



Seedlessness and Parthenocarpy in *Pistacia vera* L. (Anacardiaceae): Temporal Changes in Patterns of Vascular Transport to Ovules

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Pistacia vera 'Kerman' (pistachio nut) typically produces high numbers of seedless or blank fruits. Patterns of vascular transport into fruits and ovules were studied over 3 years by following the movement of disodium fluorescein solution from cut branches into developing fruitlets. Early in the season, vascular conductivity is intact through to the chalazal end of the ovule. Soon afterwards, the percentage of ovules with vascular conductivity through to the chalaza declines, and in a variable fraction of fruits, movement of the fluorochrome solution becomes blocked either at the placenta or in the funiculus. Six to 9 weeks after anthesis there is blockage in 90 (1 year) to 100% (2 years) of fruits. Subsequently, vascular conductivity resumes in 83.3% (3 year mean) of ovules, a percentage that correlates well with the mean percentage of seeded nuts at harvest (77.5%). Ovules from fruits with dysfunctional vascular conduction early in the season are smaller than those with fully functional vascular tissue. At the time conductivity declines, a high percentage of those ovules with blocked vascular movement lack endosperm and appear to be unfertilized; none of the ovules that retain full vascular flow lack endosperm. Pollination using gamma-irradiated pollen (^{60}Co , 1.0 kGy) led to a nearly three-fold increase in the production of blank nuts. The results indicate that fluorescein transport may be a valuable tool to predict the fate of ovules, and are consistent with the hypothesis that parthenocarpic fruit set may be an important factor in blank nut production in pistachio.

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Key words: Pistachio, *Pistacia vera* L., fluorescein, seed set, seedlessness, parthenocarpy, blanking, ovule, funiculus, chalaza, embryo.

INTRODUCTION

Pistacia spp. produce single-seeded fruits. Seedless fruits, in which the pericarp has grown to full size but no embryo growth has occurred, are common (Grundwag, 1975). In commercial pistachio nut (*P. vera*) orchards, these unfilled nuts (termed 'blanks' in the industry) will often occur at relatively high levels. Blank production varies among pistachio cultivars; for 'Kerman', the cultivar that provides the basis for pistachio production in California, blanking is considered 'excessive' (Crane, 1986). There are two phenomena associated with seedlessness. Parthenocarpy, the production of fruit without fertilization, is common; seedless *Citrus* cultivars are, for example, typically parthenocarpic. Other species or cultivars produce seedless fruit by undergoing post-fertilization embryo abortion; here, fertilization and some degree of embryo growth is required to set fruit. This latter phenomenon, termed stenospermy or stenospermocarp, characterizes most seedless grape (*Vitis vinifera*) cultivars. Both of these phenomena have been suggested to play a role in pistachio (Grundwag and Fahn, 1969; Crane, 1973, 1975; Bradley and Crane, 1975).

Pistachio seed growth is unusual compared to that of other nut crop species. The pericarp grows during the period following pollination and the fruit attains nearly full size in 5 to 6 weeks (Crane, 1986). During this time, however, there

is little growth of the ovule, primarily elongation of the funiculus and some proliferation of nucellar tissue (Lin, Polito and Crane, 1984). Embryo growth begins after pericarp expansion is complete. The time of the first division of the zygote has been variously reported as occurring between 4 and 18 weeks (Grundwag, 1975), 6 weeks (Lin *et al.*, 1984), and 4 to 8 weeks (Shuraki and Sedgley, 1996) after anthesis. In any case, it is only after the pericarp has grown to nearly its full size and endocarp lignification has begun that seed growth commences. In seeded nuts, the seed grows to fill the open locule formed by the pericarp thereby forming the kernel of the pistachio nut. In blanks, there is a breakdown in development sometime prior to this point—the pericarp attains full size, but the kernel never grows to fill it. This, too, is unusual; in most fruits, particularly single-seeded fruits, fruit set and subsequent fruit growth is dependent on the presence of a growing embryo. Pistachio's marked deviation from typical patterns of fruit and seed growth makes it difficult to extrapolate information or methods of investigation from studies of seedlessness in other species. Additionally, this unusual pattern of fruit and seed development makes the blanking phenomenon in pistachio difficult to study, resulting in a literature on the subject that is marked by considerable ambiguity.

