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SEXUAL AND ASEXUAL REPRODUCTIVE CHARACTERISTICS OF THE NORTH AMERICAN PAWPAW [*ASIMINA TRILOBA* (L.) DUNAL]

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ABSTRACT OF THESIS

SEXUAL AND ASEXUAL REPRODUCTIVE CHARACTERISTICS OF THE NORTH AMERICAN PAWPAW [*ASIMINA TRILOBA* (L.) DUNAL]

The North American Pawpaw [*Asimina triloba* (L.) Dunal] shows great potential as a new fruit crop. Kentucky State University in Frankfort, Ky. is the site for the USDA National Clonal Germplasm Repository (NCGR) for *Asimina* species. Both the fruit and the trees themselves are of high value to growers and nursery producers. Pawpaw cultivars are currently propagated by grafting or budding onto seedling rootstock; no method currently exists to clonally propagate pawpaw on its own roots. Three methods of layering were attempted in this study to clonally propagate pawpaw: trench layering, pot layering, and mound layering. Both trench layering and pot layering experiments showed the importance of both juvenility and auxin application in adventitious rooting of pawpaw. Although rooting of more mature pawpaw shoots in these experiments did not exceed 30%, these propagation methods were more successful then previous attempts at rooting more mature pawpaw stems. Mound layering was less successful, but an easier-to-root genotype of pawpaw in the KSU-USDA NCGR for *Asimina* spp. was discovered that may show promise for future propagation studies.

Diversity in reproductive characteristics of pawpaw was also assessed in this study. Accessions in the KSU-USDA repository orchard collected from six different geographic regions were selected and trunk cross sectional area, total number of flowers, length of flowering, flowering peak, fruit set, total number of clusters, total number of fruit, number of fruit per cluster, average fruit weight, yield by weight, yield efficiency, length of harvest, harvest peak, and growing degree days required for ripening were evaluated. Significant differences were found among the regions in most of the characteristics evaluated. Correlations were also found between several vegetative and reproductive characteristics. This indicates that a significant level of reproductive diversity exists within KSU's repository collection, and between pawpaws collected from different regions of the native range.

KEYWORDS: *Asimina triloba*, pawpaw, propagation, layering, fruit.

Sheri Beth Crabtree

December 10, 2004

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THESIS

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The Graduate School

University of Kentucky

2004

SEXUAL AND ASEXUAL REPRODUCTIVE CHARACTERISTICS OF THE NORTH AMERICAN PAWPAW [*ASIMINA TRILOBA* (L.) DUNAL]

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant and Soil Science in the College of Agriculture at the University of Kentucky

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2004

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Chapter 1 – Thesis introduction

Pawpaw taxonomy and biology

The pawpaw [*Asimina triloba* (L.) Dunal] is classified as a member of the Annonaceae, or Custard Apple family. Annonaceae is the largest primitive family of flowering plants, and includes approximately 130 genera and 2300 species (Cronquist, 1981). Other economically important members of Annonaceae include custard apple [*Annona reticulata* (L)], cherimoya [*A. cherimola* (Mill.)], and soursoup [*A. muricata*(L.)]. Most *Annona* species are native to Central and South America and the Caribbean, with two *Annona* species [*Annona glabra* (L.) and *A. squamosa* (L.)] and two *Deeringothamnus* species [*Deeringothamnus rugelii* (B.L. Robins.) and *D. pulchellus* (Small)] found in southern Florida (Kral, 1960).

Asimina is the only temperate genus in this otherwise tropical plant family. There are 9 species included in the genus *Asimina*, seven of which are found only in Florida and southern Georgia (Kral, 1960; Callaway 1990). *Asimina parviflora* (Michx.) Dunal, reaches farther north, into the coastal piedmont areas of the southeastern U.S., including the Carolinas, Virginia, and southern Tennessee. *Asimina triloba*, or the North American pawpaw, is indigenous to 26 states in the Eastern United States, ranging from New York, southern Ontario, and southern Michigan on the northern boundaries, south to northern Florida, and west to eastern Texas, Nebraska, and Kansas (Callaway, 1990). The North American pawpaw is native to USDA zones 5 (-25°C) through 8 (-10°C) (Peterson, 1991). Pawpaws normally are diploid (2n=18) with triploid mutants (2n=27) found in several sampled populations (Bowden, 1949).

What is now known as *Asimina triloba* has undergone many name changes throughout the years. Linnaeus originally classified this plant as *Annona triloba*. Later, Micheaux renamed the species *Orchidocarpum arietinum*. Only 4 years later, Persoon again transferred the species to *Porcelia triloba*. Finally, in 1817, Dunal reclassified the plant as *Asimina triloba*. Torrey and Gray then moved the species to the genus *Uvaria*, but it was returned to its current and proper name, *A. triloba*, by Gray in 1886 (Kral, 1960).

The pawpaw is generally found as a small deciduous tree in the understory of mesic forests, reaching 5 to 10 meters in height (Kral, 1960). As an understory tree, the pawpaw can be somewhat shrubby or lanky, with a rather thin trunk and branches. However, in full sun, the pawpaw is a dense, pyramidal tree. The leaves are dark green with a golden yellow fall color, large, glabrous, entire, and obovate-oblong (Dirr, 1990). The bark is smooth and grey in color. Buds and leaves are alternately arranged. The pawpaw grows best in soils which are moist but well-drained, high in organic matter, and of slightly acidic pH (Lagrange and Tramer, 1985; Bonney, 2002). The pawpaw naturally reproduces predominantly through root suckering and forms groves or patches, which can be quite large (Willson and Schmeske, 1980).

Pawpaw flower buds are dark brown, pubescent, and formed the previous fall on one-year-old wood (Kral, 1960). The flowers are light green upon emergence, but gradually turn maroon in color, with a slightly fetid odor. Flowers are 2-4 cm in diameter, with 3 outer petals, 3 smaller inner petals, and 3 sepals. Flowers open before the leaves have emerged and expanded, approximately mid-April in central Kentucky. The carpels contain one to several ovules, which give rise to single fruit or multi-fruited clusters (Bailey, 1960). There are usually 3 to 7 carpels per flower, and a round mass of many stamens (Lampton, 1957); however, we have counted up to 12 fruit per cluster on a tree in the KSU orchards (per obs). Pawpaw flowers are protogynous, with the stigmatic surface receptive before pollen is shed from the anthers. This is a mechanism which can force outcrossing.

In the wild, pawpaw has been found to have very low fruit set (Peterson, 1991), with a variety of factors likely responsible. Native stands of pawpaw may be genetically identical as they spread by root suckers. Since pawpaws are believed to require cross-pollination, if genetically different patches or genotypes are not close enough for cross-pollination, fruit set in isolated patches could be low. Willson and Schemske (1980) examined flowering, pollination, and fruit production in a native Illinois pawpaw patch and found a large number of flowers (up to 500 per stem), but low fruit set, ranging from 0.01% to 1.38%. These authors suggested that the low fruit set may have been due to low light levels as an understory tree and thus a low rate of photosynthesis. They also suggested that pawpaws may be self-incompatible, thus leading to low fruit set in a largely clonal patch. Pawpaws are primarily pollinated by flies and beetles (Faegri and van der Piji, 1971). Lagrange and Tramer (1985) also reported low fruit set in wild pawpaw patches (3.4%) and noted that pollinators were rarely observed visiting flowers; therefore, limited availability of pollinators may also contribute to low fruit set. The same study also found that trunk diameter and tree height were not correlated with flower or fruit production.

The pawpaw fruit itself can range from round to oval to kidney-shaped. It is classified botanically as a berry (Dirr, 1990). The fruit can weigh up to 500 grams, with the average pawpaw weighing around 150-200 g, although weight varies greatly by variety (Pomper *et al*., 1999). Seed size and shape vary from flat to rounded, approximately 2 cm long on average. The fruit is very sweet, with a strong aroma when ripe and soft custard-like flesh. The flavor of the pawpaw fruit is unique, having been described as resembling banana, mango, pineapple, or melon. The fruit are also highly nutritious, containing double the vitamin C of apples, peaches, or grapes, amounts of vitamin A similar to apples and grapes, higher levels of many minerals, including potassium, phosphorus, magnesium, calcium, and iron, than apples, peaches, or grapes; and high levels of several essential amino acids (Peterson *et al*., 1982).

New pawpaw varieties have been selected from the Midwest, as well as the southern regions of the native range of pawpaw (Jones et al., 1998). Neither the number of chilling units required for pawpaw bud emergence nor the number of growing degree days required for fruit ripening have been reported in the literature. The fruit on a single tree or even within a single cluster may ripen many days apart. Fruit may be harvested from a single tree over a period of 3-4 weeks (Archbold and Pomper, 2003).

The Domestication of Pawpaw

The pawpaw has a rich history as an important food source to Native Americans, early European explorers, and early settlers of North America (Pickering, 1879). Native Americans likely aided in the distribution of pawpaw across North America, with evidence of the Iroquois tribe bringing pawpaws into central New York, where they were not found previously (Keener and Kuhns, 1997). The pawpaw was first formally noted in 1541, by the De Soto exploration group in the Mississippi River valley (Sargent, 1890). Lewis and Clark wrote in their journal that when rations were low, their exploring party subsisted mostly on pawpaws, and enjoyed the taste (Pomper and Layne, 2005). Early settlers depended partially on pawpaw to sustain them in times of crop failure (Peterson, 1991). Pawpaw is also an important food source for many native animals, including white tailed deer (*Odocoileus virginianus*), raccoon (*Procyon lotor*), and opossum (*Didelphis virginiana*).

Pawpaw is still in an early stage of domestication as a tree fruit crop. Interest in pawpaw increased in the early 1900s, when a contest was held by the American Genetics Association looking for “The Best Papaws” (Popenoe, 1916). In the early 1900s, around 56 pawpaw varieties were in cultivation (Peterson, 1991; Peterson, 2003). However, most of these were lost through neglect, abandonment, or lack of record-keeping, and only 20 of these early cultivars are in collections today (Peterson, 2003). Over 40 named cultivars are available today, with a recent resurgence of interest in pawpaw. None of these cultivars are available on their own roots, with cultivars currently propagated by grafting or budding onto seedling rootstock.

As early as 1916, it was observed that widespread shipping and marketing of pawpaw was hindered by the softness of the fruit and short shelf life (Popenoe, 1916). Since then, great progress has been made in understanding the postharvest physiology of pawpaw and developing handling and storage recommendations, but little progress has been made in controlling these factors. Archbold and Pomper (2003) determined that pawpaw is a climacteric fruit, therefore control of ethylene production or sensitivity could help regulate the ripening process. These authors also noted that cold storage had potential in delaying ripening, and studies continue to determine the quality of fruit held in cold storage.

