Completed

FINAL REPORT

Project title: Effects of four levels of applied nitrogen on three fungal diseases of almond trees.

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STATEMENT OF OBJECTIVES

Our objectives were to determine the relationship between tree nitrogen status and

1) the natural incidence of brown rot blossom and twig blight of almond and infection of flowers and shoots inoculated with *M. fructicola* and *M. laxa*

2) infection of green fruit of almond by *B. cinerea*, *M. fructicola* and *M. laxa**

3) the natural incidence of hull rot of almond and hull infection and leaf death following inoculation caused with *M. fructicola* and *R. stolonifer.*

*Effects of nitrogen levels on green fruit rot, an original objective of the research, was deleted because data were inadequate. Bloom and early fruit growth were early, about three weeks ahead of normal, thus inoculations occurred after fruit had passed the stage of susceptibility. In lieu of

this information, data collected over several years on scab and shot hole diseases are in included in this report. Objective 2 is amended as follows:

2a) the natural incidence of shot hole and scab of almond.

EXECUTIVE SUMMARY

The effect of four levels of applied nitrogen on brown rot blossom and twig blight, shot hole, scab, and hull rot diseases of almond trees were investigated. The diseases span the growing season from bloom through harvest and occur in all almond growing districts of the state. Brown rot, shot hole, and scab usually are effectively controlled by fungicide applications, and chemical control is not available or likely for hull rot. Cultural practices in general, and nitrogen fertilization in particular, are known to affect plant disease incidence and severity. Demonstration that nitrogen levels similarly affect almond diseases should encourage growers to include evaluation of their nitrogen practices when designing fungicide programs for disease control. Even though reduction of nitrogen alone may not produce acceptable control of plant diseases, conditions that render plants more susceptible to disease, e.g. nitrogen fertilization, could negatively affect efficacy of fungicide treatments. Diseases not controlled by fungicides, such as hull rot, in part may be managed by changes in nitrogen fertilization

Our methods included inoculation of flowers (brown rot), young fruit(green fruit rot), and maturing, dehiscent fruit (hull rot) with the appropriate pathogens followed by measurement of the responses, and evaluation of natural incidence of diseases. All diseases responded similarly in that disease increased with increasing amounts of applied nitrogen. These results agree with that found in other plant systems, and further support the contention that excess nitrogen use can exacerbate disease problems and may compromise effectiveness of fungicides. These data should be useful in educational efforts directed at improved disease control, reduced nitrogen use, or both.

WORK DESCRIPTION

Location and general procedures: Experiments were conducted in commercial almond orchards located in Stanislaus County. The Salida orchard was planted 1 row: 1 row with cultivars Nonpareil and Carmel, and the Ceres orchard was planted 1row:1row:1row with cultivars Price:Nonpareil:Carmel. Trees were mature, sprinkler irrigated, and treated once annually by the growers with a fungicide at bloom to control brown rot blossom and twig blight. There were four nitrogen treatments: 500, 250, 125 and 0 pounds nitrogen per acre applied as a split application: two thirds the amount was applied in fall, one-third in spring. These treatments were applied to the same trees for four years. Each plot consisted of four adjacent trees of each cultivar in three adjacent rows with the Nonpareil row in the center. Fertilizer was positioned around and within this 4 X 3-tree plot so that one adjacent row and both sides of the Nonpareil row and the two end Nonpareil trees functioned as buffer trees for the two central Nonpareil trees. Some experiments were conducted in both orchards in 1992 or 1993, and all experiments

were located in the Ceres orchard in 1994 and 1995. Each June, 100 leaves per tree were taken from each of the central Nonpareil trees and analyzed to determine percent nitrogen content. Additional leaf and fruit samples were collected from the Nonpareil and Carmel trees for nitrogen analysis. Results of nitrogen analyses may be found in Table 1; those for which results have not been received as of this writing will be forwarded later.

Data were analyzed using a two-way factorial analysis of variance and means separated by orthogonal contrasts or Duncan's multiple range test.

TASK 1. To determine the relationship between tree nitrogen status and the natural incidence of brown rot blossom and twig blight of almond and infection of flowers and shoots inoculated with *M. fructicola* and *M. laxa*.

