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INVESTIGATIONS ON NAVEL ORANGEWORM OVIPOSITION DISRUPTION

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I. Ovipositional Disruption of Navel Orangeworm

Ovipositional disruption is similar in concept to mating disruption in that the purpose of both is to permeate the air with the odor of an object in order to make it impossible for the insect to locate the object by flying toward its odor. To disrupt navel orangeworm oviposition, the air surrounding the almond tree could be permeated with the odor of almond nut, the ovipositional stimulant, by spraying the tree with materials such as crude almond oil or powdered reject almonds. The female then would not be able to "smell" the nut (mummy or sound-split) on which to oviposit. Such materials could be applied early in the spring during the first ovipositional period, when the available ovipositional sites and populations are low, to make it difficult for the navel orangeworm to reproduce. They could also be applied at hull-split to protect the sound nuts and lessen the severity of infestation.

Research conducted during the past two years has shown that emulsified crude almond oil or a wettable powder formulation of powdered almond press cake and crude almond oil causes a disruption of the nut-finding ability of NOW. Reported here are the results of studies on the removal of aflatoxin from reject almonds, an interim report on the long-term phytotoxicity of crude almond oil and wettable powder of reject almonds, and the results of laboratory bioassays of crude almond oil and wettable powder of reject almonds on navel orangeworm ovipositional behavior.

A. Aflatoxin Removal from Reject Almonds

Aflatoxins are produced by certain species of mold, especially *Aspergillus flavus*, in almonds, walnuts, corn, peanuts, and cottonseed and various oilseed meals. The *A. flavus* mold is likely to occur in damaged nuts with an intermediate moisture level. The aflatoxin produced by *A. flavus* is extremely toxic and is carcinogenic. For this reason the FDA has set an informal guideline level of 20 ppb of aflatoxin. Products with aflatoxin levels greater than this may be seized. Reject almonds may have very high levels of aflatoxin, ranging from parts per million to non-detectable levels. If a wettable powder formulation of reject almonds is to be developed to disrupt navel orangeworm ovipositional behavior, the aflatoxin level should be reduced to below 20 ppb. Thus, studies were conducted in cooperation with Hy-D Co. and Duchi Nut Co. to reduce or eliminate the aflatoxin in reject almonds.

A procedure to detoxify aflatoxin using hot gaseous ammonia has been developed by Hy-D Co. This procedure was tested on eight samples of reject almonds. The subsequent aflatoxin levels of the treated samples and four untreated samples were determined by DFA of California. The results showed that the hot ammonia treatment did not eliminate the aflatoxin. The treated samples had a mean of 258.8 ppb aflatoxin, with a range from none detected to 900 ppb. The untreated samples had a mean of 576.4 ppb, with a range from none detected to 2300 ppb.

The apparent reason the aflatoxin was not removed was that some aflatoxin is produced within the nut meat, and the ammonia treatment detoxified only the surface aflatoxin without penetrating the nut meat. To elmiminate this problem, the reject almond meats were first diced into small pieces before the ammonia treatment. Eight samples of diced almonds were treated with ammonia by Hy-D and analyzed for aflatoxin by DFA. The results showed that the hot ammonia treatment following nut dicing would reduce the aflatoxin levels below 20 ppb. The treated samples had a mean of 9.5 ppb, with 5 samples at 0.0 and 1 sample each at 3, 5, and 58 ppb.

Thus, in the production of a wettable powder from reject almonds, the use of the hot ammonia treatment will reduce the aflatoxin levels to below 20 ppb. The cost of treatment on a commercial basis is expected to be around $.02 to $.03 per lb.

B. Phytotoxicity Studies

We observed in last year's large-plot disruption studies that some leaf burn occurred when 5 gal of crude almond oil were applied in 100 gal water per acre. Thus, additional phytotoxicity studies were conducted in Fresno County on 6-year-old Nonpareil trees. The treatments were 0.5, 1, and 2 gal crude almond oil and 10, 20, and 40 lb wettable powder of reject almonds in 200 gal water per acre, applied on 4/2, 4/22, and 5/21, with plans to apply the same on 7/11, 8/6 and 9/4. Each treatment and an untreated control were replicated five times in a randomized complete block design. The effect of the various treatments was evaluated at approximately monthly intervals by inspecting the foliage for phytotoxic reaction. Since no phytotoxicity was observed in any treatment by June, the oil rates were doubled for the last three applications. Yield data was obtained on 8/20. The treatments will be further evaluated next spring for fruit set and development. Also, next year a similar phytotoxicity study will be conducted on the same trees.

