metrical root system than cuttings treated with auxin-talc preparation (11). Roots were regenerated on 4 sides by inserting 2 toothpicks at right angles to each other, as in this study. Also, the toothpick method has been used to successfully transplant large caliber (8–15 cm) trees bare root (5). The toothpick method might be used also to stimulate adventitious root initiation in hard-to-root stem cuttings.

The response of scarlet oak seedlings to auxin treatments outlined in this study suggests that these techniques have potential for increasing the ease by which this difficult-to-transplant species may be established successfully following transplanting.

Literature Cited


Postfertilization Hybrid Seed Failure in Lycopersicon esculentum × Lycopersicon peruvianum Ovules

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Additional index words. incompatibility, interspecific cross, endosperm deterioration, tomato

Abstract. Lycopersicon esculentum Mill. × Lycopersicon esculentum (E × E) and interspecific Lycopersicon esculentum × Lycopersicon peruvianum (L.) Mill. (E × P) ovules were studied to determine the sequence of events leading to embryo and endosperm deterioration in E × P ovules. At 7 days, the single-celled zygote and multicellular endosperm stage, the E × P ovule showed no signs of abnormality. Endosperm deterioration was the first indication of anatomical abnormality. The endosperm had disintegrated totally by 10 days after pollination. The E × P embryo did not follow normal stages of embryo development but divided randomly, and at 17 days filled the embryo sac with an undifferentiated mass of cells. Deterioration of both the E × P embryo and the cells of the proliferated endothelium was complete by 24 days. The embryo sac cavity size of 14-day E × P ovules and the weight of 13-day E × P ovules also indicated that endosperm and embryo breakdown began in this interspecific cross earlier than the 30- to 40-day age reported by other researchers.

Lycopersicon peruvianum is a wild species of tomato that is resistant to root knot nematode (12), mosaic virus (2), bacterial spot, leaf mold, fusarium wilt, and septoria and alternaria blights (3). It is also a genetic source of high ascorbic acid content (12, 21). The incorporation of such desirable germplasm into a tomato breeding program is the underlying goal of our work. Attempts to introduce new germplasm from L. peruvianum into L. esculentum have been complicated by a post-fertilization barrier which prevents E × P ovules from developing to maturity. The hybridization has been achieved via embryo culture (1, 4, 20) and embryo callus (21), but the same postfertilization barrier exists in the first backcross of the hybrid to L. esculentum (2, 21).

Normal fertilization occurs in many interspecific crosses (7, 16, 17, 22), and the hybrid embryo deteriorates before differentiating cotyledons. Abnormal nutrient distribution between maternal tissue and the subnormal endosperm in Nicotiana interspecific hybrids (7) and failure of the primary endosperm nucleus to be fertilized in the L. esculentum × L. peruvianum cross (5) have been proposed as causes of postfertilization hybrid seed failure. Endosperm failure is a consistent characteristic, and in many unsuccessful interspecific crosses, the endothelium or innermost layer of the integument proliferates to form a tumoral tissue (11, 16). The primary cause of endosperm and consequently embryo deterioration is unknown, but the reason
may be a metabolic incapability or growth regulator imbalance.

Current understanding of the specific role of endosperm and the detailed path of nutrient movement within the ovule to the embryo at the earliest stages of embryonic development is limited. Ultrastructural evidence is accumulating to support the view that the suspensor is involved actively in absorption and transport of nutrients from the integument and endosperm (13, 19, 24, 25). The suspensor also may be the source of specific hormones or metabolites which are required for very early development of the embryo (6, 8, 14, 18). Histochemical studies of Haq et al. (9) indicated that embryo failure in interspecific hybrids of *Phaseolus* may be due to abnormalities in suspensor function.

Abnormally large E × P hybrid seeds are formed occasionally under optimal growing conditions. It is only from these abnormally large seeds that pseudo-heart shaped embryonic masses have been excised and cultured to mature plants (1, 2, 20). Embryos from E × P seeds which are routinely small have not been cultured successfully. However, routinely small E × P seeds have been cultured to yield embryo callus from which diploid E × P hybrid plants have been regenerated (21). Smith (20) and Choudhury (4) have reported that most E × P embryos start to collapse 30 to 40 days after pollination. Although the embryo and endosperm deteriorate, E × P fruit are comparable in size to E × E fruit (10, 20).

