December 6, 1977

Project 77-T2 Pollination

R. W. Thorp, U. C. Davis

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

Progress: Beeline® was applied twice to an eastern block of eight rows in an orchard near Yuba City at about 50% bloom of each variety (NePlus, Nonpareil). NePlus had more bloom and bee activity in all counts. Bee colonies were on the east and north. Significantly greater bee activity was found in these areas in NePlus, but only in pretreatment counts. Observations during and just following the sprays showed the bees were deterred from flower visitation for several seconds. They made no attempts to feed on the droplets of Beeline, but as soon as these evaporated, bees returned to normal foraging behavior. Percent fruit set was slightly higher for treated rows. Harvest yields were slightly lower for treated rows, confirming our 1976 results.

Fluorescent nectar was simulated in liquid form in artificial flowers for training honey bees. When tested, trained bees made over 71% correct choices. This confirms our 1976 results with dry models and supports our hypothesis that bees see and their foraging behavior is effected by the fluorescence or UV absorption of almond nectar. Spectrofluorometer analyses indicate peaks for excitation near 370nm and emission near 475nm, both corresponding to peaks of visual sensitivity in honey bees.

Nectar quantity increased, sugar concentration decreased, but fluorescence appeared unaffected by increasing humidity. These lab tests simulated effects of using plastic bags over flowers for field collections of nectar. Many samples were collected for future chemical analyses (e.g. effects of varieties, rootstocks replenishment, and nitrogen treatments).

Floral phenology, developmental stages from anthesis to senecence, was recorded by photos and observational descriptions. This can be correlated with pollen and nectar production, stigma receptivity, pollen tube growth, and post pollination changes.

Pollen morphology comparisons using the scanning electron microscope demonstrated differences within almond varieties (Nonpareil, Mission) and between tree fruits (peach, plum, pear, almond). Further studies would provide a valuable means for determining bee foraging patterns within and between orchards.

Fruit set data were gathered on early, mid, and late blooms of several varieties. In Jordanola, which blooms early, the percent set increases considerably from early to late bloom; in Peerless, a variety overlapping several others, there was only slightly better set in early bloom; in Mission, a late variety, there were only slight differences with set being highest in mid bloom and lowest in early bloom.

Bouquet pollination, placement of flowers in trees of other varieties, combined with analyses of pollen tube growth, provide a powerful tool for evaluating varietal discrimination and preferences by bees. In preliminary tests with hand held bouquets, 46-70% pollination was obtained.

Annual report on research sponsored by the Almond Board of California

Title: Tree Research: Pollination (Project No. 77-T2)

Prepared by: Dr. Robbin 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), environmental conditions, and the proportion of bees foraging for nectar. Our current and future research focuses on attempts to identify these varietal differences, 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 and Stanger 1976 U. C. Div. Agr. Sci. Leaflet 2465).

Spray applications of Beeline® to attract and increase bee activity confirmed our 1976 results. Treated rows did not show higher percent fruit set nor total yield in either test. Bees were deterred by the sprays and made no attempt to feed on the materials. 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.

Preliminary studies of fruit set in early, middle, and late blomming varieties of almonds indicate that it may not be necessary to place bees in

orchards prior to mid bloom of the earliest varieties (i.g. Jordanola). However, the late bloom of the late variety (Mission) showed significant set. Further testing will be required to define the earliest time for removal of bees from the orchard.

Our hypothesis that honey bees can see the fluorescence or UV absorption of almond nectar and that this influences their foraging behavior was tested with liquid models. The strongly positive results confirms our previous tests with dry models and supports our hypothesis.

The observational descriptions and photographic records of floral phenology serve as a basis against which pollen availability, nectar production and replenishment, stigmatic receptivity, pollen tube growth and post pollination changes can be measured.

Differences in pollen morphology found with the scanning electron microscope suggest it is possible to identify the varieties of almond pollen carried by bees. This would provide a valuable tool for determining foragin patterns within and between orchards.

Preliminary tests indicate bouquet pollination, placement of flowers i trees of other varieties, can be combined with analyses of pollen tube growt This would provide a powerful tool for evaluating varietal discrimination an preferences by bees.

