Propagation of Pawpaw (*Asimina triloba*)©

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INTRODUCTION

The North American pawpaw is a temperate member of the mostly tropical Annonaceae or custard apple family. Pawpaw has commercial value both as a small landscape tree and as an orchard fruit crop (Layne, 1996). It is also the source of several novel botanical and medicinal extracts (Zhao et al., 1994). Nurseries commonly propagate pawpaw from seed or chip budding. Seed propagation of pawpaw is important to the nursery industry as a source of seedlings for both ornamental and understock production. Currently, chip budding is used to propagate superior fruiting cultivars. One problem with budding is the propensity for pawpaw understocks to sucker and potentially compete with the desired cultivar. Cutting or tissue culture propagation would be a desirable way to establish pawpaw cultivars on their own roots. The objective of our research program in pawpaw is to develop propagation methods for seedling and clonal establishment of plants for commercial production.

MATERIALS AND METHODS

Seed Propagation. Fruits were collected from six sites in Kentucky during the fall of 1996. Seeds were cleaned and combined into one seed lot. Stratification of seeds occurred in rolls of moistened germination paper at 5°C in darkness. At 7-day intervals for 14 weeks, one set of 50 seeds was moved from stratification to germination conditions and germination percentage determined after 14 days.

To determine pawpaw seeds' ability to withstand drying, 50 fresh seeds were slowly dried at approximately 25°C. At 3-day intervals, seeds were weighed to calculate the approximate change in seed moisture content. Seeds at various moisture contents were stratified for 120 days at 5°C as previously described.

Storage life was determined in seeds extracted from fruits after macerating the pulp and floating off the fruit flesh. Seeds were washed, surface sterilized using a bleach solution, rinsed, and stored in hydrated Terrasorb™ (hydrophyllic polymer) at 5°C. Seeds were not permitted to dry out prior to storage. At various times during storage, stratified seeds were surface-sterilized with 10% bleach solution and rinsed three times with sterile deionized water prior to germination testing.

In all germination experiments, seeds were placed in three sheets of germination paper (30 cm × 38 cm, Anchor Paper Co., St. Paul, Minnesota) moistened with approximately 250 ml of deionized water. Ten seeds were placed between the second and third sheet of rolled paper. Rolls of seeds were placed in 0.5-mil polyethylene bags. Germination was conducted in 25°C growth chamber in darkness. Fifty to 100 seeds were evaluated per treatment.

Cutting Propagation. Softwood cuttings were taken from either mature or seedling stock plants. Cuttings were stuck in a peat-lite medium and placed under intermittent

mist (5 sec every 10 min) with bottom heat 75°F (25°C). Cuttings were treated with a quick dip using IBA at 0, 1000, 5000, or 10,000 ppm dissolved in 50% ethanol.

Tissue Culture Propagation. Establishment of tissue cultures was attempted from seedling, mature, or rejuvenated explants. Mature explants were taken from new growth on established, fruiting trees. Rejuvenated explants were from root suckers from mature plants. Tissue culture conditions were MS medium with 5 to 10 µM BA and 1 to 3 µM NAA. Photoperiod was 16 h at 20 $\mu mol \cdot sec^{-1} \cdot m^{-2}$ of light provided by cool white fluorescent bulbs. Culture room temperature was 25°C.

RESULTS AND DISCUSSION

Seed Propagation. Pawpaw seeds have a small rudimentary embryo embedded in a large ruminant endosperm (Finneseth, 1997). A small proportion (12%) of the seed population used in this study germinated after removal from the fruit. The remaining seeds required 8 weeks of chilling stratification to satisfy dormancy (Fig. 1). In addition, pawpaw seeds displayed a moderate form of recalcitrance. Seeds lost 50% viability when seeds were dried from their initial 37% to 25% moisture. Total loss in viability was between 15% and 5% moisture. There was no significant effect of light on germination. For germination, pawpaw seeds should be stratified for 100 days at 5°C. Seeds stored cold (above freezing) and moist retain good viability for 2 years (Fig. 2).