The visual rating of the trees for phytotoxic burn showed no significant differences among the various treatments. Although the differences in mean kernel weight among the treatments were also not significant, there was a trend toward lower kernel weight with the 1 and 2 gal/Ac crude almond oil and 40 lb/Ac wettable powder (Table 1). This will be investigated further next year.

Table 1

Phytotoxicity Study of Crude Almond Oil and Wettable Powder of Reject Almonds in Fresno, CA. 1985

Treatment[1/]	Mean kernel wt (lb) per tree[2/]
0.5 gal/Ac crude almond oil	16.7 ± 5.6
1 " " " "	14.4 ± 1.9
2 " " " "	14.5 ± 3.6
10 lb/Ac W.P. reject almonds	15.5 ± 1.4
20 " " " " "	16.0 ± 3.6
40 " " " " "	14.0 ± 3.8
Control	16.3 ± 3.4

1. Six applications. The last 3 applications (7/11, 8/6 and 9/4) of the crude almond oil were doubled to 1, 2, and 4 gal oil/Ac.
2. The differences among the means were not significant.

Based on this study and previous phytotoxicity studies, it appears that the phytotoxic reation observed in last year's disruption trials resulted from the application of too much oil per acre and a too concentrated solution. By reducing the oil to 1 to 2 gal instead of 5 gal per acre and diluting in 200 instead of 100 gal water, the phytotoxic effect should be eliminated. Multiple applications of low amounts of almond oil should result in disruption of NOW oviposition while at the same time eliminating the phytotoxic problem.

C. Oviposition Behavior of Navel Orangeworm

In previous field studies on the ovipositional disruption of navel orangeworm, decreased numbers of eggs were laid on egg traps or mummy nuts following the application of crude almond oil. It is not known whether this decrease was due to females ovipositing their eggs indiscriminately on the foliage and twigs rather than on the nuts and traps or to a general reduction in total oviposition. Laboratory ovipositional behavior studies to answer those questions were proposed.

Preliminary cage studies were conducted in the laboratory using almond foliage and nuts and gravid females. The females did not oviposit on the foliage or nuts but laid eggs indiscriminately on the cage. A modification of the cage to try to direct more oviposition onto the foliage and nuts was not successful. However, when the foliage was treated with crude almond oil during these preliminary studies, the oviposition in the cage was not suppressed below that in a cage of untreated foliage. This would indicate that the decrease in navel orangeworm oviposition on traps in field trials resulted from indiscriminate oviposition. Further studies in this area are needed and are being conducted.

Samples of crude almond oil and wettable powder of reject almonds were sent to Dr. Tom Baker at UCR for study in his wind tunnel. Drs. Baker and Phelan tested the materials for their attractiveness to gravid female navel orangeworms. The information presented here is by permission of Dr. Baker.

A number of studies were conducted by Drs. Baker and Phelan using crude almond oil; refined almond oil; press cake; and a wettable powder of reject almonds made of 30% press cake, 30% crude almond oil and 40% inert ingredients. The materials were presented to gravid female navel orangeworms in a wind tunnel where a positive response was recorded when a female traveled 1.5 m upwind and touched the source.

When gravid females were exposed to increasing amounts of crude almond oil, their response increased up to 250 mg of oil (Table 2). The response seemed to level off at higher levels of oil (500 to 2000 mg). There was also an increasing response of females to increasing amounts of press cake (Table 2). However, a much greater amount of press cake than crude almond oil was needed to elicit a similar level of response. Thus, the active component is much less abundant in the press cake than in the oil. When no attractant (press cake or oil) was placed in the wind tunnel, females would not respond by traveling upwind.

When refined almond oil, crude almond oil, press cake or a combination of crude almond oil and press cake was presented to gravid females, only crude almond oil and crude almond oil plus press cake elicited a strong positive response (Table 3). Thus, crude almond oil appears to contain the active component. Similar results were observed in field trials with crude and refined almond oil. The combination of press cake plus crude almond oil (10% by weight) is the current recommended bait for the navel orangeworm egg trap.

