1991 ANNUAL RESEARCH REPORT TO ALMOND BOARD OF CALIFORNIA

Project No. 91-F16 - Tree Research: Pollination

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Objectives: 1) To develop information on pollination by bees which will result in increased production and greater grower returns. 2) To improve pollination efficiency of rented honey bee colonies. 3) To evaluate and improve management techniques for alternative pollinators.

Summary

Weather for pollination during spring 1991 at our study sites was warm and sunny between the start of bloom on 15 February until the rains set in on the 27th. NePlus was past peak bloom; Price, Nonpareil, and Peerless had reached peak bloom and Mission had just initiated bloom by 27 February. Initial nut set was higher in NePlus, Price, and Mission, but lower in Nonpareil and Peerless in 1991 than in 1990.

Pollen hoarding honey bees--During the bloom season of February-March 1991, we cooperated with Dr. R. E. Page, Jr. on evaluation of high versus low pollen hoarding selections of honey bees. We participated in counts of pollen versus non-pollen carrying bees returning to their hives. We collected returning bees and measured pollen, nectar and water loads. These data will be included in Dr. Page's report. Flight data from the two strains of bees will be added to our data-base for correlation of entrance activity with colony strength.

Bud counts--Numbers of buds per meter of branch was lower in 1991 than in 1990 for all cultivars except Peerless in our test orchard.

Pollen dose--Hand pollinations of Nonpareil and Mission flowers were made in the lab to determine effect of pollen dose on a stigma on pollen tube growth through the style. No pollen tubes of the single grain dose reached beyond the middle of the style. As dose increased from 10 to 50 to 100 grains so did the proportion of pistils with pollen tubes penetrating at least 95% of the length of the pistil. These tests show the need for getting sufficient compatible pollen to each stigma.

Orchard Mason Bee--Small populations of *Osmia lignaria propinqua* collected from southern Sierra Nevada foothills and Carmel Valley in spring 1990 were incubated and released in a test orchard on 24 February 1991. A cold rainy period started on the 27th and continued through most of the bloom season. The bees did not nest during almond bloom nor after being moved to another location on 8 March. *Osmia* at the source locations had their nesting seasons interrupted and delayed by the late winter rains in 1991, but populations were collected and tested for ability to survive the summer in Davis. They are being overwintered in refrigeration and will be incubated and introduced into an almond orchard in 1992 to determine their percent of emergence, pollination efficacy and renesting success.

Introduction

Our studies in the 1991 bloom season were conducted in two different orchards near Davis. The same 30 acre orchard used in our studies of the past several years was used for bud counts, source material for pollen dose tests, and field release of orchard mason bees. This orchard contains five cultivars: NePlus, Nonpareil, Peerless, Price and Mission in a hexagonal planting with every other row being Nonpareil. The second orchard, where we evaluated high versus low pollen hoarding colonies of honey bees, contained Nonpareil, Peerless, and Butte in a hexagonal planting with Nonpareil every other row. Weather was warm and sunny between the start of bloom on 15 February until rains started on 27 February.

Pollen Hoarding Honey Bees

In cooperation with Dr. R. E. Page, Jr., Department of Entomology, University of California, Davis, we evaluated his selections of honey bee colonies for high versus low pollen hoarding.

Methods: Studies were conducted in a 20 acre orchard planted in 1983 near Davis. The orchard contains Nonpareil, Peerless and Butte in a hexagonal planting with Nonpareil every other row. Strength of colonies was determined before and after our evaluations of foraging populations by making detailed counts of frames of bees and measuring areas of pollen, honey and brood. Numbers of bees returning with and without pollen to 60 colonies, 30 each high and low pollen hoarding selections, were counted twice each morning on 25 and 26 February and 6 March. Samples of foragers were captured on 8 March to determine foraging profiles of colonies. Pollen loads and honey stomach contents (nectar, water, or empty) of these bees were measured in the lab after the bloom season. By 26 February, Peerless had just passed peak bloom (83% flowers) with few buds remaining and more senescent flowers than buds; Nonpareil was slightly behind (79% flowers) with less than 1% senescent bloom; Butte (75% flowers) had many buds remaining. By 8 March when we collected samples of returning foragers, very little almond bloom remained.

Results: Detailed results of these studies will be reported by Dr. R. E. Page, Jr. Summarily, we found greater numbers and proportions of returning bees were pollen foragers at high pollen hoarding colonies than at low pollen hoarding colonies.

Discussion: These results show that selection for high pollen hoarding does increase pollen foraging. Since pollen and honey storage seem to be inversely related, one of these will tend to reduce the other. Historically beekeepers have selected for colonies with the greatest honey production, thus selecting against pollen hoarding and pollen collection. Further evaluations are needed to determine whether the high pollen hoarding selections are more efficient in pollination of almonds than are currently available commercial colonies. We plan to conduct these evaluations in spring 1992 in cooperation with Dr. Page.

