fungicide treatments.

Time table: 1985-1988. Delayed because of lack of disease during fall 1986 but some data being obtained from simulated conditions.

C. Test models for forecasting based on information developed in the laboratory and in the field.

Time table: 1985-1988. Schedule based on disease incidence in the fields during 1987 and 1988 for comparison with data obtained from simulated conditions.

II. Summary

The goal of this research is to develop an effective shot hole disease control forecasting program. The data required include leaf wetness period, temperature parameters for infection, inoculum potential, and susceptibility of host stage as well as cultivar differentials. Dew chamber data indicate the required period of free moisture to be 12 to 16 hr at 20 C with high inoclum levels. Almond cultivars Nonpareil, Carmel, Ne Plus Ultra, and Peerless were found susceptible to the shot hole disease. A portable overhead misting system was developed (Wetness Simulator and Environmental Monitoring System) which was constructed to provide continuous moisture on leaves as well as to monitor the environmental parameter required for development of a forecasting model. Two infection cycle with disease symptoms developed following the first fall rains. However, the last four rains failed to induceshot hole symptoms. Sporodochial formation was observed only from infections occurring after the first rain on September 19, 1986. The first plannedimplementation of forecasting will be in 1988 based on limited field data obtain during the previous two years.

III. BUDGET FOR 1985-86: $14,200

BUDGET FOR 1986-87: $16,500

BUDGET FOR 1987-88:

Personenl (PGR or graduate student)	$13,000	
S & E	1,000	
Equipment (Environmental sumulator)	1,000	
Travel to test plots	3,000	
Computer time	1,000	
Total		$19,000

BUDGET FOR 1988-89 (Needed if insufficient precipitation during spring 1987 and 1988 to obtain disease data for the forecasting model)

IV. ACCOMPLISHMENTS FOR THE CURRENT YEAR

A. Biological basis.

1. Justification: The biological data required for the forecasting of shot hole are the sources of inoculum or the spring infections, mechanisms of retention of viability of Stigmina spores, and infection period index for certain almond cultivars. This information is being sought by using nursery trees in potted containers for incubation in the dew chamber and in the young orchard where the leaves are inoculated and exposed to certain environmental conditions.

2. Materials and methods.

Spore germination and mycelial growth tests at various temperatures (0,4, 5, 9, 12, 15, 18, 21, 24, 27, 30, and 33 C) were conducted on Difco potato-dextrose agar medium. Four Stigmina carpophila isolates were used. Isolates were from Kern County (ST), Butte County (SC), Yolo County (SD), and a stock culture (S1-79) which had been cultured on artificial medium for five years. The PDA medium was incubated for a period of 6 hr before placement of spores or mycelial plugs. Each experiment consisted of four replicated petri-plates except for the SD culture with three replications. All cultures were single spore isolates. Infection periods on leaves of potted trees were determined by exposure to free moisture in the dew chamber, and followed by incubation for disease development in an environment chamber. Field infection periods were attempted by using a Wetness Simulator and Environmental Monitoring System (WSEM).

The wetness simulator and environmental monitoring system: The system was constructed to aid in the artificial inducement of disease while monitoring field environmental conditions. The WSEMS consists of two components, a spraying or misting system and a data logging monitoring system. The spraying system consists of a 55 gallon distilled water reservoir, a 12 volt battery operated pump and pressure tank maintained between 25 and 40 psi, filters, valves connecting hose line and misting nozzles which are turned off and on with a relay and solenoid. The monitoring system is a battery powered automated weather station consisting of a Campbell Scientific Inc. CR21X

micrologger and a variety of weather sensors which measure temperature, wind speed, wind direction, humidity, rainfall, and leaf wetness. The micrologger reads the sensors at one minute inatervals and calculates hourly and daily averages of the weather data. In addition, the micrologger is programed to trigger the relay on the misting system in accordance with changes in the leaf wetness sensor readings. The leaf wetness sensor is placed within the influences of the misting nozzles and hence as the sensor drys the system turns on and once wet the system turns off. This method has been successful in keeping foliage of trees wet for periods up to 24 hr while simultaneously collecting evironmental data.

Infection parameters on almond leaves were studied by three methods under artifically controlled or under monitored conditions. In each situation, leaves on almond trees were inoculated with spores and exposed to wetness periods. Wetness periods studied were induced in the dew chamber or by the WSEMS equipment or developed under natural rainfall. In these studies, the important factors governing the germination, infection, and disease development were host susceptibility, presence of inoculum, wetness period, and temperature. The integration of these parameters plus other factors such as postinfection environment will be considered in the parameters required for prediction of infection establishment and subsequent disease development.

Plant material: Potted trees (first leaf from nursery) trimmed to about 24 inches in height with 4-8 active shoots were used for dew chamber and WSEMS studies. Almond cultivars tested included Ne Plus Ultra, Nonpareil, Carmel and Peerless. Young Drake almond trees planted in the Plant Pathology orchard on the Davis Campus were used for field studies. These trees were in its second leaf and had not shown shot hole disease symptoms during two previous years.

Inoculum: Stigmina carpophila spores were produced on mycelial cultures and frozen in water for storage. For use in inoculation studies, unfrozen spores were diluted to a concentration of 100,000 spores per ml. Viability tests showed that the on water agar germination rates within a 24 hr period ranged from 55 to 90%.

Dew chamber tests: The dew chamber was used to create condensation of water on the leaves of the potted trees. This was achieved by heating water in the base of the chamber (30C) while the cooling the side walls of the chamber (9 C. Water condensed on the cool leaves and the result was a near steady state of leaf wettness (under 100% RH) with relatively uniform temperature of 20 C. The precise control of the chamber temperature eliminated the dripping of free water and possible washing of the spores from the leaves. Actual leaf temperature was measured using thermocouples placed on the leaf tissue. Leaves on potted trees were inoculated by placing three 10 ul drops of spore suspension on the underside of each of five leaves on three or more shoot tips to provide replications and subsamples for each treatment. After exposure to leaf wetnes periods, trees were allowed to dry and then were moved into a chamber held at 20 C in constant light and humidity where no new infections were established. Disease development was recorded under this condition. In one instance, trees were placed under natural outside atmosphere to determine if differences in disease development could be detected.

Field tests: Spore suspensions (100,000/ml) were sprayed with a hand held aspirating sprayer onto leaves on the terminal parts of the shoots.

Examination of the leaves showed approximately 320 spores per cm -2. Trees in potted plants were provided wetness periods with the use of the WSEMS equipment whereas trees planted in the orchard were exposed to wetness periods with the WSEMS or under natural precipitation.

3. Results and discussion:

Temperature requirements for spore germination and mycelial growth in vitro: All four single spore isolates collected from different major almond growing regions behaved similarly except for the SD isolate which grew slower and the cultures appeared somewhat abnormal. Spore germination data after 8 hr and 24 hr incubation periods (Fig. 1 & 2) show that the spores have the ability to germination at 0 C as well as at 33 C. Within 8 hr, the germination rate was 50 to 90% at temperataures between 15 to 30 C and within 24 hrs over 50% germination to almost 100% percent germination was observed at temperatures between 5 to 30 C. Stigmina carpophila spores can germinate well at temperatures found during foliation of almond trees in February and March. Yet the data on mycelial growth shows a slightly different trend with growth occurring at 0 C with the optimum at 20 C but at 30 C no growth was observed. The implications of reduced to no growth at high temperatures requires further study on germination and infection studies on almond leaf surfaces.

Dew chamber incubation tests: The feasibility of inducing shot hole disease on potted Nonpareil almond trees using wetness periods of 8, 12, and 16 hr, showed positive disease symptoms with the 12 and 16 hr wetness period but not the 8 hr period. Isolations from the lesion provided cultures of Stigmina. Nonwet leaves on potted trees inoculated and placed in the post dew chamber incubation chamber failed to develop disease symptoms.

In another test where the role of postinfection environmental conditions on symptom expression was tested by inoculation and wetness period of 16 hr followed, disease symptoms were expressed whether the postinfection incubation was made in the 20 C chamber, in the lathe house (moderate sun and temperature), or in the field (full exposure to direct sunlight). Greatest numbers of lesions were formed on plants incubated in the 20 C chamber, followed by plants held in the lathe house and in the field. This study will be continued to assess the significance of the postinfection environment on disease expression.

Cultivar susceptibility was tested on potted plants exposed to 12 and 16 hr wetness period in the dew chamber. Judging from the number of lesions formed, the initial ranking placed Nonpareil and Carmel as equally susceptible followed by Ne Plus Ultra and the Peerless least susceptible. If this result is significant, then the tree growth and phenology would be important in the rating of tree susceptibility in the orchards as the Ne Plus Ultra cultivar has been rated to be most susceptible. This cultivar starts to grow earlier and shows a more denser type growth characteristics than the other cultivars tested.