Pollination experiments and observations-

Beeline® Application

For the second year in a row, a field trial was conducted near Yuba City in cooperation with Dave Chaney, Sutter/Yuba County Farm Advisor, by spraying Beeline® (a reputed bee food attractant) on almond flowers to test its effect on bee activity in blooming almonds and on subsequent almond production.

Experimental procedure- The 20 to 25 year old, 20 acre test orchard was planted in two row blocks of Nonpareil and NePlus. The rows and trees within the rows were 24 ft. apart. There were a total of 24 rows running North and South in the orchard. The first seven rows on the East were not used because they were of unequal lengths due to a road running diagonally along the East side of the orchard. Rows 8 through 15 were treated with Beeline® once when the NePlus was in about 50% bloom and again when the Nonpareil was in about 50% bloom. Rows 16 through 23 were untreated controls and row 24 was left as a buffer zone. There was an average of 32 trees in each of the treated and untreated rows. Beeline® was applied at 5 lb. in 100 gal. water on February 24 and March 3. There were 6 hives on the southeast corner, 8 hives on the northeast corner, and 12 hives on the northwest corner of the orchard giving 1.3 hives per acre.

Bee counts were taken at about 11 a.m. just prior to treatment and at about 2 p.m. following treatment. Counts involved 15 second visual sweeps in ten trees at each end of all rows.

Open blossoms and buds were counted in half the rows of each variety at treatment on February 24 and in the other half of the rows on March 3. A section of limb with about 100 flowers on each of five trees at each end of a row was counted and tagged to obtain percent bloom and fruit set. Fruit counts were taken on 20 April 1977.

The orchard was harvested by variety and treatment so that in-shell weights could be obtained.

Results- The percent blooms came close to the manufacturers recommended 50% bloom at application:

	NePlus	Nonpareil
Feb. 24	42.7%	1.3%
Mar. 3	92.1	54.5

None of the parameters measured: bee visitation (Table I), fruit set (Table II), total yields (Table III) showed any consistant increases in trees treated with Beeline®. Total bee counts were higher in the treated NePlus rows, but only significantly in the pretreatment counts. This may be due to the fact that the treated rows had a slightly higher number (1.4 versus 1.2 colonies per acre) and a more even distribution of bee colonies, and NePlus had more bloom at each count. The critical observations on bee behavior made during and just after the sprays showed that bees disappeared from the flowers for several seconds. They did not attempt to feed on the spray droplets, but as soon as these evaporated, bees resumed normal foraging behavior ignoring the Beeline® material. Although the NePlus had a higher yield in the treated versus nontreated rows the opposite was true with the Nonpareil. The total average yields for the treated versus nontreated were not significantly different indicating that the Beeline® was not effective in increasing yield in this test.

Nectar Fluorescence-Training Experiments

A field test was set up to test our hypothesis that the fluorescent or ultraviolet absorption characteristics of nectar from almonds (and some other bee visited flowers) is perceived and used by foraging bees. We used liquid targets which exhibited specular reflectance (shiny reflections) which more closely simulates nectar in flowers than the dry targets used last year.

Experimental procedure- Bees were trained to forage from artificial feeders containing a scented sugar syrup (20 and 40% sucrose plus 3 drops of Neuroli oil per liter). These feeders consisted of a 4 dram vial with a hole in the center of the plastic cap through which a 10 microliter pipette was inserted. A target with a hole in the center large enough to accomodate the pipette was placed over the vial. The target consisted of a 1/8 in. thick octagonal piece (one inch each side) of orange plexiglass with six 3/32 in. well drilled around the center hole. Inside the wells of the fluorescent targets, was placed a mixture of silicone grease, alcohol, zinc sulphide and Helecon 2205 (a fluorescent pigment). The non-fluorescent targets contained the same materials with the exception of the fluorescent pigment. Zinc sulphide (a white powder) was added to the fluorescent and non-fluorescent targets until they could not be distinguished from each other with the naked eye. A 1/16 in. clear piece of plexiglass of the same dimensions was sealed to the orange plexiglass with silicone or acrylic cement. During the initial trials saran wrap covers were put over the targets and replaced with a new piece after each bee had foraged to prevent footprint odors from acting as cues to the bees. However, later on, .08" clear plexiglass was used because it was easier to manipulate.