Subtask 1.1. Monitor production of sporodochia on twig lesions of *M. fructicola* and *M. laxa*. (These infections were established in spring, 1994).--Ceres orchard--Four lesions on one tree each Nonpareil and Carmel in each plot were observed every two to three weeks during winter and number of sporodochia present counted. (November 1994 through February 1995)

Results: No sporodochia were found on any naturally infected flowers and spurs (40 observed) or on any shoot lesions (128 observed).

Discussion and Conclusions: Apparently, production of sporodochia, the sources of primary inoculum for spring flower infection, was at a low level in the orchard this season thus none were observed in our sample. We did not identify any condition or circumstance to explain this lack of obvious inoculum production. The requirements for sporodochial development under natural conditions have not been determined.

Subtask 1.2. On 2 March 1994, 5 to 10 shoots on one Nonpareil tree in each replication were covered with paper bags during the fungicide applications made by the growers. Open flowers (any unopened flowers were removed from the test shoots) on these shoots were inoculated on 4 March with a 10^3 conidia per ml suspension of *M. laxa*, and covered for 24 hr with plastic bags. Fifty flowers per replication were collected, and percent infected stamens was determined on 11 March. On 10 and 15 February 1995, 10 to 15 twigs bearing open flowers were removed from one Nonpareil and one Carmel tree in each replication of each treatment and transported to the laboratory. Twenty-five open flowers from each sample were removed from the twigs and placed face-up on sterile moist sand in plastic sweater boxes. The flowers were inoculated with a 10^3 conidia/ml suspension of *M. laxa*, incubated at room temperature for three days, and percent infected stamens calculated. The first collection was made before the grower-applied fungicide treatment of Rovral 50W, 1.0 lb per acre, on 11 February. The trees were at pink bud to 5% (Carmel) and 10% to 15% (Nonpareil) and at 60% to 80% (Carmel) and 80% 100% (Nonpareil) bloom for the first and second flower collections, respectively.

Results: Percent infected stamens increased with increasing levels of applied nitrogen, and infection percentages were greater on the second than on the first collection date in 1995 (Table

2). In both years, the percent infected stamens was similar in the two highest nitrogen treatments (500 and 250 pounds per acre). Increase in nitrogen beyond the 250 pounds per acre level did not increase the amount of brown rot.

Discussion and Conclusions: Aside from the general response of increased disease with increase in nitrogen application, comparison of disease levels between years cannot be made because flowers were *in situ* in 1994 and detached in 1995. The much higher infection found in the flowers from the second collection in 1995 is of interest in that these flowers had been sprayed with Rovral by the grower 4 days before collection. Undetermined differences in inoculation technique, fungicide application or other unidentified factors also may have affected percent infection.

Subtask 1.3. Fifty flowers at full bloom were to be collected from the periphery of each central Nonpareil, Carmel and Price trees, dried, ground, and submitted for nitrogen analysis.

This was not done because the amount of flower tissue required to run the analysis was far greater than we were able to collect.

Subtask 1.4 Shoot inoculation--Four 2- to 3-year-old shoots on one each Nonpareil and Carmel tree per replication were inoculated with *M. fructicola* and *M. laxa* by placing a mycelial plug from agar cultures of these fungi under small flaps of bark then wrapping the wound with tape. Tape was removed after two weeks. In 1993, detached shoots were collected on 26 February, the basal ends submerged in beakers of water, and covered with plastic bags to prevent drying. Shoots were inoculated on 27 February, and kept at room temperature on the laboratory bench until 10 March when lesions were measured. In 1994, shoots on trees were inoculated 10 May and measured 20 June.

Results: No significant differences in lesion length were found among the nitrogen treatments or between pathogens (Table 3). Both cultivars responded similarly.

Discussion and Conclusions: Apparently elements other than nitrogen play a major role in determining the extent of shoot lesions or nitrogen content of shoot tissue does not vary in the same manner as it does in tissues such as leaves, flower parts, and fruit.

Subtask 1.5 Natural incidence of brown rot--(Not listed in the original project)--Ceres and Salida orchards. All brown rot strikes in all Carmel trees in each plot were counted on 26 May 1993 (Salida) and 10 May 1994 (Ceres).

Results: Brown rot incidence increased with increasing levels of applied nitrogen (Table 4).