Pawpaw has potential as a new fruit crop for farmers in the southeastern United States. Tobacco is the predominant crop grown by many farmers in the southeast U.S., and as tobacco profits continue to decline, farmers may be interested in supplemental or alternative crops (Gale, 1999). Pawpaw is native to all of the top 10 tobacco-producing states and could be a supplemental niche crop for growers currently dependent on tobacco. The pawpaw fruit has a high value, with fruit selling for around $3 a pound at local farmers markets (Pomper and Layne, 2000). Frozen pawpaw pulp has sold for $6 a pound (per comm. Chris Chmiel, Albany, Ohio), and may be more viable considering the perishability of the fresh fruit. In addition to uses in products such as ice creams, cookies, cakes, and beverages, the pureed pulp has also been found to be an acceptable fat substitute in baking (Wiese and Duffrin, 2003).

Pawpaw trees themselves also have a high value, showing potential for Kentucky nursery production. Pawpaw seedlings generally sell for around $5-$10 a tree, with grafted trees bringing $20-$25 a tree (Northwoods Nursery/Jim Gilbert, per comm.). As a native plant, pawpaw is well-adapted to regional growing conditions and has few pests, lending itself to potential organic production, which could further increase retail value to the consumer and also decrease chemical costs to the producer. For the homeowner, the pawpaw makes an ideal component in the edible landscape, with its attractive form, beautiful fall color, and delicious fruit.

Pawpaw is the exclusive host of the zebra swallowtail butterfly (*Eurytides marcellus* Cramer), which is not necessarily a pest as it does little damage to the trees, well below economic threshold except in some cases of very small seedlings, and is also a very attractive butterfly. Other insect pests include the pawpaw peduncle borer (*Talponia plummeriana* Busck), which bores into flower stalks, causing flower drop (Peterson, 1991); the leafroller (*Choristoneura parellela* Robinson), and the Japanese beetle (*Popillia japonica*), which may feed heavily on interveinal leaf tissue (Pomper and Layne, 2005). None of these pests affect the pawpaw severely enough to be considered serious problems at this time. Diseases which may affect pawpaw include a leaf spot caused by several fungi, *Phyllostica asiminae*, *Mycocentrospora asiminae*, and *Rhopaloconidium asiminae* (Peterson, 1991). This disease complex can also affect the skin of the fruit.

The leaf, bark, and twig tissues of pawpaw produce natural defense compounds known as annonaceous acetogenins (McLaughlin, 1997; McCage *et al*., 2002). More than 40 of these long-chain fatty acids have been extracted and identified, including asimin, asiminacin, and bullatacin. These compounds have been found to have pesticidal properties, which could explain the relatively low number of insect pests attacking pawpaw. Therefore, these compounds are being developed as a component in botanical pesticides and could be used in organic production systems. These same compounds also have been demonstrated to be toxic to cancerous tumor cells and thus have potential as anti-cancer agents (Ahammadsahib *et al*., 1993).

Pawpaw Genetic Diversity

The genetic diversity of pawpaw has also been investigated. M13 DNA fingerprinting probing (Rogstad *et al*., 1991), isozymes (Huang *et al*., 1997), allozymes (Huang *et al*., 1998), RAPD-PCR (Huang *et al*., 2000), and ISSR-PCR (Pomper *et al*., 2003) are all methods which have been utilized in determining genetic diversity in pawpaw. The last four of these studies agreed that pawpaw exhibits at least a moderate level of genetic diversity, similar to that found in other long-lived woody perennial species. The study of Pomper *et al*. (2003) utilizing ISSR-PCR found a higher level of genetic diversity than previously thought. This could be due to the ISSR-PCR methodology being more discriminating in finding genetic differences between samples. These authors suggested that high levels of genetic diversity exist in pawpaw populations, despite previous studies and the supposition that pawpaw's clonal reproductive habit would suggest otherwise (Rogstad *et al*., 1991).

In 1994, Kentucky State University (KSU), an 1890 land grant university located in Frankfort, Kentucky, was designated by the United States Department of Agriculture (USDA) as a National Clonal Germplasm Repository (NCGR) for *Asimina* species. The orchards at the KSU research farm contain over 1700 pawpaw accessions, collected from 17 different states across the native range. This collection includes over 40 named cultivars, many seedlings, hybrids of *A. triloba, A. parviflora,* and crosses between *A. triloba* and *A. reticulata* and *A. longifolia,* planted in the KSU orchards; and *A. longifolia* and *A. tetramera,* subtropical *Asimina* species, stored in the KSU greenhouses. As a USDA-NCGR site, one of our objectives is to maintain and build a diverse collection of *Asimina* species. A major goal toward this objective is to develop a research propagation method to propagate cultivars and other genotypes on their own roots. This would protect the germplasm collection in case of dieback or storm damage, and would ease the storage of valuable germplasm. To accomplish this goal, we are investigating and developing various methods of propagation.

Propagation of Pawpaw

Sexual propagation, or propagation by seed, is the predominant way many plants in the wild reproduce, and has been used and manipulated by humans throughout history (Hartmann et al., 1997). Advantages to seed propagation include high genetic diversity and lower costs, labor, and materials required. Some disadvantages to propagating plants by seed are that a desirable plant will not be propagated true to type, and many seeds require special treatments such as scarification, chilling, or specific storage requirements. Most vegetables, bedding plants, and agronomic crops are propagated by seed (Hartmann et al., 1997). Some ornamental woody plant species are propagated by seed, and seedling rootstocks may be used in *Prunus* spp., asian pear [*Pyrus pyrifolia* (Nakai)], citrus species (*Citrus* spp.), and several nut species (Webster, 1995). Clonal propagation of rootstocks is generally preferred, but seedling propagation does have some advantages. First, seedling rootstocks may be more economical and easy to mass produce. Clonal rootstocks may be infected with various pathogens, including viruses and root-borne pathogens such as *Agrobacterium* (crown gall) (Webster, 1995). In some cases, the root systems of seedling rootstocks may grow deeper and more vigorously than rootstocks from cuttings (Hartmann et al., 1997). However, there are many disadvantages to using seedling rootstocks in grafting, such as high variability in vigor and lack of desirable qualities that may be imparted by a rootstock such as dwarfing or drought resistance; and generally clonal rootstocks are preferred in tree fruit production.

The pawpaw industry depends heavily on seed propagation. Many pawpaw seedlings are sold by nurseries, and pawpaw cultivars are grafted or budded onto seedling rootstock. Pawpaw seeds are recalcitrant. Finneseth et al. (1998) found that germination percentages dropped below 50% when seed moisture was dropped to 25%. Pawpaw seeds also must be stratified prior to germination. These authors obtained the highest germination percentage, 84-90%, after 14 weeks of chilling at 5°C. Pawpaw seeds also cannot withstand freezing (Pomper et al., 2000). Hence, storage recommendations for pawpaw are to keep seed at 5°C in moist sphagnum peat moss to prevent desiccation and obtain the necessary stratification (Jones et al., 1998). Pawpaw seeds can be stored for approximately two years before a reduction in viability occurs. Given the recalcitrant nature of pawpaw seeds, it would be desirable to develop a clonal propagation method to reduce the dependence on seedling production.

Vegetative propagation has also been exploited by humans throughout history. For example, there are records of grafting being performed over 2000 years ago in ancient Greece (Webster, 1995); and plants that naturally reproduce vegetatively through runners, suckering, or tip layering; or plants whose stem cuttings root readily have also been clonally propagated by humans early in agricultural history (Hartmann et al., 1997). Various methods of vegetative, or asexual, propagation have many advantages over seed propagation. First, you can propagate superior genotypes true to type, and maintain desirable qualities such as ornamental value, fruit size and quality, pest resistance, or low vigor for dwarfing capabilities. Clonal propagation also increases uniformity in plantings by decreasing genetic variability, and can shorten the time to flowering and fruit production in fruit crops, if mature tissue is used in propagation (Hartmann et al., 1997). The most common methods of clonal propagation in horticulture are stem cuttings and grafting or budding onto a clonal rootstock, which would often be produced by mound layering or stooling. Other methods of vegetative propagation include tissue culture, root cuttings, and various methods of layering such as air layering, trench layering, or pot layering.

There is currently no feasible method for propagating pawpaw germplasm clonally on its own roots. This is true of many members of the *Annonaceae*. Most *Annona* species, including *Annona squamosa* and *A. cherimola*, have proven difficult to root and are propagated by grafting or budding onto seedling rootstock (George and Nissen, 1987). Mitra (1986) reported 25% rooting of *Annona squamosa* softwood cuttings treated with 5000 ppm IBA, and 10% of micropropagated *A. squamosa* shoots produced roots and survived. An earlier study (Venkataratanam and Satyanaranaswamy, 1956) found *A. squamosa* very difficult to root, with stem cuttings not exceeding 2% rooting. However, these cuttings were not treated with IBA, indicating the influence of IBA on rooting of *A. squamosa*. Odilo and Rafael (1976) found that only juvenile *Annona cherimola* cuttings produced roots, with 25% rooting at 5,000 ppm IBA. George and Nissen (1987) reported ease of rooting in *Annona muricata* stem cuttings, but noted that most other *Annona* spp. are difficult to root. Tazzari et al. (1990) concluded that *Annona* is a low rooting capacity species.

Many propagation methods have been attempted in pawpaw with limited success. Propagation by stem cuttings was attempted by Finneseth (1997), and it was found that juvenility plays a strong role in adventitious rooting capacity of pawpaw. Stem cuttings taken from 7 month old pawpaw seedlings were treated with IBA concentrations ranging from 0 to 80,000 ppm. Cuttings treated with 40,000 or 80,000 ppm IBA exhibited necrosis of tissue, but all other treatments rooted at a low level, not exceeding 16% (at 10,000 ppm IBA). However, cuttings from a mature source (5 year old trees) largely failed to root, with only one cutting out of 5100 producing one root (at 20,000 ppm IBA). A later unpublished study (Geneve, per comm.) reinforced the role of juvenility in rooting of pawpaw stem cuttings. Stem cuttings taken from 2, 4, 5, and 7 month old pawpaw seedlings were treated with IBA ranging from 0 to 10,000 ppm. Only 6% of cuttings taken from 7 month old seedlings treated with 5000 ppm IBA rooted; no other treatments initiated roots. Cuttings from the 2 month old seedlings produced adventitious roots more readily, ranging from 39% of cuttings not treated with IBA rooting, to 74% of stems treated with 10,000 ppm IBA producing roots. Attempts to root etiolated stem cuttings have also been unsuccessful (Geneve, personal comm).

Studies attempting pawpaw propagation by root cuttings showed that root diameter had a strong effect on shoot production (Finneseth, 1997). No shoots formed on root cuttings with a diameter less than 5 mm; however, 56% of root cuttings with a diameter of 5 mm or greater produced shoots. Unfortunately, currently available cultivars are not available on their own roots, so root cuttings are not a feasible propagation method for current varieties.