The objective of this study was to determine the anatomical sequence of events leading to endosperm and embryo deterioration in the E × P hybrid and to determine if the earliest indication of anatomical abnormality is correlated with reduced ovule weight and smaller embryo-sac cavity size. The specific timing of endosperm and embryo deterioration in an E × P cross may depend on the cultivar of *L. esculentum* and the accession or cultivar of *L. peruvianum*.

### Materials and Methods

Plants of *L. esculentum* cv. Vendor and *L. peruvianum*, plant introduction (PI) 126944, were grown with a day/night temperature regime of 27°C/18°C in a greenhouse. *L. esculentum* plants were grown in Cornell Mix A in 11-liter nursery pots. Plants were staked and pruned to one stem. Fruit was set on the first 3 inflorescences and 3 flowers per cluster were hand-pollinated. All other flowers were removed as they appeared. Flowers of *L. esculentum* were emasculated and hand-pollinated with either *L. esculentum* or *L. peruvianum* pollen at a stage approximately 1 to 2 days before anthesis. The anther cone is light yellow and the sepals and petals have reflexed about 45° at this preanthesis stage. Ovule ages are expressed as days from pollination.

**Growth analysis.** E × P ovules were weighed in groups of 10 and E × E ovules were weighed in groups of 5 to determine mean weight per ovule. Ovule and embryo-sac cavity lengths of sectioned material were measured with a calibrated microscope eyepiece micrometer. The shape of the true embryo sac would have been susceptible to distortion during anatomical preparation because the endothelial layer in some of the treatments was bounded both internally and peripherally with zones of deteriorated tissue. For this reason, the length and width of the embryo-sac cavity (Table 2) were measured from the first intact layer in the integument across the endothelial layer and true embryo sac to the first intact layer of the integument on the other side of the embryo sac. Measurements were taken on median longitudinal sections passing through the micropylar and chalazal ends of the embryo sac.

Data with equal variances were analyzed by Student’s *t* test; Cochran’s *t* modification of Student’s *t* test was used if the variances were not equal.

### Results

**Growth analysis.** The mean weight of E × P ovules was significantly different than E × E ovules at 13, 17, and 22 days after pollination (Table 1). The ovule and embryo-sac cavity dimensions of E × E and E × P ovules (Table 2) were not significantly different at 3 days or 6–7 days after pollination. The width and length of the E × P embryo sac cavity was half the width and length of the E × E sac cavity at 14 days after pollination. This difference was statistically different at the 1% level.

**Anatomy.** Individually excised ovules were fixed in 5% gluteraldehyde in 0.1 M sodium cacodylate buffer pH 6.8 for 3 hr, postfixed in 2% aqueous osmium tetroxide for 4 hr at 4°C, dehydrated in a water-acetone series through dry acetone, and gradually infiltrated and embedded in Epon-Araldite mixture. Sections (2µm) were cut with a glass knife on a OA rotary microtome and serially transferred from the water surface of the boat to glass slides with a platinum loop. Sections on glass slides were dried, stained with 0.05% toluidine blue 0 for 1 min at 80°C, rinsed, dried, and mounted under coverslips with a permanent mounting medium.

**Table 1.** *L. esculentum × L. esculentum* (E × E) and *L. esculentum × L. peruvianum* (E × P) ovule weight at 13, 17, and 22 days after pollination.

<table>
<thead>
<tr>
<th>Days after pollination</th>
<th>E × E</th>
<th>E × P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. observations</td>
<td>Ovule wt (mg)</td>
</tr>
<tr>
<td>13</td>
<td>15</td>
<td>1.17**</td>
</tr>
<tr>
<td>17</td>
<td>200</td>
<td>4.79**</td>
</tr>
<tr>
<td>22</td>
<td>75</td>
<td>7.16**</td>
</tr>
</tbody>
</table>

**E × E ovule weight was significantly different from E × P by Cochran’s *t* test at 1% significance level.**

Table 2. Ovule and embryo-sac cavity size of *L. esculentum* x *L. esculentum* (E x E) and *L. esculentum* x *L. peruvianum* (E x P) ovules at anthesis and 3, 6–7, and 14 days after pollination.

