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Cell and Tissue Damage Associated with Pistillate Flower Abscission of Persian Walnut

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Abstract. **Pistillate flowers from walnut trees having >80% pistillate flower abscission (PFA) were examined from the time of anthesis until shortly before abscission. In addition to normally developing flowers, two types of abortive flowers were found. One abnormal flower type, seen in only two cases, lacked a developed embryo sac and had cellular degeneration in the nucellus. The second type of damaged flowers, which were more commonly observed, had apparently normal development of the nucelli and embryo sacs, but cell and tissue necrosis became evident beginning at the tip of the stigma, in the integuments, and throughout the placental evaginations. No cell or tissue damage was observed until after ovary growth in these flowers had stopped. We conclude that this second type of damage is associated with PFA.**

Pistillate flower abscission (PFA) of Persian walnut, *Juglans regia* L., recently has been described as a problem that can seriously reduce yields (Catlin et al., 1987). In some cases, losses in excess of 90% of flowers initiated have occurred. PFA was recorded to varying degrees in seven cultivars, with 'Serr' most seriously affected. When PFA was first recognized in 1978, 'Serr' was rapidly becoming a dominant cultivar in California. Because of PFA, 'Serr' has lost favor and some orchards have been topworked to other cultivars. Although yield potentials have generally not been realized with 'Serr', outstanding edible yield and quality can result in dollar-per-hectare returns near the highest for all cultivars (Merrill, 1986).

Eight years of monitoring the occurrence of PFA and attempts to identify its causes were not successful (Catlin et al., 1987). No association could be made with any physiological, cultural, pathological, or entomological factor. Differences occurred in PFA of 'Serr' with location or planting site, and, in some cases, years. Catlin et al. (1987) suggested that soil environmental–root physiological interactions might be involved; however, the nature of these interactions is not known.

Pistils appeared to develop normally upon emergence from terminal mixed buds. Ovary enlargement in affected flowers ceased at ≈3 to 4 mm in diameter. Normal reflexing of styles and stigmas stopped in a partially completed position at a stage of middle to late receptivity to pollen (Forde and Griggs, 1975; Polito, 1985). Affected pistils remained attached to shoots for up to 2 weeks. When abscission occurred, separation was between the peduncle, with one to several pistils attached to it, and the shoot. Normal, but unpollinated, pistils continued to enlarge rapidly, much beyond the 3- to 4-mm size before abscising (Polito, 1985), with separation usually between the base of the ovary and peduncle. Thus, PFA, where loss of the entire inflorescence occurs at an early developmental stage, is attributed to different causes from the later abscission that results from lack of fertilization.

There was no indication of external damage to pistils. Observations of pistils cut through the ovary near the time of abscission revealed black and necrotic ovular and placental tissues. Similar observations made shortly after ovary growth had stopped did not show such discoloration. It appeared that damage might have originated within the pistil rather than from external causes. It was also possible that defective tissues had originated during pre-anthesis development. Thus, it seemed important to determine if histological changes within the pistils might be associated with events leading to PFA. This report describes the occurrence of tissue damage in walnut pistils affected by PFA.

Pistillate flowers of 'Serr' walnut were collected in 2 years from two trees with more than 80% PFA. In the first year, samples were taken at about the start of receptivity (Forde and Griggs, 1975) and after ovary growth stopped (OGS). In the second year, samples examined were those obtained at 2-day intervals for 10 days, beginning at maximum receptivity. Samples consisted of six to eight shoot apices with two pistils each.

Samples were fixed in formalin–acetic acid–ethanol under vacuum, dehydrated in a tertiary butyl alcohol series, embedded in Paraplast Plus, sectioned longitudinally at 8 to 10 μm, mounted on slides, and stained with safranin and fast green.

The pistillate flower of walnut contains a single orthotropous ovule enclosed by one integument (Fig. 1). The ovule is subtended by winged outgrowths of the placental axis referred to as evaginations (Nast, 1935). As the gynoecium develops, the evaginations grow to fill the cavity between the septa and the ovary walls.

Three classes of flowers were noted among those examined: normally developing flowers and two types of abortive flowers. In one type of abortive flower, seen in only two cases, an embryo sac failed to develop and cell degeneration was evident only in the nucellus; stigma tips, evaginations and integuments of these flowers appeared normal. These flowers appeared to undergo ovule abortion early in development in a manner distinct from the other abortive flowers.