Juvenility also has a strong effect on pawpaw bud and shoot development in tissue culture (Finneseth et al., 1997). No explants from mature sources showed bud elongation after 8 weeks in vitro, and the 4% of these explants that survived showed only minimal tissue proliferation after 7 months. In the same study, explants from juvenile pawpaw seedlings responded quickly in culture. Approximately 60% of these explants had expanded axillary buds after only 4 weeks, and by 8 weeks, 100% of these explants had expanded axillary buds, had formed adventitious buds and shoots, and were large enough to subculture. Attempts to root pawpaw microcuttings from 2 year old pawpaw cultures had limited success (Geneve et al., 2003). Limited air layering experiments with pawpaw both in the greenhouse and the field were also unsuccessful (KSU, unpublished data).

Currently, grafting or budding onto a seedling rootstock is the only viable method of clonally propagating pawpaw. Budwood is obtained in March, once the trees' chilling requirements have been met. The cut ends of the stems are dipped in paraffin to prevent water loss, and the stems are stored at 4°C until use. Seeds are sown in greenhouses in February, and seedlings from vigorous sources can be ready to graft onto by July. A success rate of approximately 80-90% is obtained with chip budding dormant wood onto an actively growing seedling rootstock (KSU, unpublished data).

While grafting onto a seedling rootstock successfully preserves the character of the scion, it does have disadvantages. First, the seedling rootstock will impart some variability in vigor. Secondly, understock suckering and winter kill of the scions in harsh winters can be a problem in pawpaw, and the root suckers would not be of the desirable genotype. Therefore, it would be preferable to develop a method of clonally propagating pawpaw on its own roots. In case of winter kill of the scions, having the trees on their own roots would prevent complete loss of the desired genotype as the remaining root system could produce clonal shoots. Pawpaw produces many root suckers, which on a grafted tree would be of the seedling rootstock's genotype rather than the desirable scion. Obtaining cultivars on their own roots would eliminate this problem. Other advantages to clonally propagating pawpaw on its own roots would be a possible reduction in labor compared to grafting, and less time to reach plantable size. Potential graft incompatibility problems would also be eliminated. These goals would not only be advantageous to the commercial pawpaw industry, but to KSU as a USDA-NCGR site.

Layering

Layering is a form of clonal plant propagation in which stems form adventitious roots while still attached to the mother plant (Hartmann et al., 1997). Once rooting has occurred, the rooted stem is separated from the mother plant. Layering has been an important method of clonally propagating many plant selections throughout horticultural history, especially of difficult-to-root woody plant species. In recent years, layering has been replaced by other, more recently developed, plant propagation techniques such as micropropagation; however layering is still commonly used to propagate difficult-to-root species, especially tree fruit rootstocks (Hartmann et al., 1997; Armstrong, 2000). There are many modifications of layering, including simple layering, French layering, serpentine layering, air layering, mound layering (or stooling), trench layering, and pot layering.

A study by Solar et al. (1994) investigating stooling of hazelnut, a difficult-to-root nut species, found that mound layering of girdled shoots was successful on both one-year-old woody shoots and the current year's "green" shoots. One-year-old woody stems were girdled in February by tying a copper wire around the base of the shoot. The current season's stems were girdled in late May using the same method, and all were covered with soil. This study found that 40% of the current year's girdled shoots rooted well enough in the mound layering system to be planted that year, and another 35-45% of the shoots were capable of transplanting the following year. Stooling of one-year-old woody stems resulted in 57% rooting.

Another difficult-to-root nut species in which mound layering has been successful is pecan. Pecans, like pawpaw, are extremely difficult to root and are often propagated as a scion cultivar grafted onto a seedling rootstock (Wood, 1989). A study conducted in India (Sharma and Rosanglura, 1987) utilized one-year-old pecan shoots, cut back to 10 cm above ground level. In June, a 2-3 cm ring was cut into the bark of the shoots, and a lanolin paste containing either no IBA or 2500 ppm IBA was applied to the ringed area. This area was covered in sphagnum moss, and the entire plant was covered with soil. Upon unearthing the plants the following January, it was found that even with no IBA application, the pecan shoots reached 31% rooting, and at 2500 ppm IBA, 81% of shoots produced roots.

In a study by Rashid (1978) examining the effect of plant growth regulators in walnut stooling, it was found that some form of auxin applied to shoots was essential for rooting. Combinations of three concentrations of three auxins (0, 5,000 ppm, and 10,000 ppm; and IAA, IBA, and NAA in all possible combinations) were examined in this study, and the treatment combination most successful at promoting rooting in walnut layers was 5,000 ppm IAA + 10,000 ppm IBA + 5,000 ppm NAA; 0 IAA + 10,000 ppm IBA + 5,000 ppm NAA; and 10,000 ppm IAA + 10,000 ppm IBA + 5,000 ppm NAA, all with 100% rooting. No control shoots produced roots.

Rooting can be further enhanced in a layering system by girdling or wounding the stem to cause accumulation of carbohydrates and auxins above the girdled area, as the downward movement of these compounds are blocked by the girdle (Taiz and Zeiger, 2002). Wounding helps plant growth regulators applied to the shoot be absorbed more easily, and can also aid rooting by cutting through the tough sclerenchyma ring and/or lignified tissue which may be a physical barrier to adventitious root initiation (Hartmann et al., 1997).

A study of pecan stooling by Wood (1989) found that girdling was essential to rooting. Four-year-old pecan seedlings were severed 6 cm above the ground in February. In July, treatments were applied to shoots. The study was a 2 x 3 factorial, with girdled vs. non-girdled, and 0 IBA, 3000 ppm IBA, and 6000 ppm IBA being the treatment combinations. Shoots were girdled by cutting a 1 cm ring around the shoot with a knife. IBA was applied in a lanolin paste. Soil was mounded around the plants, and shoots were uncovered and evaluated in March. Girdled shoots with no IBA application reached 46% rooting, and 100% of girdled shoots treated with 3000 or 6000 ppm IBA in a lanolin paste produced roots. None of the ungirdled shoots rooted, regardless of IBA concentration. The author concluded that girdling triggered rooting in mound layered pecans, whereas IBA increased rooting percentages. Survival studies were also conducted, and it was found that ramets that had been girdled but had no IBA applied had the greatest survivability, at 83%, versus girdled shoots with 6000 ppm IBA at 45% survival and girdled shoots with 3000 ppm IBA at 33% survival after the first growing season.

A study by Dunn and Cole (1995) that examined mound layering of *Pistachia chinensis* Bunge. found that both wounding and auxin application were essential to rooting. Ten-month-old trees were cut back to 18 cm above the soil line in May. This study was a 2 x 2 factorial, with wounded or not wounded and 0 IBA or 17,500 ppm IBA being the two treatments. Wounding was performed by cutting into the shoot approximately 1mm deep. IBA was applied as a liquid solution rubbed into the wound. Sawdust was mounded around the shoots and they were uncovered in April. It was found that the treatment combination of wounding plus 17,500 ppm IBA was the most successful, while the other three treatment combinations were not significantly different from each other. In the two years the study was conducted, 77% and 75%, respectively, of wounded shoots treated with 17,500 ppm IBA produced roots. The authors concluded that wounding of shoots and auxin application were necessary for root production, but only when used in combination; shoots that were only girdled or only treated with IBA did not root.

Plant shoots used in layering are usually etiolated, due to the exclusion of light from the shoots as they grow through the medium covering them. Etiolation enhances rooting by causing a number of physiological and anatomical changes in the plant's stem, including increasing carbohydrate accumulation in the etiolated area, increasing the number of parenchyma cells above the girdle, reducing sclerenchyma cell formation, reducing lignin production, and increasing the stem's sensitivity to auxin (Hartmann et al., 1997). Etiolation in combination with wounding or girdling can be especially effective in promoting rooting in difficult-to-root species.

A study examining the rooting of apple (Bramley's seedling) cuttings found that only etiolated cuttings produced roots, and that girdling of the etiolated stem further increased adventitious root formation (Delargy and Wright, 1978). The effect of girdling in this study was secondary to the effect of etiolation, as all etiolated treatments produced roots, even un-girdled cuttings, whereas girdling without etiolation did not enhance rooting. A later study by Delargy and Wright (1979) found that auxin application to etiolated cuttings enhanced rooting. Auxin application to unetiolated cuttings resulted in very low rooting percentages, whereas auxin applied to etiolated cuttings resulted in 76% rooting. Studies of cutting propagation of *Carpinus betulus* found that rooting increased in etiolated cuttings taken from plants which had been subjected to light exclusion (Maynard and Bassuk, 1992). Cuttings taken from shaded stock plants were also more responsive to auxin application. Another study by Biricolti et al. (1994) investigating rooting of chestnut in a stooling system determined that the effects of girdling and etiolation were found to have an interaction, and thus the effects of each could not be separated, suggesting a synergistic effect on adventitious rooting in chestnut.

Trench layering is a little-utilized method of propagation in which mother plants are planted in a horizontal position and covered with soil, sawdust, or some other type of rooting medium. One advantage of trench layering is that with a larger number of buds remaining as the plant is laid horizontally, one would have a larger number of shoots to work with in a smaller area. A study by Suriyapananont (1990) comparing air and trench layering in apple rootstocks found that trench layering was a superior method for some rootstocks. Shoots in both layering systems were wounded and treated with 8000 ppm IBA. Trench layering was performed by bending branches of one-year-old wood and pegging them into a trench. Treatments were then applied. Air layered MM106 shoots rooted at 77.5%, while 100% of trench layered MM106 shoots produced roots. There were no significant differences in treatments in the other two rootstocks.

Pot layering and air layering are two propagation methods that have been attempted with *Annona squamosa* (Venkataratanam and Satyanaranaswamy, 1956). To conduct pot layering, one year old plants were cut to expose a tongue near the base of the plant, which was then covered with another pot filled with soil. Shoots were gradually separated after 4 to 5 months. Air layers were formed by girdling one-year-old shoots and covering with soil packed around it and tied around the shoot. The authors reported 2.57% success with pot layering and only 0.69% rooting with air layering and concluded that there was no method superior at that time to budding in the propagation of *A. squamosa*.

However, George and Nissen (1986) had greater success in the layering of *Annona cherimola*. One-year-old seedlings were cut back in summer, and when new shoots emerged, a growth constricter ring was placed around the base to girdle the shoots. The shoots were then covered with a 1:1 sand:sawdust mixture contained in a polyethylene sleeve. These authors reported 100% rooting of shoots within 4-5 months of treatment.

Air layering has been attempted in pawpaw with no success in obtaining roots (KSU, unpublished data). Other layering methods such as stooling, pot layering and trench layering to clonally propagate pawpaw have not been reported in the literature.