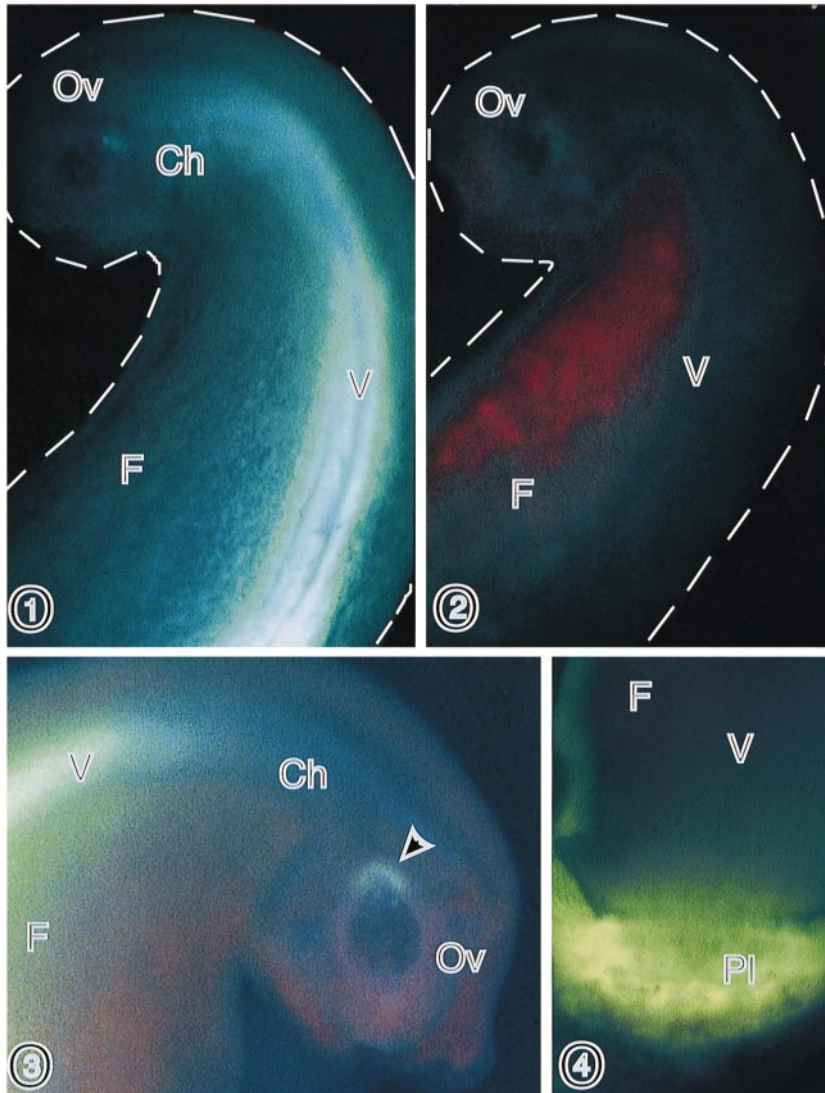
One objective of this research was to develop methodologies to study the dynamics of pistachio kernel development in a way that might enable predictions regarding the potential fate (i.e. filled or blank nut) of a given fruit. An

additional objective was to attempt to elucidate the relationships between parthenocarpic fruit set and blank nut production.

MATERIALS AND METHODS

Pistachio (*Pistacia vera* 'Kerman') trees used in these experiments were growing in an established pistachio orchard at the University of California's Wolfskill Experimental Orchards in Winters, California. Random nut samples were taken from the same trees at normal harvest

in September, and the percentage of blank nuts was determined. In each of 3 years (1987–1989), shoots bearing one or two inflorescences were collected and brought into the laboratory. They were recut under water and inserted into a 0.25% aqueous solution of disodium fluorescein (Mogensen, 1975, 1981; Pimienta and Polito, 1982), placed in a growth chamber at 28 °C with a small fan directed to the leaves of the cut shoot. After eight to 12 h, fruitlets were hand-sectioned longitudinally and observed in a fluorescence microscope using a filter set (Zeiss 09) appropriate for fluorescein excitation and emission. Under these conditions,