Field tests: Fall rains provided an opportunity to observe the effects of rainfall on disease incidence. Before each suspected rainfall, spores were sprayed onto Drake almond shoots. Ten shoots with five leaves each were inoculated while 10 similar nearby shoots on the same tree served as noninoculated controls. By inoculating shoots at different times during the

fall we hope to show a relationship between the amount of rain and temperature at that time to incidence of disease development. Unfortunately, the fall 1986 was relatively dry and limited data were obtained for analyses. The following chart summarizes the gross results of the six field experiments conducted:

Experiment number	Date	Rainfall inches	Date symptoms expressed		Air Temp. F	
			Lesion	Sporodochia	Hi	Lo
1	9/16	0.16	9/26	11/13	74	51
2	9/23	0.47	9/29	--	73	51
3	9/26	0.12	--	--	68	49
4	11/18	0.50+	__	__	67	40
5	11/28	0.16	__	__	60	36
6	12/2	0.20	__	__	64	33

Only the first experiment on 9/16 showed disease development followed by sporodochial formation on 11/8. During this period, sporadic rain occurred during the 10 days from 9/16 to 9/26. Experiment 2 with inoculations on 9/23 provided only a few lesion formation but no sporodochial development. The four later experiments did not provide any disease data. The only differences observed were those inoculation during low temperatures.

4. Plans for next year. Data on results on infection and disease development will be made on detached leaves under the various temperature ranges. Infection periods for disease made on leaves in Fall 1986 will be repeated in Spring 1987 on newly emerging leaves of different cultivars.

B. Monitoring for disease

1. Justification: Field data from natural incidences of disease are required on disease development and environmental parameters recorded for correlation with simulated laboratory and field data.

2. Materials and methods: During spring 1986, observations of orchards with diseases indicated that lesions on leaves and fruit can occur from Stigmina carpophila as well as other possible causes such as Botrytis cinerea, Monilinia fructicola, Monilinia laxa, and Alternaria alternata. Such we have made changes in the method for obtaining field data. Stigmina spores have been produced in culture and young third leaf trees in the orchard where diseases have not been observed will be spray inoculated during the winter 1986. These trees will be monitored for presence of viable spores, spore population, and natural infections with rains during the leafing period. In this experiment fungicides will be applied to determine the inactivation ability of such chemicals as ziram and captan.

3. Results and discussion. Laboratory studies have indicated that high concentrations of ziram can inactivate spores of Stigmina spores, yet field sprays of ziram or Kocide (fixed copper) during the winter months failed to prevent lesion formation on leaves or the developing fruit. Isolations from the lesions revealed evidence of a number of organisms. Inoculation experiments indicated pathogenicity of such organisms as Stigmina, Botrytis, and Monilinia. Alternaria which were isolated from lesions.

4. Plans for the next year. For the 1987 season, inoculations of leaves and fruits with specific pathogens is planned in the orchard. The orchard will receive free moisture with the WSEMS equipment for extended periods or preferably natural precipitation will occur during the experimentation.

C. Testing of the models for forecasting.

1. Justification. A model for forecasting is required. Such model will determine environmental parameters required for infection as well as for disease development. The ultimate goal would be to determine if single sprays can control the shot hole disease during years with low disease incidences in the fall and spring.

2. Materials and methods. Data on environmental conditions favorable and unfavorable for infection and disease development are being collected. Data from simulated disease developed with the WSEMS are also being collected. Data will continue to be collected from simulated as well as natural conditions during spring and fall of 1987 and 1988 for analayses.

3. Results and discussion. With current data, the only parameter of note is that under low ambient temperatures less disease was observed under natural disease incidences monitored.

VI. DATA COLLECTION AND FILING

Data obtained on the micrologger is available for examination on a new computer. Funds for the computer was generated by Ogawas various donor fund plus Marois' project.

VII. PRELIMINARY ANALYSIS AND LINKAGE ASSESSMENT

A. For data on biology, required are spore germination and infection data on detached leaf under various temperature regimes. This is required because data on nondetached leaves are difficult to acquire with only a single dew chamber held at one temperature. Also we only one incubation chamber to simulate environment for disease development. Thus laboratory data are intended as guides for simulating environmental conditions for disease in the field to determine conditions which will promote disease development after infection periods. Models for disease forecasting are planned based on natural conditions in the field for infection as well as disease development.

B. Data on infection in disease development are obtained from natural field conditions and from simulated conditions developed with the wetness simulator and environmental monitoring system. Complilation of this data must be continued each year until a forecasting model is perfected.

C. Developing a forecasting model may present problems in line with the possibility of a complex of pathogens causing a similar disease to shot hole such as that caused by Botrytis and Monilinia.

VII. IMPLEMENTATION IN FIELD

A. Field implementation in forecasting for the shot hole disease is planned for the 1989 season. Data on forecasting could influence growers on the need for additional cover sprays to control theshot hole disease.

B. Information for the Statewide Computer System will be ready when a forecasting model is made. At this time data obtain are insufficient to develop a model.

C. Pilot testing by area IPM Farm Advisors should be ready in 1989 or 1990.

D. None so far.

E. Implementation of research can be done by Farm Advisors. The difficulties in implementation could result by other diseases which must be controlled at the same time such as brown rot caused by Monilinia species, jacket rot and green fruit rot caused by Botrytis, and blast caused by Pseudomonas. Chemicals currently used by growers are somewhat specific in controlling certain diseases and require further management schemes as given in Fig. 7.

IX. PUBLICATIONS

Highberg, L. M., and Ogawa, J. M. 1986. Yield reduction in almond related to incidence of shot hole disease. Plant Disease 70:825-828.

Highberg, L. M., and Ogawa, J. M. 1986. Survival of shot hole inoculum in association with dormant almond buds. Plant Disease 70:828-831.

UC IPM Workgroup Assignment: Commodity-pest interactions

ANR/CE (Commodity) Workgroup: Almonds

Copies to: Mary Louise Flint, Chair Technical Committee
IPM Manual Group
Statewide Integrated Pest Management Program, UCD