<table>
<thead>
<tr>
<th>Time after pollination (days)</th>
<th>Measurement</th>
<th>E x E</th>
<th>E x P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. observations</td>
<td>Size (mm)</td>
<td>No. observations</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthesis</td>
<td>Width</td>
<td>12</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Length</td>
<td>12</td>
<td>0.19</td>
</tr>
<tr>
<td>3</td>
<td>Width</td>
<td>12</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>Length</td>
<td>12</td>
<td>0.26</td>
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<tr>
<td>6–7</td>
<td>Width</td>
<td>2</td>
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</tr>
<tr>
<td></td>
<td>Length</td>
<td>2</td>
<td>0.39</td>
</tr>
<tr>
<td>14</td>
<td>Width</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Length</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Embryo-sac cavity size*<sup>a</sup><sup>*b</sup><sup>c</sup><sup>d</sup>

| Anthesis                     | Width       | 12       | 0.03         | Width    | 5         | 0.04 |
|                               | Length      | 12       | 0.07         | Length   | 5         | 0.06 |
| 3                             | Width       | 12       | 0.04         | Length   | 5         | 0.06 |
|                               | Length      | 12       | 0.08         | Length   | 5         | 0.06 |
| 6–7                           | Width       | 2        | 0.10         | Length   | 5         | 0.10 |
|                               | Length      | 2        | 0.15         | Length   | 5         | 0.10 |
| 14                            | Width       | 7        | 0.44**       | Length   | 5         | 0.22 |
|                               | Length      | 7        | 0.82**       | Length   | 5         | 0.42 |

<sup>a</sup>Measured on median longitudinal sections passing through the micropylar and chalazal ends of the embryo sac.

<sup>b</sup>*L. esculentum* ovules at anthesis stage.

<sup>c</sup>Because of the limitation on the size of the block face that can be successfully sectioned, this information was sacrificed in trimming the block.

<sup>d</sup>Measured from the first intact layer in the integument across the endothelial layer and true embryo sac to the first intact layer of the integument on the opposite side of the embryo sac.

**E x E embryo-sac cavity width and length were significantly different from E x P by Student’s *t* test at 1% significance level.

The *E x E* embryo at 12 days was at a full globular stage of development and endosperm development was extensive (Fig. 8). The 12-day *E x P* embryo had continued to divide but was not following normal stages of embryo development (Fig. 9).

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Fig. 1. Longitudinal section of 3-day *E* ovule at fertilization showing central cell (C), zygote (Z), and entrance of *E* pollen tube (arrow). Triple fusion has occurred. The integument has not started to breakdown (559 ×).

Fig. 2. Longitudinal section of 3-day *E* ovule at fertilization showing entrance of *P* pollen tube. Two nucleoli are prominent in the nucleus of the central cell (C). The integument has not started to breakdown (437 ×).

Fig. 3. Longitudinal section of 7-day *E x E* ovule with single-celled zygote (Z) and well-established endosperm (EN); endothelium (ED) (281 ×).

Fig. 4. Longitudinal section of 7-day *E x P* ovule with single-celled zygote (Z) and well-established endosperm (EN). This section passes through the chalazal end (CE) of the embryo sac where the endothelium (ED) is discontinuous (281 ×).
Fig. 5. Longitudinal section of 10-day E x E ovule. A very early globular stage embryo (E) is surrounded by well-developed endosperm (EN) (116 ×).

Fig. 6. Longitudinal section of 10-day E x P ovule showing the club-shaped embryo (E) and space within the embryo sac. Note remains of the endosperm (arrows) (225 ×).

Fig. 7. Longitudinal section of 10-day E x P ovule. Several viable endosperm cells (arrows) can be seen. There is evidence of endothelial (ED) proliferation; embryo (E) (360 ×).

Fig. 8. Longitudinal section of 12-day E x E ovule. The embryo (E) is in the full globular stage of development; endosperm (ED) (53 ×).

Fig. 9. Longitudinal section of 12-day E x P ovule; abnormal embryo (E), proliferated endothelium (ED) (169 ×).

