

POLLINATION BIOLOGY AND FRUIT SET IN AVOCADO

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Summary

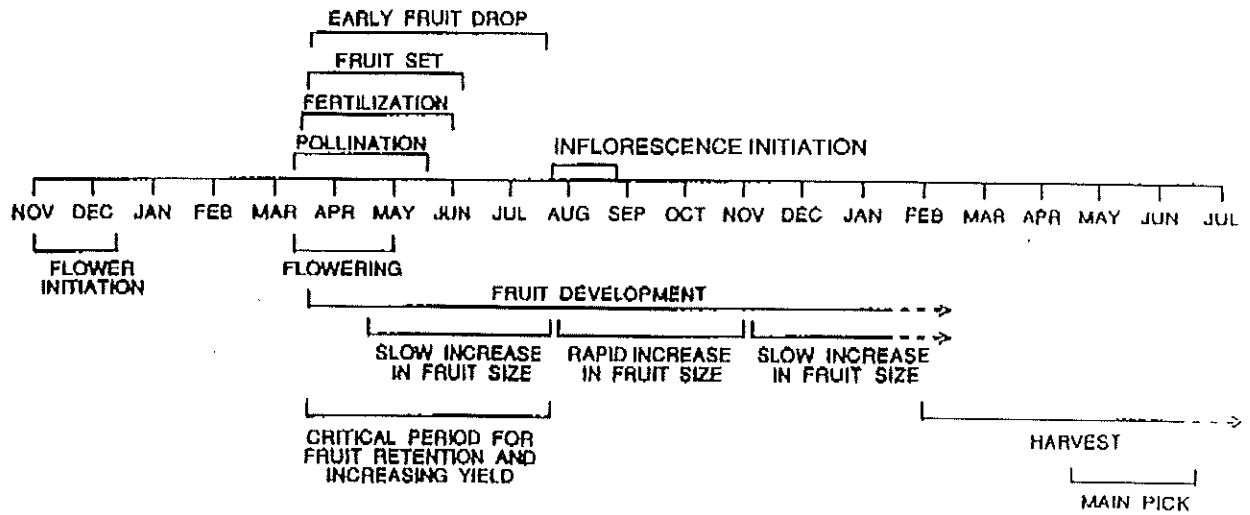
The study of pollination biology has resulted in several cultural practices that increase pollination, fertilization, and yield, including but not limited to, properly timed boron canopy sprays, foliar or soil applied nitrogen fertilization strategies, interplanting pollinizers to enhance cross-pollination, importing bees into an orchard, and increasing bee activity throughout the orchard with pheromones. Despite the success of these efforts, results are often variable due to environmental factors. The fact that research into the roles of endogenous plant hormones in fruit set, fruit development, and fruit abscission processes in avocado has been limited makes this area of investigation a resource that will likely provide new methods for increasing fruit set and yield, and perhaps a means to counter the effects of adverse climatic conditions. Recent research results regarding plant growth regulators suggest new strategies to influence avocado fruit set and size will be forthcoming.

Introduction

The seasonal cycle of flowering, fruit set, and fruit development of the 'Hass' avocado in California is depicted chronologically in Figure 1. (Please note that in this review, all references to months of the calendar are for the northern hemisphere.) As illustrated in Figure 1, during flowering, pollination and fertilization also occur. The successful completion of these two biological processes influences fruit set. The fruit set/early fruit drop period is the most critical stage of ovary (fruit) development from the grower's/producer's point of view. Pollination is defined in this review as the arrival of the pollen (the male part of the reproductive cycle) on the stigma of the pistil (the female part of the flower) (Figure 2). Under optimal conditions, the stigma is in a receptive condition and the pollen germinates, producing a pollen tube which grows through the stigma, style, and ovary tissues, to the ovule, which contains the egg. The pollen tube delivers the sperm to the egg. The fusion of sperm and egg is fertilization. The product of fertilization is the embryo, which develops into the young avocado seedling within the ovule. After fertilization, the ovule develops into the seed within the ovary, which develops into the avocado fruit (Figure 2).