FIGS 1–4. Pistachio (*Pistacia vera* 'Kerman') ovules from shoots collected 4 weeks after bloom and incubated in 0.25% aqueous disodium fluorescein solution under conditions that allowed uptake of the fluorochrome solution in the vascular tissue. Figure 1 shows an ovule in which the fluorochrome solution has been transported through the funiculus into the chalazal end of the ovule. Figure 2 shows an ovule in which there is no evidence of fluorochrome in the funiculus (the red fluorescence is autofluorescence). The same autofluorescence is present in the ovule in Fig. 1, but it is not seen because a much longer exposure time was required to obtain the image in Fig. 2. Figure 3 shows the distal end of the funiculus where it enters the chalazal end of the ovule. Fluorochrome movement to the chalaza is evident, as is the presence of fluorescence at the antipodal end of the embryo sac (arrowhead). Figure 4 shows the placenta-funiculus juncture from a fruit that had fluorochrome movement blocked at the placenta. Copious fluorescence is evident in the placental region of the pericarp, but the fluorochrome has not moved into the funicular vascular trace. Ch, Chalaza; F, funiculus; Ov, ovule; Pl, placenta; V, vascular tissue.

movement of the fluorochrome solution into the fruitlet and the ovule could be readily observed (Figs 1–4). Fruits were scored according to extent of movement of the fluorescein solution which moved through to the chalazal end of the ovule or was blocked in the placenta or the funiculus.

Ovules were examined for the presence of fluorescein fluorescence as an indicator of the extent of vascular transport at 7 to 10 d intervals beginning 14 (1987) or 7 (1988) d after anthesis, or at anthesis (1989). Sample sizes varied among sampling dates with 28–50 fruitlets being examined. Fruitlets were divided into two classes: those with fluorescence extending to the ovule, i.e. at or past the chalaza, and those with fluorescence not extending beyond the placental region or the funiculus. In 1988, ovule dimensions [ovule length (from the base of the chalaza to the micropyle) and width (from the outer epidermis of the integument at the ovule's widest point)] were determined for ovules for which transport of the fluorescein solution was complete to the chalazal end of the ovule or was blocked in the funiculus or at the placenta. Ovules were hand-sectioned longitudinally at approx. 0.5 mm thickness, and mounted on slides in glycerol solution. Measurements were made from the hand sections of the ovules with the aid of a digitizer tablet interfaced to a computer. The cursor for the tablet was fitted with a red light emitting diode (LED). The image of the LED was projected onto the microscope image by means of a drawing tube. With this apparatus, the image of the LED was visible as a red dot over the microscope image of the ovule viewed through the oculars. The LED image was traced over the ovule and the dimensions were determined by analysis of the digitized points (Polito, 1983). In 1989, ovules from each category were sectioned and mounted similarly, and examined for the presence of endosperm as an indicator of fertilization.

Pollen was obtained from *P. vera* 'Peters' trees growing in the same orchard. Pollen collection and *in vitro* germination was conducted according to Polito and Luza (1988). Pollen irradiation involved exposing pollen to a ^{60}Co gamma irradiation source at the Crocker Nuclear Laboratory at the University of California, Davis. A radiation dose response curve for pollen germination was generated from 0 to 3.21 kGy. Inflorescences were pollinated using irradiated (1.0 kGy) and non-irradiated pollen from the same collection. Twenty four 'Kerman' shoots with inflorescences were enclosed in pollination bags at least 1 week prior to their receptive period and, when the earliest pistillate flowers were fully receptive, 12 were pollinated with gamma-irradiated pollen and 12 were pollinated with untreated pollen. Pollen was introduced into the bag from a syringe fitted with a 22 gauge needle. The bag was punctured with the needle, pollen injected into the bag, the hole sealed with tape, and the bag shaken to disperse the pollen. After 3 weeks (by which time the period of pistillate flower receptivity had passed) the bags were removed. At harvest, the inflorescences were evaluated for numbers of fruits and percent blank fruits.

Data on ovule sizes were analysed by ANOVA using Student-Newman-Keuls method for multiple comparisons. Distribution data were analysed by Chi-square tests.

RESULTS

Blanking percentages for harvested nuts for the trees used in these experiments are shown in Table 1.

P. vera ovule morphology is described by Grundwag and Fahn (1969). The single ovule is anatropous (i.e. the funiculus is curved such that the micropyle points to the placenta) and somewhat basifixed. There is a single vascular trace that runs from the placental region of the ovary wall into and through the funiculus. This strand terminates basal to a well defined, cup-shaped hypostase at the chalazal end of the ovule. There is no differentiated vascular tissue distal to the hypostase; thus, transport to the ovule beyond the funiculus is symplastic. Figures 1–4 illustrate movement of fluorescein into pistachio fruitlets.

