27 December 1976

Annual report on research sponsored by the Almond Board of California

76-T1

Title: Tree Research: Pollination (Project 76-T1)

Prepared by: Dr. Robbin W. Thorp, Department of Entomology, University of California, Davis.

Objectives: To develop information on pollination by bees which will result in increased production and greater grower returns.

Interpretive Summary: Our previous research has shown that almond varieties differ in their attractiveness to bees and in percent fruit set. These are probably related to other varietal differences we have noted including nectar and pollen production, qualitative characteristics of nectar (e.g. amount of fluorescence), and the proportion of bees foraging for nectar. Our current and future research focuses on these differences in an attempt to identify their bases, to determine how they effect bee visitation behavior, and to determine whether any of these differences can be manipulated to improve pollination and yields. Since our studies in this new direction are just beginning, it is too premature to modify existing recommendations on almond pollination (see Thorp & Stanger 1976 U. C. Div. Agr. Sci. Leaflet 2465).

Our tests of blower applications of materials to improve orchard pollination included pollen to supplement that available (1967) or of "Beeline" to attract and increase bee activity (1976). In each test the orchard contained Nonpareil and a slightly later blooming cross-compatible variety (~~Texas~~ Mission or Harvey respectively) and sufficient bees. Treated rows did not show higher percent fruit set in either test. These materials are not recommended for orchards with adequate cross-pollinating varieties. We do not have data to indicate whether they would be useful for solid block plantings, however.

Pollination experiments and observations -

"Beeline" Application

A field trail was conducted near Yuba City in cooperation with Dave Chaney, Sutter/ Yuba County Farm Advisor, to test the effects of spraying "Beeline" (a reputed bee food attractant) on bee activity in blooming almonds and on subsequent almond production.

Experimental procedure - The 8 year old, 15 acre test orchard consisted of 2 rows of Nonpareil to one of Harvey. The 24 rows were aligned East-West and were 26 feet apart and 80 trees long with 13 feet between trees within each row. Alternate groups of three rows received applications of "Beeline" at 5 lbs. in 50 gal. water per acre from a ground spray rig on 23 February starting with rows 4-6 numbering from south to north. Bees were applied at the rate of 10 hives per acre with all 150 hives placed to the west and just outside the orchard.

Bee visitation counts were made on 20, and 27 February. Counts involved 15 second visual sweeps of a tree. Bees in ten trees at each end of 3 treated and 3 nontreated rows were counted by each observer twice during the peak activity period each day.

Open blossoms and buds were counted on 20 and 25 February to obtain percent bloom before and after treatment. Limbs used in these counts were also tagged for comparison with later fruit set counts to get percent fruit set. A limb on each of 5 trees was counted at each end of 8 rows on 20 Feb and 4 rows on 25 Feb. About 70 flowers and buds were counted on each limb. Pistils of 100 flowers of each variety and treatment were collected and autoclaved for evaluations of pollen tube growth by fluorescence microscopy.

The orchard was harvested so that in shell weights could be obtained from each pair of rows of Nonpareil and each row of Harvey trees.

Results - None of the parameters measured: bee visitation (Table I), fruit set (Table II), total yields (Table III) showed any increases in trees treated with "Beeline". The percent bloom on 20 February was 20.4% (Nonpareil) 0.5% (Harvey),

and on 25 February was 58.8% (Nonpareil) and 20.6% (Harvey).

We found differences in several of our measurements related to position in the orchard. An unexplained lag in percent bloom at the east end of most rows may have caused a similar reduction in percent fruit set at the east end by exposing more bloom to less favorable weather conditions in late February. Lower yields and percent fruit set (Table II) in rows at the north end (higher numbers)is accounted for in part by more nonbearing and missing trees and an unexplained lower amount of bee visitation.

Although percent fruit set was higher in Harvey (Table II), yield per tree was greater in Nonpareil (Table III). This is due in part to the generally larger size of Nonpareil trees and probably to their earlier bloom in relation to weather patterns during the pollination period.