Bud and Nut Set Counts

Bud and nut set counts were continued in the 30 acre orchard used in previous studies.

Methods: Buds and flowers per meter of branch were counted on 26 and 27 February. Initial nut set was counted on 18 June. Buds and flowers were counted for 10 clusters on each of 10 trees per cultivar. Numbers of clusters per meter of branch were counted on five branches of 10 trees per cultivar. Results were compared with 1990 data.

Results: Buds per meter were less in 1991 than in 1990, except for Peerless (Figure 1). Nut set was higher in 1991 than in 1990 for Price, Mission, and NePlus, but lower for Peerless and Nonpareil (Figure 2).

Discussion: In general, there was an inverse relationship between bud production in 1990 and in 1991. Peerless, which had the fewest buds per meter among the five cultivars in 1990, showed the most in 1991 while Price which had the most buds per meter in 1990, had the fewest in 1991. The nut set in Price and Mission, the two cultivars that had the lowest bud production in

1991, showed the greatest increase in comparison to nut set in 1990, especially Price. Since nut set is based on the percent of flowers that become fruit, it is to be expected that the fewer the buds the greater the set if pollination conditions are adequate. Price reached peak bloom before the rains, while Mission had reached only about 34% bloom. Thus, the poor pollination weather probably decreased some of the potential set for Mission. NePlus reached peak bloom during the warm dry weather of mid February so that most of its early blossoms had plenty of opportunity to be pollinated. Peerless and especially Nonpareil produced most of their blossoms during the rainy period of late February and early March. As a result poor pollination weather was probably the dominant determinant of nut set.

Pollen Dose

Although it should require only one cross-compatible pollen grain to fertilize the single ovule of an almond, previous field hand pollinations suggested that there may be some dose response. That is, the amount of compatible pollen grains on a stigma might influence the probability of fruit set in almonds. Laboratory experiments were conducted in 1991 in order to test this further under more controlled conditions.

Methods: Small branches from ten or more trees each of Nonpareil and Mission with blossoms in the "popcorn" stage were cut in an orchard near Davis and transported in water buckets to the lab. Flowers with a few inches of stem were clipped underwater from the branches and placed in individual water vials. Vials were sealed with parafilm and petals and most leaves were removed to reduce transpiration. Anthers were removed to prevent contamination by self-pollen and to serve as the source of pollen for hand cross-pollinations. Pistils were cross-pollinated one day later using pollen from the other cultivar. Pollen was applied with a stiff hair. Pollen doses (1, 10, 50 or 100 grains) were picked up by touching the hair to dehiscing anthers. The hair was then dragged over the stigma simulating bee contact. Loading and depositing pollen were done under a dissecting microscope. Pollinated pistils were kept at room conditions and water was added to the vials as needed (about once per day). After allowing 3.5 to 4.5 days for pollen tube growth, pistils were removed and preserved in ethanol (95%) and glacial acetic acid (3:1 by volume). Pistils were clarified with NaOH, sliced lengthwise with a razor blade, and stained with aniline blue. The stained callose tissue of pollen tubes fluoresces brightly under a UV microscope (Figure 3). Each style was divided into quarters and the numbers of pollen tubes penetrating to each quarter style length were counted.

Results: Pollen tubes from single grain applications did not penetrate more than 50% of the length of any styles in either cultivar (Figures 4 & 6). Some pollen tubes from applications of 10, 50, or 100 pollen grains to stigmas of each cultivar penetrated at least 95% of the length of the styles. A dose-related response is apparent for both cultivars (Figures 4 & 6) with nearly 30% of the pistils of each cultivar receiving 100 grains having some pollen tubes reach the base of the style. The pattern of penetration and the percent of pistils with pollen tubes at each depth differed between the two cultivars. Nonpareil showed a more gradual and uniform decrease (Figures 4 & 5) while Mission produced a sharp initial decrease (Figures 6 & 7). The pollen dose effect is apparent in the percent of pistils with full length pollen tubes in comparison to the numbers of pollen grains applied to the stigmas (Figure 8).

Discussion: Previous experiments in the field have suggested a pollen dose effect in almonds. However, those trials were subject to many uncontrollable environmental variables. In order to attain better control, we conducted experiments in the laboratory. However, constraints of time for nut set with cut flowers forced us to use pollen tube growth as the measure of potential nut set. These experiments confirmed our earlier results that pollen dose is important in assuring nut set. The differences in pattern of penetration between the two cultivars may be related to the texture of the styles. Pistils of Mission were harder and more solid while those of Nonpareil were softer and more diffuse. Now we have a basis for exploring further questions regarding the numbers of pollen grains deposited during bee visitation, the numbers of cross-compatible grains carried by bees, and the amount of pollen carry-over from one flower to the next.