The pattern of movement of the fluorochrome solution over time in each of the 3 years is shown in Fig. 5 which illustrates two categories of ovules, those with transport through to the chalaza and those with transport blocked at a point basal to the chalaza. There was transport of the fluorochrome solution to the fruitlet wall and placenta in every sample examined. As one would expect in a developmental event in a tree species, there is considerable year to year variation in the temporal dynamics of vascular movement beyond the placenta. However, within years distributions vary significantly ($P \leq 0.01$) among collection dates, and for the 3 years a general pattern is apparent (Fig. 5). There was an early period during which the fluorochrome solution moved to the chalaza of all, or nearly

TABLE 1. Blank nut percentages at harvest for pistachio (*Pistacia vera* 'Kerman') trees used in this study

Year	Blanks (%)
1987	22.4
1988	27.3
1989	17.8

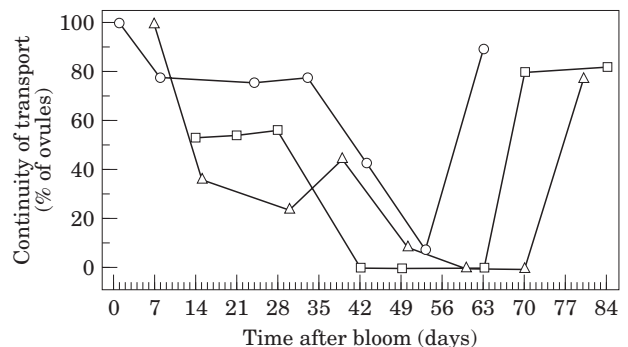


FIG. 5. Patterns of vascular transport into pistachio (*Pistacia vera* 'Kerman') ovules as indicated by movement of fluorescein. In all cases the fluorochrome solution moved into the pericarp/placenta. Ovules were scored on the extent of transport, either through to the chalazal end of the ovule, or incomplete transport not extending past the placenta or the funiculus. □, 1987; △, 1988; ○, 1989.

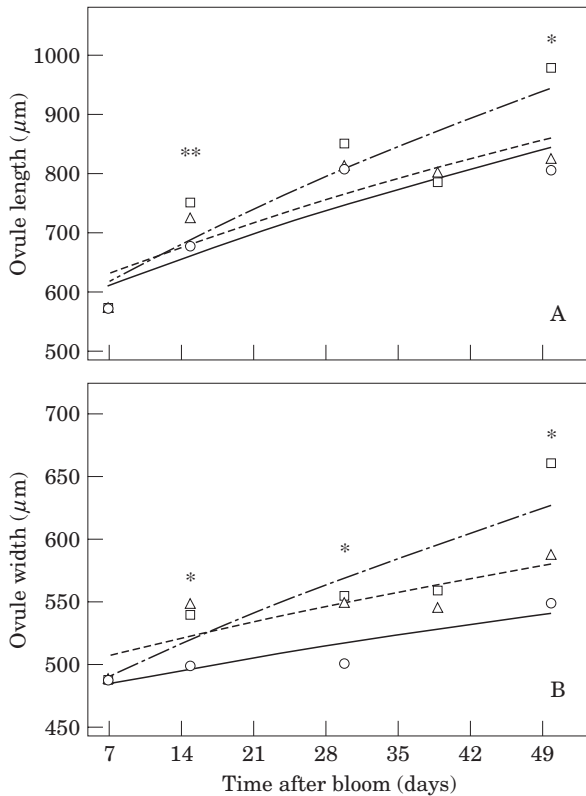


FIG. 6. Size of pistachio (*Pistacia vera* 'Kerman') ovules (1988) separated into three classes of vascular transport: to the chalazal region of the ovule; into the funiculus; and to the placenta. A, Ovule length; B, ovule width. Data points represent mean values. Lines represent log regressions of ovule size *vs.* time for each of the three categories of ovules. Correlation coefficients (r^2) for ovule length are 0.82, 0.81 and 0.83, and for ovule width are 0.81, 0.73 and 0.84, for ovules with transport to the ovule, funiculus and placenta, respectively. Asterisks indicate a significant treatment (ovule category) effect ($*P \leq 0.05$, $**P \leq 0.01$) as determined by analysis of variance. Multiple comparisons indicate that for each date having a significant treatment effect, ovules with transport through to the chalaza were significantly larger than ovules in the other two categories which did not differ from each other ($P \leq 0.05$).

all, of the ovules. By 2 weeks after bloom, differences became apparent as 53 (1987) and 63% (1988) of the ovules failed to show transport of the fluorochrome solution past the funiculus. At about 7 to 8 weeks after bloom, movement to the chalaza became blocked. This blockage occurred in all samples from 1987 and 1988, and in 89.6% of the samples from 1989. Transport into the ovule resumed between 9 and 11 weeks after bloom. At this time, transport of the fluorescein solution into the ovule was apparent in 82 (1987), 78 (1988) and 90% (1989) of the samples examined.