## Nectar production and quality

Nectar samples were collected from 21 trees of the Mission and Nonpariel Varieties. These nectar samples are being analyzed in cooperation with Dr. Eric Erickson of the USDA and WARF laboratories in Wisconsin and a team at U.C. Berkeley. In the process of collecting these samples, data were generated on the effects of bagging, of nectar extraction method, and of variety, rootstock, and environmental conditions on nectar volume and quality.

Experimental procedure - Limbs were bagged after removal of open flowers. When at least 20 blossoms reached early dehiscence, the bagged limbs were excised from the trees and brought back to the laboratory where the nectar was extracted and the volume and sugar concentration measured.

Different methods of excluding the bees from the blossoms were tried. We compared Kraft bag, cloth bag, screen "sleeve"cage exclosures with unbagged flowers collected early in the morning prior to bee foraging.

Two methods of extracting the nectar from the flower were tried. In one method, the petals and anthers were removed and the flower was centrifuged in a 3 ml sedimentation tube. In the other method, the nectar was sucked from the flower using a

small aquarium pump.

The method of maintaining the flowers prior to collecting the nectar seemed to affect the volume and concentration of nectar produced. Excised branches were immersed in water immediately, or after 15 minutes. Some had an inch cut off the bottom under water.

Temperature at which the excised branches were held prior to centrifugation also affected the amount of nectar produced. Branches were held in water in the laboratory at 20°C for 45 min. prior to centrifugation or removed from temperatures of 4-14°C and kept at 20°C from 0-30 min.

Wind and dew seemed to affect the quantity and concentration of nectar also. One tree was sampled over a four day time period including a foggy day preceded by heavy dew and two windy days with no dew from the night before. To test the affects of wind on concentration and volume of nectar, a fan (simulating a constant 6-8 mph breeze) was set in our lab. in front of some almond blossoms part of which had been previously sampled.

In another test blossoms were sampled to detect differences due to branch size or location on different parts of the tree in relation to the main trunk.

Results - Flowers in Kraft bags, screen sleeves, and unbagged flowers yielded very nearly the same amounts of nectar. However, flowers in cloth bags yielded about 1/4 more nectar than the others for some unknown reason. Because of the ease of application, cost, and availability Kraft bags were used in most of the tests.

The centrifuge removed about twice the volume of nectar from the flowers as the pump did so the centrifuge was used in all of the other tests.

Flowers excised and not placed in water within 15 minutes apparently began to resorb nectar. Flowers put in water maintained their nectar at least up to 6 1/2 hours but not as long as 24 hours. Cutting an inch off the bottom ends of the excised branches under water did not maintain nectar volume in the flowers any better than just placing the excised flowers in water.

Flowers held for at least 45 min. at 20°C produced almost twice as much nectar

as those removed from lower temperatures and held less than 30 minutes at 20°C, but their sugar concentration was lower.

Following the heavy dew nectar concentration decreased by 1/3 while volume increased by 1/4. Following the winds and lack of dew the concentration quadrupled while volume decreased to almost nothing. In the lab with exposure of flowers in front of a fan, concentration nearly doubled while volume decreased by 1/3.

Samples taken in 1974 and 1975 had suggested a difference in nectar volume produced by the 4 different root stocks in the orchard. However, data this year did not show any differences consistant with the previous two years. Also, fruit set counts were taken to see if there was any correlation with volume of nectar produced. There did not appear to be any.

No significant differences in volume or concentration were found with position in the tree.

## Nectar Fluorescence - Training Experiments

A field test was set up to test our hypothesis that the fluorescent or ultraviolet absorbant characteristics of nectar from some bee visited flowers such as almonds is perceived and used by foraging bees.

Experimental procedure - Bees were trained to forage from artificial feeders containing a scented sugar sirup (40% sucrose plus 3 drops of Neuroli oil per liter). These feeders consisted of 4 dram vials with a hole in the center of the plastic cap through which a 10 microliter pippette was inserted. A target with a hole in the center large enough to accomodate the pippette was placed over the vial. The target consisted of two 2 1/2 inch square, 1/8 inch thick pieces of plexiglass. Sandwiched in the center between the pieces of plexiglass was a 1/2 inch diameter donut-shaped piece of #1 filter paper. Surrounding the donut in a ray-like fashion were eight 1/8 x 5/8 inch strips of filter paper. The donut-shaped filter paper was soaked in either sugar sirup or a fluorescent solution and allowed to dry prior to being placed between the plexiglass. The 2 pieces of plexiglass were then sealed together to prevent escape of any odors associated with the soaked papers.