When crude almond oil (50 mg) or increasing amounts of a wettable powder of reject almonds was presented to gravid females, the responses were nearly equal (Table 4). However, in previous studies with 50 mg of crude almond oil, female response was at maximum. Thus, the wettable power, which contains only 30% crude almond oil, appears to be as attractive as crude almond oil and could substitute for crude almond oil in a disruption field trial. The wettable powder of reject almonds should be considerably less expensive than crude almond oil.

Table 2

Gravid female navel orangeworm response to various rates of crude almond oil and press cake in a wind tunnel

Crude almond oil 1/		Crude almond oil 2/		Press cake 3/	
Amount (mg) on filter paper	% response 4/	Amount (mg) on cotton wick	% response	Amount (mg)	% response
5	14.4 a	200	35.0	50	8.0 a
25	28.3 b	500	35.0	100	10.0 ab
50	40.0 b	2000	32.5	500	25.0 ab
250	42.5 c			1,000	27.5 b
				10,000	30.0 b

1. 90 females tested.
2. 80 females tested.
3. 40 females tested.
4. Values in a vertical line followed by the same letter are not significantly different after arcsin transformation (DMRT).

Table 3

Gravid female navel orangeworm response to various almond products in a wind tunnel

Material and amount[1/]	% response[2/]
Refined almond oil, 100 mg	5.5 a
Press cake, 1,000 mg	8.8 a
Crude almond oil + press cake, 100 mg + 1,000 mg	22.9 b
Crude almond oil, 100 mg	24.4 b

1. 90 females tested.
2. Values followed by the same letter are not significantly different after arcsin transformation (DMRT).

Table 4

Gravid female navel orangeworm response to crude almond oil and various rates of a wettable powder of reject almonds

Material and amount[1/]	% response
Crude almond oil, 50 mg	28.0
Reject almond WP, 50 mg	22.0
" " " , 100 mg	23.0
" " " , 250 mg	27.0

1. 90 females tested.

II. Tenlined June Beetle

The tenlined June beetle, Polyphylla decemlineata (Say), is causing considerable damage to sporadic almond orchards in San Joaquin County. The larvae feed on almond roots causing severe injury and death to mature almond trees. The beetle has a very clumped distribution in the orchards and is always associated with orchards grown on very sandy soil. The beetle appears to have 1 generation every 2 to 3 years.

Although not part of our formal research project for the Almond Board, a secondary research project was initiated in cooperation with Don Rough, San Joaquin County Farm Advisor. Our findings are presented here.

A. Adult Emergence

Eight emergence cages covering approximately 4.9 sq ft were placed in an infested orchard on May 13 and monitored weekly until Oct. 14. Adult emergence began in mid June and continued until mid October, with peak emergence occurring on Aug. 5 (Fig. 1). The total number of beetles captured was 235, or 6.0 beetles per sq ft. The total number of beetles per cage ranged from 23 to 46. With continuous adult emergence over a 4-month period, control of the adults with an insecticide application seems unlikely.

B. Soil Profile

The larval distribution in the soil was determined on 4/17, 5/13 and 10/16 by removing soil in 2-inch layers down to 2 ft from a trench approximately 1.5 ft wide by 3 ft long around the base of heavily infested mature almond trees. Three samples were taken on each sampling date and the larvae were classified into small, medium and large.

The majority of larvae were found in the top 12 in of soil and were always associated with almond roots (Fig 2.). Only approximately 17% of the larvae were found between 1 and 2 ft. However, when a dead tree was removed, larval feeding could be found below 3 ft in depth. In the first two soil samples (April and May), only medium and larger larvae were found (Table 5). However, by the October sample, after adult emergence and oviposition, small larvae were found in greater numbers than medium and large larvae combined. Thus, these small larvae become medium in size by the following spring and summer and will probably emerge the following season. This would indicate that there is 1 generation every 2 or possibly 3 years. Also, larger larvae were not found deeper in the soil than small or medium larvae, contrary to the findings of other workers.