Growth of ovules where transport was blocked at the placenta, the funiculus, or was intact through to the chalazal end of the ovule is shown in Fig. 6. Analysis of variance indicates a significant effect of ovule transport category on ovule length for all but one date and on ovule width for each of the dates where the three ovule transport categories are present. Multiple comparisons indicate that for each of these dates, ovules that have intact transport to the chalaza are significantly larger (length and width) than ovules where transport is blocked at the placenta or funiculus ($P \leq 0.05$).

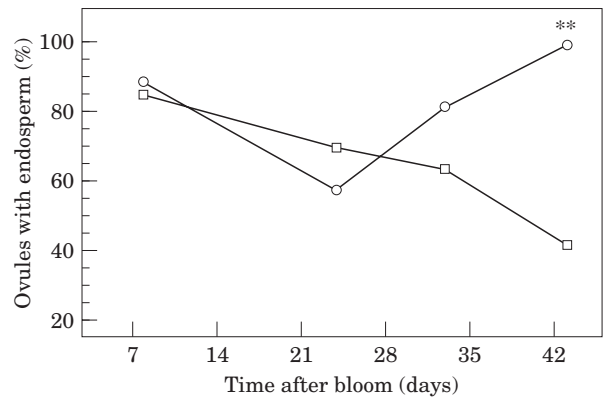


FIG. 7. Pistachio (*Pistacia vera* 'Kerman') ovules with endosperm present (1989). Ovules are separated into two classes: those with functional vascular transport into the chalazal end of the ovule and those where transport does not continue beyond the funiculus. These are the same ovules as analysed in Fig. 5. Asterisks indicate significance at $P \leq 0.01$. \circ , Chalaza; \square , Funiculus or placenta.

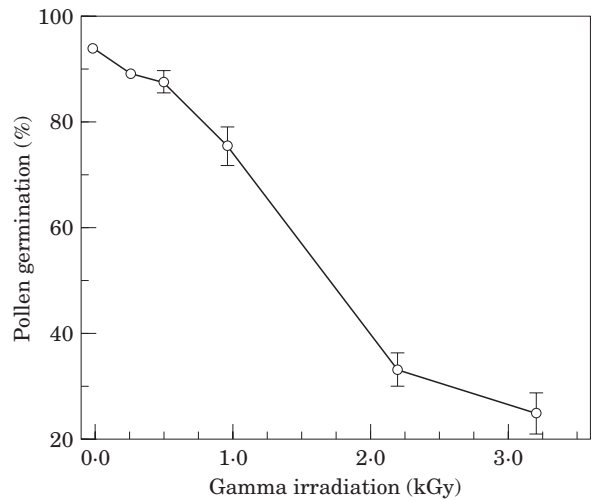


FIG. 8. Dose response curve to gamma irradiation (^{60}Co) by pistachio (*Pistacia vera* 'Peters') pollen. Bars = s.e.m.

Figure 7 shows results of analyses for presence or absence of endosperm in ovules showing intact vascular transport compared to ovules where transport is blocked at some point in the placenta or funiculus. This data set indicates that there were similar percentages of samples that lacked endosperm in both classes of ovules until 34 d after bloom. In early June, 6 weeks after bloom, 100% of ovules in fruits with fully functional vascular transport contained endosperm, whereas endosperm was lacking in 59% of ovules with vascular blockage.

Results of dose response of pollen germination to gamma irradiation are presented in Fig. 8. Based on these results, 1.0 kGy, the dosage at which germination begins to show a decline, was selected as the dosage most likely to damage pollen sufficiently to minimize fertilization, but retain sufficient pollen germination to provide a potential pollination stimulus to trigger parthenocarpic fruit set (Vardi, Frydman-Shani and Weinbaum, 1988; S. Weinbaum, pers. comm.). Results of pollination with irradiated and non-

TABLE 2. Fruit set and blank production for pistachio (*Pistacia vera* 'Kerman') after controlled pollinations using gamma-irradiated (~ 1 kGy) and non-irradiated control pollen injected into pollination bags

Pollen source	Fruits per pollination bag	Blanks (%)	n
Irradiated	18.5*	44.6**	222
Control	14.2	15.7	170

* $P \leq 0.04$.