In the second type of abortive flowers, embryo sacs developed normally and there was a consistent pattern of cell and tissue degeneration that appeared concurrently with

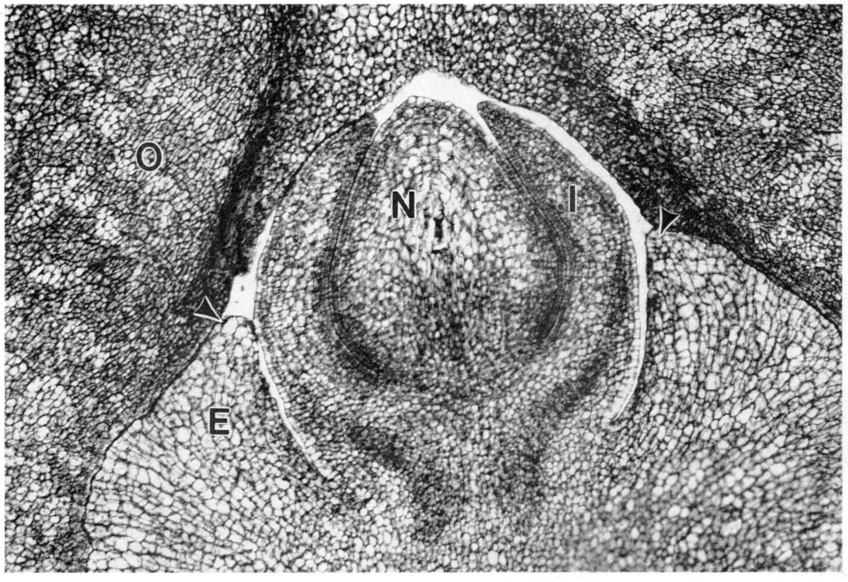

Fig. 1. Walnut ovule before fertilization. No tissue damage is evident in this sample. Note the cells at the tips of the evaginations (arrowheads) and the close contact of the evagination to the ovary wall and the integument. E, evagination; I, integument; N, nucellus; O, ovary wall.

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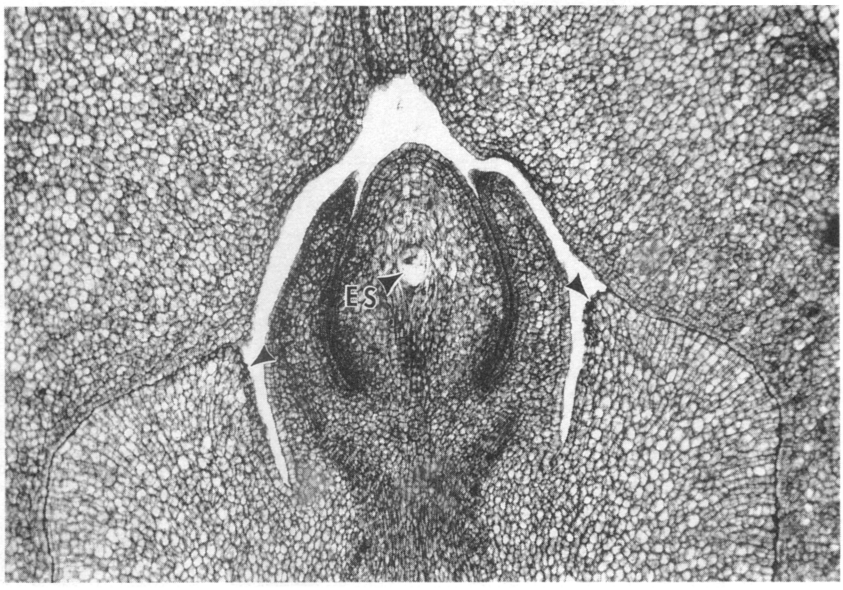

Fig. 2. Walnut ovule showing early signs of damage. Arrowheads indicate necrotic cells at the tips of the evagination. Note that a distinct space has developed between the evagination and the integument. ES, embryo sac.

