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Physiological factors affecting flower and fruit abscission of 'Hass' avocado

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ABSTRACT

'Hass' avocado (*Persea americana* Mill.) is characterized by distinct periods of excessive flower and fruit drop. Determining the cause(s) of these abscission events is critical for the development of strategies to increase fruit set and yield. Therefore, the objectives of this research were to determine pollination and fertilization rates of abscising flowers and to quantify the viability of developing ovules (seeds) and hormone concentrations of abscising versus persisting fruit through early and June drop of 'Hass' avocado. Experiments were conducted during sequential off- and on-crop years. Pollination, pollen germination, pollen tube growth and ovule viability of abscising flowers were determined by microscopic analysis. During early and June drop, cellular deterioration of developing ovules of abscising and persisting fruit was visualized with Evan's blue stain. Abscisic acid (ABA), indole-3-acetic acid (IAA), and isopentenyladenine (IPA) concentrations were determined by radio-immuno assay (RIA) during the on-crop year only. On average ≥ 70% of abscising 'Hass' flowers were pollinated but due to the failure of the pollen grains to germinate and produce pollen tubes, fertilization never occurred and the ovule deteriorated. Fruit abscission during early and June drop was also due primarily to a lack of fertilization. Fruit abscission was associated initially with deterioration of the nucellus within the developing ovule in early drop, and subsequently with deterioration of the integument (seed coat) in June drop; both occurred more frequently in abscising than persisting fruit. Abscising fruit had greater ABA concentrations during early drop, June drop and fall fruit drop compared to persisting fruit; IAA and IPA were greater in abscising fruit during June drop, but not early drop or fall fruit drop. Only 25% of the total number of fruit that abscised per crop year were fertilized. Taken together, these results provide strong evidence that the majority of flowers and fruit of 'Hass' avocado abscise due to a lack of pollen germination and subsequent fertilization. In addition, the results suggest that ABA accumulation is related to ovule (seed) abortion and reduced fruit growth in abscising fruit.

1. Introduction

The avocado is characterized by excessive flower and fruit abscission (Cameron et al., 1952; Garner and Lovatt, 2008; Inoue and Takahashi, 1990; Lahav and Zamet, 1975; Slabbert, 1981), resulting in extremely low fruit set (<0.1%) even in healthy, well-managed orchards (Whiley and Schaffer, 1994). As with all seeded fruit, mature avocado fruit are the product of a long series of events that include successful pollination, fertilization, and embryo, endosperm, and seed coat development simultaneous with transition of the ovary to the fruit. Even if pollen is received on a receptive stigma, fertilization will not occur without successful pollen germination, pollen tube growth and pollen tube penetration of the stigma, style, ovary and ovule to complete double fertilization. During this period, the ovule must remain viable. The rate of failure of each of these processes, and therefore, their impact on flower abscission, is currently unknown for avocado.

Developing fruit often abscise due to the lack of proper development of the ovule into the seed. Researchers have noted that many abscised fruit are enlarged ovaries (Sedgley, 1980; Tomer and Gazit, 1979) that resulted from stimulated parthenocarpic growth, during which pollination without fertilization briefly stimulated ovary enlargement prior to abscission. However, even with successful fertilization, the embryo and endosperm of some fruit fail to develop properly, resulting in seed abortion (Blumenfeld and

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Gazit, 1974; Steyn et al., 1993; Tomer et al., 1980). The persistence of these seedless fruit, typically referred to as "cukes", is rare in most avocado cultivars but is enhanced by girdling (Garner, 2004). Loss of avocado fruit might occur even if fertilization and early embryo and endosperm development are successful. In anatomical studies, abscised avocado fruit are often characterized by premature deterioration of the developing integument into the seed coat (Cowan et al., 1997; Cutting, 1993; Davenport and Manners, 1982; Moore-Gordon et al., 1998). Whether deterioration of these tissues is the cause of fruit abscission or a symptom thereof remains to be determined.

Differences in fruit growth rate and endogenous hormone concentrations between young abscising and persisting fruit have been investigated for many fruit tree crops, including 'Valencia' orange (*Citrus sinensis* L.) (Pozo, 2001), mango (*Mangifera indica* L.) (Bains et al., 1999), almond (*Prunus dulcis* Mill.) (Koukourikou-Petridou, 2003), and peach (*Prunus persica* Sieb. and Zucc.) (Ramina and Masia, 1980, 1982). Demonstration of the changes that occur in seed development and hormone concentrations of abscising and persisting fruit during key stages of 'Hass' avocado fruit development will provide fundamental information required for developing and implementing horticultural strategies to decrease abscission and increase yield.

Physiological factors contributing to the major periods of flower and fruit abscission in the phenology of the 'Hass' avocado tree have not yet been fully identified. Therefore, the objectives of this study were to determine whether flower abscission is due to a lack of pollination or fertilization and to determine whether differences in ovule (seed) viability or hormone concentrations distinguish abscising from persisting 'Hass' avocado fruit.