** $P \leq 0.0001$.

irradiated pollen are presented in Table 2. Note that irradiated pollen produced a 30% increase in overall fruit set; however, this was accompanied by a nearly three-fold increase in percentage of blank fruits.

DISCUSSION

The question of blanking in pistachio has attracted a little research over the years (Grundwag and Fahn, 1969; Crane, 1973, 1975; Bradley and Crane, 1975; Shuraki and Sedgley, 1996) but the problem has proven difficult to study and the accumulated results are difficult to interpret. Grundwag and Fahn (1969), for example, noted several abnormalities in pre- and post-pollination ovule differentiation and development; however, it is impossible to tell if their 'abnormal' samples were destined to become unfilled nuts, or if they would have abscised during the post-pollination drop that characterizes this species. Similar uncertainties are evident in the contributions of Bradley and Crane (1975) and Shuraki and Sedgley (1996). The present research attempts to determine a means to predict, at the time of sampling, the fate of a given fruit i.e. whether or not the fruit was likely to produce a filled or blank nut. This would allow subsequent analysis of the fruit to be done with some indication as to whether or not it was likely to produce a blank or filled nut.

The fluorescent dye, disodium fluorescein, is a useful indicator of vascular continuity: it is readily transported in functional vascular tissue and is highly fluorescent, and can therefore be easily detected in tissues at very low levels (Mogensen, 1975, 1981). As a result, presence or absence of fluorescein fluorescence is a reliable indication that transport is or is not occurring. Mogensen (1975, 1981) demonstrated its value as an analytical tool in his research on ovules of *Quercus*. Subsequently, it was used by Pimienta and Polito (1982) in a study of secondary ovule abortion in *Prunus dulcis* (almond). They found that the secondary or abortive ovule could be identified well before the onset of structural changes by two fluorescence techniques: alkaline aniline blue showed that callose deposition at the chalazal end of one of the almond ovules had begun by 2 d after anthesis, and patterns of disodium fluorescein transport showed that vascular transport to that ovule became blocked at the same time. These responses enabled prediction of which ovules would ultimately abort. The same techniques were applied to the present research on ovule dysfunction in *Pistacia*.

Aniline blue staining did not show any patterns comparable to those seen in *Prunus* (data not shown); however, patterns of fluorescein transport did provide information on ovule function.

Movement of the fluorochrome solution to the fruit wall occurred in all samples examined, and in many cases transport through the funicular vascular bundle to the chalaza was clearly evident. Blockage of dye movement, when it occurred, was apparent at the juncture of the funiculus and the placenta (Fig. 4), or in the funiculus itself. In the latter cases, the fluorochrome solution moved beyond the point of attachment of the funiculus to the placenta, into the funicular vascular strand, but failed to move through the funiculus to the chalaza. The transport of the fluorochrome was apparent in the symplast as well as the apoplast, as it could be seen in the embryo sac (Fig. 3) some distance distal to differentiated tracheary elements of the funicular xylem trace. Symplastic transport of fluorescein into the embryo sac was also noted by Mogensen (1975, 1981).

The pattern of vascular transport among fruitlet populations varied over the period examined (Fig. 5). Early in the growth period the dye solution moved to the chalaza of a large fraction of the ovules. In the second year, analysis began 1 week after bloom at which time vascular transport was evident the entire length of the funiculus through to the chalazal region of all ovules. In the third year, transport was apparent for the full length of the funiculus in all ovules at full bloom, and in 78% of the ovules 1 week after bloom. An interesting, and unexpected, result was the complete or near complete cessation of fluorescein transport at about the time the pericarp reached full size and endocarp lignification began. The basis for the year-to-year variability is unclear; however, it is not inconsistent with well known patterns of year-to-year variation that characterize the timing of many developmental events in tree species. One example of this variation in pistachio is that the occurrence of the first division of the zygote ranges from as early as 4 to as late as 18 weeks after bloom (Grundwag, 1975; Lin *et al.*, 1984; Shuraki and Sedgley, 1996).