L. T. Wilson, Entomology Department, UCD

Mark Freeman, Cooperative Extension, Fresno County

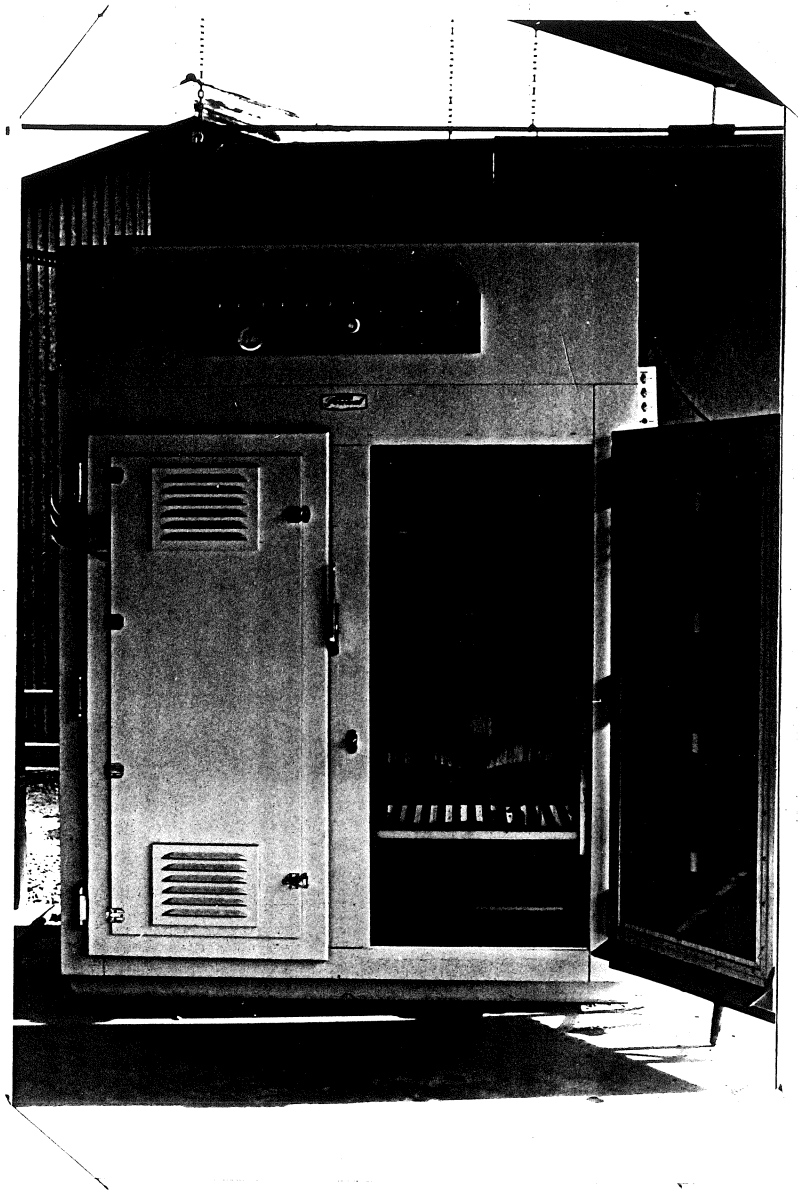

Fig. 1. Dew chamber

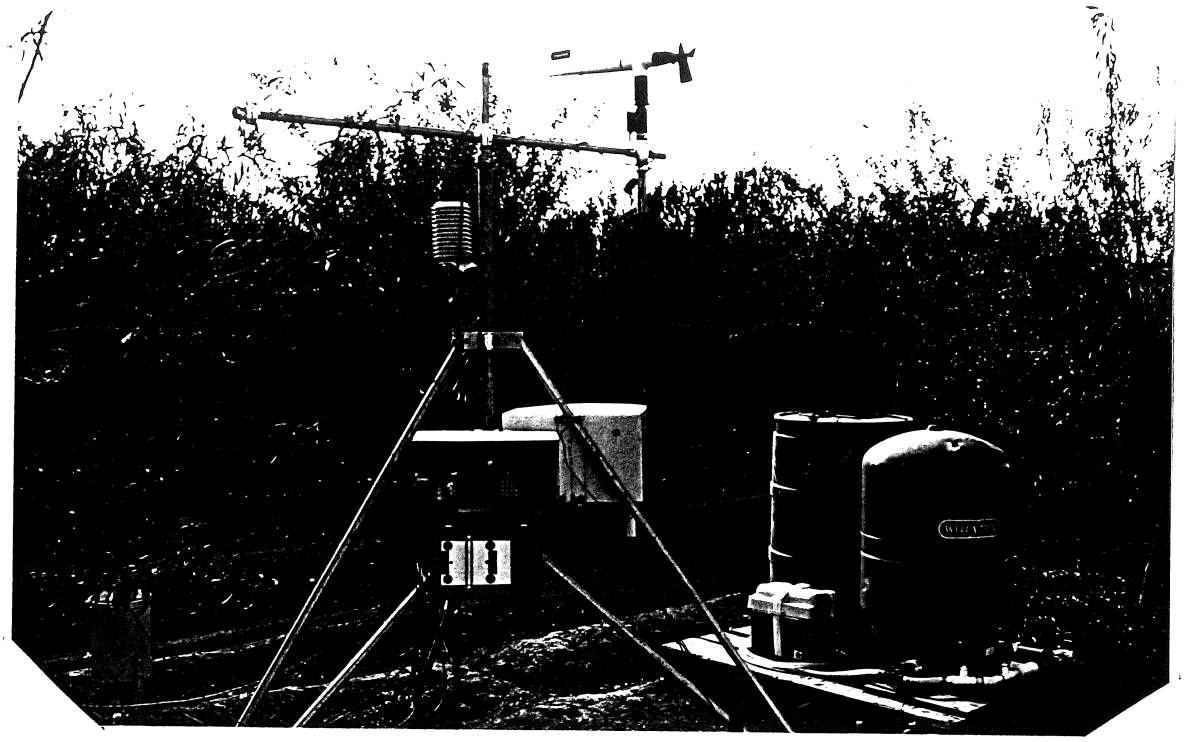

Fig. 2. Wetness simulator and environmental monitoring system (WSEMS)

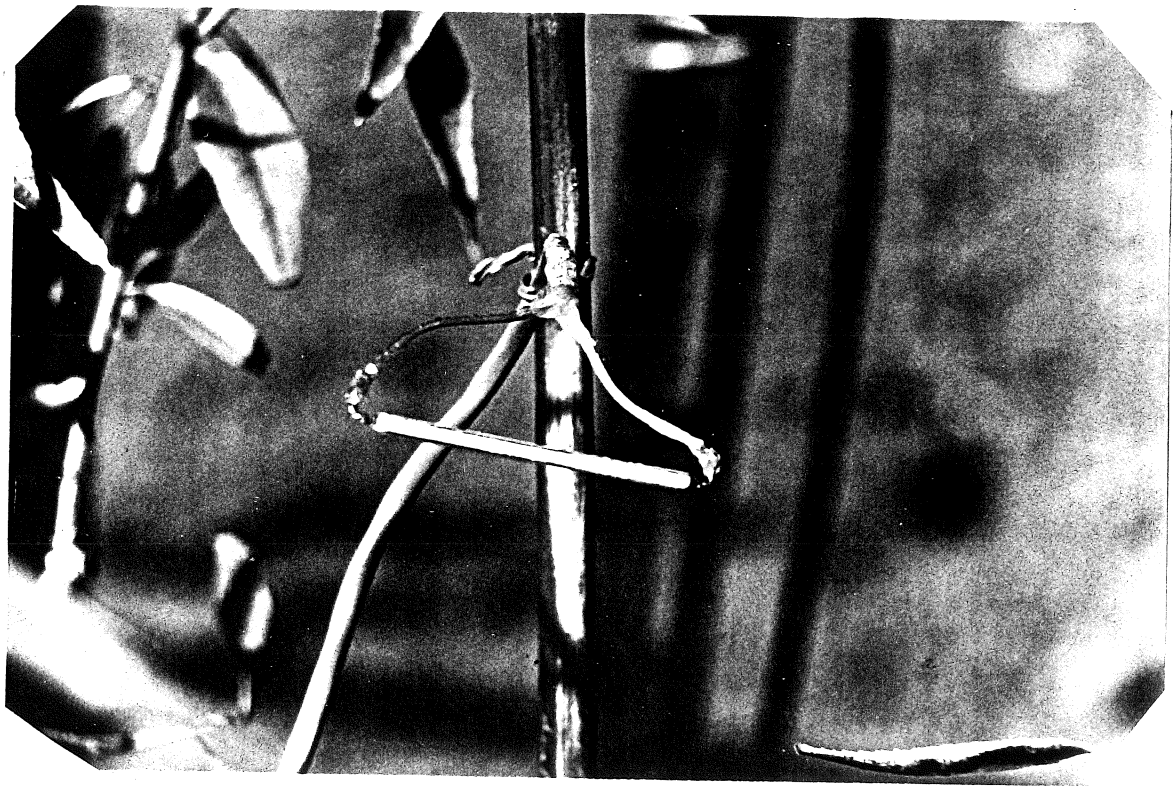

Fig. 3. Dew sensor

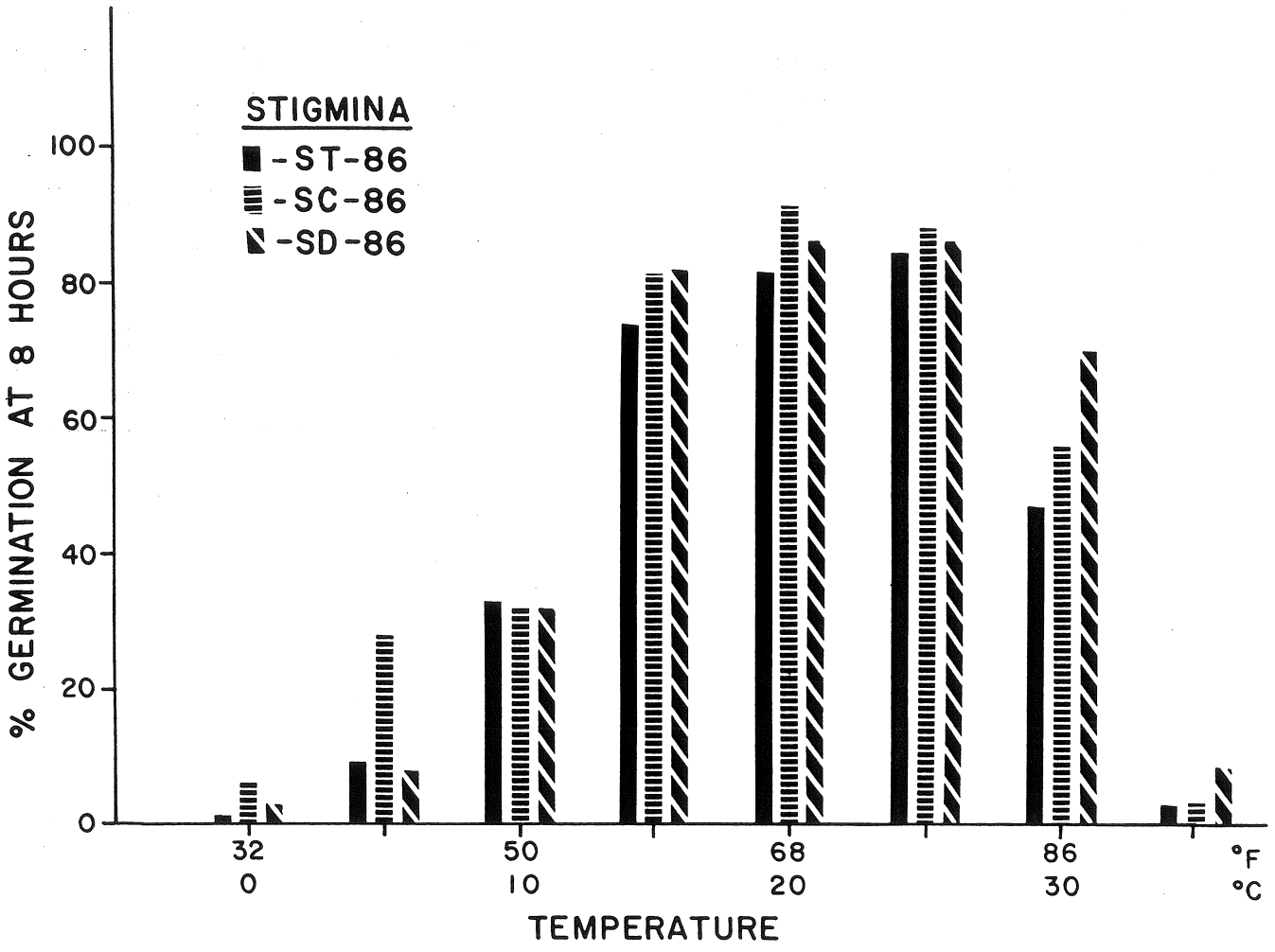

Fig. 4. Germination percentages of Stigmina spores on Difco-PDA at various temperatures within 8 hr incubation period.