Avocado fruit that fail to set can be divided into two categories: (i) fruit resulting from flowers in which pollination occurred, but subsequent fertilization failed to take place (in some crops, pollination in the absence of fertilization is sufficient to stimulate development of the ovary into a mature, seedless fruit; this process, called stimulated-parthenocarpy, rarely occurs in avocado); and (ii) fruit resulting from flowers in which both pollination and fertilization occurred, initiating the development of the embryo and seed.

FLOWERING, FRUIT SET, AND FRUIT DEVELOPMENT OF THE 'HASS' AVOCADO IN CALIFORNIA*



*BASED ON SAN DIEGO - RIVERSIDE ENVIRONMENTAL CONDITIONS

Figure 1

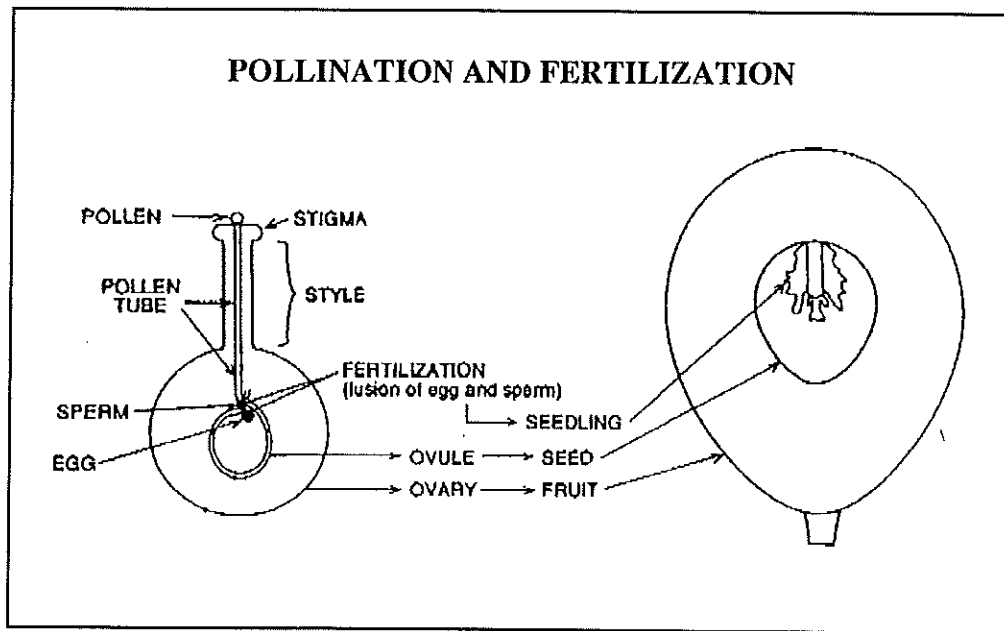


Figure 2

Factors affecting pollination and fertilization (fusion of egg and sperm)

Weather impacts both pollination and fertilization in a number of ways. Both low and high temperatures adversely affect pollen development. For tropical fruit trees, the period from meiosis to the pre-vacuolate stage of pollen development appears to be the most sensitive to temperature (Issarakraisila and Considine (1994). Temperatures below 15°C or above 33°C reduced pollen viability. Night temperatures below 10°C reduced pollen germination by more than 50%. For the 'Hass' avocado in California, this developmental period occurs around the end of January beginning of February (Salazar-Garcia *et al.*, 1997). At temperatures between 12 to 17°C, only a small percentage of flowers open in the female stage, i.e. with receptive stigmas; the majority open as males only (Sedgley, 1977). When flowers do open in the female stage, there are only a few hours during which successful pollination can occur. Low temperatures, especially if accompanied by fog, drizzle or rain, dramatically reduce bee activity, which further compromises pollination. In addition, at these temperatures pollen tube growth ceases before reaching the ovule, and thus no fertilization occurs. Pollination stimulates the ovary to start developing into a fruit, but the stimulation is exhausted within a couple of weeks and the fruitlet abscises (Sedgley, 1977). Pollination has recently been found to increase gibberellin levels in developing ovaries of seeded varieties of citrus (Ben-Cheikh *et al.*, 1997). A similar study in avocado could identify the complement of endogenous hormones necessary to sustain stimulated-parthenocarpy. The most successful fruit set occurs at temperatures between 20 to 25°C. At these temperatures, the opening of flowers in female and male stages overlaps for several hours. At temperatures above 28°C, abscission of individual floral buds and flowers is accelerated; entire inflorescences abscise before flowers open (Sedgley, 1977).