During the training phase of the experiment, 8 fluorescent and 8 non-fluorescent targets were distributed randomly around a 22 in. diameter circle on the training table. The fluorescent targets had Neuroli scented sugar water in them whereas the non-fluroescent targets had only Neuroli scented water in them. After a sufficient number of bees had been marked (7 to 27 in 50 trials) with airplane Dope®, any new recruits were aspirated up and sacrificed in detergent water. The bees marked were trained by allowing them to collect sugar water at the targets from 1 to 4 hr. (mostly 3 hr.) prior to testing.

During the test phase of the experiment the number of targets was reduced to 4 each of the fluorescent and non-fluorescent type. The targets

were again placed randomly in a circle, but during this phase all targets contained scented sugar water. Two observers recorded on tape which targets bees chose. Once a bee landed and fed, it was aspirated from the target and sacrificed in detergent water, to prevent additional recruitment. The cover of each target landed on was removed and replaced with a clean cover before other trained bees were allowed to choose a target.

Results- As in tests last year with dry targets, these liquid targets gave encouraging results. Out of a total of 49 tests 81.6% of the tests were positive, 12.2% were equal, and 6.1% were negative for the fluorescent targets. Some of the results in early tests may have been high because of difficulty in making the liquid media uniform. This was probably compensated for in some of the later tests in which some bees were not learning to go to the fluorescent targets because they were exhibiting "robbing" (nectar stealing) behavior. Robbing behavior occurs in the late summer and early fall when there are very few natural sources of nectar present.

Nectar Analyses

Nectar samples were taken from various rootstocks and varieties at the U.C. Davis experimental orchards and the Nichols Ranch at Arbuckle, and from the Beeline® test orchard at Yuba City. Most of these samples are being analyzed in cooperation with Dr. Eric Erickson of the USDA and WARF laboratories in Wisconsin. In the process of collecting these samples, data was generated on the effects of sampling procedure, nectar depletion, variety, rootstock, nitrogen application, and humidity on nectar volume and quality.

Experimental procedure- Limbs were bagged after the removal of open flowers. When a sufficient number of blossoms (usually about 20) had reached early dehiscence, the bagged lumbs were excised from the trees and brought back to the laboratory where the nectar was extracted, and volume and sugar concentration measured.

Kraft paper bags and plastic bags were used to exclude bees from the blossoms. We discovered that blossoms under plastic bags had several times the volume of nectar that blossoms under Kraft paper bags had (i.e., an average of 20.8 ul (microliters) per flower for 32 flowers under plastic versus less than 1 ul for the same number of flowers under paper). We thought that possibly higher humidity in the plastic bags was preventing the nectar from evaporating from the flower. To test this, we placed almond branches inside large garbage bags with about 2 in. of water in the bottom in which the cut stems rested. Half of the bags had the top wired shut with twistems while the other half were left open. At the end of 24 hr., the flowers were centrifuged.

Results- The humidity in the closed bags varied from 88 to 95% whereas in the open bags it was 37 to 45%. When 20 flowers of each treatment were centrifuged, we obtained an average 45.2 ul per flower for the high humidity treatment opposed to 2.5 ul for the low humidity. Refractometer reading for the high humidity showed 4% sugar versus 23% sugar for the low humidity. This indicates that the nectar evaporates from the flowers under low humidity conditions giving a more concentrated nectar solution. Fluorescence appears unaffected by increasing humidity.

Samples of Mission and Nonpareil nectar were analyzed with a spectrofluorometer which indicated peaks for excitation near 370 nm and emission near 475 nm, both corresponding to peaks of visual sensitivity in honey bees.

Many nectar samples were collected for future chemical analyses. These samples are being processed by the WARF Laboratories on a "space available" basis, and consequently we have no data on them yet.

Floral Phenology

Developmental stages from anthesis to senescence were recorded by photos and observational descriptions. This can be correlated with pollen and nectar production, stigma receptivity, pollen tube growth, and post pollination changes.