2. Materials and methods

2.1. Plant material

Commercially bearing 6-year-old 'Hass' avocado trees on Mexican race rootstock in an orchard in Carpinteria, CA (lat. 34°23′N, long. 119°31′W, alt. 8 m above sea level) were selected for uniform health, size, and vigor and were subjected to the same standard cultural practices as the remaining orchard trees. The research was conducted during two sequential years, representing an off-crop and an on-crop. The experiment was initiated at the beginning of the off-crop bloom. Abscising flowers and fruit were collected in nets placed under 10 trees. Collections were made every 2 weeks starting in January and continuing through the following February each year. Samples were bagged and transported in a cool box to the laboratory, where reproductive structures were sorted into flowers and fruit, weighed and counted. All trees were harvested in October each year. Total yield was determined as kilograms per tree. At harvest, a randomly selected sample of 100–150 fruit per data tree, representing ~30% to 100% of the mean total number of fruit on a tree for each year of the experiment, was collected and the mass of each fruit in the subsample was determined. These data were used to estimate the total number of fruit harvested per tree for comparison to the number of reproductive structures that abscised per tree.

2.2. Weather conditions

Weather data for the period of the research was downloaded from the California Irrigation Management Information System (CIMIS) website (California Department of Water Resources, 2003). Daily wind speed, precipitation, solar radiation, and maximum, minimum, and average air temperatures were obtained from the closest coastal station, Santa Barbara #107 (lat. 34°26′N, long. 119°44′W, elevation 76 m). CIMIS quality control criteria and comparisons with data from the National Climatic Data Center (NCDC) Santa Barbara Station (COOP ID 047902; lat. 34°25′N, long. 119°41′W, elevation 2 m (National Oceanic Atmospheric Administration, 2004)) were used to validate the data.

2.3. Pollination, pollen germination and ovule viability of abscising flowers

Once abscised reproductive structures were removed from the nets under the 10 data trees, branches approximately 1.5 m above ground in four quadrants (N, E, S and W) of each data tree were shaken gently to collect abscising flowers. To ensure that collected flowers abscised at the time the branch was shaken and not earlier, only flowers without browning at the base of the pedicel were collected. This criterion was based on the results of an earlier study in which flowers were removed and observed overtime. Browning of the flower pedicel was observed within 1 h after flower removal (Garner, unpublished data). Ovaries with a diameter ≥2 mm were assumed to be the result of fertilization or parthenocarpic development (Sedgley, 1980) and flowers with such ovaries were rejected. The flower abscission rate was sufficient to provide samples of 10 flowers per tree collected approximately weekly from 7 May through 30 June and 7 April through 31 May of the off- and on-crop years, respectively, for a total of 8 sample dates per year. Flowers were fixed immediately in formalin-acetic acid solution (FAA) solution (70% ethanol: formaldehyde: concentrated glacial acetic acid at a ratio of 90:5:5 by volume) for subsequent analyses. Persisting flowers were not collected because it was not possible to determine if individual abscising and persisting flowers were at the same phenological stage of development due to avocado's protracted bloom and dichogamous floral development.

The number of pollen grains per stigma, germinated pollen grains per stigma (pollen tube visible), pollen tubes per style, and viable or degenerate ovules per carpel were determined microscopically using a method modified from Jaganath (1993). For each sample date, 10 flowers were evaluated (one from each data tree). Flowers were rinsed in 70% ethanol and, using fine forceps and a razor blade, the gynoecium was dissected from each flower and its ovule was detached from the gynoecium at the base of the style under a Leica dissecting scope (MZ125; Bannockburn, IL). The ovule was softened for 10 min in 1 m NaOH at 70 °C; the stigma and style required 4 h for softening under the same conditions. After softening, samples were rinsed in distilled water, squashed in aniline blue (0.01% in 0.1 m K_3PO_4, pH 10) for 10 min and observed with a Zeiss fluorescent microscope (Thornwood, NY). Aniline blue stains selectively for callose, which allows for the visualization of the pollen tube walls, callose plugs in the tips of the pollen tube in stigmatic and stylar tissues, indicative of arrested pollen tube growth (Dumas and Knox, 1983; Martin, 1958), and callose deposition in the ovule, which is indicative of avocado ovule degeneration (Jaganath, 1993).

2.4. Ovule (seed) viability of abscising and persisting fruit

During early and June drop, 10 abscising and 10 persisting fruit were collected weekly from 17 May to 25 August and 20 April to 11 August for the off- and on-crop years, respectively. Branches approximately 1.5 m above ground in each of the four quadrants of the tree were shaken gently to dislodge abscising fruit, which were collected at random for immediate examination. To ensure the collection of currently, not previously, abscised fruit, only those fruit without browning at the base of their pedicel were collected. This criterion was based on the results of a preliminary study, which demonstrated that browning at the base of fruit pedicel begins within 30 min after fruit removal from the shoot (Garner, unpub-