Discussion and Conclusions: The relationship between increased nitrogen and increased brown rot was observed in both orchards even though the amount of brown rot was much less in the Ceres orchard in 1994. As was found with the flower inoculation tests, the incidence of brown rot in the two highest nitrogen treatments (250 and 500 pounds per acre) were similar.

TASK 2. To determine the relationship between tree nitrogen status and infection of green fruit of almond by *B. cinerea*, *M. fructicola*, and *M. laxa*.

Although we inoculated fruit as expected, the progress of bloom and fruit development was so early and advanced that fruit apparently had passed the stage of susceptibility by the time we began this experiment. In place of information on this subject, we offer data on two other diseases: shot hole and scab.

TASK 2a. To evaluate the relationship between tree nitrogen status and natural incidence of shot hole and scab diseases of almond trees.

Shot hole (*Wilsonomyces carpophilus*) and scab (*Cladosporium carpophilum*) diseases affect leaves and fruit of almond trees. Both cause defoliation, and early severe shot hole causes young fruit to drop. We evaluated natural incidence of shot hole and scab on 50 fruit, scab on 80 leaves of Nonpareil and Carmel trees per replication on 14 July 1993, and 50 leaves per replication of Carmel trees in the Ceres orchard 21 August 1995.

Results: Incidence of scab and shot hole increased with increasing levels of applied nitrogen (Table 4).

Discussion and Conclusions: These two important diseases of almond also are exacerbated by higher levels of nitrogen.

TASK 3. To determine the relationship between tree nitrogen status and the natural incidence of hull rot of almond and on hull infection and leaf death caused by inoculation with *M. fructicola* and *R. stolonifer*.

Subtask 3.1. Fruit with firmly attached hulls in early dehiscence and associated with healthy leaves were inoculated with 10^4 conidia per ml suspensions of *M. fructicola* and *R. stolonifer* on 7 August 1995. Inoculum was delivered into the open hull using a hand pump atomizer. Noninoculated healthy fruit at the same stage of dehiscence served as controls. Twenty-five fruit per hull rot treatment were inoculated in each of the experimental nitrogen plots. Hulls of ten fruit at similar stages of maturity as those inoculated were collected, weighed immediately in the field, returned to the laboratory, and air dried at 130 F for 72 hours to determine the percent hull moisture content. Selected hulls were submitted for nitrogen analysis. On 21 August, before trees were shaken for harvest, the condition (healthy, dead, or missing) of leaves associated with each test fruit were recorded and all test fruit collected and returned to the laboratory and examined for hull lesions and presence of the pathogens.

All hull rot strikes (clusters of dead leaves) in each tree were counted 25 August, after trees were shaken (24 August 1995) A random samples of fruit were collected from beneath the data trees, returned to the laboratory, the number per 100 fruit having hull rot lesions was counted, and the hull rot pathogen(s) present identified.

Results: Natural incidence of hull rot increased with increasing levels of nitrogen, and Monilinia was the more common hull rot pathogen found in all years except 1992 (Tables 5 and 6) In inoculation tests, percent leaf strikes tended to be greater near inoculated fruit on trees in the higher nitrogen treatments (250 and 500 lbs per acre) but this relationship was not as clear as in natural infections (Table 7).

Discussion and Conclusions: There was great difficulty in inoculation tests throughout the course of this work. Fruit in the higher nitrogen treatments tended to ripen more slowly, and trees within a treatment also varied. Thus it was impossible to select fruit at similar stages of abscission and dehiscence in all replications of all treatments on the same day. Inoculation of same-stage fruit meant inoculation on different days. We tried this approach but it did not appreciably change the results. Consequently, we report here results of inoculations that occurred and were evaluated on the same days even though fruit were not at the same stages of development when inoculated.

The natural infection data provide better information about the relationship of hull rot to nitrogen regimes. The inoculation tests do, however, show that *Monilinia* generally caused more hull rot than did *Rhizopus*, and that both fungi responded similarly to the nitrogen treatments.