Fig. 10. 17-day E x P ovule. Embryo (E) is an undifferentiated mass of cells filling the embryo sac and surrounded by proliferated endothelium (ED) (232 ×).
Cell division appeared to be random. No residual evidence of the endospERM’s existence remained. The innermost layer of the integument, the endothelium, had started to proliferate.

The 17-day E X E embryo was at an intermediate stage of development. The 17-day E X P embryo had formed a mass of undifferentiated cells (Fig. 10) surrounded by multiple layers of endothelium.

The 24-day E X E embryo was at an inverted-U shape stage of development, whereas the E X P embryo and proliferated endothelium had disintegrated by 24 days.

Discussion

Growth analysis. The weight of 13-day E X P ovules was less than 13-day E X E ovules, indicating that abnormalities occurred in the interspecific cross earlier than the 30- to 40-day age previously reported as the beginning of endosperm and embryo breakdown (5, 20). Data on embryo-sac cavity size also supported this conclusion. The width and length dimensions of the 14-day E X P embryo-sac cavity were half the dimensions of the E X E embryo-sac cavity, but there was no significant difference between the E X E and E X P 7-day ovule in terms of ovule or embryo-sac cavity size. It is apparent, based on these observations, that the growth of E X P ovules must have been suppressed earlier than 14 days in order to explain such a large difference between the E X E and E X P ovule and embryo-sac cavity size at 14 days.

Anatomy. Endosperm failure was the first indication of anatomical abnormality in the E X P ovule. The endosperm had deteriorated completely at 10 days after pollination. Remains of the endosperm were evident in most 10-day E X P ovules and occasionally a few viable endosperm cells could be seen among degenerating endosperm cells. Endosperm deterioration, which left a space within the embryo sac, preceded extensive endothelial proliferation. Therefore, it is unlikely that endothelial proliferation is a causal factor in endosperm deterioration in this interspecific cross.

The E X P embryo continued to develop without any endosperm, and it was evident by 12 days that the pattern of embryonic cell division was random. The fact that E X P embryos had been cultured successfully is usually cited as evidence that the embryo is not inherently defective but is victimized by a failing nutritional support system or is subjected to an unfavorable developmental environment. However, it is only embryos excised from rare, abnormally large seeds that have been cultured successfully (1, 4, 20). Our study indicated that most interspecific E X P embryos deteriorated without differentiating cotyledons. The failure of embryos to make the transition from heterotrophy to autotrophy makes their in vitro culture extremely difficult (15). It is of interest to observe that the E X P embryo did not simply stop growth at a specific developmental stage, but continued to develop as an undifferentiated mass. It is unknown whether this indicates a hormonal or a nutritional deficiency. Whether embryo failure is a consequence of endosperm deterioration or other unknown factors has not been determined.

Choudhury (5) studied E X E and E X P hybrid embryos 30-40 days after pollination. He reported that the reputed 3n E X P endospermic nuclei and the 2n embryonic nuclei were equal in diameter, rather than the 3n nuclei being proportionately larger in diameter. He concluded, consequently, that triple fusion failed, and that the endospermic cells were 2n. In general, we found embryonic and endospermic nuclei to be nonspherical and we could not confirm Choudhury’s results. The E X P endosperm had deteriorated completely by 10 days in our study.

Conclusions

Embryo-sac cavity size at 14 days (E X E 0.44 mm width; 0.82 mm length; E X P 0.22 mm width, 0.42 mm length) and ovule weight at 13 days (E X E  = 1.17 mg; E X P  = 0.49 mg) indicated that endosperm and embryo breakdown began in this interspecific cross earlier than the 30- to 40-day age reported by other researchers (5, 20). Fertilization occurred in E ovules 3 days after pollination with either E or P pollen. The E X P ovule showed no anatomical signs of abnormality at 7 days after pollination, the single-celled zygone and multi-cellular endosperm stage. The dimensions of the E X P embryo-sac cavity were also not significantly different from E X E at 7 days. Endosperm failure was the first indication of anatomical abnormality in the E X P ovule. The endosperm had disintegrated completely by 10 days after pollination. Endosperm deterioration (10 days) preceded extensive endothelial proliferation (12 days), so it is unlikely that endothelial proliferation is a causal factor in endosperm deterioration in this interspecific cross. The E X P embryo did not follow normal stages of embryo development, but divided randomly forming an undifferentiated mass of cells by 17 days. Deterioration of both the E X P embryo and cells of the proliferated endothelium was complete by 24 days.