Eight to sixteen feeders were used in each trial. Half the feeders had targets with fluorescent centers and half non-fluorescent centers, and were distributed in a random manner on the training table. During the training phase of the trial, either all of the fluorescent or all of the non-fluorescent feeders had scented sugar sirup placed in them while the other group had scented water placed in them. Bees coming to the feeders were marked with airplane Dope$^{R}$. After a sufficient number of bees had been marked (11 to 70 in the 18 trials), any new recruits were aspirated off and sacrificed in detergent water. The bees marked were trained from 1.25 to 6 hours prior to testing in the various trails.

During the test phase of the trial, the table with targets redistributed randomly was placed out. In testing, all feeders had scented sugar sirup. Two observers noted on tape recorders which targets incoming marked bees alighted and fed. Once the bee had alighted and fed, it was aspirated from the target and sacrificed in detergent water, to prevent additional recruitment. Each target landed on was removed, quickly wiped with alcohol to remove any bee odor signals, and replaced before other trained bees were allowed to choose a target.

Results - Preliminary tests showed no spontaneous preference for our fluorescent, UV-absorbing models, but training tests supported our hypothesis. All but three of the eighteen trials (85.7%) gave 59 to 91% positive response to the targets to which the bees were trained. Of the 407 bees trained and recaptured in all 18 trials, 72% correctly chose the targets to which they had been trained.

## Osmia lignaria: (Blue Orchard Bee)

In 1976, we continued our cooperation with Phil Torchio of the USDA in the study of the blue orchard bee as a potential commercial pollinator of almonds. The bee managed to increase in population in an almond orchard near Davis in spite of unfavorable weather conditions during bloom this year. During the previous year, the bee was found to collect very high percentages of almond pollen.

## Disposable Pollination Units

Research on DPU's was concluded in 1975 and summarized in a paper now in press.

Experimental procedure - In our final test single Nonpareil almond trees with 1/3 of their limbs grafted to the compatible variety 'Milow' were caged with disposable pollination units (DPU's) or with colonies of honeybees to determine the effectiveness of pollination of bees in DPU's.

Results - Fruit set as a percentage of total flower production on selected limbs of each variety was not significantly less with DPU's than with colonies. Also, more fruit was set on Milow by DPU bees, which forage almost exclusively for nectar, than by colony bees, an indication of a potential negative effect of extensive pollen collection by bees on the extent of almond pollination. The data also demonstrate the usefulness of DPU's as a tool in pollination research.

General Discussion - Although the Beeline test gave negative results, it should be repeated in other orchards and in another year, with possibly different weather conditions, to get comparative data.

More work needs to be done in comparing different sampling methods in order to develop a uniform technique for comparative studies of almond nectar. It would be helpful to analyze in detail the qualitative differences between almond nectars (i.e. sugars, amino acids, volatiles, fluorescent compounds). These methods would aid in finding what factors such as weather, rootstock, variety, soil fertility and pruning are the most important influences on nectar quantity and quality and hence pollination and yield.

Our training experiments showing discrimination by honeybees of dry fluorescent target feeders is very encouraging, but we need to determine whether bees can discriminate fluorescence in the liquid phase in relation to specular reflectance. If our hypothesis continues to be supported, we will have a new tool for evaluating and better understanding bee foraging behavior. This would also provide us with a tool to evaluate varietal and rootstock differences in almonds in relation to pollination. These differences might then be manipulated genetically or environmentally to improve pollination and grower yields.

We plan to continue cooperating with the U.S.D.A. in their cage tests and overwintering in California of the blue orchard bee.

We have no immediate plans to conduct further studies on potential commercial uses of DPU's for almond pollination since price and availability of packaged bees and queens are drastically less favorable than rental of overwintered colonies.

Publications:

Thorp, R. W., W. Stanger, T. Aldrich 1967. Effects of artificial pollination on yield of Nonpariel almond trees. Calif. Agric., 21:14-15 (Sept. 67).

