Anatomy Differs for Aborting and Nonaborting Pistillate Flowers in Pecan

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Abstract. Comparative anatomical features of nonaborting (normal) and aborting pistillate flowers were examined with light and scanning electron microscopy during the first of four physiological drops characteristic of pecan [*Carya illinoensis* (Wangenh.) C. Koch]. Flowers sampled over a 3-year period from a protandrous and a protogynous cultivar (Desirable and Wichita, respectively) did not have any tissue necrosis. Diameter, length, and weight of aborting intact flowers were significantly less at 65%, 55%, and 30%, respectively, in aborting than nonaborting intact flowers. A common anatomical deviation in aborting flowers was that the integument was less extended over the nucellus of the ovule than in nonaborting flowers. The number of parenchymal nucellus cell layers lateral to the embryo sac often was less in aborting than nonaborting flowers. Embryo sacs were inflated in nonaborting flowers, but appeared deflated in aborting flowers. Both sacs had a conspicuous central nucleus, egg, and synergid, with a second synergid evident in the embryo sacs of some nonaborting flowers. Thus, aborting pecan flowers had incompletely developed ovules with no evidence of necrosis.

Four distinct periods of flower and fruit abortion occur within the pecan growth cycle and are referred to as Drops I to IV (Sparks and Madden, 1985). Drop I consists of aborting pistillate flowers from a spike borne terminally on the shoot. Abortions are more common on the distal than the proximal portion of the spike. Pistillate flower abortion is especially prevalent during "off" years (Woodroof et al., 1928), with widespread abortion of the entire spike in severe "off" cycles. Alternate bearing is not due to the absence of pistillate flowers in such years, but to massive abortion of pistillate flowers. Thus, pistillate flower abortion has major economic significance.

Pistillate flower abortion is not due to lack of pollination (Sparks and Madden, 1985), although opinions to the contrary persist (McEachern, 1993; Wood and Reilly, 1993). Indirect evidence supports the hypothesis of an association between Drop I and underdeveloped pistillate flowers (Sparks and Heath, 1972; Sparks and Madden, 1985; Woodroof et al., 1928), although documentation is lacking to substantiate this hypothesis. The number of pistillate flowers in the spike is positively correlated with shoot vigor (Finch and Crane, 1931; Gossard, 1933; Sparks, 1988; Sparks and Heath, 1972; Sparks and Madden, 1985), and pistillate abortion is inversely related to shoot vigor (Sparks, 1988; Sparks and Heath, 1972; Sparks and Madden, 1985). Correlations with shoot length have been verified experimentally by demonstrating that increasing shoot vigor increases pistillate flower production and decreases abortion (Sparks, 1988). Spring growth of the pecan shoot, leaves, and pistillate inflorescence depends on substrates accumulated during the previous growing season. Labeling experiments with ^{14}C indicate a decreasing allocation of substances from the base to the apex of the elongating shoot; thus, pistillate flower development may be prevented or impeded simply because of the terminal position of the inflorescence when assimilate reserves are low (Lockwood and Sparks, 1978).

The inverse relationship of pecan pistillate flower abortion to shoot length and the ^{14}C labeling experiments imply that nutrients for flower development are limited, as previously suggested (Sparks and Heath, 1972). Nutrient limitation apparently has the greatest effect during the "off" year, when low-vigor shoots are common, abortion is high, and assimilate reserves necessary for shoot growth and pistillate flower development (Lockwood and Sparks, 1978) are low (Sparks, 1983). Under limited nutrients, aborting flowers should be underdeveloped. The objective of our study was to determine if aborting pecan pistillate flowers have underdeveloped floral structures.

Materials and Methods

Source of plant material. Flowers were collected over three growing seasons at Watkinsville, Byron, and Waycross, Ga., from 'Desirable' trees at all three sites and 'Wichita' trees at the two former sites for analyzing growth and anatomy. Flowers also were collected in Crystal City, Texas, from 'Western Schley' trees for analyzing the development of integuments and nucellus. Nonaborting and aborting flowers with macroscopic stigmas were collected at peak anthesis from the same inflorescence. Collections represented a wide gradient in pistillate flower development, as floral maturation within an inflorescence or tree and among trees is variable (Yates and Sparks, 1992). However, apical flowers without visible stigmas were excluded from our study, as it is generally accepted that their abortion is due to incomplete development.