C. Control

Control of the larvae in the soil was attempted by applying 20 lb of Diazinon 14 G per acre around 5 heavily infested trees. The insecticide granules were evenly applied over 729 sq ft around each tree on April 9. The material was disced and watered in with approximately 2 in of water applied by sprinklers. Five heavily infested untreated trees were used as a check, and the experiment was conducted in a completely randomized design. A pre-treatment evaluation was made by counting larvae in 4 1-sq ft x 2 ft deep soil samples on April 9. The post-treatment evaluation was made on April 17 by counting larvae in 8 1-sq ft x 2 ft deep soil samples around each tree.

The pre-treatment counts, which did not differ significantly ($p \leq 0.05$, DMRT) averaged 0.85 larvae per sq ft for the diazinon-treated trees and 1.05 larvae per sq ft for the check trees. The 8-day post-treatment counts were 0.25 larvae per sq ft in the diazinon-treated trees and 0.84 larvae per sq ft in the check trees, and were significantly different ($p \leq 0.05$, DMRT). Thus, approximately 40% control was obtained with the diazinon treatment. However, the soil was very sandy which may have allowed greater penetration of the diazinon than would occur in heavier soils. Further studies this fall and winter are underway to determine the effectiveness of various rates of diazinon and Furadan.

Table 5

Distribution of tenlined June beetle larvae

in the soil, San Joaquin Co., 1985

Soil depth (in)	No. and size of larvae found on: 4/27			5/13			10/16		
	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large
0-2	0	2	1	0	0	0	0	0	0
2-4	0	4	2	0	3	3	4	0	0
4-6	0	5	6	0	1	5	8	1	0
6-8	0	4	5	0	0	1	17	1	0
8-10	0	2	2	0	0	1	13	3	1
10-12	0	0	3	0	1	0	13	1	5
12-14	0	0	1	0	0	0	4	0	1
14-16	0	0	1	0	10	0	2	0	0
16-18	0	0	0	0	0	1	4	0	1
18-20	0	0	1	0	0	0	1	0	0
20-22	0	0	2	0	0	0	1	0	0
22-24	0	0	2	0	0	0	0	0	0

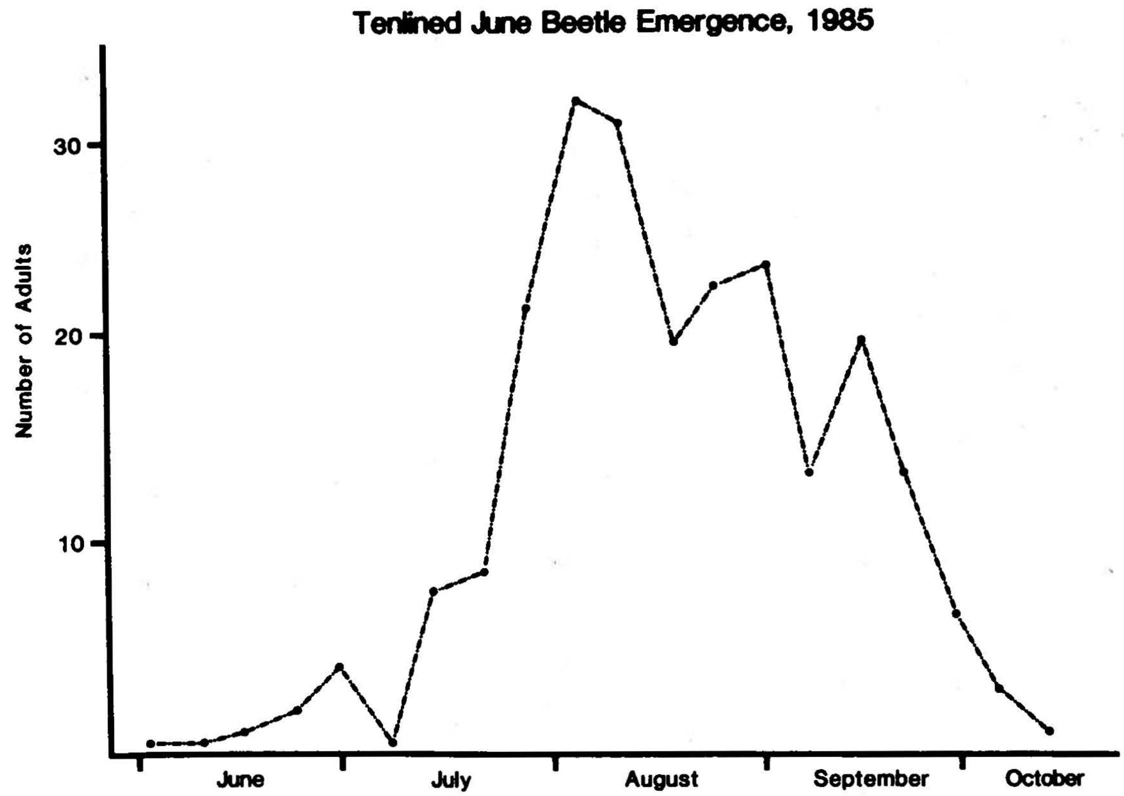
Tenlined June Beetle Emergence, 1985
Number of Adults
30
20
10
June
July
August
September
October