Marks, F. and R. W. Thorp. 1972. Something new: D.P.U.'s for almonds. Almond Facts, 37(2):22-23.

Thorp, R. W., E. H. Erickson, F. E. Moeller, M. D. Levin, W. Stanger, D. L. Briggs. 1973. Flight activity and uniformity comparisons between honey bees in disposable pollination units (DPU's) and overwintered colonies. Environ. Entomol., 2(4):525-529.

Thorp, R. W., E. H. Erickson, F. E. Moeller, M. D. Levin, W. Stanger, D. L. Briggs. 1974. Disposable pollination units test for almond pollination in California. Amer. Bee J. 114(2):58-60.

Erickson, E. H., R. W. Thorp, D. L. Briggs. 1975. Comparisons of foraging patterns among honey bees in disposable pollination units and in overwintered colonies. Environ. Entomol. 4:527-530.

Thorp, R. W., D. L. Briggs, J. R. Estes, E. H. Erickson. 1975. Nectar fluorescence under ultraviolet irradiation. Science 189:476-478.

Thorp, R. W., W. Stanger. 1976. Honey bees in almond pollination. Univ. Calif., Div. Agri. Sci. Leaflet 2465, 3p.

Thorp, R. W., D. L. Briggs, J. R. Estes, E. H. Erickson. 1976. Fluorescent Nectar. Science 194:342.

Torchio, P. F. 1976. Use of Osmia lignaria Say (Hymneoptera: Apoidea, Megachilidae) as a pollinator in an apple and prune orchard. J. Kans. Entomol. Soc. 49(4):475-482.

Erickson, E. H., R. W. Thorp, D. L. Briggs. 1977. Cage Tests of Disposable Pollination Units in Almonds. J. Apic. Res. In press.

## Beeline Trial

Table I Bee visitation counts before and after application of Beeline on 23 February 1976 based on 480 counts per day.

| | 20 Feb. | | 27 Feb. | |
|---|---|---|---|---|
| | Treated | Not Treated | Treated | Not Treated |
| Nonpareil (160)[a/] | 1003(6.27)[b/] | 1040(6.5) | 865(5.41) | 865(5.41) |
| Harvey(80) | 65(0.81) | 74(0.93) | 236(2.95) | 234(2.93) |
| Total (240) | 1068(4.45) | 1114(4.64) | 1101(4.59) | 1099(4.58) |

a/ (# counts/variety/treatment/day)
b/ (# bees/tree/15 sec. count)

Table II Fruit set in Beeline test based on fruit production on limbs on which previous blossom counts were made.

| | Treated | | | | | | Not Treated | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nonpareil | | | Harvey | | | Nonpareil | | | Harvey | | |
| Row numbers | 5 | 11 | 17 | 6 | 12 | 18 | 8 | 14 | 20 | 9 | 15 | 21 |
| Blossom prod. | 871 | 1018 | 912 | 1192 | 999 | 1232 | 631 | 1253 | 656 | 1337 | 1288 | 1000 |
| Fruit prod. | 89 | 101 | 71 | 248 | 183 | 130 | 64 | 104 | 55 | 254 | 328 | 135 |
| % set/row | 10.2 | 9.9 | 7.8 | 20.8 | 18.3 | 10.6 | 10.1 | 8.3 | 8.4 | 19.0 | 26.7 | 13.5 |
| % set/var. | | 9.3 | | | 16.4 | | | 8.8 | | | 19.8 | |
| % set/treatment | | | 13.2 | | | | | | 15.3 | | | |

Table III Yields in Beeline test based on pounds of nuts harvested from pairs of rows of Nonpareil and single rows of Harvey.

| | Treated | | Not Treated | |
|---|---|---|---|---|
| | Nonpareil[b/] | Harvey[c/] | Nonpareil | Harvey |
| Total | 21,201(lbs.) | 4976 | 21,347 | 5350 |
| Avg./Row | 2650 | 1244 | 2668 | 1338 |
| Avg./Tree[a/] | 35 | 18 | 37 | 19 |

a/(nonbearing trees and skips accounted for)
b/(8 rows per treatment)
c/(4 rows per treatment)