Temperatures prevailing during bloom also affect the viability of the egg and ovule, as well as pollen germination and pollen tube growth and thus, influence avocado fruit set (Sedgley, 1977). The relationships among the ovule, pollen, and fruit set are integrated in the concept of "effective pollination period" (Williams, 1965), which is the length of time the ovule is viable minus the length of time necessary for the pollen tube to reach the ovule in order to deliver the sperm to the egg. Cool temperatures during the flowering period decrease the viability of the ovule and increase the length of time it takes for the pollen tube to grow from the stigma to the ovule. Thus, the duration of the effective pollination period is significantly shortened and fruit set is reduced. Warm temperatures during flowering increase both ovule longevity and the growth rate of the pollen tube. This correspondingly increases the effective pollination period and fruit set.

Tree nutritional status and fertilizer applications influence the effective pollination period in avocado. Prebloom canopy application of nitrogen, as low biuret urea, to the 'Hass' avocado during the cauliflower stage of inflorescence development [during this stage, secondary axes of the inflorescence are elongating, processes leading to the formation of the sperm and egg are initiated (Salazar-Garcia *et al.*, 1997)] significantly increased the number of viable ovules, the number of pollen tubes that successfully reached the ovule, and cumulative yield (Jaganath and Lovatt, 1995). Boron is another nutrient that influences the effective pollination period. It is well documented that boron is essential for pollen germination, for successful growth of the pollen tube through the stigma, style, and ovary to the ovule, and for the mitotic divisions necessary to produce the sperm and egg (Lovatt and Dugger, 1984). Boron sprays applied either during fall or spring to deciduous fruit tree species not deficient in boron based on leaf analysis have proven effective in increasing fruit set and yield (Hanson, 1991), especially when cool temperature and overcast or wet weather prevail during bloom (Callan *et al.*, 1978; Hanson and Breen, 1985). Benefits from boron sprays are less likely when conditions are optimal for fruit set. Thus, there has been considerable interest in the

use of boron fertilization to increase fruit set in the avocado (Robbertse *et al.*, 1990, 1992; Miyasaka, 1992; Jaganath and Lovatt, 1995). Robbertse *et al.* (1990, 1992) demonstrated that when pistils (female reproductive structures) harvested from avocado trees receiving a foliar application of boron were pollinated with pollen from trees also sprayed with boron, pollen germination and pollen tube growth were significantly better than in flowers from untreated trees. Fruit set and yield also increased in response to the boron sprays if the concentration of boron in the leaves close to the shoot tip was sufficiently high well in advance of flowering. In South Africa (Robbertse *et al.*, 1990, 1992), there was always an increase in yield in response to boron, but in some experiments or in some years, only a marginal improvement over the control trees was obtained (Coetzer *et al.*, 1993). Boron ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}$) applied to the canopy during the cauliflower stage of inflorescence development significantly increased the number of pollen tubes that reached the ovule, increased ovule viability, and increased cumulative yield (Jaganath and Lovatt, 1995). In California, yield increases in response to prebloom canopy applications of nitrogen or boron were variable from year to year and represented only a 25% increase in kg fruit per tree over three years compared to untreated control trees (Jaganath and Lovatt, 1995). Applying boron in high boron orchards (180 ppm B by leaf analysis) resulted in statistically significant decreases in yield in some years. Yield increases are greater with canopy applications of boron. The results of Robbertse *et al.* (1992) provided evidence that root absorption of boron is very restricted and Jaganath and Lovatt (1995) could not increase yield with trunk injections of boron despite the fact that trunk injections increased the boron status of the trees to a greater degree than the canopy sprays.