Experimental procedure- Mission and Nonpareil flowers were observed periodically and photographed every 24 hr. for five to six days per week throughout their bloom period. In another experiment, three groups of branches were cut from a Mission almond tree. The branches were placed in a hot water bath (105°F for 2 hr.) where about one more inch was cut off the cut end of the stem to help maintain fluid flow to the flowers. One third of the flowers were then left in a laboratory at about 70°F while the other 2/3 were placed in a breezeway where the temperature vacillated with the ambient. Half of the flowers in the breezeway and all of the flowers in the laboratory were followed through successive bloom stages and times were recorded. The other half of the flowers in the breezeway were observed after eight days.

Results- Seven stages of floral development were noted in Nonpareil almonds as follows:

1) Bud- petals overlapping curled and pink at tip, pistil bent and shorter than stamens

2) Opening- outside petals recurve and open more (half of the petals recurve first), pistil lengthens to the length of the stamens which change from recurved inward to straight up

3) Open- pink petals open forming cup-shaped flower, pistil as long or longer than stamens, anthers not dehisced

4) Early dehiscence- petals open fully, pistil green and equal or longer than stamens, outside anthers dehisce, inside anthers shorter and non-dehisc

5) Late dehiscence- stigma green and about equal in length to stamens, anthers yellow and fuzzy with pollen

6) Early senescence- petals dark pink at bottom only, yellow anthers become bare of pollen starting with the outside anthers, creases appear in anthers, filaments turgid

7) Late senescence- petals fall, stigma browns, anthers whiten at creases, and filaments curve and wither.

In the experiment with the cut flowers, the length of time in all stages was the same in each treatment except stage 4 where the flowers at constant 70°F developed in less than 20 hr., the 2 day flowers at ambient temperature in 36 hrs., and the 8 day flowers in 24 hrs. The times for stages 2 and 3 were four hrs. and less than four hours respectively. The flowers did not progress much beyond stage 4 possibly because of rainy, cool weather and lack of insect visitation. These times compare favorably with times noted on trees in the field except in stage 4 which ranged from 1 to 3 days but was usually 3 days in the 10 flowers observed.

Pollen Morphology

Pollen morphology comparisons were made between almond varieties and between other species of tree fruit in order to develop a tool for determining bee foraging patterns within and between orchards.

Experimental procedure- Flowers were collected from Nonpareil and Mission almonds and from peach, plum and pear trees. Pollen was removed from the flowers and prepared for viewing and photographing with the scanning electron microscope as described by S. Lynch and G. Webster (1975. Grana 15:127-136).

Results- The surface sculpturing (length and width of striae) differed among pollen grains of Nonpareil almond, peach, plum, and pear. The relatively nonstriate, but micropunctate surface of Mission almond pollen was very distinct from any of these.

Fruit Set

Fruit set data was gathered on early, mid, and late blooms of Jordanola,

Peerless, and Mission varieties to determine the most effective times to move bees in and out of the orchard.

Experimental procedure- Buds, blooms and old flowers were counted on limbs of the tree varieties. All other stages from bud to developing fruit were removed except those which would give us viable flowers at early, mid or late bloom for each variety. The limbs were tagged and fruit set counts were made 1 1/2 to 2 months later.

Results- In Jordanola, which blooms early, the percent fruit set increases considerably from early to late bloom (early-4.2%, mid-24.6%, late 65.0%). In Peerless, a variety overlapping several others, there was slightly better set in early bloom (early-12.9%, mid to late, 10.4%). In Mission, a late variety, there was only a slight difference (early-18.1%, mid-24.4% and late 22.7%). These preliminary data indicate that it is not necessary to have bees in orchard during the early bloom of the earliest blooming varieties until another variety begins to bloom. The significant set in the late bloom of the last variety suggests further testing is needed to define the earliest date for removal of bee colonies from an orchard.

Bouquet Pollination

Placement of cut almond flowers in trees of other varieties, combined with analyses of pollen tube growth, provide a powerful tool for evaluating varietal discrimination and preferences by bees.

Experimental procedure- Large Mission branches were cut and held in deionized water in a breezeway at ambient temperature. Any opened flowers were removed and the remaining buds allowed to develop to anthesis. The branches were then taken to a Nonpareil tree and held up in the canopy. While one person observed the number of bees visiting each flower and whether the bees were pollen or nectar collectors, another person recorded the observations. The branches were then returned to the breezeway for 4 to 6 days after which the

pistils were excised and processed to determine pollen tube growth acc to the methods of Griggs and Iwakiri (Calif. Agric., 29 (7):4-7) and I (pers. comm.).