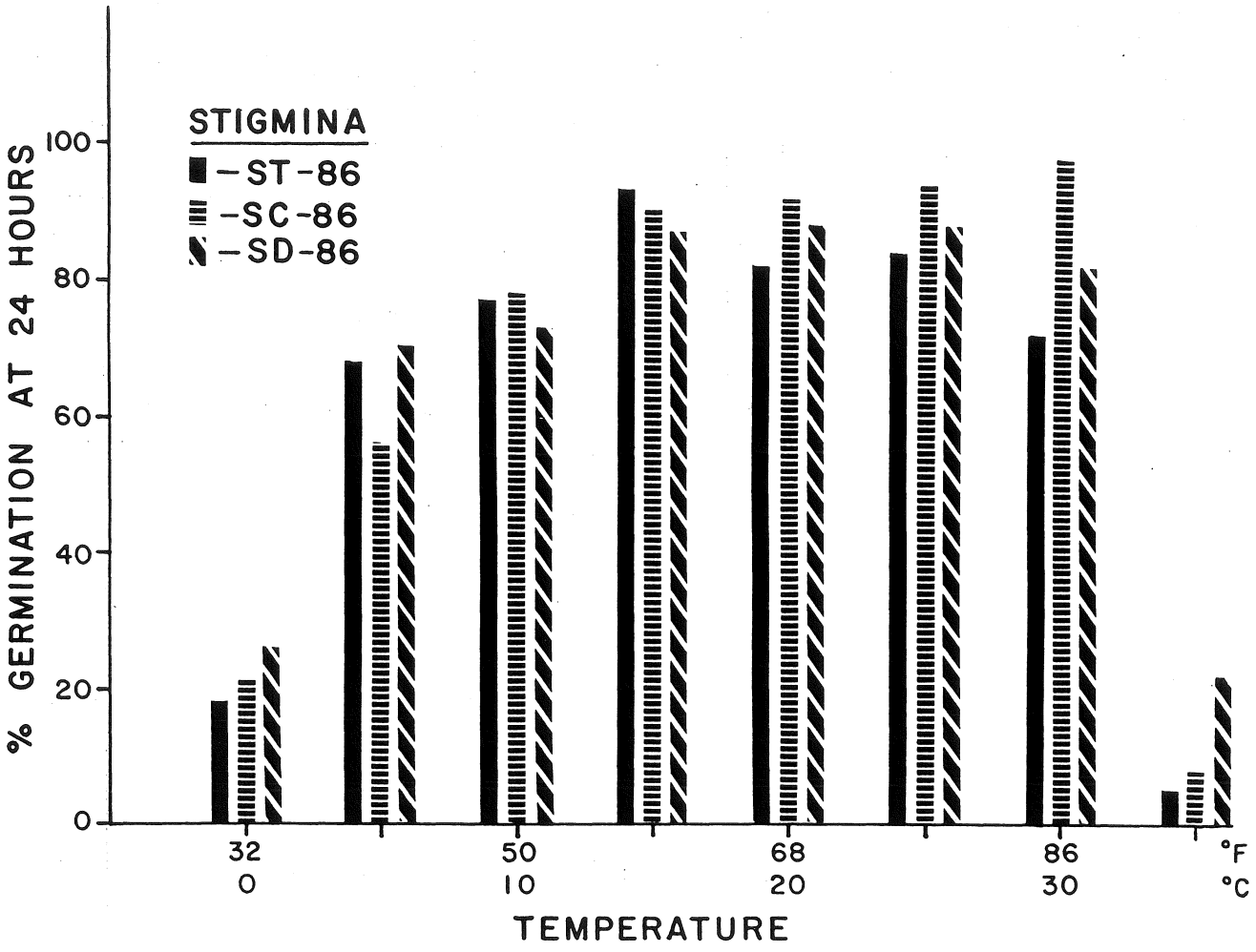

Fig. 5. Germination percentages of Stigmina spores on Difco-PDA at various temperatures within 24 hr incubation period.

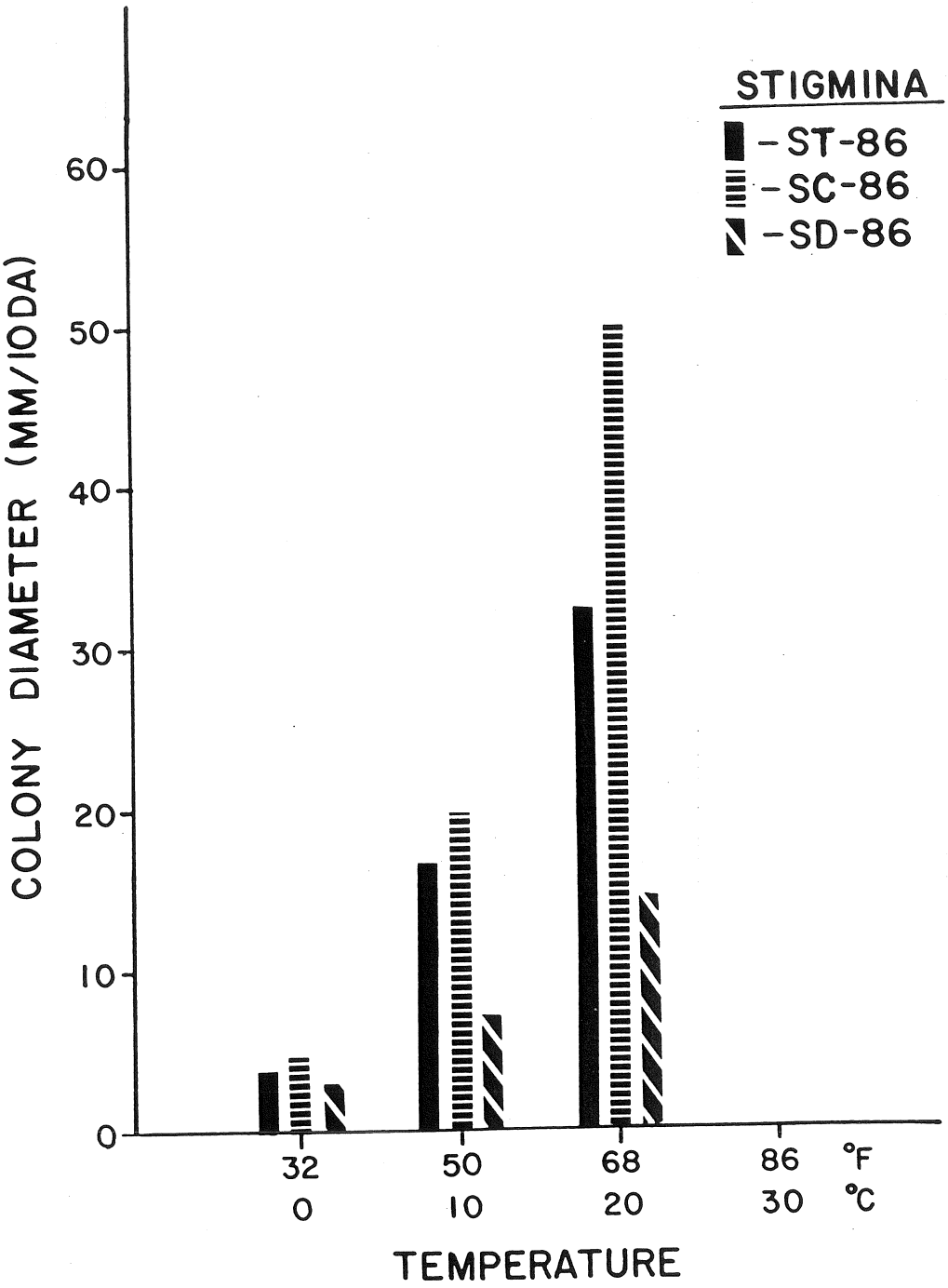

Fig. 6. Collony diameter of Stigmina colony at vrious temperatures within 10 day period.

MANAGEMENT STRATEGY

FUNGICIDE	SHOT HOLE	BROWN ROT	BOTRYTIS	BLAST
ZIRAM	+++	+	+	o
CAPTAN	+++	++	++	o
BENLATE TOPSIN	o	+++(R)	+++(R)	o
ROVRAL	++	++	+++	o
FUNGINEX	o	++	o	o
FIXED COPPERS	++	++	+	++°
BRAVO	++	++	++	o

Fig. 7. Management strategy on controlling diseases of almonds with fungicides.

Survival of Shot-Hole Inoculum in Association with Dormant Almond Buds

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ABSTRACT

Highberg, L. M., and Ogawa, J. M. 1986. Survival of shot-hole inoculum in association with dormant almond buds. Plant Disease 70:828-831.