Orchard Mason Bees

The Orchard Mason Bee, Osmia lignaria propinqua, has been shown to be an efficient pollinator of almonds and has been used commercially in apple pollination. Sufficient populations of this native solitary bee are not commercially available for almonds currently. Additional study is needed to refine management techniques for reproducing these bees in almond orchards. Current studies resulted from a spin-off from other long-term ecological research on diversity of bees in California wildlands from which small populations of Osmia lignaria became available.

Methods: Small populations of Osmia lignaria propinqua were collected in stick trap-nests from the southern Sierra Nevada foothills and Carmel Valley in spring 1990. These were incubated and released in an almond orchard near Davis on 24 February 1991. Newly emerged adult bees were released into a footlocker-size domicile with straws in redwood or styrofoam blocks for nesting material. Sticks containing nests were also placed in the domicile to permit emergence of any remaining bees. On 8 March, after most of the bloom had finished, the bees and nesting materials were transferred from the orchard to Davis to allow the bees to finish their nesting. All 1990 sticks were dissected in early August 1991 to determine mortality. Additional bees were trap-nested from the southern Sierra in April and May 1991. Nests were X-rayed to determine contents and stage of development and the population divided equally. Half were returned to the trap site, and the other half were set out in Davis to test their ability to survive summer temperatures in the Central Valley. All were placed in a cold room in mid-November for winter storage prior to incubation and release in 1992.

Results: Male bees started emerging from the 1990 nests in incubation in mid-February. When the nests were installed in the field females had begun to emerge. No nests were successfully completed in the almond orchard or in Davis. Parasites from the 1990 Carmel Valley population included a blister beetle, Tricrania stansburyi (Meloidae) and a wasp Sapyga sp. (Sapygidae). No difference was found in summer survival between bees held in Davis versus those returned to the site where the nests were originally obtained. Overall survival was just over 71%. Bees had transformed to adults in natal cocoons, the normal overwintering stage, by the time they were placed into cold storage (Figure 9).

Discussion: The onset of cold wet weather shortly after the populations were installed in the almond orchard in February 1991 interfered with nesting by Osmia. Summer temperatures in 1991 at Davis were milder than usual which may account for the good survival in the Central Valley test group.

Publications

DeGrandi-Hoffman, G., R. Thorp, G. Loper, and D. Eisikowitch. 1991. The influence of nectar and pollen availability and blossom density on the attractiveness of almond cultivars to honeybees. Proc. VIth Internat. Symp. Pollin. Acta Hort. 288:299-302.

DeGrandi-Hoffman, G., R. Thorp, G. Loper, and D. Eisikowitch. 1991. Identification and distribution of cross-pollinating honeybees (Apis mellifera L.) on almonds [Prunus dulcis (Mill) D.A. Webb]. J. Appl. Ecol. [In Press]