Literature Cited

Ethylene, Cellulase, 2,4-D, and Summer Fruit Drop of Navel Orange in Florida

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Additional index words. Citrus sinensis, abscission, growth regulators

Abstract. Secondary-fruit yellowing (SFY) of navel orange [Citrus sinensis (L.) Osbeck], a major cause of summer fruit drop, was induced artificially by girdling the stem 5 to 10 cm from the fruit. Girdling from 26 to 28 May 1982 increased ethylene levels at the stylar end and induced SFY in 90% of treated fruit. Girdling at other times, or at 30 to 45 cm from the fruit, however, caused minor SFY, particularly if leaves were present between the fruit and girdled area, indicating some material is produced by the leaves that reduces the incidence of SFY. Increase of ethylene levels and cellulase activity in the abscission zone of the secondary fruit prior to increases in the abscission zone of the primary fruit indicate that secondary-fruit abscission leads to primary fruit abscission. Water stress or changes in nonstructural carbohydrate levels within the leaves induced by branch sawing did not cause SFY. Application of 2,4-dichlorophenoxyacetic acid (2,4-D) prior to or during induction lessened the severity of SFY and significantly decreased ethylene levels at the stylar end of the fruit.

Yields of navel oranges in Florida typically are lower than those of other sweet orange cultivars because of poor fruit set (2, 10, 22) and extensive fruit drop following June drop (15, 16). Summer drop, which occurs between early June and early August, significantly reduced yields in 3 of 5 seasons from 1978 to 1982, with as much as 15% of the crop being lost in one location during 1981 (14).

Most fruit that abscise during summer drop show symptoms of secondary-fruit yellowing (SFY) (14, 15, 16). A separation zone that develops at the base of the secondary fruit causes the navel to become yellow, atrophy, and decay. The fact that fungi (20) and insects (15) are not causal factors suggests a physiological basis for the problem; however, little is known about the time of SFY-induction or the physiological nature of the secondary-fruit separation zone. Water stress (2, 4, 22) and carbohydrate levels (7, 13) influence abscission of young fruit. Neither factor, however, has been studied in relation to SFY.

Fruit abscission typically is promoted by ethylene, and delayed or prevented by auxins (3, 5, 6, 21). Secondary-fruit yellowing can be induced by ethylene treatment (20) and the severity reduced by 2,4-D sprays (15), but effects of 2,4-D on the induction of SFY and the secondary abscission zone have not been studied.

Our objectives were to study factors associated with the incidence of SFY and possible solutions to the problem. Experiments included: 1) inducing SFY artificially at various times by fruit-stem girdling to determine time of natural induction; 2) determining the role of leaves in SFY by removing them at various distances from the fruit; 3) measuring ethylene and cellulase activity in primary and secondary abscission zones to ascertain if secondary-fruit abscission precedes, or is associated with primary-fruit abscission; 4) determining the role of leaf water and carbohydrate status in SFY; and 5) testing the influence of 2,4-D on the induction and severity of SFY.

Materials and Methods

Plant material. Navel orange trees on sour orange (Citrus aurantium L.) rootstock, planted in 1957, were used for experiments in 1980, and a 2nd group of trees, planted in 1969, were used in 1981 and 1982. Trees were located near Eustis, in the north central citrus region of Florida.

Induction of SFY. Ten to 30 fruit stems per tree were girdled by removing a 1-cm ring of bark 5-10 cm from the fruit on 6, 21, 26, and 28 May, 3 June, and 6 Aug. in 1982. Percentage of SFY was evaluated 25–40 days after treatment. A 2nd group of treatments consisted of girdling 5–10, 15–20, or 35–40 cm from the fruit with all leaves being retained between the fruit and girdled area, girdling at 5–10 or 30–45 cm with removal of leaves, and no girdling. Two to 6 fruit were treated in each of 4 to 5 replications during late May to early June, 1980–1982. Percentage SFY was determined 20–35 days after treatment.

Ethylene, cellulase, and fruit removal force. Ethylene and cellulase levels in the abscission zone at the base of the primary