External visual criteria used for differentiating aborting and nonaborting flowers were size, color of bracts adnate to the ovary, and firmness of flower attachment to the peduncle. Most aborting flowers were smaller than nonaborting (normal) flowers in the same cluster. Bracts adnate to the ovary in aborting flowers had a yellowish tinge compared to the vivid green of nonaborting flowers. Aborting flowers were detached from the inflorescence by touching, whereas nonaborting flowers required severing at the junction to the peduncle. Aborting flowers partially detached from the peduncle and/or darkened in color were excluded to ensure that abortion-related events were investigated, not dehydration and/or secondary malady. Each flower was examined for pests to avoid

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confounding the results of abortion with those of abscission-induced pest damage, as emphasized by Sparks and Madden (1985).

Growth analyses. Diameter, length, and weight were determined for four or five intact aborting and nonaborting flowers collected from six trees each of 'Desirable' and 'Wichita'. Diameter was measured at the midpoint of the involucre, and length was measured from the junction of the involucre with the stigma to the flower base. Cultivars were compared by unpaired *t* tests, and aborting and nonaborting flowers of the same cultivar were compared by paired *t* tests (Gomez and Gomez, 1984).

Microscopy. Flowers for scanning electron microscopy (SEM) and light microscopy (LM) were fixed overnight at 4°C in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, and dehydrated in an ethanol series. SEM samples were critical-point dried with CO₂, mounted on stubs, coated with gold-palladium, and examined with an SEM (model 505; Phillips, Mahwah, N.J.) at 15 kV. LM samples were infiltrated and embedded in resin (Spurr, 1969). Sections were cut using an ultramicrotome (model Ultracut E; Reichart-Jung, Vienna, Austria) and were stained with a mixture of 1% azure II and 1% sodium borate (1:1) by applying gentle heat to the glass slides.

Embryo sacs were examined in cleared dissected ovules (Herr, 1982). Ovules were fixed for 24 to 48 h in FPA₅₀ [5 formaldehyde : 5 propionic acid : 90 ethanol (50%)], stored in 70% ethanol, and cleared for 24 h in BB-4 1/2 [2 lactic acid : 2 chloral hydrate : 2 phenol : 2 clove oil : 1 xylene : 1 benzyl benzoate (by weight)]. Cleared ovules were hand-sectioned to remove the outermost

Fig. 1. Weight, length, and diameter of nonaborting and aborting 'Desirable' (A) and 'Wichita' (B) flowers. Weight of nonaborting flowers was the only significantly different growth criterion between flowers from the two cultivars as determined by the unpaired *t* test, $\alpha \leq 0.05$. Abortive flowers within a cultivar were significantly smaller than nonaborting flowers for all three growth criteria as determined by the paired *t* test, $\alpha \leq 0.05$.

Fig. 2. External (A and B) and internal (C and D) gross morphology of physiological aborting (A and C) and nonaborting (B and D) 'Desirable' flowers were comparable. Points on intact flowers indicate plane for sectioning flowers in C and D. b = Bract, bt = bracteole, i = involucre, ov = ovule, ow = ovary wall, p = central partition wall, s = stigma, sy = style. Bar = 1 mm. Variations in light reception account for the differential color of the ovules in C and D. Bar = 1 mm.

layers of nucellar cells. Samples were mounted in BB-4 1/2 on Raj slides (Herr, 1982). Optical sections of embryo sacs were examined with a microscope (model 22 EB; Leitz Dialux, LEICA, Wetzlar, Germany) equipped with differential interference contrast. Integument length and nucellus thickness lateral to the embryo sac were measured in dissected ovules with a stereo microscope (Zeiss, Goettingen, Oberkochen, Germany) equipped with a micrometer disk, and data were analyzed by unpaired *t* tests (Gomez and Gomez, 1984).

Results

Growth of pistillate flowers. 'Desirable' nonaborting and aborting flowers were not statistically different for either length or diameter than those of 'Wichita', but weight of 'Desirable' nonaborting flowers was almost double that of 'Wichita' (Fig. 1). Aborting flowers in both cultivars were suppressed significantly for all growth parameters measured, with weight influenced most, then length, and last diameter. Size of aborting flowers compared to nonaborting flowers of 'Desirable' and 'Wichita' was 26% and 35% in weight, 50% and 60% in length, and 66% and 67% in diameter, respectively.

Gross morphology. Aborting and nonaborting flowers had a distinctive bifurcated stigma and an involucre with projecting bracts and bracteoles (Fig. 2 A and B). The stigma on some aborting flowers had a smoother surface and less color than that on nonaborting mature flowers in the same inflorescence; however, these gradients were not universal to aborting flowers, as they

occurred to some extent in nonaborting flowers. Thus, external structure did not differ consistently between nonaborting and aborting flowers.