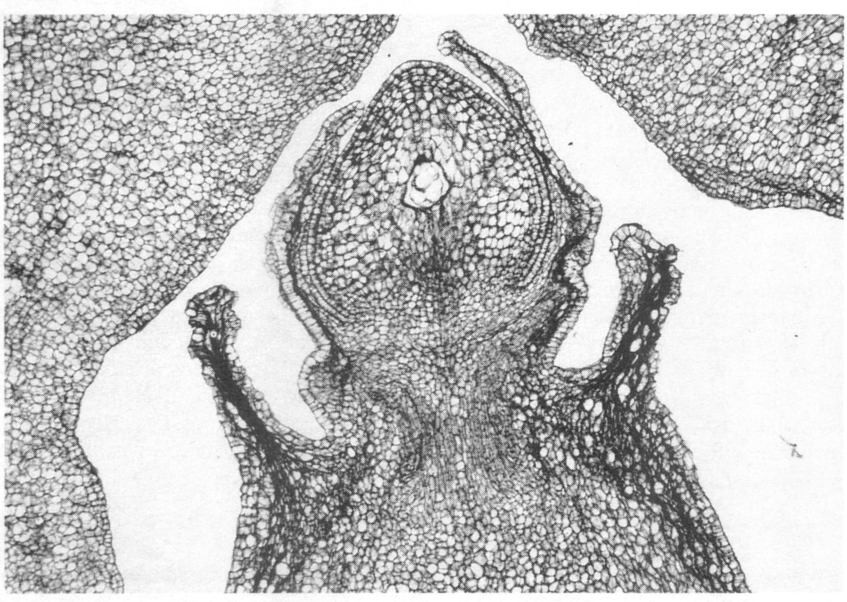

Fig. 3. Walnut ovule showing advanced stages of damage. The evagination has collapsed leaving empty space throughout the locule. Cell necrosis is most evident in internal cells of the evagination and integument. The epidermal layer of the integument and part of the evagination appear to remain healthy.

embryo sac development. No disintegrating or necrotic cells were observed in flowers that were in a stage of ovule development before the first division of the megaspore. Tissue damage was first observed in flowers with ovules at the two- to four-nucleate stage of embryo sac differentiation. This damage was manifested as necrosis of cells at the apical portion of the evaginations (Fig. 2) and at the tip of the stigma. This defect was followed by degeneration and collapse of cells in the apical cortical region of the integument. Tissue damage progressed basipetally throughout the evaginations, where all cells ultimately degenerated (Fig. 3). Similarly, all the cortical cells of the integument ultimately collapsed; inner and outer epidermal cells of the integuments remained intact and turgid until late in the degeneration of the integuments.

The nucellus developed more or less normally with no indication of cell necrosis or degeneration evident. Embryo sac differentiation and, in some cases, early embryogeny also appeared to progress normally, even in markedly deteriorated flowers. In at least two cases, flowers with severe cellular degeneration in the integument and evaginations contained fertilized ovules with a large vacuolate zygote at the micropylar end of the embryo sac and endosperm, with eight to 16 nuclei apparent (Fig. 4).

It is noteworthy that the stigma and the evaginations, which incur the earliest and ultimately most severe damage, are ephemeral tissues that degenerate during the normal course of development, although at a later stage than that seen in the abortive flowers. Because fertilization had occurred in some of the affected flowers, it is apparent that the stigmatic surface cells retained at least partial functional integrity and the ability to support pollen germination and tube growth. This retention may have occurred at the basal portion of the stigma where degeneration occurred later than at the tips. The significance of the early degeneration of cells of the evaginations is unclear, mainly because the function of this tissue is not known. It may serve as a temporary storage tissue (Nast, 1935). Another view considers the evaginations to function as an obturator, directing pollen tubes to the chalazal region of the ovule (Luza, 1986). Although the tissue does degenerate during normal development, it does so in a different manner and at a different developmental stage from that observed in abortive flowers. Normally, this tissue degenerates as the ovule expands after fertilization, during which time the cells become broken and separate. The early cell necrosis and collapse seen in abortive flowers does not occur.

The exact time of ovary growth stoppage (OGS) was not determined. With repetitive monitoring of pistils at 3- to 4-day intervals (Catlin et al., 1987), OGS is evident ≈3 to 4 days after its occurrence. Estimates that OGS had taken place were consistent with abscission occurring later (Catlin et al., 1987). In the first year, pistils obtained well in advance of OGS were without tissue abnormalities. After OGS had occurred, extensive damage of the second type described above was observed.

In the second year, samples examined were those obtained at 2-day intervals between early indication of OGS and when OGS was certain with many pistils. No tissue damage was observed with the two earliest samples. With the third sample, two of 13 flowers had damage of the second type. The fourth sampling revealed the only instance of first-type damage; two flowers were affected and 13 appeared normal. The fifth sample contained nine normal flowers and nine with extensive second-type tissue disintegration. The first occurrence of PFA was 4 days after the last sample. In both years, a high incidence of tissue damage was present only after pistil enlargement had stopped for several days. It seems clear that the second type of tissue damage, that involving stigma necrosis and damage to integuments and evaginations, is characterstic of PFA-affected flowers.

Tissue damage was not evident before OGS began or for a few days after its occurrence. Thus, PFA does not result from abnormalities occurring during differentiation and development before OGS. PFA of walnut appears different than early postanthesis abscission of pecan, where losses were attributed to weak and underdeveloped flowers (Sparks and Madden, 1985).