Anatomical studies of pawpaw seed revealed a small, linear embryo that does not change in length during cold or warm stratification (Finneseth et al., 1998). Cotyledons grew through a specialized channel of cells extending above the cotyledon tips, but never emerged from the seed. The time required for the development of the cotyledons delayed seedling emergence more than 50 days. The cotyledons appear to be haustorial and translocate storage material from the endosperm to the growing embryo. Seedling development could be divided into four distinct stages, including radicle protrusion, hypocotyl emergence, epicotyl elongation, and seedcoat abscission.

Cutting Propagation. Over 1000 softwood cuttings were taken from mature flowering trees throughout the spring and summer. All failed to form adventitious roots. However, seedlings up to 2 months old showed a capacity to root. Cuttings treated with IBA (10,000 ppm) rooted at 75% and averaged 2 roots per cutting (Fig. 3). Seedlings beyond 2 months old lost the capacity to form roots. These data suggest that strategies to revert stock plants to a more juvenile state (like tissue culture or mound layering) will be required before a reliable method for cutting propagation can be obtained.

Micropropagation. The effect of juvenility on explant performance was seen with the inability of explants from 26 mature sources to respond in culture. Of the 551 mature explants, 72% were successfully disinfested, but only 4% survived in the culture environment (Finneseth et al., 2000). Most of the mature explants turned black and lost tissue integrity. The explants that were alive did not respond in culture to produce axillary shoots or adventitious buds. Only the small percentage of explants from mature sources that survived showed some tissue growth after approximately 7 months in culture.

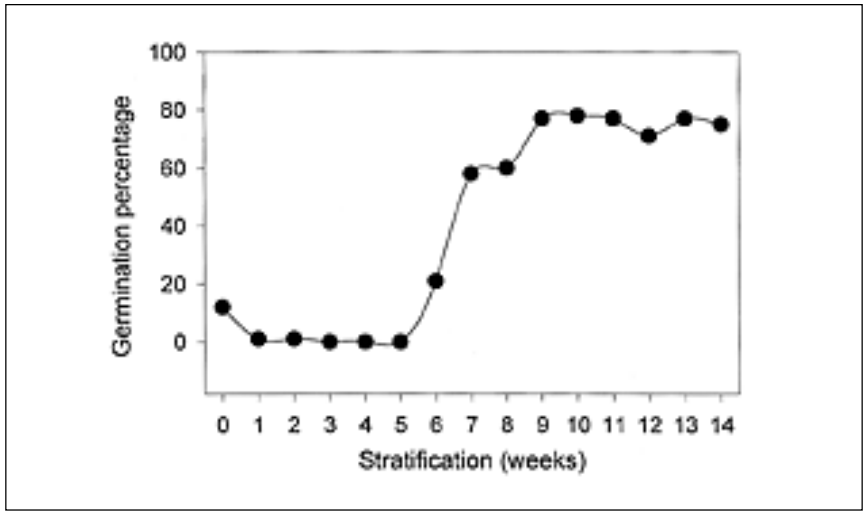

Figure 1. Germination percentage of pawpaw seeds after stratification at 5°C.