Subtask 3.2. and 3.3 Because leaf and shoot death is thought to be caused by a toxin that is produced in the hull and transported to the shoot and leaves, the rate of hull abscission (and therefore interruption of toxin transport) may play a role in severity of hull rot. We investigated the relationship of nitrogen status to hull loosening. Fifty non-split Nonpareil fruit on one tree in each replication were tagged at early hull split 20 July 1995. Fruit were observed weekly and the percent of visible separation from the hull estimated for each fruit. (Subtask 3.2) Abscission was rated for each fruit as 1= no abscission or dehiscence, 2=no abscission, slight dehiscence, 3=<25% abscission, 4=25 to 50% abscission, 5=50 to 75% abscission, and 6=>75% abscission, hulls fully open and yellowing. Fruit in categories 3 through 6 were counted to determine percent hull dehiscence (split) (Subtask 3.3).

Results: Hull abscission and dehiscence tended to progress more rapidly in the lower nitrogen level treatments (Table 8).

Discussion and Conclusions: Fruit maturity apparently is delayed by higher nitrogen regimes and this in turn may allow more opportunity for the hull rot pathogens to generate the toxin which causes leaf death.

GENERAL RESULTS, DISCUSSION, AND CONCLUSIONS

In every disease studied, disease incidence increased with increasing nitrogen application. This was true regardless of the general level of disease present, and responses were similar where disease levels were low as well as when levels were higher. In several instances, the amount of disease did not differ much between the two highest nitrogen rates, 250 and 500 pound per acre treatments. This pattern occurred in the percent leaf nitrogen content as well. Thus there seems

to be a direct response to leaf nitrogen content. We found no evidence of threshold values for leaf nitrogen content that might signal an upsurge in disease. The many other factors that govern severe disease outbreaks must play out against the background of tree susceptibility. Hence, in any given set of circumstances, higher nitrogen content appears to foster relatively more disease.

Any grower could use this information to advantage. Coupled with optimum nitrogen levels for general productivity and growth, measures could be taken not to exceed these levels. Growers with very serious disease problems may consider reducing nitrogen treatment at least slightly as this should help reduce disease.

Evaluation of fungicide programs probably should take into account the nitrogen status of the test trees. At least in part, differences in nitrogen status may help explain some of the variability found among different years and locations in the efficacy of fungicide treatments.

PROJECT EVALUATION

Not applicable

OUTREACH

Results of this work were presented in radio interviews conducted by Patrick Cavanaugh and aired three times during the year, at the annual conference sponsored by the Almond Board of California (December 1995), the Fertilizer Research and Education Conference (December 1995), and in an article in the California Arizona Farm Press (January 1996). Other articles will be published in Pacific Nut Grower, Almond Facts, and California Agriculture. The information also will be presented at grower meetings sponsored by farm advisors (variable, spring of 1996 and later).

I want to impress on the reviewers that this work will not sit on the shelf gathering dust. I speak on fungicide programs for almonds many times in any year, both formally and informally. I am convinced that review of the nitrogen program in an orchard is no trivial matter in developing good disease control practices, and I will urge growers to include re-evaluation of their nitrogen fertilization practices when designing their fungicide programs.

Table 1. Leaf nitrogen content of almond cv Nonpareil trees fertilized with four levels of nitrogen, Stanislaus County.

Nitrogen,	Ceres orchard[y]						Salida orchard[y]			
lbs/acre	1990	1991	1992	1993	1994	1995[z]	1989	1990	1991	1992
500	2.67	2.52	2.48	2.74	2.72		2.54	2.42	2.36	2.61
250	2.70	2.48	2.44	2.72	2.74		2.60	2.36	2.24	2.52
125	2.68	2.48	2.30	2.51	2.63		2.65	2.34	2.21	2.42
0	2.71	2.48	2.24	2.36	2.51		2.63	2.27	2.12	2.28
$P =$	.820	.800	.003	.001	.010		.707	.173	.009	.024
Linear contrast, $P =$	NS	NS	.001	.000	.005		.319	.039	.001	.004

[y] 100 leaves per tree collected in June each year, four replications.

[z] Not available at this time.

Table 2. Infection of flowers inoculated with *Monilinia laxa* from almond trees fertilized with four levels of nitrogen, Stanislaus County.