Fig. 1. Buds per Meter for Five Almond Cultivars: 1990 versus 1991

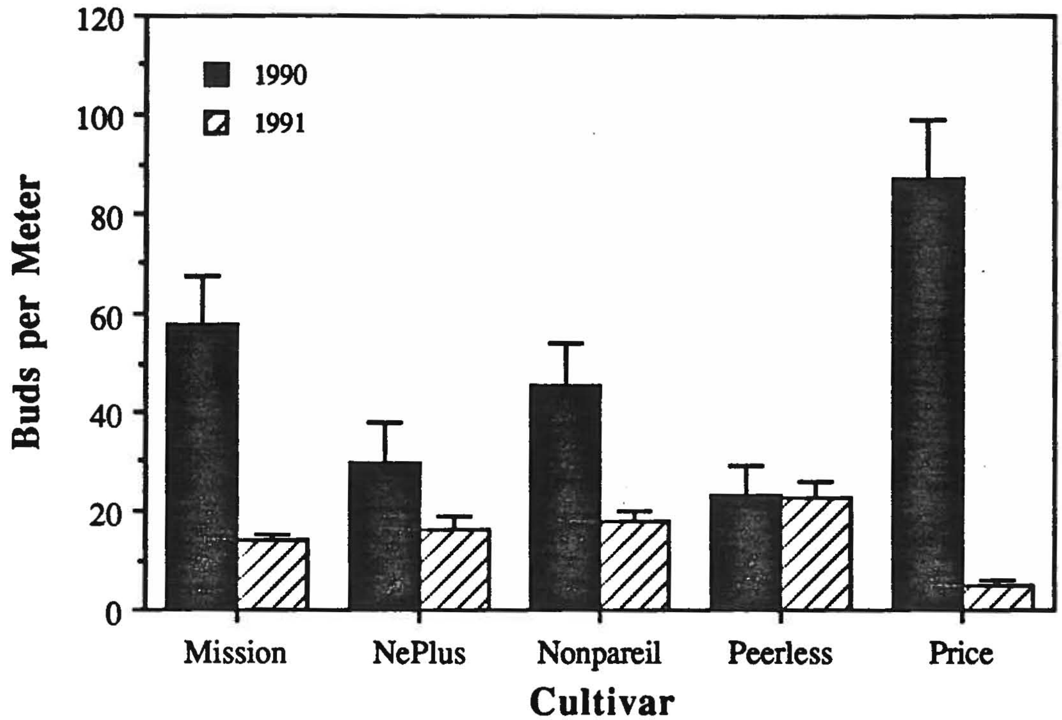
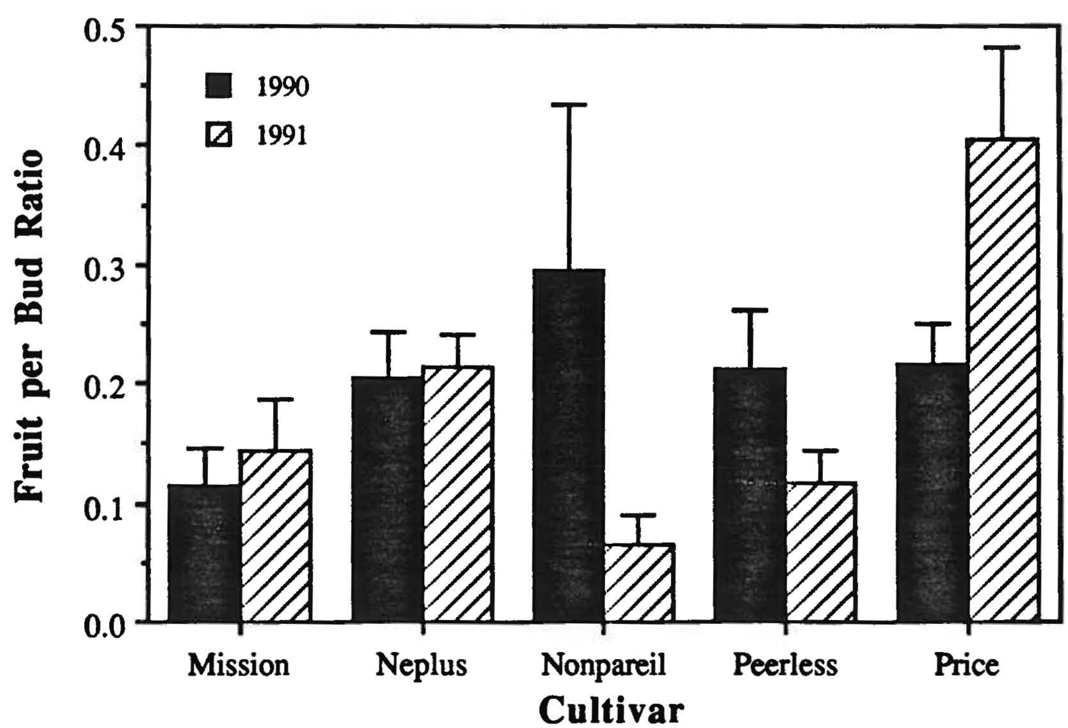


Fig. 2. Initial Fruit per Initial Bud for Five Almond Cultivars: 1990 versus 1991