Viable conidia of *Stigmina carpophila* were found associated with dormant almond buds collected throughout the 1982 dormant season in a commercial almond orchard in Merced County, California. Samples taken from trees where shot-hole disease levels were high during the growing season had significantly more conidia associated with dormant buds than did buds from trees in which disease levels had been significantly lower. In addition, during the 1-mo period between bud swell and bloom, 15- and 10-fold increases in numbers of conidia associated with dormant buds were observed, over previous sampling dates, in samples from trees with high and low disease levels, respectively. Viability of conidia, as determined by germinability, ranged from 65 to 96% for samples throughout the dormant season. In a second study, in which the survival of inoculated conidia on dormant buds was monitored, viability of recovered conidia ranged from 52 to 100% throughout the dormant season. These observations indicate that *S. carpophila* conidia survive the dormant season in association with healthy dormant buds, thereby contributing to the overwintering population of the fungus on the almond tree.

Additional key words: *Coryneum beyerinckii*

Shot-hole disease of stone fruit caused by the fungus *Stigmina carpophila* (Lév.) Ellis (*Coryneum beyerinckii*) has recently been shown to cause significant yield losses in California almond orchards (2). Although bloom-time fungicide applications for shot-hole control are a standard orchard practice in almond production, the number and timing of applications necessary to maintain economically profitable yields have not been related to overwintering population levels of the pathogen.

Unlike other stone fruit hosts where *S. carpophila* overwinters as mycelium in twig infections and blighted buds, on almond, neither type of infection was found in sufficient abundance to constitute the major source of primary inoculum for severe spring leaf and fruit infections (6,7). Although Vuillemin (5) reported the formation of a sexual stage (*Ascospora beyerinckii*) for *S. carpophila* in infected leaf debris, subsequent workers in California, Germany, and Australia failed to find the sexual stage and concluded that only the asexual stage served as an inoculum source (3,4,6).

Despite low frequencies of dormant twig infections and bud blighting on almonds, primary inoculum for spring infection of blossoms and emerging leaves is thought to originate from overwintering sources on the tree, because conidia are disseminated through splashing and windblown rain (6). The occurrence of numerous leaf infections and inoculum buildup in unsprayed almond orchards during fall rains, together with information on the durable nature of the dark, multicelled conidia (3,6), suggest conidia of *S. carpophila* may be deposited on bud scales of healthy dormant buds during fall rains and there survive the subsequent dormant season. If conidia were found to survive the dormant season in association with healthy dormant buds, a control program aimed at preventing fall buildup of inoculum could reduce the amount of primary inoculum the following spring.

The purposes of this study were to determine if viable conidia of *S. carpophila* are associated with healthy dormant almond buds collected from the orchard and to test the survival of *S. carpophila* conidia throughout the dormant season when inoculated onto healthy dormant buds.

MATERIALS AND METHODS

Natural association of *S. carpophila* conidia with dormant buds. Dormant bud samples were collected from 23-yr-old Nonpareil almond trees in a

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commercial almond orchard in Merced County, California, where a study on the effect of shot-hole disease on almond yields had been conducted (2). Plots within the orchard were arranged in a randomized, complete block (RCB) design with eight treatments and three replicates. Treatments consisted of various bloom-time fungicide applications applied over a 4 yr period.

Initial observation of dormant buds collected from lower branches of trees throughout the orchard indicated the presence of *S. carpophila* conidia. To determine whether conidial populations associated with dormant buds parallel disease levels, buds were obtained from trees within the two treatments where disease levels had been the highest (100% infected fruit in group 1) and lowest (11% infected fruit in group 2) when rated earlier in the 1982 season (2). Results from preliminary testing indicated that a sample size of 60 buds was optimal for conidial detection and sample processing.

Ten twigs from each of three Nonpareil almond trees within treatments were randomly collected on 8 December 1982 (leaf fall), 12 January 1983 (bud swell), and 11 February 1983 (early pink bud). For each treatment, 60 apparently healthy flower buds were removed from collected twigs, pooled, and all inner and outer bud scales removed. Scales from each 60-bud sample were placed in glass centrifuge tubes containing 5 ml of sterile distilled water and subsequently mixed on a Vortex mixer for 3 min, centrifuged at 3,000 rpm for 5 min, and remixed for 30 sec to suspend conidia. Washings containing the suspended conidia were removed from the bud scales with a Pasteur pipette and transferred to a clean centrifuge tube. The entire washing procedure was repeated three times for each sample, then suspensions containing conidia were pooled and centrifuged for 5 min at 3,000 rpm. Pelleted materials were resuspended in 0.5 ml of sterile distilled water and spread onto water agar plates. Plates were incubated at room temperature (24 C) for 8 hr, then the total number and viability (determined by germination) of conidia in each sample were recorded (Table 1). Under these conditions, 98% of *S. carpophila* conidia derived from culture germinated when placed on water agar.

Preliminary tests in which a wetting agent was added to bud scale samples before the successive washings did not show an increase in conidia removed from buds previously washed with distilled water. Thus, no wetting agent was used to remove conidia from buds.

Survival of *S. carpophila* conidia inoculated onto dormant buds. Studies on the survival of *S. carpophila* conidia on dormant buds were conducted on 6-yr-old Nonpareil almond trees at the U.C. Davis Armstrong Experimental Field Station. One hundred apparently healthy dormant buds on each of 12 trees were tagged for inoculation.

Conidial suspensions of *S. carpophila* used to inoculate dormant buds were derived from naturally infected almond leaves collected in fall of 1982 and held in cold storage at 32 C. Conidial masses from the centers of sporulating leaf lesions were removed with a sterile dissecting needle, placed in a test tube with 5 ml of distilled water, and mixed on a Vortex mixer to disperse conidia. Conidial suspensions were then adjusted to concentrations of 1.5×10^4 and 1.5×10^5 conidia per milliliter with sterile distilled water.

Inoculations were made on 12 December 1982 by pipetting 20-μl aliquots of the conidial suspension onto the surfaces of tagged dormant buds. Buds on five trees were inoculated with a suspension containing 1.5×10^4 conidia per milliliter (300 conidia per bud) and buds on five trees with a suspension containing 1.5×10^5 conidia per milliliter (3,000 conidia per bud). Buds on two trees were inoculated with sterile distilled water and served as controls.

Beginning 5 January 1983, eight buds per tree were removed at biweekly intervals through pink bud stage of bloom, and the number and viability of conidia present were determined according to the procedure outlined in the previous section.

RESULTS

Viable ungerminated *S. carpophila* conidia were detected in all bud samples collected from December 1982 through February 1983. The average numbers of conidia in 60-bud samples collected from group 1 trees on 8 December, 12 January, and 11 February were 194, 213, and 3,301, respectively. Corresponding figures for group 2 samples were 53, 147, and 1,535 (Fig. 1).

Regression analysis performed on collected data revealed a positive correlation ($P = 0.01$) for the general model between numbers of conidia and time. Regression equations for group 1 and group 2 samples and corresponding coefficent of correlation (r) values were $y = 2.0754 + 0.01826x$, $r = 0.85$, and $y = 1.5759 + 0.0223x$, $r = 0.94$, respectively.

The number of conidia detected in bud samples was significantly correlated ($P = 0.05$) with initial disease severity levels for plots from which samples were obtained. Greater numbers of conidia were consistently detected in samples from trees with higher disease severity levels (group 1) than in those from trees with lower disease severity levels (group 2) (Fig. 1).

A significant correlation ($P = 0.01$) also existed between numbers of conidia and time; increased numbers of conidia were detected in both group 1 and group 2 samples as the dormant season progressed. The greatest increase in numbers of detected conidia occurred during the 1-mo period between bud swell and early pink bud stage of bloom, where 15- and

Table 1. Number and viability of conidia inoculated onto dormant almond buds in 1982–1983

Inoculum density[a] (conidia/ dormant bud)	Sample date[b]								
	5 January			19 January		2 February		16 February	
	Tree no.	No. of conidia[c]	Viability[d] (%)	No. of conidia	Viability (%)	No. of conidia	Viability (%)	No. of conidia	Viability (%)
0 (control)	1	2	100	0	···	3	67	1	100
	7	0	···	0	···	2	100	1	100
300	2	17	76	53	94	7	100	21	95
	3	29	52	20	90	9	100	12	92
	4	59	90	49	86	10	90	28	89
	5	40	72	36	83	26	88	15	100
	6	43	84	51	94	7	100	9	89
3,000	8	>800	82	>800	91	>800	84	>800	90
	9	746	82	>800	86	>800	92	>800	88
	10	>800	75	>800	85	>800	92	>800	87
	11	>800	83	>800	88	>800	93	>800	91
	12	>800	84	>800	84	570	90	>800	95

[a] Conidial suspensions were obtained from sporulating leaf lesions.
[b] Eight tagged inoculated buds were randomly sampled at each sample date during the dormant season.
[c] Total number of conidia present in eight pooled buds.
[d] Percentage of total number of conidia that germinated on water agar after 8 hr.

10-fold increases over previous sampling dates were observed for group 1 and group 2 samples, respectively. There was not a significant correlation between number of conidia and the interaction term, date by disease, for collected samples ($P = 0.05$).