Likewise, most internal gross morphological features of aborting and nonaborting flowers were similar (Fig. 2 C and D). The major floral organs of stigma, style, and ovary were present and had no evidence of necrosis or developmental anomalies. The ovary wall and central partition wall were conspicuous, but the ovule was less prominent. One distinction between nonaborting and aborting flowers was the presence of white and cream ovules in the respective flowers because of different external tissues, as evident from the microscopic analyses described below.

Integuments. Differences between nonaborting (Fig. 3A) and aborting flowers (Fig. 3B) were observed by SEM in the integument, the tissue forming the thin brown membranous seed coat or testa of the kernel in the mature pecan nut. The integument caused a nonaborting ovule to appear white (Fig. 2D) and was more completely developed over the cream nucellus in nonaborting (Fig. 3A) than in aborting pecan flowers (Fig. 3B). Longitudinal sections of ovules from nonaborting flowers of a protandrous ('Desirable') and protogynous ('Wichita') cultivar (Figs. 4A and 5A, respectively) showed that integuments of both cultivars had an outer and inner epidermis surrounding at least five central layers of parenchyma cells. Cytoplasm of parenchyma cells toward the micropyle was less dense and more vacuolated than that toward the chalaza or the epidermal cells. In comparison, the integument in aborting flowers (Figs. 4B and 5B) was underdeveloped. Furthermore, length of the integument in nonaborting ovules significantly

Fig. 3. Scanning electron micrograph of ovule in nonaborting (A) and aborting (B) 'Wichita' flower. The integument of the nonaborting flower had almost enclosed the nucellar tissue, but integument development was incomplete in the aborting flower. p = Central partition wall, i = integument, n = nucellus. Bars = 0.2 mm.

exceeded that in aborting ovules. Thus, underdevelopment of the integument in aborting compared to nonaborting flowers was substantiated by LM, SEM, and statistical treatment of length measurements of integuments from dissected ovules.

Nucellus. A multicellular layer of nucellus surrounded the embryo sac in nonaborting (Figs. 4A and 5A) and aborting (Figs. 4B and 5B) flowers of 'Desirable' and 'Wichita', respectively. The nucellus of aborting and nonaborting flowers was similar in that a

Fig. 4. Light micrograph of 0.5- μ m sections of fixed ovules comparing nonaborting (A and C) and aborting (B and D) 'Desirable' flowers during Drop I for integument growth (A and B) and development in the embryo sac (C and D). Ovules are oriented with the micropyle to the top and the chalaza to the bottom. eg = Egg cell with point indicating nucleus; em = embryo sac; fa = filiform apparatus; i = integument with point to left and right designating outer and inner epidermis, respectively; ne = nucellus epidermis; np = nucellus parenchyma; c = central nucleus; s = synergid; sg = starch granule. Bars = 10 μ m (A, B, and D) and 5 μ m (C).

greater proportion was located beneath the embryo sac toward the chalaza than above the embryo sac toward the micropyle, but was different in the number of cell layers and cytoplasmic density. The number of parenchymal nucellus cell layers lateral to the embryo sac was about twelve in nonaborting flowers, but as few as five in aborting flowers. A difference in the number of cell layers was reflected in thickness measurements, which were statistically different in aborting and nonaborting flowers. Cytoplasm of the epidermis compared to that of the parenchyma was more dense in nonaborting flowers, but was of similar density in aborting flowers. Starch granules were prevalent in the nucellus of nonaborting and aborting flowers (Figs. 4 C and D, 5 C and D). Thus, the nucellus in aborting compared to nonaborting flowers had fewer layers of

parenchyma cells lateral to the embryo sac and a more uniform cytoplasmic density in the epidermal and parenchymal cells.

Embryo sac. Nonaborting and aborting flowers of 'Desirable' (Fig. 4) and 'Wichita' (Fig. 5) had a conspicuous embryo sac located in the micropyle region of the ovule. Embryo sacs were ovate, with an inflated appearance in nonaborting flowers (Figs. 4A and 5A), but often appeared irregular and deflated in aborting flowers (Figs. 4B and 5B). Size and orientation of embryo sacs in sections of fixed ovules limited visualization to part of the structural features, such as only the egg cell and synergid (Fig. 4 A and C). The egg cell had a polar orientation, with the nucleus and cytoplasm concentrated in the chalaza direction. The filiform apparatus, wall ingrowths at the micropyle end of the synergids, was

Fig. 5. Light micrograph of 0.5- μ m sections of fixed ovules comparing nonaborting (A and C) and aborting (B and D) 'Wichita' flowers for integument growth (A and B) and development in the embryo sac (C and D). Ovules are oriented with the micropyle to the top and the chalaza to the bottom. eg = Egg cell with point indicating nucleus; em = embryo sac; fa = filiform apparatus; i = integument with point to left and right designating outer and inner epidermis, respectively; ne = nucellus epidermis; np = nucellus parenchyma; c = central nucleus; s = synergid; sg = starch granule. Bars = 10 μ m (A, B, and C) and 5 μ m (D).

assumed to be the intensely stained region apparent in aborting and nonaborting ovules (Figs. 4C and 5C and D). A central nucleus was conspicuous in aborting and nonaborting flowers (Figs. 4D and 5C and D), but antipodal cells were not identified in ovules of either nonaborting or aborting flowers (Figs. 4C and D and 5C and D).