	Infected stamens (%)[x]				
Nitrogen,	4 Mar 1994[y]	10 Feb 1995[z]		15 Feb 1995[z]	
lbs/acre	Nonpareil	Carmel	Nonpareil	Carmel	Nonpareil
500	15.1	9.5	5.3	68.4	14.2
250	14.6	9.2	7.2	68.9	10.6
125	8.2	5.2	3.7	51.9	5.4
0	7.5	4.8	3.0	36.3	3.2
$P =$	.076	.040	.096	.004	.020
Linear contrast, $P =$	.019	.012	.032	.001	.009

[x] Twenty-five flowers per each of four replications.

[y] Flowers were inoculated 4 March, on tree, and examined 11 March 1994.

[z] Flowers were removed and inoculated on 10 and 15 February 1994, and incubated 3 days at room temperature. Trees treated by grower with fungicide 11 February 1995.

Table 3. Effect of four levels of applied nitrogen on shoot infection of almond trees inoculated with *Monilinia laxa*, Stanislaus County.

Nitrogen, lb/acre	Canker length, mm			
	1993[y]		1994[z]	
	Carmel	Nonpareil	Carmel	Nonpareil
500	95.2	76.0	116.6	111.3
250	105.5	79.0	99.5	90.3
125	71.5	78.2	96.4	117.9
0	77.0	69.0	92.9	88.1
$P =$	.097	.393	.485	.296
Linear contrast, $P =$	.091	.107	..148	.193

[y] Detached 2-yr old shoots, collected 26 Feb 1993 from each of four replications, inoculated 27 Feb 1993, incubated at room temperature, cankers measured 10 Mar 1993.

[z] Two-yr old shoots on trees in each of four replications, inoculated 10 May 1994, cankers measured 20 June 1994.

Table 4. Natural incidence of brown rot, shot hole, and scab of almond cv Carmel trees fertilized with four levels of nitrogen, Stanislaus County.

Nitrogen, lbs/acre	Brown rot[x]		Shot hole[y]		Scab[z]		
	Strikes/ tree		Infected fruit (%)		Infected leaves (%)		Infected fruit (%)
	Salida	Ceres	Salida		Salida	Ceres	Salida
	Carmel	Carmel	Carmel	Nonpareil	Carmel	Carmel	Carmel
500	60.8	4.7	97.6	60.0	3.1	50.0	22.4
250	61.2	4.0	82.8	48.5	2.5	44.0	18.4
125	24.7	3.5	78.5	46.5	1.5	16.0	14.7
0	19.0	2.5	69.0	40.5	2.1	19.5	9.9
$P =$	.003	.494	.069	.003	.146	.002	.005
Linear contrast	.001	.153	.012	.001	.046	.001	.001

[x] All brown rot strikes in eight Carmel trees per replication counted 26 May 1993 (Salida orchard) and in four Carmel trees 10 May 1994 (Ceres orchard).

[y] Fifty fruit collected from each of eight Carmel and two Nonpareil trees, per replication, 14 July 1993.

[z] Fifty leaves and fruit collected from each of eight trees per replication 14 July 1993 (Salida orchard) and 21 August 1995 (Ceres orchard).

Table 5. Natural incidence of hull rot in almond cv. Nonpareil trees fertilized with four levels of nitrogen, Ceres orchard, Stanislaus County.

Nitrogen,	Number leaf strikes/tree[z]				Hull infection (%)[z]			
lbs/acre	1992	1993	1994	1995	1992	1993	1994	1995
500	22.8	39.5	34.5	23.2	12.6	28.5	35.0	12.9
250	22.0	33.5	32.7	15.5	11.0	30.2	32.3	10.6
125	19.2	24.5	21.5	11.2	9.6	26.0	25.1	9.1
0	17.0	17.2	14.0	9.2	8.5	17.0	21.5	6.6
Linear contrast *P*=	.044	.012	.007	.000	.016	.074	.010	.030
Monilinia					8.9	49.7	51.7	17.8
Rhizopus					12.0	1.1	5.1	1.8
P =					NS	.000	.000	.000
Trees shaken for harvest	19 Aug	28 Aug	29 Aug	21 Aug				
Data collected	21 Aug	18 Aug	31 Aug	25 Aug				

[z] All hull rot strikes in two trees per replication counted 21, 18, 31, and 24 August 1992, 1993, 1994, and 1995, respectively. One hundred fruit from beneath trees collected from orchard floor after trees shaken for harvest, hulls examined for lesions, and pathogen(s) identified.