Juvenility

Another way layering works to overcome a plant's difficulty in rooting is by rejuvenating the stock plants (Couvillion, 1988; Howard et al., 1988). Plant material taken from mature sources often has reduced rooting capabilities, and this has been found to be true in pawpaw as well (Finneseth, 1997; Geneve, unpublished data). Lack of adventitious rooting due to maturity can be overcome by rejuvenating the plant. Shoots that emerge from nearest the base of the plant are ontogentically more juvenile, and usually have greater rooting capacity than plant material from ontogenetically more mature sources, such as branch tips. This concept is illustrated in the cone of juvenility (Hartmann et al., 1997). Since plant shoots utilized in mound layering come from severely pruned mother plants, and emerge from buds near the base of the plant, these shoots will have a more vigorous, juvenile character and may be easier to root. The same would apply to pot layering and trench layering, if plants are pruned back to the 2-3 buds nearest the base of the plant.

Many examples exist in the literature demonstrating the effect of maturity on adventitious rooting. A study comparing cuttings taken from one-year-old seedlings, cuttings from 3-year-old plants, and cuttings taken from previously rooted, immature cuttings of *Persoonia virgata* found 32.4% of cuttings from young seedlings rooted; 36.7% of cuttings taken from juvenile rooted cuttings produced roots, and only 3.2% of cuttings from mature sources rooted (Bauer et al., 1999). The researchers found both anatomical and chemical barriers to rooting in mature cuttings. Extensive fibers were found in the phloem of mature cuttings, whereas juvenile cuttings had limited phloem fiber bands. Using high performance liquid chromatography (HPLC), the researchers found several unknown compounds in the leaves that were present in higher levels in mature cuttings than in juvenile cuttings. These compounds could be potential rooting inhibitors. A study by Swamy et al. (2002) investigated the effects of maturity and auxin application on rooting of *Robinia pseudoacacia* found that cuttings from mature trees were not only more difficult to root, but that juvenile cuttings also responded better to auxin application. A study on the effects of IBA and juvenility on the rooting of *Rhamnus caroliniana* softwood cuttings also found a significant interaction between the effects of IBA and juvenility (Graves, 2002). Juvenile cuttings rooted at 88% when treated with 3000 ppm IBA, compared to only 17% of mature stem cuttings rooting at the same IBA concentration. Higher levels of IBA were able to induce rooting even in the mature cuttings, with 70% of cuttings producing roots at 8000 ppm IBA.

Endogenous rooting inhibitors have been found in chestnut leaves from biologically mature and juvenile sources (Vieitez and Ballester, 1988). Plant compounds extracted from the leaves that were separated by HPLC indicated the presence of two ellagic acid derivatives, 3,3’4-tri-0-methyllellagic acid, and 3,4,4’tri-0-methylellagic acid. The first compound was found to have inhibiting effects on root initiation by interfering with IAA metabolism. An earlier study of chestnut found physical barriers to root induction as well, with more extensive sclerification of mature cuttings than juvenile (Vieitez and Ballester, 1976).

Phase change is initiated in the shoot apical meristem (Sussex, 1976). Bitonti et al. (2002) examined whether DNA methylation occurs in mature, but not juvenile, shoot apical meristems of peach (Bitonti et al., 2002). DNA methylation changes may cause alterations in gene expression and gene regulation during phase change in plants. These researchers found a much stronger zonation of chromatin condensation, DNA methylation, and distribution of zeatin in adult peach apical meristems than in apical meristems from juvenile shoots. The authors speculated that the zonating patterns in mature tissue could mark change on a cellular level of gene expression that controls shoot identity.

Juvenility is still somewhat of a mysterious concept in horticulture. It is still unknown what causes these phase changes to occur in plants, and what physiological and biochemical changes the plant exhibits when this phase change occurs (Hackett, 1976). Pawpaw for example, loses rooting capability once the plant reaches over 4 months of age (Geneve, unpublished data). At this age, the plant is not sexually mature, not capable of flowering and fruit production, but apparently the plant has undergone some physiological changes that render it incapable of rooting. It seems likely that there is a gradation in maturity as the plant develops. However, since chronological age and biological, or ontogenetic age are not the same in plants, this issue could be overcome by propagating the ontogenetically most juvenile parts of the plant, such as root cuttings or shoots from the base of the plant, such as shoots generated in stooling. Haissig and Riemenschneider (1988) state that in many plants, the ability to root, which is a juvenile trait, and the ability to flower, a mature trait, compete in some plant species to the point that they are mutually exclusive of each other.

Hypotheses and Objectives

The hypotheses for the asexual propagation portion of this thesis research project were that trench layering, mound layering, and pot layering would be viable research propagation methods for pawpaw, that juvenile pawpaw shoots root more readily than more mature pawpaw shoots in a layering system, and that both auxin and wounding enhance rooting of pawpaw shoots. The objectives for this project were to examine various layering systems to develop a clonal propagation method for pawpaw for research purposes, determine if juvenility plays a role in the rooting of pawpaw, and examine the effects of auxin and wounding on the rooting of pawpaw shoots in various layering systems.

The hypotheses for the sexual propagation portion of this thesis research project were that high levels of diversity of reproductive characteristics exist among accessions contained in the KSU-USDA NCGR orchards for *Asimina* species. The objectives for this project were to examine the diversity in vegetative and reproductive characteristics of pawpaw populations contained in the KSU-USDA NCGR orchards that may be useful in future pawpaw breeding efforts.

Chapter 2- The Influence of Juvenility and IBA Concentration on Rooting of the North American Pawpaw in a Trench Layering System

Introduction

The pawpaw [*Asimina triloba* (L.) Dunal] is a tree fruit native to the eastern and Midwestern areas of the United States (Callaway, 1990; Layne, 1996). The pawpaw is a member of the Annonaceae, or custard apple family, and *Asimina* is the only temperate genus in this otherwise tropical family. The pawpaw produces large fruit with a custard-like texture and flavor that resembles a blend of mango, banana, and pineapple (Peterson, 1990). Pawpaw grows wild in hardwood forests of 26 states in the eastern United States, usually in river-bottom lands where it grows as an understory tree and often in large patches due to root suckering (Kral 1960; Callaway 1990; Callaway 1993). The trees can reach 5 to 10 meters in size (Dirr, 1990). Pawpaw has shown great potential as a new fruit crop in the southeastern U.S. (Layne, 1996; Pomper and Layne, 2005).

Kentucky State University in Frankfort, Kentucky, is the site of the USDA National Clonal Germplasm Repository (NCGR) for *Asimina* species (Pomper et al., 2003). As a USDA repository site, developing clonal propagation techniques is a very important research objective in order to build and maintain a diverse collection of unique plant material. There are many reasons that developing a clonal propagation system of pawpaw on its own roots would be beneficial. Both KSU, as a germplasm repository site, and commercial pawpaw growers, have a need to propagate superior genotypes true to type. In order for the KSU-NCGR to maintain its diverse *Asimina* collection, it would be advantageous to develop a propagation system to obtain currently grafted germplasm on its own roots. In case of scion dieback due to winter injury or storm damage, self-rooted plants would be more desirable than grafted selections. Due to high variability in seedling rootstocks, it would also be desirable to clonally propagate pawpaw varieties on their own roots to reduce tree to tree variability in vigor, and possible other factors, for pawpaw growers.

Pawpaw has proven to be extremely difficult to clonally propagate. Previous studies have examined stem cuttings, root cuttings (Finneseth, 1997), tissue culture (Finneseth et al., 1997), and air layering (KSU, unpublished) as possible clonal propagation techniques, and none have been extremely successful at promoting adventitious rooting in pawpaw. Pawpaw is a naturally suckering species, readily forming adventitious shoots from roots (Pomper and Layne, 2005). Propagation by root cuttings shows the most promise, with 56% of root cuttings greater than 5 cm in diameter producing shoots (Finneseth, 1997). However, clonal propagation by root cuttings requires the genotype to be on its own roots. Pawpaw cultivars are not currently self-rooted, but are budded or grafted onto a seedling rootstock (Pomper and Layne, 2005).

Studies utilizing many different methods of propagation have shown the effect of juvenility on adventitious rooting in pawpaw and other species. Odilo and Rafael (1976) found that only juvenile *Annona cherimola* cuttings produced roots, with 25% rooting at 5,000 ppm IBA. Stem cuttings from mature sources of pawpaw fail to root, whereas stem cuttings from more juvenile seedlings root more readily (Finneseth, 1997; Geneve, unpublished data) Finneseth (1997) found that stem cuttings taken from 7-month-old seedlings rooted at up to 16% whereas only one out of 5100 mature stem cuttings rooted. In preliminary studies at the University of Kentucky, stem cuttings from 2-month-old pawpaw seedlings rooted at up to 74%, while virtually no cuttings taken from older seedling sources (4-7 months) produced roots (R. Geneve, unpublished data).

Layering is a form of clonal plant propagation in which the shoot of a plant is induced to form roots while still attached to its mother plant. Various modifications of layering systems are often used to propagate more difficult-to-root species (Hartmann et al., 1997). Layering can enhance rooting by causing etiolation of the shoots as they grow through the medium placed on top of the mother plants. Etiolation enhances rooting by causing physiological changes in the plant (Hartmann et al., 1997). Several studies of various fruit and nut crops have shown the positive effect etiolation has on adventitious rooting, including apple (Delargy and Wright, 1978) and chestnut (Biricolti et al., 1994).

Trench layering is a modification of layering that is not often used in the commercial horticultural industry. To perform trench layering, the mother plants are planted and bent over so that they are in a horizontal position, and then are covered with soil, sawdust, or some other type of rooting medium. While trench layering is fairly uncommon, it has been used in extremely difficult-to-root plants, and has the advantage of a larger number of buds left to emerge as it is laid horizontally, and therefore this has the potential for a larger number of shoots to form plants in a small area. Trench layering could also be utilized in grafted plants, as buds from the scion could be rooted, and emerging shoots would be more distant from any shoots emerging from the rootstock and thus easier to differentiate from root suckers, unlike mound layering. Suriyapananont (1990) compared air and trench layering in apple rootstocks and found that trench layering was a superior method for at least one rootstock. Both air layered and trench layered shoots were wounded and treated with 8000 ppm IBA. Trench layered shoots were bent and pegged to lay horizontally in a trench. Wounding and IBA application was then performed. About 77% of air layered shoots of the MM106 rootstock rooted, while 100% of trench layered MM106 shoots produced roots. However, no significant differences in treatments were found in the other two rootstocks.

Our long-term goal is to develop a clonal propagation method where pawpaw genotypes can be propagated on their own roots. The hypotheses of this study were that pawpaw seedlings will root in a trench layering system in the greenhouse, increasing concentrations of auxin applied to girdled shoots will enhance rooting, and that shoots of ontogenetically more juvenile pawpaw seedlings will have greater rooting potential than shoots of ontogenetically more mature seedlings. The objectives for this study were to examine the role juvenility plays in rooting of pawpaw and determine if trench layering with wounding and auxin applied to shoots would promote rooting of shoots.