Fig. 1

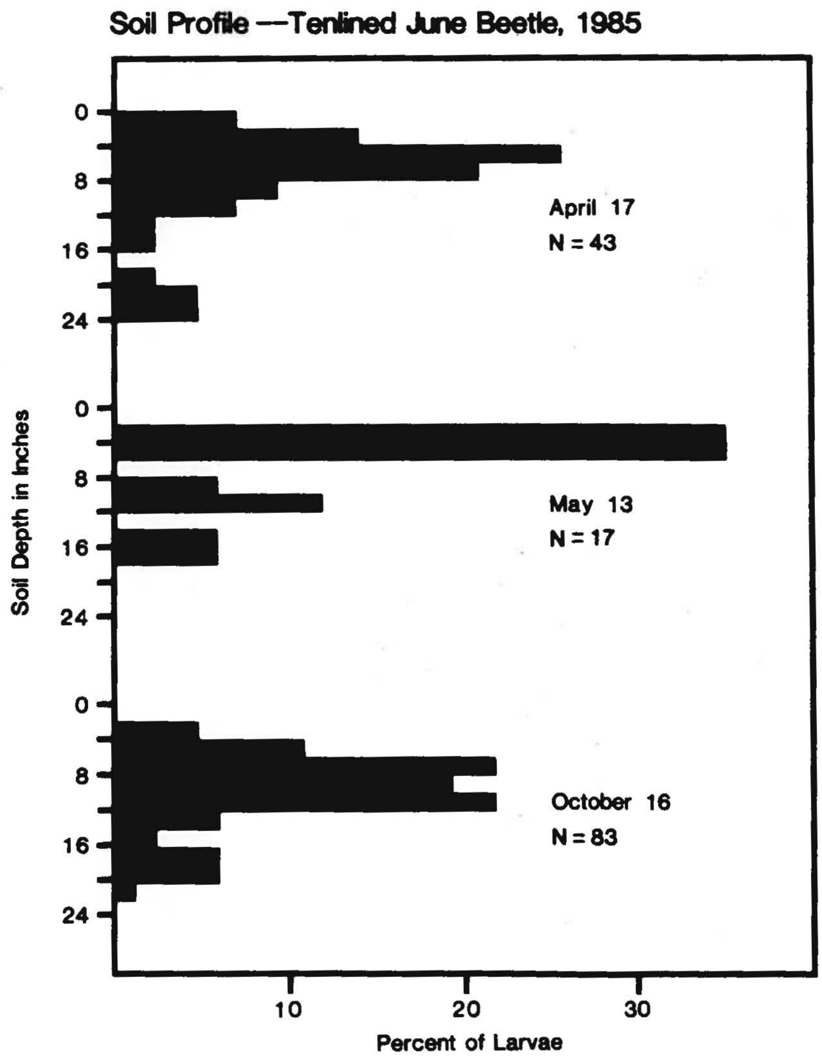
Soil Profile —Tenlined June Beetle, 1985
0
8
16
24
0
8
16
24
0
8
16
24
Soil Depth in Inches
April 17
N = 43
May 13
N = 17
October 16
N = 83
10
20
30
Percent of Larvae

Fig. 2