An interesting observation was made in the study of Jaganath and Lovatt (1995). Open pollinated 'Hass' avocado flowers from untreated field trees had many pollen grains, which had successfully germinated but growth of the pollen tubes had ceased mid-style. The prebloom canopy sprays of nitrogen and boron, to a significantly greater degree than nitrogen, increased the number of pollen tubes that grew completely through the style to the opening in the ovule, which is consistent with the break down of the barrier preventing self-fertilization (Lord, personal communication). Thus, in the absence of cross-pollination under adverse climatic conditions, boron or nitrogen sprays might facilitate self-pollination and increase yield, a response that would be without effect if cross-pollination occurred.

Recently, there has been increased effort on the part of researchers and growers to increase pollination, outcrossing, and fruit set by increasing the number of bees in an orchard, increasing bee activity and movement through the orchard by the use of pheromones, and by planting or grafting other avocado varieties into an orchard to enhance cross-pollination. Vrecenar-Gaduz and Ellstrand (1985) reported that outcrossing rate and yield per tree were significantly higher in interplanted versus pure groves of 'Hass' avocado and Guil and Gazit (1992) documented that yield of 'Hass' avocado trees in orchards planted next to 'Ettinger' orchards decreased as the distance of the 'Hass' trees from the 'Ettinger' orchard increased. By isozyme analysis, 90% of the harvested fruit had been cross-pollinated by 'Ettinger'. Within the inland regions of California, a clear correlation was found between outcrossing and yield when 'Hass' trees were located near 'Bacon', 'Fuerte' or 'Zutano'. Outcrossing and yield declined as the distance between 'Hass' trees and the alternate pollen source increased. In the coastal avocado growing areas of Santa Barbara and Ventura, outcrossing and yield were not correlated (Clegg *et al.*, 1997). The effect of temperature differences between the inland and coastal orchards during bloom is being investigated. The possibility that other pollen sources were available cannot be ruled out. The best variety for cross-pollinating 'Hass'

under California conditions has not been thoroughly investigated as of yet.

Recent research in California confirmed that honey bees are the primary insects in avocado orchards (Visscher, 1997). They remain the most likely candidate for improving pollination since they can be easily manipulated. However, Visscher's (1997) data provides evidence that it may not be the lack of pollinators in cool springs that limits fruit set, but that temperature itself negatively impacts flower opening, pollen tube growth, or ovule viability as discussed above or that successfully fertilized (normal) fruit drop due to limited resources or abiotic stress as discussed below.

Factors Affecting the Abscission of Normal Fruit (i.e., Those in Category 2)

The abscission of normal fruit during the early drop period is a critical factor in avocado production. The limited research that has been conducted to study early drop in avocado has concentrated on the competition between the young fruit and vegetative shoot growth occurring during the period critical to fruit retention and yield (Figure 1). Early work by Kalmar and Lahav (1976) was the first to suggest that mineral nutrient applications could stimulate shoot growth during the period critical to fruit retention resulting in increased fruit drop and loss of yield. The optimal time and quantity of nutrient applications for improved fruit set still has not been adequately investigated for avocado. Yields were significantly increased over a period of four harvests in California by applying 1/3 of the total annual nitrogen to the soil in April during fruit set and shoot flushing or in November [which we now know to be the time of flower formation (Salazar-Garcia *et al.*, 1997)]. Mid-February and mid-June nitrogen applications significantly reduced yield (Lovatt, 1995). The results suggest that if nitrogen is not limiting, vegetative growth does not negatively impact fruit set. Sustaining vegetative shoot growth has the advantage of increasing photosynthetic area and flowering and fruiting points for the following season. Reducing vegetative growth during the period critical to fruit retention either by pruning (Cutting and Bower, 1990) or by the use of paclobutrazol (Köhne and Kremer-Köhne, 1987; Köhne, 1988; Wolstenholme *et al.*, 1989) resulted in increased fruit set and yield. The effect on fruit size has been variable. Pruning increased the calcium content of the fruit (Cutting and Bower, 1990), making them less susceptible to postharvest disorders (Bower, 1988). Combining a pre-anthesis soil application of nitrogen with a mid-anthesis foliar application of paclobutrazol increased yield without a reduction in fruit size and improved fruit storage quality (Whiley, 1994). Gibberellic acid₃ (GA₃) canopy sprays in November or January resulted in precocious development of the vegetative shoot of indeterminate inflorescences, such that the majority of leaves were sources at the time of fruit set rather than competing sinks; the GA₃ treatments increased yield without reducing fruit size (Salazar-Garcia and Lovatt, 1997).

