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Title: Tree Research: Pollination (Project No. 78-T3)

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

Interpretive summary: The application of supplemental pollen to be redistributed by adequate numbers of bees was tested in an orchard with solid blocks of four rows of Nonpareil bordered by NePlus and Mission. This was the first controlled test in which we have seen a positive result of artificial pollination.

Although there is considerable variation as to the rate of development of almond flowers from one phenological stage to the next the early stages are the most ephemeral. Bees exhibit a preference for flowers in late dehiscence, one of the longer developmental periods, with nectar foragers more frequently visiting later stages of development than pollen foraging bees. We also found an increasing percentage of nectar gatherers foraging on the later blooming varieties in 1978.

The earliest blooming flowers of all varieties tested in 1978 set more fruit than later opening flowers on the same varieties. Flowers of earlier blooming varieties tended to exhibit better pollen tube growth in 1978 bouquet trials than late varieties. The best combination was Peerless and Nonpareil.

Chi Square analyses of 1977 and 1978 honey bee training experiment data were significant and support our hypothesis that bees can see the flourescence and/or UV absorbance and use this visual cue among others to detect the presence of nectar in almond flowers.

Nectar and pollen samples were collected from over 20 varieties for subsequent chemical and physical analyses. Comparisons of pollen grains with the aid of the scanning electron microscope indicate that there are ultrastructural characters by which different almond varieties can be distinguished thus providing a useful tool for determining bee foraging patterns.

<u>Pollination experiments and observations</u>:

Artificial Pollination

Previous controlled test of applications of supplemental pollen to almond orchards with adequate bees to move it around, have not demonstrated increased yields in orchards with recommended plantings of cross-compatible varieties (e.g. 1:2:1 NePlus: Nonpareil: Mission). We conducted an experiment in an orchard with an unfavorable varietal combination planting (1:4:1 with 4 contiguous rows of Nonpareil) to determine if the addition of compatible pollen would improve yields.

<u>Experimental procedure</u>: The 60 acre test orchard had four rows of Nonpareil bordered by a row of NePlus on one side and Mission on the other. Pollen from Merced and Jordanolo with <u>Lycopodium</u> spores as the carrier was applied by ground blower with a special auger to meter the material uniformly. Nonpareil rows in the north half of the orchard were treated three times (Table 1) at the rate of 50 grams per acre, the south half of the orchard served as the check plot. Bees at the rate of 2 3/4 hives per acre were

distributed throughout the orchard. Bouquets were placed in trees to increase pollination, but these were distributed equally throughout the orchard so as not to interfere with the pollen application test. Bud and flower counts were made on a tagged limb in each of 10 trees in both areas in the pretreatment bloom count, 17 February. On each treatment day an additional 10 limbs each on a separate tree were tagged, receptive flowers were counted and all others were removed. Fruit set counts were made on all tagged limbs in May. Four limbs (2 NePlus, 2 Nonpareil) were bagged and flowers hand pollinated with the application material as an in vivo test of viability.

Results: Fruit set data (Table 2) show that the treated rows had gnerally higher set, averaging 4% more than set in nontreated rows. Hand pollination tests for viability of the pollen applied were inconclusive since only one of the four limbs produced any fruit. This was a Nonpareil limb which set 11.4% of 73 original flowers. Harvest data (Table 3) based on about 1/5 of the Nonpareil rows in each plot showed a 19% higher yield in treated over the nontreated plots. Another harvest sample of treated rows not included in the original test since they were border rows supported the increase with artificial pollination and gave 11.25 bins and 1735 lbs. per acre of shelled meats or about 24% increase in yield over the nontreated plot.

This constitutes the first evidence we have seen of an increase in fruit set and harvest yield which might be attributed to artificial pollination. This test needs to be repeated with the treated and nontreated plots reversed to reduce the possibility this increase may have been an artifact of orchard effects. Closer investigations of pollen viability, pollen dispersal during application, and subsequent redistribution of applied materials by bees need to be conducted. The nontreated rows should be exposed to the blower without pollen to make them more of a control and rule out the effects of air movement alone.

Floral Phenology

The purpose of these observations was to determine the rate of development of almond flowers under field conditions through the seven phenological stages identified and described in 1977.

Experimental procedure: Flowers on selected tagged limbs of Nonpareil and Mission trees were observed twice daily throughout the bloom period and their phenological stage was recorded. On most limbs all but the flowers under observation were removed. On one limb of each set all flowers were left on as a check on effects of removal. One limb of each set was caged initially. Weather records were kept throughout the observation periods. Stages 1 (buds) and 7 (late scenescence) were used as starting points and the duration of these stages were not measured.