Rainfall data for the 2-mo period when bud samples were collected show that rain occurred on 7 days between 8 December 1982 and 12 January 1983 and on 15 days between 12 January 1983 and 11 February 1983. The total amount of rainfall during these periods was 1.63 and 6.53 in., respectively (1).

Germination percentages for conidia found within the samples ranged from 65 to 96 on water agar after 8 hr, indicating that most of the conidia associated with dormant buds were viable (Table 1).

Survival of *S. carpophila* conidia inoculated onto dormant buds. Viable conidia were recovered from buds inoculated on 12 December 1982 throughout the period from inoculation to early pink bud stage of bloom. More than 800 conidia per eight-bud sample were recovered on each sampling date from buds inoculated with 3,000 conidia per bud with two exceptions: 746 conidia from an eight-bud sample on 5 January and 570 conidia from an eight-bud sample on 2 February (Table 1). Fewer conidia were recovered from buds inoculated with 300 conidia per bud, ranging from seven to 59 per eight-bud sample.

The increase over time in number of conidia per sample observed in naturally infested bud samples from Merced County did not occur with inoculated bud samples in this study. Rain occurred on 41 days from 12 December 1982 to 16 February 1983, for a total of 10.67 in. during this period.

A few blighted buds were observed in trees in which dormant buds had been inoculated with 3,000 conidia per bud. These blighted buds were not evident before the last sampling date, 16 February 1983, when healthy buds were in the pink bud stage of development. Both mycelium and conidia were observed in the blighted buds.

Germination percentages for inoculated conidia recovered from dormant buds ranged from 52 to 100, indicating that most inoculated conidia remained viable throughout the 2-mo dormant season (Table 1).

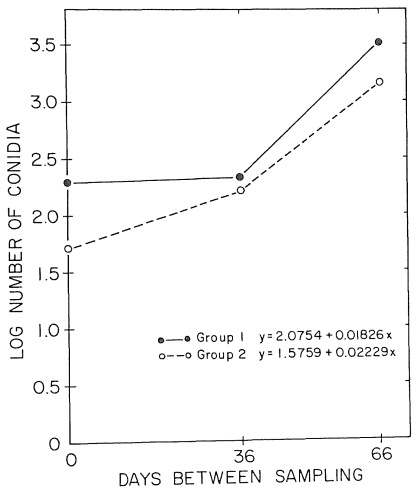

Fig. 1. Number of conidia associated with dormant bud samples collected during the 1982–1983 dormant season from a commercial almond orchard in Merced County, California. Samples were obtained from trees in which disease severity was rated at 100% infected fruit (group 1) and 11% infected fruit (group 2) on 12 May 1982. Each data point represents the mean of three replicates of three 60-bud samples each.

DISCUSSION

Results from these studies show viable conidia of *S. carpophila* were associated with healthy dormant blossom buds throughout the 1982–1983 dormant season in a commercial almond orchard. This observation, together with results from a study in which conidia inoculated onto dormant buds at leaf fall remained viable throughout the dormant season, supports the hypothesis that *S. carpophila* conidia contribute to the overwintering of the fungus on almond trees.

The significant increase in number of conidia detected in bud samples collected in the Merced County orchard over time can be explained in terms of both climatic and disease conditions that existed within the orchard during the dormant season. Rainfall data collected during the study show the frequency of rain increased twofold and total rainfall increased fourfold during the study.

Because *S. carpophila* conidia are easily dislodged and disseminated by rain (6), conidia associated with dormant buds on limbs higher in the trees were perhaps being washed downward by rain onto dormant buds lower in the tree. Limited sampling of twigs during the dormant season showed conidia present on twig surfaces as well as on buds. Thus conidia on aerial portions of the tree, in addition to buds, may have contributed to the number of conidia associated with bud samples. Because bud samples were collected from lower limbs, the occurrence of increased numbers of conidia over time would have been expected because significant amounts of rain fell as the dormant season progressed.

The infrequency and low amount of rainfall during the interval between the first and second sampling dates would account for the relatively small increase in numbers of conidia detected in samples from both group 1 and group 2 collected on 12 January compared with samples collected on 11 February. The significantly greater numbers of conidia consistently detected in group 1 samples compared with group 2 samples (evidenced by the significant difference in the midpoints of the two regression lines) would also have been expected because trees within group 1 had higher initial disease severity levels.

Although dormant bud blighting by *S. carpophila* commonly occurs on other hosts, fewer than 1% of the almond buds observed in either of our two studies were blighted despite the presence of viable conidia on buds throughout the dormant season. Although mechanism(s) that inhibit germination of conidia and bud infection are unknown, this situation does not appear unique to almond. On peach, dormant bud blighting results predominantly from early winter infection of twigs at the bases of buds with subsequent bud kill and invasion of the dead tissue rather than from direct infection of the bud itself (6). The fact that blighted buds found in the bud-inoculation study were not detected until time of bloom suggested that infection had occurred after bud swell.

Despite the low frequency of twig infections on almond and the apparent lack of sexual state formation by *S. carpophila*, results from this study suggest that the buildup of leaf infections and inoculum levels that occur during fall rains, previously considered to be of little consequence (6), is an important component in the shot-hole disease cycle on almond. These conidia, formed within leaf infections during fall rains, not only contribute to the overwintering population of the fungus but also constitute a ready source of primary inoculum for spring infections. In California, seasonal rains normally begin in November and continue through the first of May (1).

Unlike the situation found for other multiple-cycle diseases, the amount of primary inoculum present for initial infections appears to be an important factor in shot-hole disease development on almond. Because almonds begin blooming around mid-February, there is a period of about 2.5 mo in which spring infections can occur. If viable conidia are present on the bud surfaces and blossoms as the young nutlets and leaves emerge, infections and inoculum buildup can readily occur. Thus, if the amount of conidia that survive the dormant season could be reduced by preventing or decreasing the amount of fall leaf infection, perhaps spring infections and inoculum buildup could be delayed long enough to significantly reduce disease levels. Evidence for the effect of reduced amounts of fall inoculum on primary inoculum levels the following spring can be seen in results from our Merced County field plot, where both initial and overall inoculum levels were significantly lower from trees that had significantly lower disease severity levels the previous year. Control strategies such as fall applications of protectant or eradicant fungicides, antisporulants, or zinc sprays to induce defoliation should be investigated in terms of their potential for reducing fall and early winter inoculum buildup and inoculum survival.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Anonymous. 1982, 1983. National Oceanic and Atmospheric Administration Climatological Data, California. Vols. 86(12-13) and 87(1-2).
2. Highberg, L. M., and Ogawa, J. M 1986. Yield reduction in almond related to incidence of shot-hole disease. Plant Dis. 70:825-828.
3. Samuel, G. 1927. On the shot-hole caused by *Clasterosporium carpophila* and on the "shot-hole" effect. Ann. Bot. 41:377-404.
4. Smith, R. E. 1907. California peach blight. Calif. Univ. Agric. Exp. Stn. Bull. 191:73-100.
5. Vuillemin, P. 1888. L'*Ascospora beyerinckii* et la maladie des cerisiers. J. Bot. 2:255-59.
6. Wilson, E. E. 1937. The shot-hole disease of stone-fruit trees. Calif. Univ. Agric. Exp. Stn. Bull. 608:3-40.
7. Wilson, E. E. 1953. Coryneum blight of stone fruits. Pages 705-710 in: USDA Yearbook of Agricultural Plant Diseases. Government Printing Office, Washington, DC.

Yield Reduction in Almond Related to Incidence of Shot-Hole Disease

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ABSTRACT

Highberg, L. M., and Ogawa, J. M. 1986. Yield reduction in almond related to incidence of shot-hole disease. Plant Disease 70:825-828.

The effect of shot-hole disease, caused by *Stigmina carpophila*, on yield of Nonpareil almond was studied in two commercial almond orchards. Differences in disease severity among plots were obtained when various fungicides were applied over several years. Higher yields were obtained from treatments that significantly reduced disease severity. In a Merced County field plot where various fungicide treatments had been applied, disease severity in 1982 was 89 and 90% lower and yields were 283 and 240% higher, respectively, for the ziram and captan petal-fall spray treatments than for the untreated control. Similarly, in a Kern County field plot, a 2-yr treatment consisting of three bloom-time ziram spray applications resulted in 59% lower disease severity in 1982 and 36% higher yield than for the untreated control. Differences in kernel size and weight between treatments were not observed. Higher yields can be maintained with fungicide applications that significantly reduce disease incidence.

Additional key words: *Coryneum beyerinckii*

Shot-hole disease of stone fruit caused by the fungus *Stigmina carpophila* (Lév.) M. B. Ellis (=*Coryneum beyerinckii* Oud.) has been reported on *Prunus* species throughout the temperate regions of the world, including North and South America, Africa, Australia, and New Zealand. In the United States, shot-hole disease is serious on stone fruit cultivated in the Pacific Coast states, although it also is found in other parts of the country. In California, annual applications of protective fungicide sprays on almond, apricot, nectarine, and peach for shot-hole disease control are a standard orchard practice.