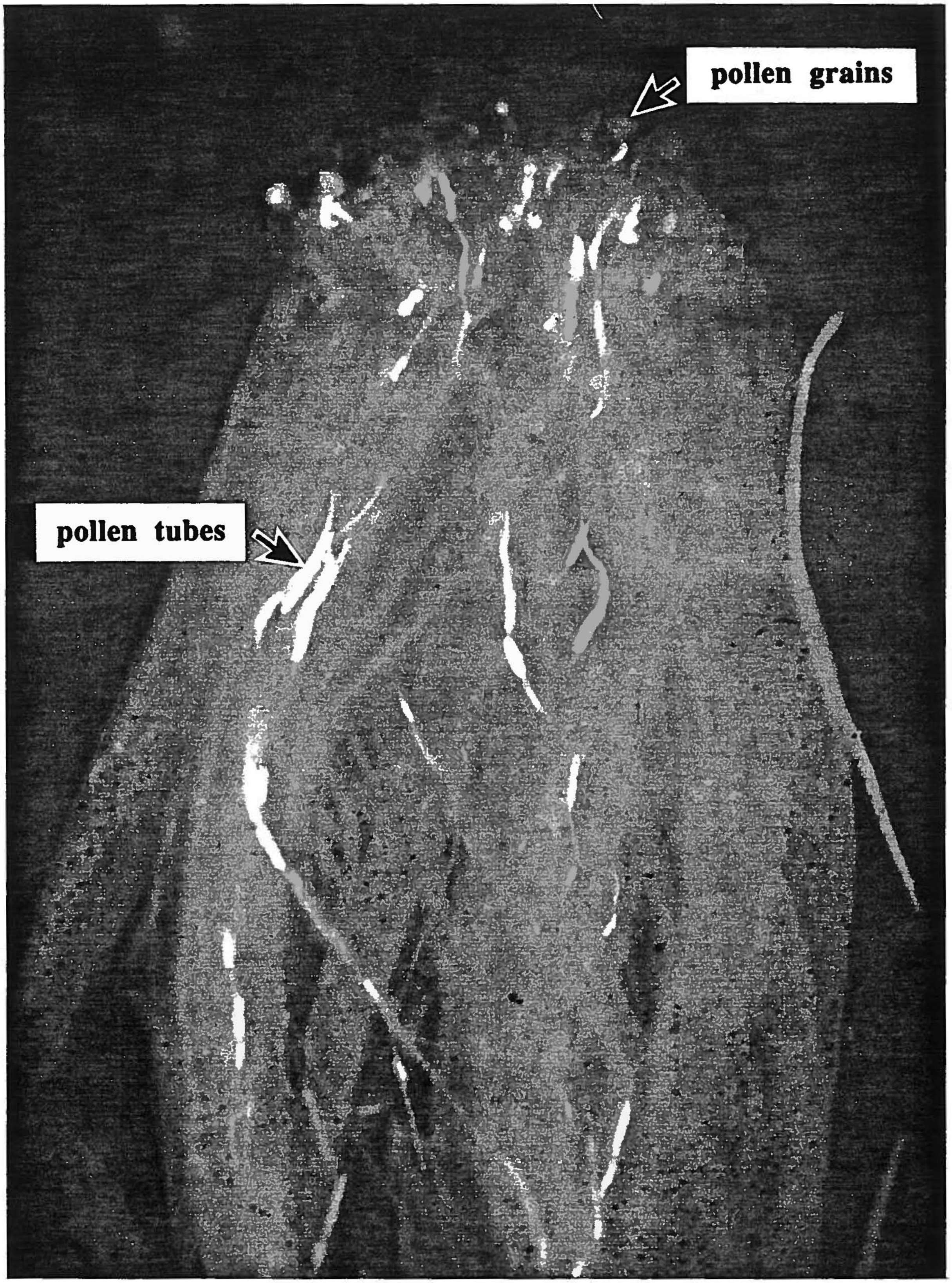


Fig. 3. Almond pistil examined with fluorescence microscopy. Pollen grains on stigma send out pollen tubes into the style. Pollen tube growth is seen as brightly fluorescing callous plugs.

Fig. 4. Categorical Pollen Tube Growth: Nonpareil

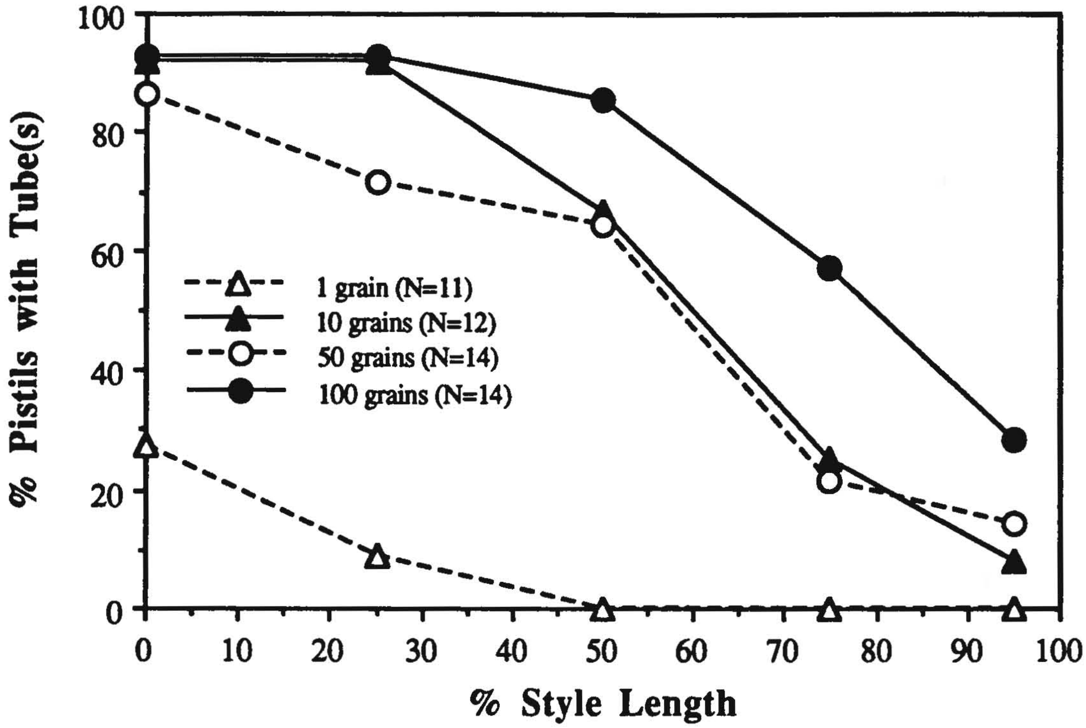


Fig. 5. % Change in Pollen Tube Numbers Among Three Pollen Load Treatments: Nonpariel