Table 6. Natural incidence of hull rot in almond cv Nonpareil trees fertilized with four levels of nitrogen, Salida orchard, Stanislaus County.

Nitrogen,	Number leaf strikes/tree[z]		Hull infection (%)[z]	
lbs/acre	1992	1993	1992	1993
500	37.5	59.2	18.7	32.0
250	36.0	47.1	18.0	28.5
125	24.5	22.5	12.2	27.6
0	35.3	27.4	18.0	22.0
Linear contrast $P =$	.066	.001	.196	.024
Monilinia			8.7	52.7
Rhizopus			24.7	2.3
$P =$			.000	.000
Trees shaken for harvest	20 Aug	23 Aug		
Data collected	21 Aug	24 Aug		

[z] All hull rot strikes in two trees per replication counted 21 and 24 August 1992 and 1993, respectively. One hundred fruit from beneath trees collected from orchard floor after trees shaken fro harvest, hulls examined for lesions, and pathogen(s) identified.

Table 7. Effect of four levels of applied nitrogen on hull rot almond fruit almond fruit inoculated with *Monilinia fructicola* and *Rhizopus stolonifer*, Stanislaus County.

	Leaf strikes[z]				Hull infection[z]			
Nitrogen	Salida	Ceres			Salida	Ceres		
lbs/acre	1993	1993	1994	1995	1993	1993	1994	1995
500	49.0	56.8	30.7	55.7	74.7	76.5	58.5	48.6
250	51.0	58.0	38.5	46.3	65.2	69.1	65.5	50.7
125	39.1	47.5	54.0	39.7	60.7	71.1	70.5	42.1
0	38.5	50.8	49.5	43.3	60.3	65.9	74.5	47.7
Monilinia	84.6 a	83.1 a	48.1 a	55.6 a	96.5 a	98.2 a	83.5 a	62.4 a
Rhizopus	33.0 b	57.2 b	38.2 b	62.7 a	54.2 b	69.2 b	51.0 b	58.8 a
Control	15.7 c	19.5 c	13.9 c	20.4 b	45.1 c	44.6 c	36.2 c	20.5 b
Nitrogen (N)	.004	.097	.009	.063	.025	.005	.323	NS
Pathogen (P)	.000	.000	.019	.000	.000	.000	.000	.000
N x P	.076	NS	.389	NS	NS	NS	NS	NS
Linear contrast, *P* =	.003	NS	.002	.017	.000	.028	NS	NS

[z] Twenty-five fruit, each situated next to healthy leaves, per replication, inoculated, and leaf strikes and fruit infection evaluated in August, within seven days before harvest each year.

Table 8. Effect of four levels of applied nitrogen on hull abscission and dehiscence of almond cv Nonpareil trees, Ceres orchard, Stanislaus County.

	Abscission[y]			Dehiscence (%)[y]		
	1993	1994	1995	1993	1994	1995
Nitrogen, lbs/acre						
500	2.5	3.7	3.0	46.5	72.5	63.7
250	3.7	3.7	3.1	70.3	72.9	65.0
125	3.9	4.0	3.2	71.7	78.9	65.5
0	3.9	4.2	3.7	71.6	79.8	71.7
Week						
1	1.2 c[z]	1.6 d	1.3 d	17.2 d	30.6 c	34.0 c
2	1.6 c	3.1 c	2.5 c	53.2 c	76.3 b	58.9 b
3	4.1 b	5.1 b	4.3 b	90.1 b	97.3 a	83.5 a
4	5.1 a	5.8 a	5.2 a	98.8 a	99.9 a	89.5 a
Nitrogen	.003	NS	.269	.021	NS	.340
Week	.000	.000	.000	.000	.000	.000
Nitrogen x week	NS	NS	.312	.001	NS	NS
Linear contrast, *P*=	.001	.265	.101	.006	.287	.327

[y] Fifty fruit tagged before hull split and monitored weekly until harvest. Abscission rated from 1 = no abscission or dehiscence to 6 = hulls barely attached, fully opened, and yellowing.

[z] Means are for main effects and those followed by the same letter do not differ significantly according to Duncan's multiple range test ($P = 0.05$).