Increased costs of fungicide application and decreased returns to almond growers in recent years have resulted in the need for careful evaluation of the costs and returns associated with disease control programs. Demonstration of yield loss caused by shot-hole disease severity is a critical factor in such analyses, because shot-hole infection does not appear to result in almond yield loss through blemished fruit, bud blight, or twig dieback (1,3–7). Despite the absence of yield loss by such direct means, studies by Wilson (6) established the efficacy of fungicide applications for reducing shot-hole disease severity in almonds and also suggested concomitant yield increases. Wilson's data presented to support this contention, however, were inconclusive.

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In this study, shot-hole disease severity and crop yield comparisons were made between unsprayed trees and trees sprayed with various protective fungicides in two naturally infected almond orchards in an attempt to document and quantify crop loss associated with shot-hole disease incidence.

MATERIALS AND METHODS

To study the association between shot-hole disease severity and crop yield in Nonpareil almond, two field plots in Merced and Kern counties (San Joaquin Valley) were maintained over a 4- and 2-yr period, respectively. Comparisons of shot-hole disease severity and crop yields were made between trees that received various fungicide applications and trees that were not sprayed.

Merced County field plot. The Merced County field plot was in a 23-yr-old almond orchard with alternating rows of Nonpareil/Mission/Nonpareil/Merced cultivars planted on a 7.63-m-square planting design (173 trees per hectare). The orchard was sprinkle-irrigated and had a sandy loam soil. Trees within the field plot were differentially treated with various fungicides and timings of application over a 4-yr period (Table 1), using a randomized complete-block (RCB) design with six treatments replicated three times. Treatments consisted of five trees in each of four adjacent cultivar rows (Nonpareil/Mission/Nonpareil/Merced).

The 1982 season treatments consisted of single spray applications of protective fungicides at pink bud (PB) or petal fall (PF). The various fungicides, timings, and rates of application are summarized in Table 2. Ziram (76%) and captan (50W) were applied as either PB or PF sprays, and chlorothalonil (40.4%) was applied as a PF spray. Dates of application for the PB and PF sprays were 27 February and 28 March, respectively. Control trees did not receive protective fungicide applications. Benomyl (0.9 g a.i./L) was applied to all trees at full bloom for control of brown rot blossom blight, and dormant oil was applied in

Table 1. Three-year fungicide application histories of 1982 treatments in Merced County[a]

	Chemical and timing[b]		
1982	**1981**	**1980**	**1979**
Ziram (PF)	Copper (DD) Ziram (PB) Ziram (PF)	Ziram (PB) Ziram (PF)	Copper (D) Copper (DD) Ziram (PB)
Captan (PF)	Copper (DD) Captan (PB) Captan (PF)	Copper (DD) Captan (PB) Captan (PF)	Copper (DD) Ziram (PB) Ziram (PF)
Captan (PB)	Copper (DD) Captan (PB)	Captan (PB)	Copper (DD) Captan (PB) Captan (PF)
Chlorothalonil (PF)	Copper (DD) Chlorothalonil (PF)	Copper (DD)	Copper (D) Copper (DD)
Ziram (PB)	Copper (DD) Ziram (PB)	Ziram (PB)	Copper (DD) Ziram (PF)
Untreated control	Copper (DD) (control)	Untreated control	Copper (DD) (control)

[a] Fungicides were applied with a semiconcentrate airblast sprayer at 935 L/ha: ziram at 7.28 g a.i./L, captan at 4.79 g a.i./L, and chlorothalonil at 3.03 ml a.i./L. Annual applications of benomyl were included in all treatments, at full bloom for brown rot blossom blight and at dormant oil for mites and aphids.

[b] Timings of fungicide applications: D = dormant, DD = delayed dormant, PB = pink bud, and PF = petal fall.

January to control mites and aphids. Fungicide applications were made with a semiconcentrate airblast sprayer traveling at 3.54 km/hr and delivering 935 L/ha.

Data on disease levels were obtained from three representative Nonpareil trees in each treatment over the 4-yr period 1979–1982. In 1979, disease levels were evaluated in terms of percent infected leaves and percent infected fruit (i.e., one or more lesions present) after spring rains. In subsequent years, disease data were obtained by rating either leaf or fruit infection. Disease levels in 1982 treatments were evaluated in terms of percent infected fruit, average number of lesions per infected fruit, and total number of lesions per treatment for 60 fruits collected from the lower limbs of representative trees on 12 May. Maximum disease levels were present at the time of fruit collection. Disease ratings were analyzed by analysis of variance (ANOVA) and means were separated by Duncan's multiple range test at $P = 0.05$.

Crop yield in 1982 was measured by mechanical harvesting of Nonpariel trees on 16 September. Gross field weights of hulls, shells, kernels, and debris for each treatment sample were obtained, and subsamples (1,814 g) were removed. Nuts in each subsample were counted, hulled, and shelled, then kernels were dried to constant weight (130–145 C for 72 hr) and final subsample kernel dry weights were obtained. Gross field weights for each treatment were adjusted to a kernel dry weight basis for subsequent statistical analysis. Data on the number, weight, and size (volume) of kernels were also obtained for each treatment. Yield and kernel data were analyzed by ANOVA and means were separated by Duncan's multiple range test at $P = 0.05$.

Kern County field plot. The second field plot was in Kern County in an 8-yr-old almond orchard with alternating rows of Merced/Nonpareil/Nonpareil/Mission cultivars planted on a 7.32-m offset planting design (185 trees per hectare). The orchard was sprinkle-irrigated and had a sandy loam soil. The 2-yr field plot was arranged as a RCB design with two treatments (a ziram treatment and an untreated control treatment) and two replicates, each consisting of two adjacent 107-tree Nonpareil rows. Trees within the ziram treatment received applications at PB, PF, and 5 wk after PF. All applications were made at a rate of 7.28 g a.i./L with a semiconcentrate airblast sprayer traveling at 3.54 km/hr and delivering 935 L/ha. Before the 2-yr study, all trees within the field plot had identical fungicide application histories.

Disease levels were assessed in 1982 by rating the level of fruit infection on 100 fruits per replicate collected from the lower portions of treatment trees. Yield data for 1982 treatments were obtained by total plot harvest. Nuts from Nonpareil trees within treatments were mechanically harvested and gross field weights were obtained. Field weights were adjusted to a kernel dry weight basis, as previously described, and the data were analyzed by ANOVA as RCB with two treatments.

RESULTS

Merced County field plot. Disease data obtained for the Merced County field plot in 1979 and 1982 treatments are shown in Table 3. In 1979, significant ($P = 0.05$) differences in disease levels existed between the various fungicide treatments and the untreated control trees; fruit and leaf infection differences were similar for a given treatment.

Severe leaf drop in late spring of 1980 and 1981 made leaf infection ratings difficult during these two seasons. Data obtained from fallen leaves collected from underneath trees, however, showed leaf infection differences between fungicide-treated and untreated control trees similar to that in 1979. Because similar disease level differences were detected in 1979 with leaf infection and fruit infection data, fruit data were collected for disease evaluation in 1982 treatments to avoid complications resulting from premature leaf drop.

Regardless of the method used in 1982 to evaluate fruit infection levels, trees within the 1982 ziram and captan PF treatments had significantly lower disease severity levels ($P = 0.05$) than those within the chlorothalonil PF, ziram PB, captan PB, or control treatments (Table 2). Although significantly greater than in ziram or captan PF treatments, disease severity within the chlorothalonil PF treatment was significantly lower ($P = 0.05$) than in ziram PB, captan PB, or control treatments. Disease severity was not significantly different for the remaining treatments.

Yield data collected in 1982 showed trees within ziram or captan PF treatments had significantly greater yield than trees within the remaining treatments (Table 2). Yield of trees within the captan PB treatment, although significantly lower than in ziram or captan PF treatments, was significantly greater ($P = 0.05$) than in the control treatment. Yields for the remaining treatments were not statistically different.

Regression analysis of 1982 Merced County data showed a negative correlation ($P = 0.01$) between yield and disease severity. The regression equation obtained for the model was $y = 85.00 - 0.522x$, and the coefficient of correlation was $r = 0.9117$.

Comparisons of kernels obtained from the various treatments revealed no significant differences in the average size

Table 2. Effects of various fungicides and application timings on disease severity and yield of Nonpareil almond, Merced County, 1982

Treatment			Disease ratings[u]			
Fungicide[v]	Timing[w]	Rate (g a.i./L)	Fruit infected (%)	Total lesions (no.)	Av. lesions per infected fruit (no.)	Kernel yield[x] (kg/ha)
Ziram (76%)	PF	7.28	11 a[y]	24 a	4 a	2,203.6 a
Captan 50W	PF	4.79	10 a	27 a	5 a	1,961.5 a
Captan 50W	PB	4.79	95 c	822 c	14 bc	1,161.0 b
Chlorothalonil (40.4%)	PF	3.03[z]	77 b	507 b	11 b	1,130.7 bc
Ziram (76%)	PB	7.28	99 c	832 c	14 bc	1,025.2 bc
Untreated control	···	···	100 c	1,141 cd	19 d	575.2 c

[u] Based on 60 fruits per replicate collected 12 May 1982.
[v] Fungicides were applied with a semiconcentrate airblast sprayer at 935 L/ha.
[w] Dates of application for delayed dormant (DD), pink bud (PB), and petal fall (PF) spray treatments were 25 January, 27 February, and 28 March 1982, respectively.
[x] Based on adjusted kernel weights for three trees per replicate in a planting containing 173 trees per hectare.
[y] In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[z] Milliliters (a.i.) per liter.