Results: Data for developmental rates of Nonpareil and Mission flowers under field conditions (Table 4) show considerable similarity in average rates of development regardless of starting dates (thus different weather patterns) and variety. The least time is spent in opening (Stage 2) followed by other early stages: open (Stage 3) and early dehiscence. The later stages: late dehiscence and early scenescence are longest in duration. These field data are comparable to the 1977 laboratory data, however, there is a considerable range of variation within and among the stages in the field. The cage and control limbs do not relate in any consistant manner to the averages.

These observations assume equal opportunity for pollination. They should be repeated to sort out effects of pollination versus no pollination to determine whether post-fertilization changes speed up or delay duration of any phenological stages.

Flower Age Preferences of Bees

Since we have established that foraging behavior of honey bees differs depending on whether they are gathering pollen or nectar, we wanted to determine whether these differences might be related to phenological stages as identified and described in 1977.

Experimental procedure: Over 3650 observations of honey bees on flowers were made on several different varieties at different times of day. The foraging activity of each bee was recorded in relation to the phenological stage of development each was working on (Table 5).

Results: Data on preferential bee visitation by phenological stage (Table 5) show that all foragers most frequently visit flowers in late dehiscence (stage 5). This includes 57.84% of pollen foragers, 59.63% of nectar foragers, and 76.30% of bees foraging for both. Over 85% of pollen foragers visit primarily flowers in early and late dehiscence (stages 4 and 5) while over 82% of nectar foragers visit primarily older flowers in late dehiscence and early senescence (stages 5 and 6). There was a dramatic reduction in overall percentage of pollen foragers and an increase in nectar gatherers in all varieties in the late season (from 1 March on). In February over 91% of the bees encountered were collecting pollen, but in March less than 20% of these bees encountered were collecting pollen, while over 69% were collecting nectar. It is not clear whether these are related to varieties or weather. Further comparative counts are needed, especially in relation to varieties and their phenological development.

Bloom Sequence and Fruit Set

In order to determine when bee hives should be introduced and removed from orchards we extended our studies initiated in 1977.

Experimental procedure: Flowers which bloomed in the early, middle and late portion of the season for each of five varieties, were counted and all other buds and old blossoms were removed from marked branches. Percent bloom counts were made at each initial count and followed by fruit counts to obtain percent fruit set for each group (Table 6).

Results: Early opening blossoms on each variety tend to set a higher percent fruit than later ones regardless of variety or time of season (Table 6).

Bouquet Studies

Bouquets of almond limbs were hung in trees in various varietal combinations to determine its value as a tool for evaluating best cross-pollination combinations.

Experimental procedure: Bouquets of limbs of many almond varieties were cut and recut under water. These were hung in various combinations in available varieties also in bloom (Table 7). One of these combinations included a bouquet of the same variety as the tree in which it was suspended. In addition a nearby limb in each test tree was used for a base line comparison or control. Flowers were harvested after several days exposure and pistils were preserved for pollen tube growth measurements as the index of effective cross-pollination.

<u>Results</u>: Although the results (Table 7) are quite variable, it is apparent that best set was obtained with the earlier varieties. This, may have been a function of the poor pollination weather during bloom of the later varieties in 1978. Among the varietal combinations, Peerless and Nonpareil seemed to be consistantly the best. The need for further tests is indicated.

Nectar Fluorescence Training Experiments

In order to test our previous hypothesis that honey bees can visually detect the presence of nectar in almond flowers by the fluorescence and/or UV absorbance characteristics of almond nectar, we continued our experiments to train honey bees to flower models with similar characteristics.

<u>Experimental procedure</u>: Essentially the same procedure used in 1977 was followed in training bees to artificial flower models with fluorescing liquid nectaries by rewarding them with sugar syrup and giving only water at the non-fluorescing models. The trained bees were then given a choice between a new arrangement of the two models in a test situation in which all models had sugar syrup and each bee was captured and its choice recorded as it landed on and probed for reward at a model.

<u>Results</u>: Although not all the test runs were consistantly positive in 1977 or 1978 a Chi Square test of the data for both years show that the selection of the fluorescent models after training deviates significantly from random supporting our hypothesis that bees can discriminate almond nectar visually:

1977: with 544 captures and 381 positive choices.

$$X^2 = \frac{(109 - 0.5)^2}{272} + \frac{(109 - 0.5)^2}{272} = 86.6 \text{ with 1 d.f} = > 0.001$$

1978: with 415 captures and 237 positive choices

$$X^2 = \frac{(29.5 - 0.5)^2}{207.5} + \frac{(29.5 - 0.5)^2}{207.5} = 8.1 \text{ with 1 d.f} = > 0.01$$

Primary Rewards for Bees

In our efforts to learn more about the primary rewards which bees seek in almond flowers we sampled almond pollen and nectar extensively from a diversity of varieties for chemical and physical analyses.