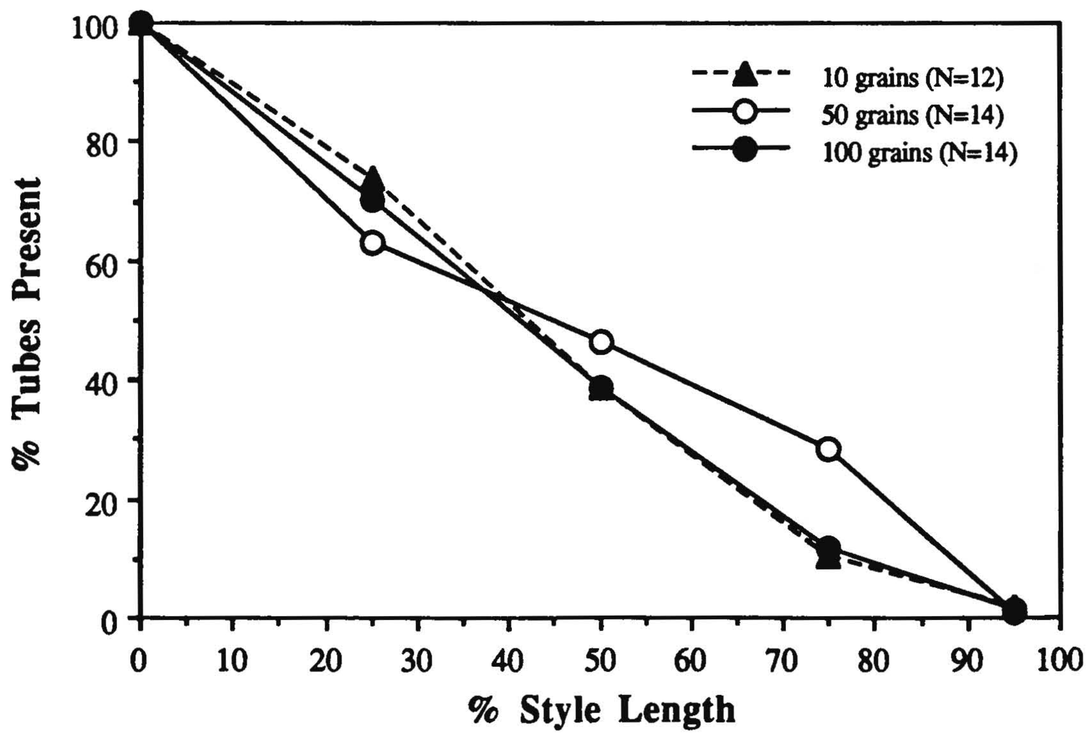


Fig. 6. Categorical Pollen Tube Growth: Mission

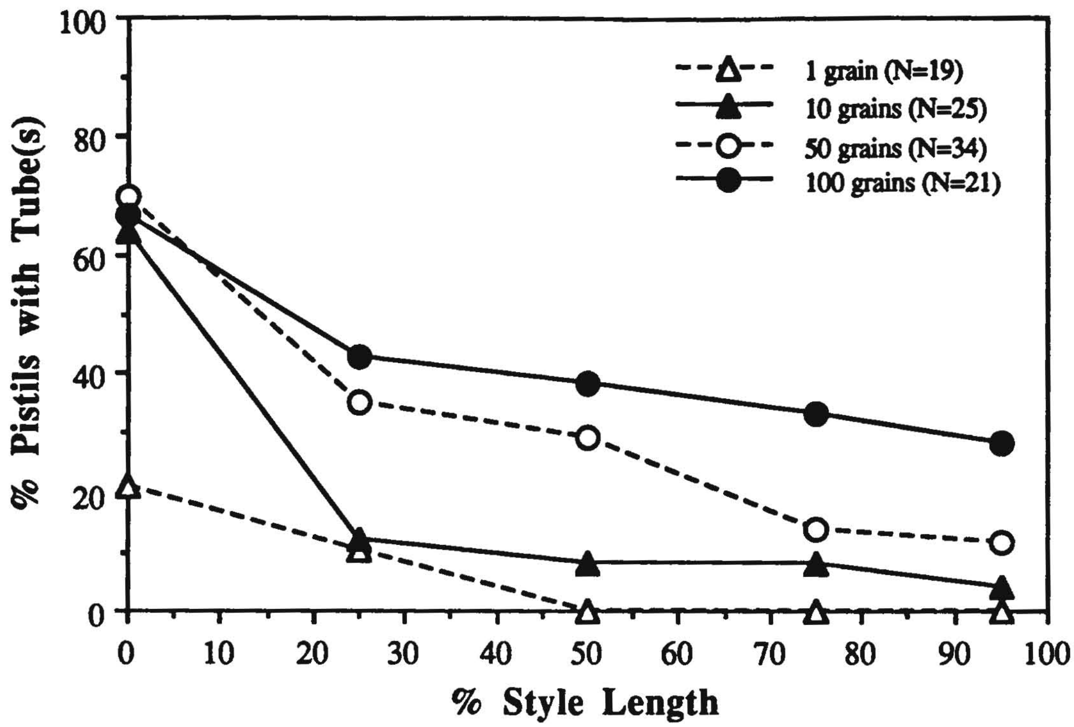


Fig. 7. % Change in Pollen Tube Numbers Among Three Pollen Load Treatments: Mission