In all cases, transport resumed in a large fraction of the ovules by 9 to 11 weeks after bloom, the approximate time that embryo growth begins and the ovule begins to grow to fill the empty pericarp. At this time, full vascular movement to the chalaza occurred in 82, 78 and 90% of the ovules. These percentages correlate closely with the percentages of filled nuts determined at harvest (Table 1). Thus, it appears that vascular conductivity to ovules ceases during the period of intense metabolic activity in the pericarp as growth and endocarp lignification proceeds, and resumes at the time the ovule begins to grow to fill the ovarian locule. Further, these results are consistent with the hypothesis that, at the time vascular conductivity resumes, it does so in those ovules that will develop into the seeds of filled nuts, but not in those that fail to grow and result in blank nuts.

Further evidence that transport of the fluorochrome solution correlates with ovule growth is seen in the data on ovule size (Fig. 6): ovules from samples that show full transport are significantly larger than those from samples where transport does not proceed beyond the placenta or the funiculus.

In 1989, fruits from two transport classes were analysed for the presence or absence of endosperm, an indication that successful fertilization had occurred. Early in the season there was little difference between fruits with full transport to the chalaza and those where transport was blocked basal to the chalaza. By 6 weeks after bloom, the last time a sufficiently large sample could be obtained from the two classes, a marked disparity between the two classes became apparent: 100% of the fruits that had complete vascular movement through to the chalaza had endosperm, whereas endosperm was present in only 41% of those with transport blocked at a point basal to the chalaza (Fig. 7). As shown in Fig. 2, 10 d after this date, only 7.7% of fruits exhibit full transport to the ovule. This indicates that this collection was taken at the time fluorescein transport had begun to decline to very low levels in all fruits. Thus, the pattern of cessation of transport that occurs during the period from 6 to 10 weeks after bloom appears to begin preferentially in those fruits with ovules lacking endosperm, presumably unfertilized ovules. These data, considered along with data on ovule size presented in Fig. 6, begin to suggest that parthenocarpic fruit set may be an important factor in the failure of 'Kerman' pistachio fruits to develop seeds. If, as the data suggest, an early failure to support normal transport into an ovule is associated with seedlessness, and if that phenomenon is preferentially seen in those ovules that lack endosperm, one can assume at least some fraction of the blank nuts result from parthenocarpic fruits. Previous workers (e.g. Crane, 1986) have recognized the potential for parthenocarpic set in pistachio but have considered it to be a relatively minor factor in blanking as compared with post-fertilization embryo abortion.

A possible role for parthenocarpy contributing to blanking is further supported by the results of experiments with gamma-irradiated pollen. The intention of these experiments was to induce parthenocarpic fruit set by supplying a pollination stimulus using pollen capable of germinating at high levels, but damaged sufficiently that it was incapable of effecting normal fertilization (Vardi *et al.*, 1988). To this end, a dosage was selected at which pollen germination is high but has begun to decrease. Pollen irradiated at 1.0 kGy retains 71% of the ability of non-irradiated pollen to germinate (Fig. 8). When pistillate flowers were pollinated using pollen irradiated at this level, both fruit set and the percentage of blank fruits increased (Table 2). Fruit set increased by 30% from 14.2 to 18.5%; the percentage of blank fruits increased from 15.7 to 44.6, almost a three-fold increase. Again, these results are consistent with the hypothesis that parthenocarpy, as a phenomenon distinct from post-fertilization abortion of embryos, is a cause of blanking of 'Kerman' pistachio.

Parthenocarpy, while not uncommon in species that produce fruits with several to many seeds, is relatively rare in species having single-seeded fruits (Roth, 1977). Crane (1973) presented results indicating that 'Kerman' will set parthenocarpic fruits, and suggested that parthenocarpy may be responsible for some of the blank fruits produced by the cultivar. However, in this and subsequent work (see, for example, Crane, 1986), he further concluded that the

primary cause of blank nuts is not parthenocarpy but post-fertilization embryo abortion, although the evidence he presents for this conclusion is not compelling. Shuraki and Sedgley (1996) found a high proportion of blank fruit from unpollinated flowers or from flowers pollinated late in their receptive period, a finding consistent with the hypothesis that parthenocarpy is a factor in pistachio blanking. When considered in light of this background, the present results suggest a more important role for parthenocarpy than previously suspected. Therefore, it will be worthwhile to re-evaluate the extent parthenocarpy contributes to blanking in pistachio.

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