Table 3. Disease ratings on Nonpareil almonds, Merced County, for 1979 and 1982

	1979		1982
1982 Treatment	Leaves infected (%)	Fruit infected (%)	Fruit infected (%)
Ziram PF[y]	22.6 b[z]	3.3 a	11 a
Captan PF	8.9 a	1.0 a	10 a
Captan PB	17.1 ab	0.7 a	95 c
Chlorothalonil PF	22.8 b	55.3 bc	77 b
Ziram PB	16.5 ab	14.7 a	99 c
Control	49.5 c	67.7 c	100 c

[y] PF = petal fall and PB = pink bud.
[z] In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

of kernels or the weight of 100 kernels per subsample (Table 4). Only kernel numbers per subsample differed significantly; the captan PF treatment had the greatest number of kernels per subsample.

Kern County field plot. Results obtained from the Kern County plot in 1982 revealed that fruit infection was significantly ($P = 0.05$) reduced by the ziram treatment (Table 5). Both percent infected fruit and number of lesions per infected fruit were significantly reduced by the ziram treatment. Sixty-four percent of the fruit in the ziram treatment compared with 12% in the control were uninfected (zero lesions per fruit). Similarly, only 6% of the diseased fruit in the ziram treatment compared with 48% in the control had more than 15 lesions per fruit.

Yield data for the Kern County plot in 1982 revealed that the ziram treatment resulted in a 36% increase in yield over the control (Table 5). No significant differences in average kernel size, number of kernels per subsample, or weight of 100 kernels per subsample (Table 6) were associated with the treatments in the Kern County plot.

DISCUSSION

Results of studies conducted in 1982 on the effect of shot-hole disease on almond yield show a significant correlation ($P = 0.01$) between yield and disease severity and support an earlier contention that higher yields are obtained where protective fungicide applications effectively control the disease (7). In the Merced County plot, highest yields and lowest disease levels were obtained in 1982 from trees that had received ziram or captan PF applications. These trees received two bloom-time spray applications of ziram or captan per year in 1979, 1980, and 1981. In contrast, a slight increase in yield and reductions in disease level resulted when trees received ziram or captan PB applications in 1982 and single bloom-time fungicide applications per year in 1979, 1980, and 1981.

The mechanisms by which shot-hole disease reduces almond yield are not known. As previously stated, shot-hole infections apparently do not result in direct yield loss through blemished fruit, bud blight, or twig dieback as is seen in other stone fruit crops (1,3–7), and they do not cause reduction in kernel size or kernel weight, as shown in this study.

Despite the lack of direct yield loss, several workers have reported premature defoliation (with or without an associated nut drop) resulting from shot-hole leaf infections (1–7). Wilson (6) presented data on the amount of defoliation within various fungicide treatments, as well as leaf infections and nut yield, and concluded that defoliation contributes to crop reduction and economic loss. However, he expressed caution in interpreting the data, stating that a severe and uneven infestation of red spider mites may have influenced defoliation and crop yield. In a later paper (7), Wilson again suggested an association between defoliation and shot-hole infections and stated that young, newly formed leaves will drop with only a few lesions per leaf, whereas older leaves remain on the tree despite larger numbers of lesions per leaf.

If leaf infections cause early defoliation (with greater defoliation where disease levels are greater) and if early defoliation adversely affects tree growth or vigor (2), then defoliation over several years could stress the trees or reduce the amount of fruiting wood. This argument would explain the apparent connection between 1982 yields and fungicide application histories in our Merced County study. The extremely low yield obtained in the control (575.5 kg/ha in the Merced County plot compared with the state average of 1,105 kg/ha for cultivar Nonpareil) possibly reflect tree stress or decline over the 4-yr period resulting from extremely high disease levels. In contrast, trees receiving single bloom-time fungicide applications over the 4-yr period had less disease and possibly more vigor than control trees. Trees treated with ziram or captan at PF, with disease

Table 4. Effects of various fungicide application timings on kernel characteristics of Nonpareil almonds, Merced County, 1982

Treatment			Kernel measurements[v]		
Fungicide	Timing	Rate (g a.i./L)	Av. kernel size[w] (ml)	Av. kernel no. per subsample	Weight of 100 kernels per subsample (g)
Captan 50W	PF[x]	4.79	109 a[y]	300 a	111 a
Ziram (76%)	PF	7.28	121 a	266 ab	122 a
Chlorothalonil (40.4%)	PF	3.03[z]	112 a	243 bc	177 a
Ziram (76%)	PB	7.28	115 a	239 bc	118 a
Captan 50W	PB	4.79	113 a	241 bc	115 a
Untreated control	···	···	107 a	216 bc	111 a

[v] Based on 1,814-g subsamples from each replicate. Nuts were hulled, shelled, and dried to constant weight before measurements were recorded.
[w] Based on the amount of water displacement (ml) of 100 kernels.
[x] PF = petal fall and PB = pink bud.
[y] In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[z] Milliliters (a.i.) per liter.

Table 5. Effects of three ziram applications on disease severity and yield of Nonpareil almonds, Kern County, 1982

Treatment	Fruit infected (%)	Percentage of infected fruit[w] (lesions per fruit)			Kernel yield[x] (kg/ha)
		1–5	6–15	>15	
Ziram (76%)[y]	36 a[z]	76	19	6	2,838 a
Untreated control	88 b	27	25	48	2,082 b

[w] Based on reading of 100 fruit per replicate collected from lower 6 ft of trees by Tejon Farming Company.
[x] Nuts were harvested from all trees within treatment replicates (214 trees per replicate).
[y] Applied in pink bud, petal fall, and 5 wk after petal fall with a semiconcentrate sprayer at 935 L/ha. Treatments were applied to the same trees for two consecutive years (1981 and 1982).
[z] In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Table 6. Effects of three ziram applications on kernel characteristics of Nonpareil almonds, Kern County, 1982

Treatment	Kernel measurements[w]		
	Av. kernel size[x] (ml)	Av. kernel no. per subsample	Weight of 100 kernels per subsample (g)
Ziram (67%)[y]	114 a[z]	427 a	118 a
Untreated control	120 a	2383 a	121 a

[w] Based on 1,814-g subsamples from each replicate. Nuts were hulled, shelled, and dried to constant weight before measurements were recorded.
[x] Based on the amount of water displacemnt (ml) of 100 kernels.
[y] Applied in pink bud, petal fall, and 5 wk after petal fall with a semiconcentrate sprayer at 935 L/ha. Treatments were applied to the same trees for two consecutive years (1981 and 1982).
[z] In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

kept at low levels by two bloom-time spray applications annually over the 4-yr period, were distinctly more vigorous than control trees.

Indirect evidence of tree stress or decline in the Merced County study was apparent in subsamples collected after mechanical harvesting of the various treatment plots. Subsamples obtained from trees where shot-hole disease levels were high contained significant amounts of tree debris along with kernels. In these subsamples, debris probably accounted for a significant proportion of the subsample weight. However, in subsamples collected from trees where disease levels were low (ziram and captan PF plots), the amount of tree debris collected along with kernels was minimal and kernels alone probably accounted for the bulk of the subsample weight. As would be expected, these subsamples contained significantly greater numbers of kernels. During mechanical harvesting, greater amounts of debris would be expected to fall from stressed trees, because weakened and dead limbs would be more abundant. Results from the Kern County study illustrate how quickly shot hole can affect yield of Nonpareil almond trees; after only 2 yr, significant yield increases were obtained for trees receiving three ziram applications over trees in the control.

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LITERATURE CITED

1. Aldrich, T., Moller, W. J., and Schulbach, H. 1974. Shot hole disease control in almonds—by injecting fungicides into overhead sprinklers. Calif. Agric. 28(10):11.
2. Anonymous. 1930. Shot-hole disease of almond trees in farming in South Africa. Union S. Afr. Dep. Agric. Reprint 37. 2 pp.
3. Luepschen, N. S., Rohrback, R. G., Core, C. M., and Gilbert, H. A. 1968. Coryneum blight infection and control studies. Colo. State Univ. Exp. Stn. Prog. Rep. 68-2. 2 pp
4. Ogawa, J. M., English, H., Yates, W. E., and O'Reilly, H. J. 1959. Chemical control of Corynemum blight. Almond Facts 23:4.
5. Ogawa, J. M., Teviotdale, B. L., and Rough, D. 1983. Shot hole of stone fruits. Univ. Calif. Div. Agric. Sci. Leafl. 21363. 4 pp.
6. Wilson, E. E. 1937. The shot-hole disease of stone-fruit trees. Calif. Univ. Agric. Exp. Stn. Bull. 608:3-40.
7. Wilson, E. E. 1953. Coryneum blight of stone fruits. Pages 705-710 in: USDA Yearbook of Agricultural Plant Diseases. Government Printing Office, Washington, DC.