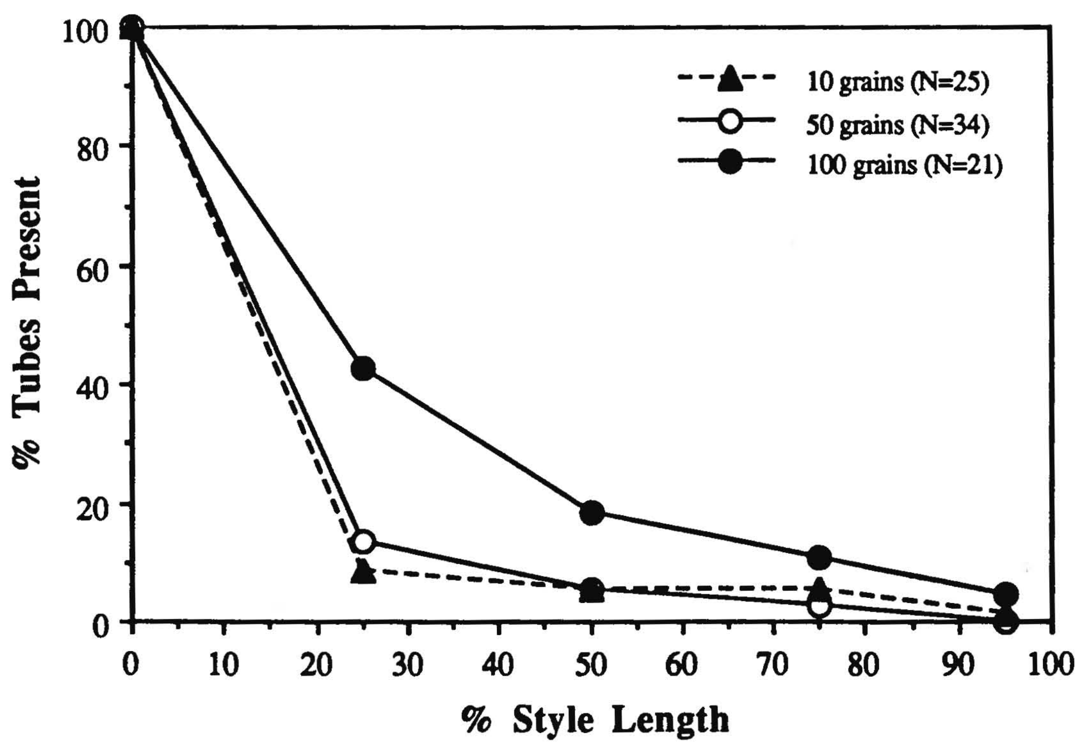
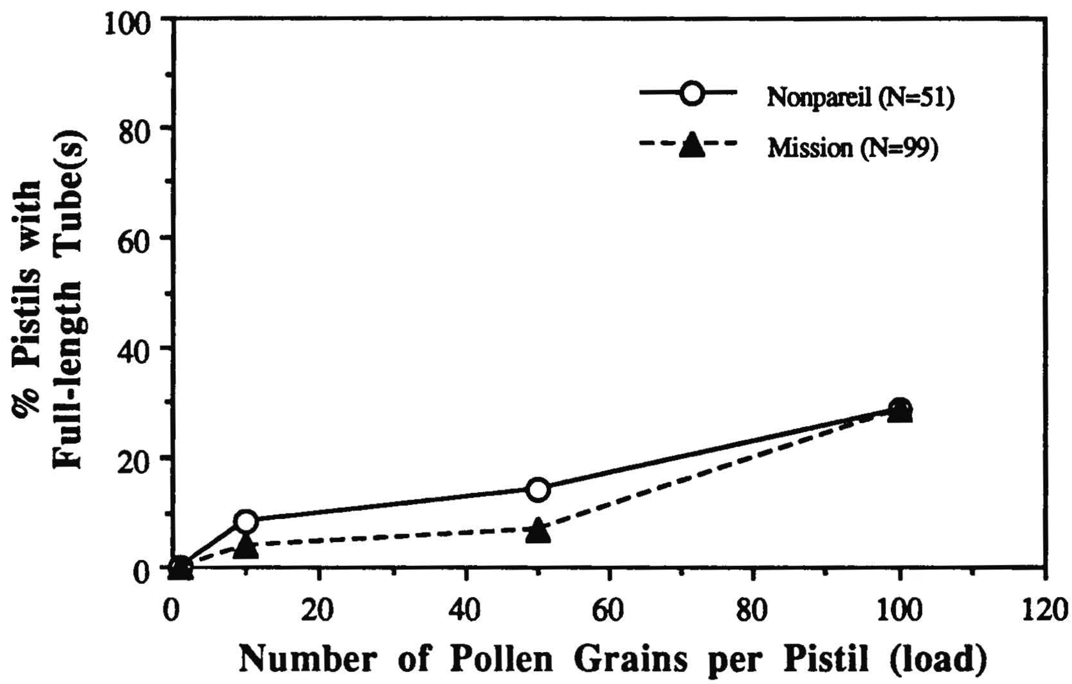