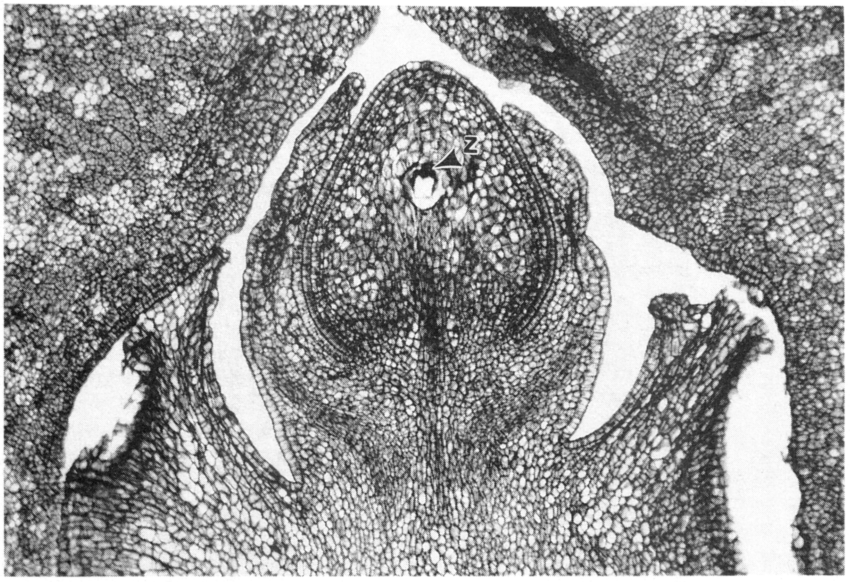

Fig. 4. Intermediate stage of tissue necrosis in fertilized walnut ovule. A zygote (Z) and endosperm are present in the embryo sac of this ovule, which has necrotic cells in the integument and evagination.

Events occur shortly after anthesis and after flowers become receptive to pollination that stop pistil enlargement and are later expressed as necrosis of internal ovarian tissues. These events then initiate the abscission process, resulting in PFA 1 to 2 weeks later. The nature of the primary causes leading to PFA remains unknown.

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Embryo Sac Development in Sour Cherry During the Pollination Period as Related to Fruit Set

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Abstract. **The development of the embryo sac was followed during the pollination period in 'Montmorency' sour cherry (*Prunus cerasus* L.) and related to fruit set. Twenty five percent to 40% of the embryo sacs were incomplete, degenerating, or contained four nuclei or fewer at anthesis and were considered nonfunctional. The effective pollination period extended for 3 to 5 days in each of two growing seasons, and fruit set ranged from 14% to 26%. The incidence of nonfunctional ovules at anthesis did not appear to be sufficient to fully account for the limited fruit set observed. Other factors, most likely physiological, play a contributing role.**

Fruit set is frequently the limiting factor in efficient production of sour cherries. Initial fruit set, as indexed by ovary enlargement after petal fall, may exceed 40%, but fruit abscission during June drop may reduce final set to ≤25% (Gray, 1934; Diaz, 1979; Retamales and Bukovac, 1986). Fertilization of the megagamete apparently takes place and embryo growth is initiated (Bradbury, 1929), but embryo abortion is often associated with the abscissing fruit during June drop (Bradbury, 1929; Stösser and Anvari, 1982, 1983). The reasons for the failure of sustained embryo development and, thus, fruit growth are not well-understood. However, nutritional and environmental stresses, particularly during early fruit development, are generally believed to be important factors.

While studying sour cherry fruit growth, we have observed (data not presented) localized discoloration (browning) at the chalazal end of the developing seed in fruits visually (color and size) considered to have a high potential for abscission. The embryo sacs of such fruits frequently appear to have normally developing embryos, but the endosperm develops poorly or may be discolored. Abscission of such fruits may be related to a failure in development of essential components of the embryo sac at anthesis, leading to incomplete development of the endosperm and/or embryo and subsequent fruit abscission.

To gain a better understanding of fruit set in sour cherry, time of controlled pollination and development of the embryo sac during the pollination period was investigated in relation to fruit set.

Our studies were performed on 6-year-old sour cherry ('Montmorency'/Mahaleb) trees receiving standard horticultural practices at the Horticultural Research Center, East Lansing, Mich. during Spring 1984 and 1985. Uniformly vigorous (25- to 35-cm) lateral flowering shoots with good light exposure at the periphery of the trees were selected. In 1984, the pistils of some flowers (≈20%) were damaged by low temperature (−2.8C) 11 days before anthesis. Flower damage appeared to be random, and injured flowers (visual evaluation) were not used.

One day before expected anthesis (early balloon stage), two flowers per node (usually the two most advanced in development) were emasculated and petals were removed to delimit the effective pollination period. All additional flowers were removed and the shoots were enclosed in glassine bags (≈15 × 50 cm). Three shoots selected at random were

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