Materials and Methods

Plant Material. For all experiments, pawpaw seed was harvested in 2001 from open-pollinated half-sib trees at the Keedysville, Md. orchard at the Western Maryland Research and Education Center. Seed was stratified at 5 ^{o}C for 3 months and was sown to a 3 cm depth in moist Pro-Mix ‘BX’ potting substrate (Premier Horticulture, Inc. Red Hill, Pa.) in 15 cm deep flats (on February 7, March 14, and April 4, 2002; and December 16, 2002, March 10, 2003, and May 12, 2003). Seedlings were grown in a white-washed greenhouse under a 16-h photoperiod supplemented by high pressure sodium lights with temperatures set at 24/30^{o}C. Photosynthetic photon flux was measured at canopy level on a clear sunny representative day at 150-350 µmol•m-2•s-1 by using a LiCor LI-185B photometer with a quantum sensor (LiCor, Lincoln, Nebr.). Flats were fertigated with Peters 20-20-20 (20N-8.6P-16.6K) water soluble fertilizer with micronutrients at 500 ppm once a week. Seedlings emerged approximately 45 days after sowing.

Trench layering methods. When seedlings had reached the appropriate age post-emergence, they were defoliated, tipped, and transplanted into 5 m x 2 m x 0.5 m trench layering beds, constructed of untreated lumber on the concrete greenhouse floor, filled 30 cm deep with ProMix-BX, in another glass greenhouse (June 18, 2002 and July 30, 2003). Temperature and light conditions were the same. Seedlings were planted slanting diagonally, so that when the pine bedding was placed on top, they would lay horizontally. Pot tags were placed by the seedlings to indicate the treatment, which had been arranged in a randomized block design, and seedlings were covered with fine pine bedding (Southern States). Seedlings were watered 3 times a week and fertigated once a week with Peters 20-20-20 water soluble fertilizer with micronutrients at a rate of 500 ppm.

When most shoots had emerged and reached approximately 5 leaves, they were subjected to treatment (September 29-30, 2002 and December 2, 2003). The pine bedding was removed from the area around the shoot, and the shoot was wounded by rubbing a small area of the stem circumferentially with sandpaper. A 3-indolebutyric Acid (IBA) solution was prepared by dissolving reagent-grade (water-insoluble) IBA (Sigma-Aldrich, St. Louis, MO) in a small amount of ethanol and diluting with the appropriate volume of double distilled water. The IBA solution was then applied to the wounded area of the shoots using a paintbrush. The shoots were re-covered with the pine bedding after treatment. Light, temperature, irrigation, and fertigation procedures remained the same as before treatment.

Approximately 12 weeks after treatment, shoots were uncovered to examine for evidence of rooting (December 2, 2002 and March 29, 2004). The pine bedding and ProMix was delicately removed from around each plant, so as not to disturb the root system. Diameter, height, vigor rating, root number and root length were recorded for each shoot.

Experimental and treatment design. The experimental design for this study was a randomized block, with the three blocks based on temperature variation in the greenhouse. Trench layering beds ran parallel to a cooling pad. There were 15 replicate plants in each treatment included per block in 2002 and 12 plants per treatment per block in 2003. The treatment structure was a 3 x 4 factorial in 2002, with the two main factors being age (3, 6, and 12 week old seedlings) and auxin concentration/wounding (0 IBA/wounded, 0 IBA not wounded, 5,000 ppm IBA wounded, and 10,000 ppm IBA wounded). The treatment structure in 2003 was a 3 x 3 factorial, with the two factors being age (3, 12, and 24 week old seedlings) and auxin concentration (0 IBA, 10,000 ppm IBA, and 20,000 ppm IBA; all wounded).

Statistical Analysis. Data for number of roots per stem, average root length, and rooting percentage were subjected to an analysis of variance (ANOVA) or general linear model of analysis of variance (GLM-ANOVA) using the statistical software CoStat (CoHort software, Monterey, Calif.). Statistical significance of differences between mean values was determined using an LSD separation with a level of significance of $P<0.05$.

Results

2002 Experiment. Although it was expected that multiple buds would break along the stem and form shoots, pawpaw seedlings displayed strong apical dominance and only what was the uppermost bud after tipping broke and formed a shoot. All other buds remained dormant. Upon analysis, data failed normality testing using Bartlett's Test, therefore these results are not reported. Two-way tables of all treatment means are reported.

Age appeared to affect rooting percentage of seedlings, with twice and three times as many shoots of three-week-old seedlings rooting than shoots of 6-week and 12-week old seedlings, respectively (Table 2.1). Rooted shoots of 6-week-old seedlings produced the most roots per stem, at 2.5, and also had the longest average root length.

IBA concentration also had an impact on rooting percentage, with only 2-3% of control shoots producing roots, compared to approximately 16% of those shoots treated with either level of IBA producing roots (Table 2.2). There did not appear to be differences in rooting percentages between the wounded and unwounded controls, nor between the two levels of IBA. Shoots treated with 5,000 and 10,000 ppm IBA respectively produced 30% and 80% more roots per stem than the control plants. Shoots treated with IBA also produced roots that were approximately 3 times longer than the control shoots.

A breakdown of rooting characteristics by age and IBA concentration can be found in Table 2.3. Only the youngest, 3-week-old shoots were capable of rooting if not treated with IBA. None of the older control shoots produced any roots. Also, the treatment combination most successful at promoting rooting was shoots of 3-week-old seedlings treated with 10,000 ppm IBA, with a rooting percentage of 31%.

2003 Experiment. As in 2002, only one bud broke at the tip and one shoot was formed per mother plant in the trench layering experiment in 2003. Again, upon analysis, data failed normality testing using Bartlett's Test, therefore these results are not reported. Two-way tables of all treatment means are reported.

Seedlings of all ages had a similar rooting percentage of about 16% (Table 2.4). Age of the plant did appear to affect the average number of roots per rooted shoot. Shoots from 12-week-old seedlings produced the most roots, greater than 3-week and 24-week-old plants by abut 2 fold and 3.7 fold, respectively. Age of seedling also appeared to have affected root length, with 3-week-old seedlings producing roots that were about 3 times longer than 12 and 24-week-old plants.

IBA concentration impacted rooting percentage, with shoots treated with 0 IBA rooting at 5%, and shoots treated with 10,000 and 20,000 ppm IBA having rooting percentages of about 20% (Table 2.5). The concentration of IBA applied to shoots also affected average number of roots per shoot, with shoots treated with 10,000 ppm IBA producing twice as many roots as control shoots, and shoots treated with 20,000 ppm IBA producing approximately 5 times as many roots as the control shoots. IBA concentration appeared to have some effect on average root length, with shoots treated with 10,000 and 20,000 ppm IBA producing roots that were approximately four and three times longer, respectively, than control shoots.

Discussion

Trench layering does show potential as a method of clonally propagating pawpaw on its own roots. Trench layering may be preferable to other layering methods in pawpaw. Trench layering was more successful in this study at rooting more mature pawpaw material than any other method of clonally propagating pawpaw studied to date. One pitfall encountered in this study was that usually in trench layering, a larger number of buds will break and allow a greater number of shoots to root in a smaller area than with other layering methods. However, apical dominance is very strong in pawpaw, causing only the bud at the tip to break and take over as a new 'leader'. Therefore only one shoot developed on most trench layered pawpaw plants limiting the plant production potential of this system; on several plants two shoots on some did form. Since the original seedling tree of most commercially available cultivars no longer exists, we are attempting to propagate cultivars budded onto a seedling rootstock. A goal of the USDA-NCGR is to propagate these cultivars, and other valuable germplasm, on their own roots. With trench layering, the scion would be more distant from the rootstock, and so it would be easier to differentiate which shoots emerged from the scion, and which shoots emerged from root suckers or the base of the plant beneath the graft, which would not be the genotype desired to propagate. Scion shoots would consist of mature tissue, likely more ontologically advanced than the oldest seedlings used in this study; however, this study does suggest that trench layering has potential for establishing a limited number of self rooting cultivars.

Wounding alone did not promote rooting. It was found that girdling was essential to rooting in pecan, with girdled shoots that were not treated with any auxins capable of producing roots (Wood, 1989). However, this does not appear to be the case with pawpaw, as girdled shoots with no auxin treatments were not capable of rooting. There is a possibility that shoots were not completely girdled using the sandpaper method, although every effort was made to completely girdle the shoots and disrupt the cambial layer each year.

Juvenility did affect rooting characteristics of pawpaw shoots. Only in 2002 was plant maturity shown to greatly influence rooting percentage, but both experiments suggested that age of the mother plant affected number of roots formed per shoot and average adventitious root length of shoots. Source plant maturity has been shown to have a strong influence on adventitious rooting in pawpaw in previous studies (Finneseth, 1997; Geneve, unpublished data). Odilo and Rafael (1976) found that only juvenile *Annona cherimola* cuttings produced roots. However, upon examining the 2003 data, it appears that perhaps the adventitious rooting-inhibiting effects of maturity could be overcome somewhat by applying very high levels of IBA to shoots. Utilizing increased concentrations of IBA that year, more mature shoots were induced to root.

Auxin application promoted rooting characteristics in pawpaw, as has been shown in previous studies of other fruit and nut crops, such as apple (Delargy and Wright, 1979), pecan (Wood, 1989), and walnut (Rashid, 1978). Control shoots not treated with IBA had great difficulty rooting, especially in more mature plants. In the 2003 experiment, a greater number of mature shoots rooted with high levels of IBA. It is possible that greater numbers of mature shoots in 2003 rooted because of the increased levels of IBA than that used in 2002, which could have promoted rooting in even the oldest seedlings. Plants were also allowed to remain in the trench layering beds for 4 weeks longer after treatment in 2003, which would have given slow- or difficult-to-root plants a greater length of time in which to root, and could have led to higher rooting percentages in the more mature plants. Also, the trend of IBA application promoting rooting was seen in both years.