Fig. 8. % Pistils with Full-Length Tubes from Varying Pollen Load Sizes: Two Almond Cultivars



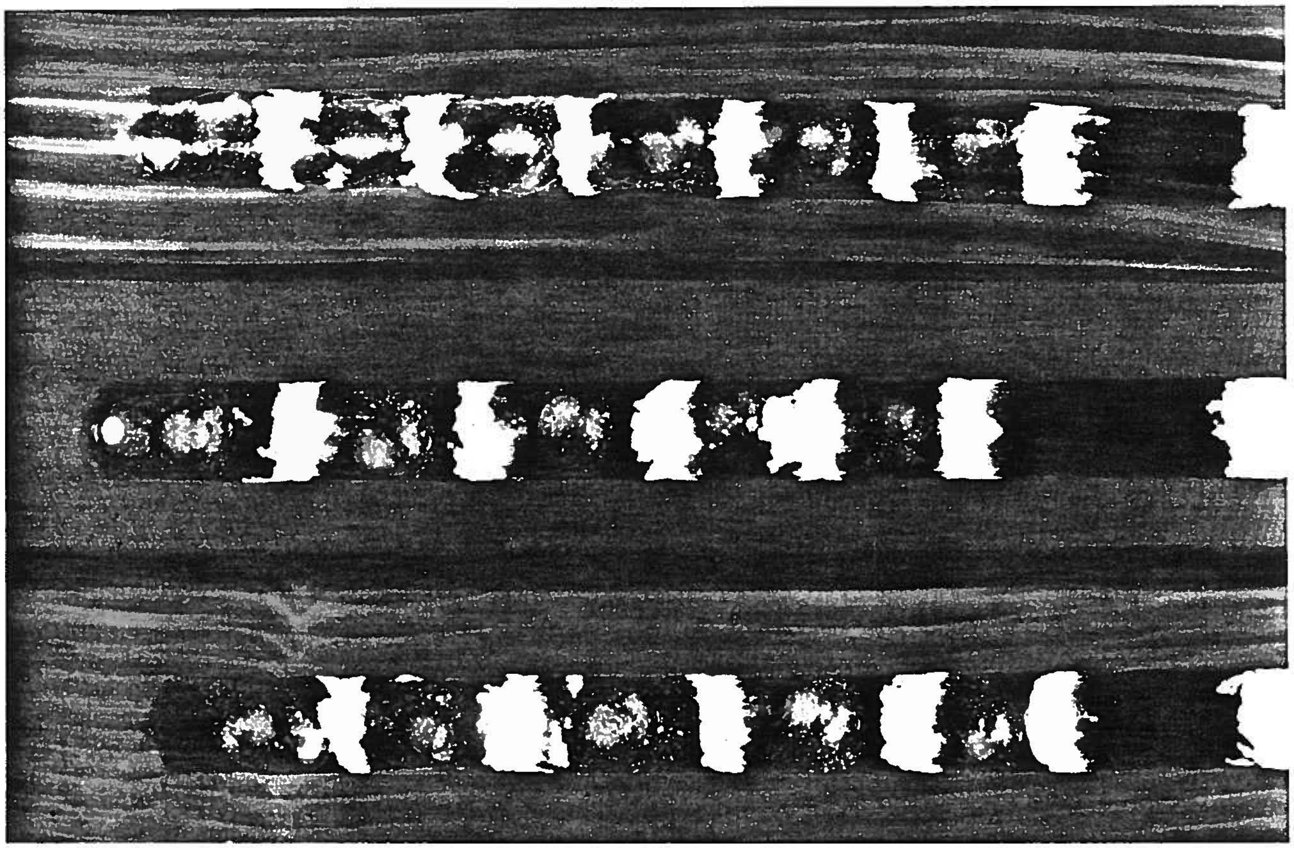


Fig. 9. X-ray of nests in holes drilled in wood blocks showing adults in cocoons for overwinter storage. (Bright areas are mud partitions).