Experimental procedure: Nectar was sampled from several trees each of 27 varieties and several of these varieties were sampled in different environments and with different cultural treatments. Samples were stored dry on filter paper to be eluted off for later chemical analyses. Anthers were collected from 21 varieties and dehisced in the laboratory for subsequent counts by a particle counter to determine pollen production by variety and for scanning electron microscope studies for identification purposes.

Results: A procedure has been developed for fingerprinting nectar samples for varietal identification in cooperation with the U.S.D.A. Bee Lab in Madison, Wisconsin and WARF Laboratories (see Erickson, *et al*. cited under publications). At present the budget for this project will not permit rapid analyses so new samples will be processed on a "space available" basis.

An available particle counter has been located in the Genetics Department at UC Davis and has been successfully tested with *Limnanthes* pollen. However, a larger aperature must be purchased before the almond pollen samples can be processed.

Pollen Morphology

Further comparisons of the ultrastructure of pollen grains from different almond varieties using scanning electron microscopy indicate that some varieties at least can be distinguished by this method. Thus providing a tool for determining bee foraging patterns.

Experimental procedure: Pollen grains from 21 almond varieties have been prepared and mounted on stubs for examination with the scanning electron microscope. For analytical comparisons standard orientations and magnifications are being examined and photographed for permanent record.

Results: Detailed photos of Jordanolo and Mission pollen show several distinguishing characters between them. These photos are being published in an article in the Proceedings of the IVth International Pollination Symposium (see Thorp, 1979 publication list). Additional comparisons indicate that pollen of NePlus is somewhat intermediate in surface sculpture between these two varieties and that of Nonpareil most closely resembles Mission and yet may be distinguished from it. Further comparisons of these and the other varieties are underway.

Publications

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

Thorp, R. W. 1978. Bee management for almond pollination. In Micke, W. and D. E. Kester, eds. Almond orchard management. Div. Agr. Sci., Univ. Calif., Man. (In Press).

Erickson, E. H., R. W. Thorp and D. L. Briggs; J. R. Estes; and C. H. Schrader, R. J. Daun, and M. Marks. 1978. Fingerprinting floral nectars via high-performance liquid chromatography. J. Apic. Res. (In Press).

Throp, R. W. 1979. Honey bee foraging behavior in California almond orchards. Proc. IVth Internat. Pollin. Symp., College Park, MD. (1978), (In Press).

Table 1. Artificial pollination. Percent of bloom before and during applications of pollen to Nonpareil trees.

	Date	Treated rows	Nontreated rows
Pretreatment:	17 Feb.	26%	20%
Treatment 1:	21 Feb.	78	71
Treatment 2:	24 Feb.	93	87
Treatment 3:	28 Feb.	98	97

Table 2. Artificial pollination. Percent fruit set on tagged limbs on each of 10 Nonpareil trees for each date and plot.

Original Counts	Treated rows	Nontreated rows
17 Feb.	11.8%	6.4%
21 Feb.	12.8	7.9
24 Feb.	18.8	11.3
28 Feb.	8.4	11.2

Table 3. Artificial pollination. Harvest data from four rows of Nonpareil trees in each plot.

	No. Bins	Shelled Meats
Treated	10.5	1619 lbs/A
Nontreated	8.5	1311 lbs/A

Table 4. Floral phenology. Average and range (in parentheses) of half days which almond flowers remained in phenological stages 2 to 6.