Results- In the first branch tested, the exact length of pollen tube growt not observed, but five of seven flowers (71%) had pollen tube growth. second test, pollen tube growth was noted to be 30 to 50% the length of style. In this case 7 of 15 flowers, 47% showed pollen tube growth. T figures are high relative to Griggs and Iwakiri who found 34% pollinati Mission. Our data is based on small samples from preliminary studies n needs to be repeated.

Publications:

Erickson, E. H., R. W. Thorp, and D. L. Briggs. 1977. The use of dis pollination units in almonds. J. Apic. Res. 16(2):107-111.

Thorp, R. W. and E. Mussen. 1978. Honey bees in almond pollination. Agr. Sci., Univ. Calif. Leaflet 2465 (revised) 3 p.

Beeline® Trial

Table I. Bee visitation: 15 second visual counts per tree on 160 trees per variety per treatment before and after applications of Beeline® on 24 February and 3 March 1977.

	Pretreatment		Post Treatment	
	Treated	Not treated	Treated	Not treated
Nonpareil	65(0.41)[a/]	65(0.41)	20(0.13)	21(0.13)
NePlus	310(1.94)	223(1.39)	254(1.59)	243(1.52)
Total	374(1.17)	288(0.90)	274(0.86)	264(0.83)

[a/] (# bees/tree/15 sec. count)

Table II. Fruit set based on production of one limb on each of 40 trees per variety per treatment on which previous blossom counts (> 100 per limb) were made.

	Treated			Not treated		
	Nonpareil	NePlus	Total	Nonpareil	NePlus	Total
Blossoms	4680	4791	9471	4927	5310	10237
Fruits	516	877	1393	435	1035	1570
% set	11.0	18.3	14.7	8.8	19.5	14.4

Table III. Yields based on in-shell weights of nuts in pounds harvested per variety per treatment.

	Treated			Not treated		
	Nonpareil	NePlus	Total	Nonpareil	NePlus	Total
Total	8820	10200	19020	12740	7500	20240
Per row	2205	2550	4755	3185	1875	5060
Per tree[a/]	73.5	83.6	78.6	98.0	56.0	76.7

[a/] (nonbearing trees and skips accounted for)

Shafter--1977

4 cages & 4 open trees

(Almond)

	Variety	# Nuts	Shell wt. (gm)	Total Meat wt. (gm)	# Shriv-elled	# with Worms	# with Double Prs.	# Graded Meats*	Wt. Graded Meats (gm)	Wt. Graded Meats (lbs)	Pound-Weight Graded Meats/ Tree	# Nuts per Pound	Average gm. Weight of Double Fruit	Average gm. Weight of Single Fruit
Cages[1]	Jef-feries	3,970	6,089	4,587	58	320	63	3,529	4,079	8.99	2.25	393	2.03	1.21
	1238	700	827	596	16	17	8	659	570	1.26	0.32	523	1.64	0.86
		4,670	6,916	5,183	74	337	71	4,188	4,649	10.25	2.57	458 (Av.)	--	--
Open	Jef-feries	1,332	1,990	1,498	28	97	16	1,191	1,344	2.96	0.74	402	1.94	1.19
	1238	527	585	436	19	29	6	473	403	0.89	0.22	531	1.53	0.98
		1,859	2,575	1,934	47	126	24	1,664	1,747	3.85	0.96	467 (Av.)	--	--
Totals	Jef-feries	5,302	8,079	6,085	86	417	79	4,720	5,423	11.96	--	398 (Av.)	--	--
	1238	1,227	1,412	1,032	35	46	14	1,132	973	2.15	--	527 (Av.)	--	--
		6,529	9,491	7,117	121 (1.85%)	463 (7.09%)	93 (1.42%)	5,852	6,396**	14.11	--	--	--	--

*#meats excluding shrivelled, wormy, and double-fruited nuts

**10.13% wt. loss due to grading

[1]Cages = *O. lignaria*-pollinated; Open = honey bee-pollinated.