Optical sections of cleared ovules provided visualization of all components of the embryo sac, but not necessarily in the same focal plane (Fig. 6). Embryo sacs of nonaborting and aborting flowers contained a large central nucleus (Fig. 6A) with a dense nucleolus. The central nucleus was embedded in a blanket of

cytoplasm with a series of thin strands extending to near the sac wall and dominating the lower half of the embryo sac. The egg apparatus in the micropyle region of the embryo sac had an egg cell as a consistent component in aborting and nonaborting flowers (Fig. 6B). Egg cell cytoplasm was more dense toward the chalaza at which the nucleus was located than in the highly vacuolated micropyle region. The nucleus was spherical and contained a dense nucleolus. As in thick sections of fixed ovules described above, antipodal cells were not detected in optical sections of cleared ovules of either nonaborting or aborting flowers. Two identical synergids (Fig. 6A) were observed in some, but not all, nonaborting flowers, with only one synergid apparent in aborting flowers. Cytoplasmic density and nucleus location differentiated synergids from the egg cell. Synergids had dense cytoplasm throughout with a nucleus located toward the micropyle in contrast to a differential cytoplasmic density and chalaza nuclear position in the egg cell. In summary, nonaborting and aborting flowers had ovules in which the embryo sac had developed and contained a central nucleus, egg, and synergid. Some nonaborting embryo sacs contained one synergid and others two, but aborting embryo sacs appeared to contain only one synergid.

Discussion

Earlier investigators hypothesized that Drop I in pecan is associated with underdeveloped flowers (Sparks and Heath, 1972; Sparks and Madden, 1985; Woodroof and Woodroof, 1926). Our results support this hypothesis and identify the underdeveloped tissues in the aborting flower. Ovular tissues, particularly the integument and nucellus, of aborting flowers were incompletely developed. Structural anomalies for aborting flowers from 'Desirable' and 'Wichita' were comparable. Because incompletely developed flowers can be greatly minimized by increasing shoot growth (Sparks, 1988), the grower can minimize abortion. In this regard, consistently producing pecan orchards are characterized by trees with vigorous growth.

Necrotic tissues were not evident either macroscopically or microscopically in aborting pecan flowers as reported for abscising walnut flowers (Catlin and Polito, 1989), a closely related nut crop. Abortive flowers in both species have embryo sacs, but other characteristics differ. Stigma tips are necrotic in walnut flowers, but not in pecan. Walnut flowers cut through the ovary have black and necrotic ovular and central partition wall tissue, but pecan flowers do not have similar necrosis. The integument of walnut ovules degenerated, whereas, in pecan, the integument has no necrosis, but is not as developed compared to the nonaborting flowers.

A common feature of plants that possess two synergids is that one synergid degenerates before the pollen tube enters the embryo sac (Mogensen, 1978; Willemse and van Went, 1984). Products may be liberated during synergid degeneration prompting the pollen tube to enter the embryo sac to discharge its contents (Kuroiwa, 1989). The direction a pollen tube grows may be controlled by a combination of factors including glucose, calcium, or a low-molecular-weight protein (Reger et al., 1992). All aborting and some nonaborting pecan flowers examined during Drop I lacked a second synergid. The absence of this second synergid could indicate that one synergid had degenerated in these flowers. On the other hand, the consistent absence in aborting flowers could indicate a developmental anomaly. Regardless, Drop I in pecan is not influenced by pollination (Sparks and Madden, 1985).

In summary, internal anatomy, but not external morphology, distinguishes aborting from nonaborting pecan flowers at Drop I. Flowers aborting from a protogynous and a protandrous cultivar

Fig. 6. Differential interference contrast micrograph of embryo sac at two focal lengths (A and B) in cleared ovule from nonaborting 'Desirable' flower during Drop I. Ovule is positioned with the micropyle to the top and the chalaza to the bottom. eg = Egg cell, c = central nucleus, s = synergid, point = nucleus. Bars = 5 µm.

were similar. Major anatomical features of aborting flowers were incomplete development of the integument and nucellus compared to nonaborting flowers, characteristics unlike the necrosis reported for tissues of abscising walnut flowers.

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