A number of problems may have influenced the results of the 2003 study. The greenhouse this study was conducted in experienced several heater failures, causing the temperatures to drop much lower than optimal. Freezing temperatures were not reached in the greenhouse, although temperatures from 10-15° were sustained for at least one day on several occasions. These cool temperatures caused the emerging shoots to have much less vigor. These cool temperatures in the rooting substrate are also not conducive to adventitious root formation. Approximately 40% of shoots rotted in 2002; however, many more shoots (64%) rotted in 2003, which could also be attributed to the soil and sawdust staying much cooler and moister due to the greenhouse conditions, which could have lead to increased disease pressure. A fungal organism likely invaded the trench layering beds and caused a shoot rot of many plants. These two factors led to having much fewer replicate plants in each treatment combination 2003 than in the original design. This lack of replication could be one reason neither main effect was shown to affect rooting percentage; a high degree of variation combined with a lack of replication caused differences to not be statistically significant. Perhaps if more plants had remained in the experiment, there would have been enough plant material to effectively show differences between treatments. No differences were seen either year between the two levels of IBA, suggesting that even higher concentrations of IBA could be tested on trench layered pawpaws with the possibility of increasing rooting. Finneseth (1997) tested various levels of IBA applied as a quick dip to pawpaw cuttings. Cuttings treated with 40,000 and 80,000 ppm IBA did not root and exhibited tissue necrosis. Therefore, IBA concentrations above 40,000 ppm should not be attempted. Finneseth (1997) obtained the highest rooting percentages at 10,000 ppm IBA with juvenile material. Various formulations of IBA or other auxins in combination could also be attempted in future experiments to promoting rooting.

Conclusions

Trench layering does show potential as a novel propagation system for the difficult-to-root pawpaw, and could be developed for rooting unique plant material at the KSU USDA-NCGR to maintain germplasm on its own roots. Both maturity and auxin concentration influenced rooting characteristics of pawpaw in a trench layering system. Trench layering was more successful in rooting more mature pawpaw material than any method examined so far, so it shows great promise in pawpaw propagation efforts.

Table 2.1: The effect of age on rooting percentage, average number of roots, and average length of roots for pawpaw in a 2002 trench layering study in the greenhouse

Age	Rooting percentage	Mean roots per rooted stem	Average root length (mm)
3 weeks	15.1%	1.9	72.1
6 weeks	7.5%	2.5	125.8
12 weeks	4.9%	1.8	76.9

Table 2.2: The effect of IBA concentration on rooting percentage, average number of roots, and average length of roots for pawpaw in a 2002 trench layering study in the greenhouse

IBA (ppm)	Rooting percentage	Average number of roots per rooted stem	Average root length (mm)
0 NW[z]	2.7%	1.0	32.5
0 W	1.9%	1.0	32.0
5000 W	16.3%	1.8	91.7
10000 W	15.7%	1.3	97.9

[z]: NW= not wounded; W= wounded

Table 2.3: The effects of seedling age and IBA concentration on rooting characteristics of pawpaw in a 2002 trench layering experiment in the greenhouse

Age	IBA (ppm)	Rooting Percentage	Average # Of Roots Per rooted stem	Average Root Length	n[z]
3 weeks	0 NW[z]	8% ±4%	1.0	32.5	25
	0 W	4%±6%	1.0	32.0	24
	5000 W	16%±11%	1.0	23.7	25
	10000 W	31%± 5%	2.8	87.4	26
6 weeks	0 NW	0	-	-	26
	0 W	0	-	-	25
	5000 W	23%±6%	2.5	109.5	26
	10000 W	8%±4%	2.5	174.4	26
12 weeks	0 NW	0	-	-	12
	0 W	0	-	-	10
	5000 W	17%±13%	2.0	98.8	12
	10000 W	13%±8%	1.5	47.7	16

z: n= total number of plants in all blocks

Table 2.4: The effect of age on rooting percentage, average number of roots, and average length of roots for pawpaw in a 2003 trench layering study in the greenhouse

Age	Rooting percentage	Average number of roots per rooted stem	Average root length (mm)
3 weeks	14.0%	3.1	178.8
12 weeks	22.2%	5.2	62.1
24 weeks	10.3%	1.4	59.8

Table 2.5: The effect of IBA concentration on rooting percentage, average number of roots, and average length of roots for pawpaw in a 2003 trench layering study in the greenhouse

IBA concentration (ppm)	Rooting percentage	Average number of roots per rooted stem	Average root length (mm)
0	5.1%	1.0	30.0
10000	20.5%	2.2	121.2
20000	20.0%	5.1	98.3

Table 2.6: The effects of seedling age and IBA concentration on rooting characteristics of pawpaw in a 2003 trench layering experiment. in the greenhouse

Age	IBA (ppm)	Rooting Percentage	Average # Of Roots Per rooted stem	Average Root Length (mm)	N[z]
3 weeks	0	0	-	-	14
	10000	20%±12%	1.0	165.3	15
	20000	21%± 2%	3.7	182.5	14
12 weeks	0	8%±11%	1.0	18.0	12
	10000	25%±15%	2.5	123.3	12
	20000	30%±17%	3.8	40.5	12
24 weeks	0	8%±8%	1.0	42.0	13
	10000	17%±17%	1.5	72.7	12
	20000	7%±8%	1.0	39.0	14

z: n= total number of plants in all blocks

Chapter 3: The Influence of age, auxin concentration, and auxin application method on rooting of pawpaw in a pot layering system.

Introduction

The pawpaw [*Asimina triloba* (L.) Dunal] is a member of the Annonaceae, or custard apple family and is the largest tree fruit native to the United States (Callaway, 1990). Pawpaw fruit are large, with a creamy texture and flavor resembling a blend of mango, banana, and pineapple. The fruit are also highly nutritious (Peterson, 1990). The trees reach 5 to 10 meters in size (Dirr, 1990) and are usually found growing in the understory of forests (Willson and Schmeske, 1980). The pawpaw is being investigated as a new alternative crop for farmers in Kentucky and elsewhere in the southeastern U.S. (Layne, 1996; Pomper and Layne, 2004).

Kentucky State University in Frankfort, Kentucky, was designated as the site of the USDA National Clonal Germplasm Repository for *Asimina* spp. in 1995. One of the responsibilities as a repository site is to build and maintain a diverse collection of plant material, therefore investigating new forms of propagation to enhance the collection is a priority. It would be desirable to develop a method to clonally propagate pawpaw on its own roots. First, it is necessary to propagate superior genotypes true to type, and it would be preferable to develop a research propagation system to obtain currently grafted trees on their own roots, so as to not lose valuable germplasm in case of scion dieback due to winter injury or storm damage. Also, due to high variability in vigor of seedling rootstocks, it would also be desirable to clonally propagate pawpaw varieties on their own roots.

There are currently no feasible methods to propagate pawpaw on its own roots. Pawpaw has proven to be extremely difficult to propagate by many methods in the past. Methods such as stem cuttings, root cuttings (Finneseth, 1997), tissue culture (Finneseth et al., 1997), and air layering (KSU, unpublished) have been investigated as possible propagation methods for pawpaw, and none have been successful. Pawpaw cultivars are currently propagated by grafting or chip budding a cultivar onto a seedling rootstock. Several studies have also shown the effect of juvenility on adventitious rooting in pawpaw. Mature pawpaw stem cuttings generally are incapable of rooting, while more juvenile cuttings (2 months old or younger) root more easily (Finneseth, 1997; Geneve, unpublished data).

One clonal propagation method that has been little-investigated in pawpaw is layering. Layering is a form of clonal plant propagation in which the shoot of a plant is induced to root while still attached to its mother plant. Layering is often successful in propagating difficult-to-root species that may be impossible to propagate by any other method (Hartmann et al., 1997). One aspect of layering that encourages rooting is the etiolation of the shoots as light is excluded due to the medium covering the top of the mother plants. Etiolation causes several physiological changes in the plant that enhance rooting (Hartmann et al., 1997). Etiolation has been shown to enhance adventitious rooting in several fruit and nut crops, including apple (Delargy and Wright, 1978) and chestnut (Biricolti et al., 1994). Wounding or girdling shoots also enhances rooting by slowing or blocking downward movement of carbohydrates, auxins, and other translocated compounds so that these compounds to accumulate just above the girdle, causing rooting to occur at this point.

Pot layering is a unique form of layering seldom mentioned in the literature. Some advantages of using pot layering over mound layering would be that pot layering is performed in a greenhouse, under more controlled conditions than in the field. Also, the plants in pot-layering would be self-contained and easy to move compared to mound layering. Pot layering was attempted in a species related to pawpaw, *Annona squamosa*. (Venkataratanam and Satyanaranaswamy, 1956). The stems of one year old plants were cut to expose a tongue near the base of the plant, then covered with another pot filled with soil. Shoots were gradually separated after 4 to 5 months. These authors reported that 2.57% of pot layered shoots produced adventitious roots. While the rooting percentage was low, this study found that pot layering was more successful than air layering, with 0.69% of shoots producing roots.

Pot layering is a method of propagation that may enable rooting in difficult-to-root plants such as pawpaw, and would be a research propagation method that would allow the KSU-NCGR to obtain and store valuable germplasm on its own roots. The hypotheses for this study were that pawpaw shoots treated with a continuous supply of auxin delivered via wick will have an increased rooting potential than shoots treated with a quick dip solution, shoots of ontogenetically more juvenile pawpaw seedlings will have greater rooting potential than shoots of ontogenetically more mature pawpaw seedlings, and increasing concentrations of auxin applied to pawpaw shoots will enhance rooting. Our objectives were to determine which of two different auxin delivery methods most enhances rooting of pawpaw shoots, and to examine the role juvenility plays in rooting of pawpaw.

Materials and methods

Plant Material. For all experiments, pawpaw seed was harvested in 2002 from open-pollinated half-sib trees at the Keedysville, Md. orchard at the Western Maryland Research and Education Center. Seed was stratified at 5°C for 3 months and was sown to a 3 cm depth in moist Pro-Mix 'BX' potting substrate (Premier Horticulture, Inc. Red Hill, Pa.). Seedlings were grown in a white-washed greenhouse under a 16-h photoperiod supplemented by high pressure sodium lights with temperatures set at 24/30°C. Photosynthetic photon flux was measured at canopy level on a clear sunny representative day at 1200 HR at 150-350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ by using a LiCor LI-185B photometer with a quantum sensor (LiCor, Lincoln, Nebr.).

2003 Seedling experiment

In a preliminary experiment to examine the effects of juvenility, IBA concentration, and auxin delivery system on rooting of pawpaw shoots in a pot layering system, 120 pawpaw seeds were sown in ProMix BX potting medium into 1 gallon TreePots (Stuewe and Sons, Inc., Corvallis, Ore.), on May 12, 2003, while one-year-old seedlings were moved into greenhouses after remaining in a walk-in cooler obtaining their chilling requirement. The young seedlings were defoliated once they had reached 3-5 leaves, approximately 4 weeks after shoot emergence, and the one-year-old seedlings were defoliated, cut back to approximately 10 buds, and the lower, more juvenile buds rubbed off, leaving 3-4 more mature upper buds. Another one-gallon TreePot, with the bottom cut off, was fitted securely upright into the existing pot containing the seedling. The bottomless pots placed on top of the larger, one-year-old seedlings were approximately 30 cm tall, while the bottomless pots placed on top of the smaller, 4-week-old seedlings were approximately 12 cm tall. The bottomless pot was then filled with fine pine shavings.