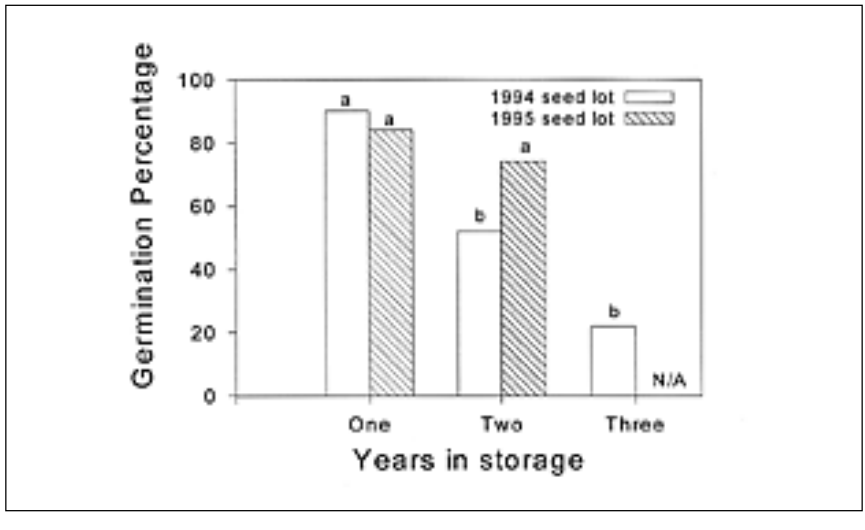

Figure 2. Germination percentage in two seed lots of pawpaw after storage.

In contrast, 88% of seedling explants showed expanded shoots (3 cm) and were suitable for subculture after 6 weeks (Fig. 4). For explants from root suckers, axillary shoot elongation began in 42% of the explants after 8 weeks. Although explants from root suckers did not respond as rapidly or at the high percentages of the seedling explants, these explants did respond in culture and would produce clones of the donor plant.

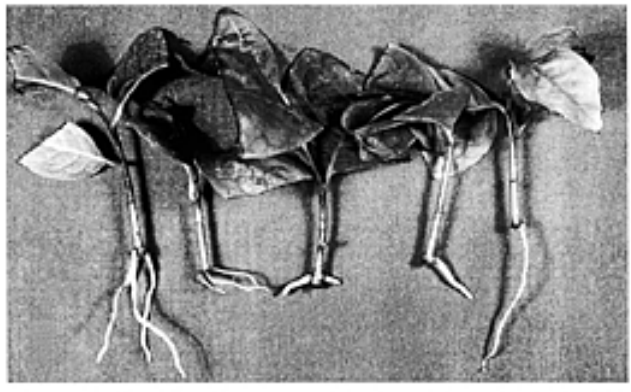

Figure 3. Root formation in seedling cuttings treated with 10,000 ppm IBA.

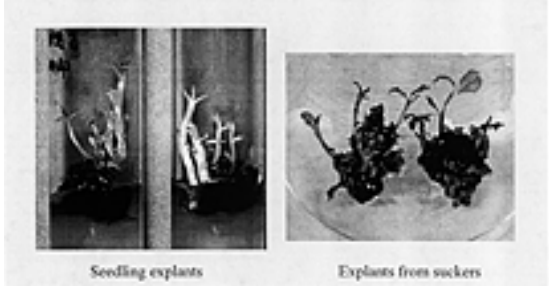

Figure 4. Shoot formation from seedling and rejuvenated explants pawpaw.

LITERATURE CITED

Finneseth, C.L.H., D.R. Layne, and R.L. Geneve. 2000. Establishment of North American pawpaw [*Asimina triloba* (L.) Dunal] shoots in vitro from mature and juvenile explants. Acta Hort. 520:97-102.

Finneseth, C.H., D.R. Layne, and R.L. Geneve. 1998. Requirements for seed germination in North American pawpaw [*Asimina triloba* (L.) Dunal]. Seed Sci. Tech. 26:471-480.

Finneseth, C.H., D.R. Layne, and **R.L. Geneve.** 1998. Morphological development of the North American pawpaw [*Asimina triloba* (L.) Dunal]during germination and seedling emergence. HortScience 33:802-805.

Layne, D.R. (1996). The pawpaw [*Asimina triloba* (L.) Dunal]: A new fruit crop for Kentucky andthe United States. HortScience 31:777-784.

Zhao, G., Miesbauer, L.R., Smith, D.L., and **McLaughlin, J.L.** (1994). Asimin, asiminacin and asiminecin: novel highly cytotoxic asimicin isomers from *Asimina triloba*. Journal of Medicinal Chemistry 37: 1971-1976.