When competition has been demonstrated to be a factor influencing fruit drop in a tree crop, lack of available carbohydrate has been assumed initially to be the cause. For avocado, this argument is supported by the fact that yield has been correlated with tree carbohydrate status (Scholefield *et al.*, 1985; Wolstenholme, 1987; Wostenholme *et al.*, 1988; Whiley, 1994). Properly timed branch girdling is a technique used to increase carbohydrate levels above the girdle to increase fruit set and size. How many and how frequently branches can be girdled without adverse effects long-term to avocado tree health and production remains to be determined.

Experiments with other tree crops, such as citrus, for which fruit set and early drop have been

studied in more depth, ultimately precluded carbohydrate availability as the key factor limiting fruit set and causing increased early drop (Erner, 1989).

Cultivars of *Citrus sinensis*, like avocado varieties, undergo a period of excessive fruit drop commencing during flowering and continuing through the first stage of fruit growth. For both crops, fruit drop is completed by approximately mid-July. During this critical period, fruit retention/fruit drop in both crops is characterized by (i) competition between the young fruit and vegetative growth; (ii) sensitivity to temperature extremes; and (iii) water-deficit stress. Studies on fruit set in citrus have yielded the current hypothesis that fruit set is favored by a greater ratio of endogenous growth promoting (GA and/or cytokinins) to growth inhibiting [abscisic acid (ABA)] hormones. Evidence is beginning to appear in support of this hypothesis in avocado. Determinate inflorescences of the 'Hass' avocado in California were confirmed to set three-fold more fruit than indeterminate inflorescences (Salazar-Garcia and Lovatt, 1997). During fruit set, fruit of determinate inflorescences had a significantly higher ratio of endogenous GA to ABA than fruit borne on indeterminate inflorescences (Bertling and Lovatt, 1995). Cowan *et al.* (1997) demonstrated that 'Hass' avocado fruit growth and abscission were related to changes in cytokinin to ABA ratio. In vivo application of ABA reduced fruit growth and increased fruit abscission, whereas co-treatment with isopentyladenine reversed the effects. March canopy applications of GA₃ increased yield and fruit size of the 'Hass' avocado (Salazar-Garcia and Lovatt, 1997). Some degree of early avocado fruit drop likely results from high temperatures and transient water-deficit stress. It remains to be determined whether ABA or ethylene levels increase in avocado and/or GA or cytokinin levels decrease in response to abiotic stresses leading to fruit drop. The lack of basic information regarding hormonal regulation of these processes in avocado leaves open the possibility for significant improvement in fruit set and yield in the future as our knowledge base increases.

Conclusions

Pollination and fertilization are two processes critical to fruit set that have been targeted to increase avocado yield to a greater degree than preventing abscission of normal fruit during the early drop period. The roles of hormones in the stimulation of fruit growth in response to self- or cross-pollination, in the subsequent development of a normal fruit, in the fruit abscission process in the presence or absence of stress, and in alternate bearing have been minimally investigated. The results of such studies would undoubtedly provide new information of practical value for improving fruit set and yield. Whereas cross-pollination of 'Hass' by 'Ettinger' in Israel is an important factor to maximize production, many 'Hass' orchards produce good yields without benefit of outcrossing. For many growers, the significant yield differences between "on" and "off" years cannot be overcome in the "off" year with our present abilities to increase yield by improving pollination. For countries in which paclobutrazol cannot be used in avocado production, canopy sprays of boron or nitrogen improve yield, but not consistently. Similarly, interplanting pollinizers to increase outcrossing, importing bees into an orchard and luring them through the orchard with pheromones increases yield more in some years than in others. It is this reviewer's opinion that our ability to dramatically increase avocado production awaits the results of further research into the mechanisms by which endogenous plant hormones regulate developmental processes and mediate the responses of the avocado tree to the environment. Recent results of Cowan *et al.* (1997) and Salazar-Garcia and Lovatt (1997) clearly demonstrate the potential of such research.

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