After etiolated shoots emerged from the top of the bottomless pot, the upper pots and pine shavings were removed and treatments were applied on September 24. This experiment had a 2 x 2 x 3 treatment structure, with the main effects being age (4-week-old seedlings vs. one-year-old seedlings), auxin delivery system (quick dip applied with paintbrush vs. auxin solution supplied longer-term via a wicking system), and IBA [potassium salt (Phytotronics, Inc. Earth City Mo.)] at a concentration of either 0 (Control-ddH_2O only), 10,000, or 20,000 ppm delivered by painting on to the wounded area of the plant, or alternatively, IBA at concentrations of either 0, 1,000, or 5,000 ppm delivered by wicking to plants. All treatments were wounded circumferentially using sandpaper. IBA concentrations were higher for the paintbrush application than for the wicking system because traditionally, IBA concentrations are higher in quick-dips, which is comparable to painting, than in longer-term auxin applications, such as soaking cuttings in an IBA solution, which the wicking system is comparable to.

The experimental design was completely randomized, with 15 plants of each treatment combination. IBA solutions were applied by either painting or swabbing the IBA solution onto the wounded area with a paintbrush ("quick-dip" method) or by placing the IBA solution into a 1.5 ml microfuge tube, inserting a wick made from cheesecloth, and wrapping and tying the wick around the wounded area of the stem. The microfuge tube was covered with parafilm to reduce solution loss into the substrate, and to secure the tube to the stem of the plant. After treatments were applied, the bottomless pots were placed back on top of the seedlings and re-filled with pine shavings. Plants were fertigated with 500 ppm Peters 20-20-20 water soluble fertilizer once a week.

The amount of auxin solution lost from the microfuge tube and the amount of solution applied with the paintbrush strokes were measured to calculate the amount of auxin applied to the stems using each method. Approximately 3 ml of IBA solution was taken up in the wicking system: 2 ml were instantly absorbed by the wick, and 1 ml was left remaining in the 1.5 ml microfuge tube. The remaining 1 ml was absorbed within 2 days. Therefore, 3 mg of actual IBA was removed from the tube in the 1,000 ppm wicking treatment. Fifteen mg of IBA was taken up in the 5,000 ppm wicking treatment. An estimated 0.33 ml of IBA solution was applied with the paintbrush strokes, which is approximately 3.3 mg of actual IBA applied in the 10,000 ppm IBA treatment, and 6.6

mg of IBA applied in the 20,000 ppm treatment. Therefore, the 10,000 ppm painted and 1,000 ppm wicking treatments had comparable amounts of actual IBA applied, whereas the 20,000 painted treatment had only about half as much IBA applied as the 5,000 ppm wicking treatment.

The shoots were uncovered and rooting data was collected on January 21, 2004. The bottomless pot and surrounding pine shavings were carefully removed from around the plants. Root number, length, and the number of rotted or dead shoots were recorded.

2003 grafted cultivar study

In a preliminary experiment to examine the effect of auxin concentration on rooting of shoots of grafted pawpaw trees treated with IBA delivered via wick, 36 pawpaw trees which had been chip-budded the previous year and stored at approximately 4° C in a walk-in cooler were selected. Eighteen of the trees had been chip-budded using PA-Golden, and the other 18 had been budded with Sunflower, both on seedling rootstocks. The trees were in 2 gallon TreePots. The scions were cut back to 3-4 buds and a 2-gallon RootTrainer which had had the bottom cut off was fit securely into the original pot and filled with fine pine shavings. Once the new shoots emerged through the surface of the sawdust, the bottomless pot and pine shavings were removed and treatments were applied.

The treatment structure was a 3 x 2 factorial, with the two factors being IBA concentration (0, 1,000 ppm and 5,000 ppm) and genotype. All shoots were wounded using sandpaper. This was a completely randomized experimental design, with 6 plants per treatment combination. Quick dip solutions were prepared at 1,000 ppm and 5,000 ppm using water-soluble IBA salts and placed in 1.5 ml microfuge tubes. A cheesecloth wick was submerged in the tube and wrapped and tied around the stem at the girdle. The tube and was covered with parafilm to reduce dispersion into the pine shavings and to tie the tube securely to the plant. After treatments were applied, the bottomless pot was replaced and shoots were re-covered with sawdust. The shoots were uncovered and data on root number, length, and shoot death and rotting was collected on January 21, 2004.

2004 seedling experiment

Based on preliminary data collected in the 2003 seedling study, an experiment with higher auxin concentrations and additional replicate plants was conducted in order to determine the effect of IBA concentration and juvenility on rooting of pawpaw shoots in a pot layering system. Approximately 168 seeds were sown in early December 2003 and late April 2004 into one gallon TreePots filled with ProMix. After the younger seedlings reached 5 leaves (about 6 weeks) they were tipped to remove apical dominance; the older plants were cut back to 10 buds. The lower buds were rubbed off, leaving 3 more mature upper buds. A bottomless one-gallon TreePot was then placed over the plant and filled with pine shavings to cause etiolation of new shoots. Once the new shoots emerged through the surface of the sawdust, the upper pot and sawdust was carefully removed and treatments were applied. All shoots were wounded using sandpaper. An IBA quick dip solution at 15,000 ppm, 30,000 ppm, or control (double distilled water only) was applied using a paintbrush. The treatment structure is a 2 x 3 factorial, with age and IBA concentration being the main effects. The experimental design was a randomized complete block, with 3 blocks and 12 plants of each treatment combination per block. IBA solutions were prepared using IBA water-soluble salts and double distilled water. After treatments were applied, the bottomless pot was replaced and shoots were re-covered with pine shavings. On November 1, 2004, the pine shavings were carefully removed from around the plant and root number and length was recorded.

Statistical Analysis. Data for number of roots per stem, average root length, and rooting percentage were subjected to an analysis of variance (ANOVA) or general linear model of analysis of variance (GLM-ANOVA) using the statistical software CoStat (CoHort software, Monterey, Calif.). Statistical significance of differences between mean values was determined using an LSD separation with a level of significance of $P<0.05$.

Results

2003 Seedling Study. In the 2003 seedling pot layering study, few shoots produced roots. Only shoots treated with IBA were capable of producing adventitious roots (Table 3.1). None of the wounded control shoots rooted. Rooting percentages were twice as high using the wicking method rather than painting the IBA solution on, although too few plants remained to perform meaningful statistical analyses to determine differences between treatments. Approximately 14-15% of shoots from one-year-old seedlings treated with either level of IBA rooted using the wicking method rooted, while only about 7% of shoots from one-year-old seedlings which had IBA painted on produced roots. 25-38% of shoots from month-old seedlings treated with wicked IBA produced roots, while 13-25% of shoots from one-month-old seedlings treated with painted-on IBA rooted. About half as many shoots from one-year-old plants rooted using either method, compared to shoots from more juvenile seedlings. IBA concentration did not appear to have a significant impact on this preliminary study. The average number of roots and root length also appeared to be higher using the wicking method of applying IBA than with the painted IBA. Rooted shoots treated with IBA via a wick produced an average of 3 roots per stem, across all treatments. Rooted shoots treated which had IBA painted on the wound produced an average of 1.6 roots per rooted stem, across all treatment combinations. Roots produced by shoots that had been treated with wicked IBA had an average length of 64.5 mm, across all treatments. Roots produced by shoots that had been painted with IBA had an average length of 28.5 mm.

2003 Grafted tree study. Very few shoots from grafted trees produced roots in the pot layering system (Table 3.2). Approximately half of the plants were lost to stem rot, leaving very few plants to work with. One shoot of a PA-Golden tree treated with 1,000 ppm IBA produced 2 roots, with an average length of 103 mm. Another PA-Golden, treated with 5,000 ppm IBA, produced more roots, 12, with a shorter average root length (43.3 mm).

2004 seedling study. Both main effects of age and IBA application were found to significantly affect rooting percentage of pot layered pawpaws in 2004 (Table 3.3). However, neither main effect significantly affected average number of roots per rooted stem or root length. Block effects were not significant in regards to rooting percentage or number of roots per rooted stem, but did significantly impact average root length. There was no interaction between the two main effects on any aspect of rooting characteristics evaluated.

Shoots of younger seedlings proved again to be easier to root than those of more mature seedlings, with approximately 6 times more shoots of 6 week old seedling producing roots than 24 week old seedlings (Table 3.4). However, age did not have an impact on average number of roots or root length.

IBA application helped to promote rooting, however, there were no significant differences between the two IBA concentrations tested on rooting percentage (Table 3.5).

Shoots treated with either 15,000 ppm IBA and 30,000 ppm IBA rooted at approximately 12%. No control shoots produced roots. However, IBA concentration did not have a significant effect on average number of roots per rooted stem or root length.

Discussion

This study confirmed the importance of juvenility in the rooting of pawpaw, with about two to six times as many of the more juvenile shoots producing roots, compared to the more mature shoots. Shoots from grafted trees were also difficult to root. Plant material used in chip budding is mature tissue, and mature pawpaw cuttings have proven extremely difficult to root in the past (Finneseth, 1997; Geneve, unpublished data). Preliminary data also shows that longer-term auxin application via wick may enhance rooting of pawpaw shoots. Other difficult-to-root plants often respond better in cutting propagation to an auxin solution soak rather than a quick-dip, because more auxin may penetrate the stem tissue (Hartmann et al., 1997).

IBA application to pawpaw shoots was also found to enhance rooting. However, no differences were seen between the two levels of IBA in either year. Therefore, higher concentrations of IBA, and possibly combinations of different auxins could be attempted to optimize rooting in pot layered pawpaws. Finneseth (1997) found that IBA concentrations above 40,000 ppm were damaging to pawpaw stem tissue, causing necrosis and failure to root. Therefore, IBA concentrations up to 40,000 ppm could be attempted. However, it does appear that some auxin application is essential to rooting of pot layered pawpaws, as no wounded control shoots produced roots in any of these three studies.

Some shoots in the 2003 preliminary seedling experiment were not covered deeply enough to be completely etiolated, which could have inhibited rooting. Etiolation has been shown to promote rooting in other fruit and nut crops, such as apple (Delargy and Wright, 1978) and chestnut (Biricolti et al., 1994). Approximately 20% of the shoots of one-year-old seedlings appeared to be slightly etiolated, but not completely white in color. The base of these only slightly etiolated shoots were buried only about 5 cm. It appears that at that depth, enough light penetrates to prevent complete etiolation, as shoots that were buried deeper were completely etiolated. However, care was taken in the 2004 follow-up study to bury shoots deeply and etiolation was achieved on all shoots.

Approximately 32% of the one-month-old seedlings and 50% of shoots from grafted trees rotted in 2003. It is possible that plants were over-watered. There were also several heater failures in the greenhouse that allowed temperatures to reach into the 10° Celsius range. These cool, damp conditions are favorable for the growth of various stem rot fungi. These environmental conditions are also not favorable for rooting, and may have contributed to rooting failure in this experiment. Only 3.5% of shoots rotted in 2004, and 5% of shoots were broken in 2004 due to deep wounding of stems.