	Limb	2 Opening	3 Open	4 Early Dehiscence	5 Late Dehiscence	6 Early Senescence
Nonpareil 2/28-3/10	2-1	1.00(0-2)	1.05(0-4)	2.18(0-5)	5.86+(1-12)	4.72(0-12)
	2-2	0.75(0-1)	1.85(0-5)	1.28(0-5)	4.55(0-10)	6.37+(2-13)
	2-3	0.46(0-3)	1.69(0-4)	1.61(0-4)	3.61(0-7)	5.03+(1-10)
	Cage	0.73(0-1)	1.13(0-3)	2.00(0-5)	3.85(1-9)	6.00+(1-11)
	Control	0.85(0-2)	1.00(0-4)	2.28+(0-5)	2.20*(0-4)	2.00*(1-3+)
	Average	0.73	1.45	1.77	4.14	5.55
Nonpareil 3/3-3/12	4-4	0.86(0-2)	1.06(0-3)	1.86(0-4)	3.50+(2-7+)	3.00*(1-6+)
	4-5	0.90(0-2)	1.09(0-3)	1.18(0-3)	4.18+(1-9)	3.37*(3-4+)
	4-8	0.75(0-1)	2.08(0-3)	0.72(0-2)	4.45(3-7)	3.00+(3-6+)
	Cage	1.00(0-2)	2.00(0-4)	0.40(0-2)	5.20(4-7)	2.23*(2-11+)
	Control	0.77(0-2)	1.44(0-3)	0.66(0-2)	3.37*(1-5+)	2.33*(2-3+)
	Average	0.85	1.46	1.12	4.02	3.09
Mission 3/4-3/17	5-12	0.50(0-1)	2.30(1-4)	2.75(2-4)	4.20(3-6)	2.60+(1-4)
	5-13	0.63(0-1)	0.72(0-1)	0.72(0-3)	3.45(1-5)	2.63(0-8)
	5-11	0.50(0-2)	0.83(0-2)	0.90(0-2)	5.41(1-8)	1.66+91-2)
	Cage	0.70(0-1)	1.50(0-3)	1.60(0-5)	7.10+(5-9)	7.44+(5-12+)
	Control	0.33(0-3)	1.88(0-2)	0.22(0-2)	4.44(2-6)	4.11(1-7)
	Average	0.54	1.40	1.12	4.61	3.22

+ = Some observations missing, figure probably an underestimate

* = Probably a larger underestimate than for +.

Table 5. Flower age preferences of bees. Phenological stages of bloom visited by honey bees foraging for pollen (P), nectar (N), or both (B) on different varieties and dates.

		1 Bud	2 Opening	3 Open	4 Early Dehiscence	5 Late Dehiscence	6 Early Senescence	7 Late Senescence	Total
Jordanolo 2/9-2/13	P		2	88	278	454	70		892
	N			1	7	23	29		60
	B					4	2		6
NePlus 2/10-2/15	P		1	109	380	813	59		1362
	N			7	9	70	21		107
	B			4	1	23	2		30
NePlus & Nonpareil 3/1	P				1	3			4
	N					90	6	1	97
	B				3	36			39
Mission 3/9-3/14	P		2	11	30	106	2		151
	N			3	12	209	74	71	369
	B		2	2	7	46	4	4	65
Thompson 3/9-3/14	P			5	10	62			77
	N		6	27	19	209	97	17	375
	B		1	3	2	23	4		33

Table 6. Bloom sequence and fruit set. Percent bloom and subsequent percent fruit set counts for early, mid, and late bloom of five almond varieties based on over 2900 initial blossoms and over 450 fruits per variety.

Variety	Stage	Date	#Trees	Bloom%	Fruit set %
NePlus	Early	2/13	18	20.5	55.0
	Mid	2/17	18	36.6	57.5
	Late	2/22	18	69.8	21.6
Peerless	Early	2/22	20	44.9	68.0
	Late	2/27	20	73.3	25.3
Nonpareil	Early	2/22	19	28.9	35.0
	Late	2/27	19	64.5	8.3
Thompson	Early	2/27	9	56.1	59.6
	Mid	3/3	9	75.5	30.2
	Late	3/7	9	66.7	9.2
Mission	Early	2/28	8	41.2	44.5
	Mid	3/3	8	77.2	28.4
	Late	3/10	8	86.9	28.3

Table 7. Bouquet studies. Percent of pistils showing full pollen tube growth from flowers of bouquets hung in almond varieties.

Early to Mid Varieties

Host tree	Test bouquet or control	% flowers with pollen tubes
Jordanolo	NePlus	72.8
	Jordanolo	53.7
	Control	29.4
NePlus	IXL	43.8
	NePlus	57.4
	Control	40.4
Nonpareil	NePlus	43.3
	Peerless	88.0
	Nonpareil	72.7
	Control	70.7
NePlus	Nonpareil	60.0
	Peerless	63.6
	NePlus	64.7
	Control	29.0
Peerless	Nonpareil	84.6
	NePlus	36.4
	Peerless	82.2
	Control	56.5

Mid to Late Varieties

Host tree	Test bouquet or control	% flowers with pollen tubes
Nonpareil	Mission	72.0
	Thompson	18.0
	Nonpareil	15.8
	Control	34.8
Mission	Nonpariel	24.4
	Thompson	48.0
	Mission	32.6
	Control	68.0
Thompson	Nonpareil	12.5
	Mission	56.0
	Thompson	30.0
	Control	48.1