Many plants were also uncovered and disturbed near the end of the 2003 experiment by repairmen moving them. This took place in late December, when most personnel were away for Christmas. Therefore, damage was not discovered until later, when any roots that may have formed and been exposed would have already desiccated. It is possible that more shoots had produced roots, but they were exposed and lost during this time.

Conclusions

Preliminary data shows that pot layering may have potential as a clonal propagation system. Modifications could be made by increasing auxin concentrations, or perhaps attempting different forms of auxins. However, in this preliminary experiment, the rooting of even a few shoots of mature seedlings and grafted cultivars shows promise for pot layering as a clonal propagation method for pawpaw.

Table 3.1. The effects of wounding, juvenility, IBA concentration, and auxin delivery system on rooting characteristics of pawpaw seedlings in a 2003 pot layering study.

age	[IBA]	IBA application method	number of plants rooted	% rooting	roots per rooted shoot	average root length(mm)	% survival
year old	0	Wicking	0/14	0.0	-	-	100
year old	1,000 ppm	Wicking	2/14	14.3	1.0	63.0	100
year old	5,000 ppm	Wicking	2/13	15.4	3.0	93.2	100
month old	0	Wicking	0/7	0.0	-	-	57
month old	1,000 ppm	Wicking	2/8	25	3.0	65.3	88
month old	5,000 ppm	Wicking	3/8	37.5	5.0	12.7	63
year old	0	Painted	0/14	0.0	-	-	100
year old	10,000 ppm	Painted	1/15	6.7	2.0	43.5	100
year old	20,000 ppm	Painted	1/14	7.1	1.0	29.0	93
month old	0	Painted	0/8	0.0	-	-	63
month old	10,000 ppm	Painted	2/8	25.0	2.5	21.4	63
month old	20,000 ppm	Painted	1/8	12.5	1.0	4.0	75

Table 3.2. The effects of wounding and IBA concentration on rooting characteristics of grafted pawpaw trees of the cultivars PA-Golden and Sunflower in a 2003 pot layering study.

Cultivar	[IBA]	Number of plants rooted	Rooting percentage	Number of roots per rooted stem	average root length (mm)	% survival
PA golden	0	0/3	0.0	-	-	50
PA golden	1000 ppm	1/5	20.0	2.0	103.0	83
PA golden	5000 ppm	1/3	33.3	12.0	43.3	50
Sunflower	0	0/3	0.0	-	-	50
Sunflower	1000 ppm	0/3	0.0	-	-	50
Sunflower	5000 ppm	0/2	0.0	-	-	33

Table 3.3 Analysis of variance effects of seedling age and IBA concentration on rooting of pawpaw in a 2004 pot layering study

Source of Variation	Rooting percentage	Average # Of Roots Per rooted stem	Average Root Length (mm)
Blocks	P=0.0536 N.S.	P=0.6601 N.S.	P=0.0040 **
Main Effects			
Age	P=0.0062 **	P=0.3143 N.S.	P=0.2272 N.S.
[IBA]	P=0.0118 *	P=0.8395 N.S.	P=0.9051 N.S.
Age x [IBA]	P=0.0827 N.S.	P=0.5447 N.S.	P=0.2913 N.S.

Table 3.4: The effect of age on rooting percentage, average number of roots, and average length of roots for pawpaw in a 2004 pot layering study

Age	Rooting percentage	Average number of roots per rooted stem	Average root length (mm)
6 weeks	12.3% a^{z}	4.2 N.S..	37.9 N.S.
24 weeks	2.2% b	1.5 N.S.	9.3 N.S.

^{z}: means separation using LSD separation at a level of significance of 0.05;

N.S$^{\cdot}$= not significant; means for age are from all IBA concentrations

Table 3.5: The effect of IBA concentration on rooting percentage, average number of roots, and average length of roots for pawpaw in a 2004 pot layering study

IBA (PPM)	Rooting percentage	Average number of roots per rooted stem	Average root length (mm)
0	0 b^{z}	-	-
15,000	10.6% a	4.0 $^{N.S.}$	39.9 $^{N.S.}$
30,000	12.3% a	3.6 $^{N.S.}$	32.7 $^{N.S.}$

^{z}: means separation using LSD separation at a level of significance of 0.05; N.S. = not significant; means for IBA concentration are from all ages.

Chapter 4 – The Influence of Juvenility and IBA Concentration on Rooting of the North American Pawpaw in a Mound Layering System

Introduction

Pawpaw [*Asimina triloba* (L.) Dunal] is a tree fruit native to the eastern and Midwestern United States (Callaway, 1990). The pawpaw belongs to the Annonaceae, or custard apple family, with *Asimina* being the only temperate genus in this family (Kral, 1960). Pawpaw fruit are large, with a texture ranging from firm and avocado-like to soft and pudding-like. The flavor resembles a blend of several fruits, including mango, banana, melon, and pineapple. The fruit also contain high levels of many vitamins and minerals (Peterson, 1990). Pawpaw is a medium-sized tree, reaching 5 to 10 meters in size (Dirr, 1990), and are usually found growing in the forest understory (Lagrange and Tramer, 1985). The pawpaw naturally produces many root suckers and can form large thickets or patches (Kral, 1960). The pawpaw is being investigated as a new alternative crop for farmers in Kentucky and elsewhere in the southeastern U.S. (Layne, 1996; Pomper and Layne, 2004).

Kentucky State University in Frankfort, Kentucky, was designated as the site of the USDA National Clonal Germplasm Repository for *Asimina* spp. in 1994 (Pomper et al., 2003). As a repository site, it is important to build and maintain a diverse collection of plant material, and investigating new forms of propagation to enhance the collection is a high priority. Methods to propagate pawpaw on its own roots are of special interest. Superior genotypes must be propagated true to type, and it would be preferable to develop a method of propagating currently grafted trees on their own roots rather than grafting onto a seedling rootstock, so valuable germplasm would not be lost due to scion dieback because of winter injury or storm damage. Also, propagating pawpaw varieties on their own roots would eliminate the high degree of variability in vigor imparted by seedling rootstocks.

Pawpaw is difficult to clonally propagate by most conventional methods. Propagation methods such as stem cuttings, root cuttings, and tissue culture (Finneseth et al., 1997), as well as air layering (KSU, unpublished) have been investigated as possible propagation methods for pawpaw, and none have been entirely successful for propagation from both juvenile and mature tissue sources (Geneve et al., 1999). Currently, pawpaw cultivars and advanced selections are propagated by grafting or chip budding onto a seedling rootstock (Layne, 1996).

One clonal propagation method that has not been reported in the literature of pawpaw is layering. Layering is a form of clonal plant propagation in which the shoot of a plant is induced to root while still attached to the mother plant. Layering may be used to propagate difficult-to-root species that may be impossible to propagate by any other method (Hartmann et al., 1997). Root initiation during layering may be induced by light exclusion (etiolation) and by various mechanical treatments (girdling or wounding) to slow or block downward movement of translocated compounds (Westwood, 1993). Rooting occurs just above the point of blockage. Etiolation causes several physiological changes in the plant that may enhance rooting (Hartmann et al., 1997). Several fruit and nut crops that are difficult to root using other methods may be induced to root once shoots are etiolated, including apple (Delargy and Wright, 1978) and chestnut (Biricolti et al., 1994). Girdling has also been shown in other studies to enhance rooting in pecan (Wood, 1989) and pistachio (Dunn and Cole, 1995).

Mound layering, or stooling, is a method of layering in which the shoots of the mother plant are cut back within a few inches above the soil surface, and soil or some other type of rooting substrate such as sand or sawdust is mounded around the plant to cause etiolation of the new shoots, and provide a medium for root growth of the shoots (Hartmann et al., 1997). After one season, if the new shoots have produced adventitious roots, they can be separated from the mother plant; and the process is begun again for the next growing season.

Mound layering is used to propagate many woody plant species, especially tree fruit rootstocks such as apple and pear (Westwood, 1993, Webster, 1995, Hartmann et al., 1997). Several nut crops that have proven difficult to root via other methods have been successfully propagated using mound layering. Hazelnut (*Corylus avellana)* cultivars are usually grown on their own roots, without a rootstock, and mound layering is commonly used by nurseries and growers for propagating additional trees (Bissal et al., 1991). Pecans, like pawpaw, are extremely difficult to root and are often propagated as a scion cultivar grafted onto a seedling rootstock (Wood, 1989). A study conducted in India found that ringed pecan shoots rooted in a mound layering system, even with no 3-

indolebutyric acid (IBA) application (31% rooting), reaching maximum success at 2500 ppm IBA applied in a lanolin paste (81%) (Sharma and Rosanglura, 1987). A later study conducted in Georgia by Wood (1989) found that girdling was essential to the rooting of pecan in mound layering. Approximately 46% of girdled shoots with no IBA application rooted, and 100% of girdled shoots treated with 3000 or 6000 ppm IBA in a lanolin paste produced roots. None of the ungirdled shoots rooted, regardless of IBA concentration. The author concluded that girdling triggered rooting in mound layered pecans, whereas IBA increased rooting percentages.

In a study by Rashid (1978) examining the effect of plant growth regulators in walnut stooling, it was found that some form of auxin applied to shoots was essential for rooting. Combinations of three concentrations of three auxins (IAA, IBA, and NAA) were examined in this study, with all shoots being girdled by cutting a 2-5 cm ring in the bark. The treatment combinations most successful at promoting rooting in walnut layers were 5,000 ppm IAA + 10,000 ppm IBA + 5,000 ppm NAA; 0 IAA + 10,000 ppm IBA + 5,000 ppm NAA; and 10,000 ppm IAA + 10,000 ppm IBA + 5,000 ppm NAA, all with 100% rooting. No control shoots produced roots.

One reason mound layering can be a successful method of propagating otherwise difficult-to-root plant species is because the process of constantly cutting the mother plant back to near the base causes rejuvenation of the plant. Shoots arising from nearer the base or roots of a plant are ontogenetically more juvenile and thus may have a greater rooting capacity than ontogenetically more mature tissue, such as cuttings taken from the shoot tips (Hartmann et al., 1997). Therefore shoots used in mound layering, since they emerge from buds near the base of the plant, have a more juvenile character and may provide a method of rooting plants which may be difficult or impossible to root under other propagation systems. Juvenility plays a major role on adventitious rooting in pawpaw. Mature pawpaw stem cuttings are extremely difficult to root, while more juvenile cuttings (2 months old or younger) root more easily (Finneseth, 1997; Geneve, unpublished data).

Since pawpaw roots easily produce adventitious shoots forming root suckers, it would seem likely that the development of stooling beds for clonal propagation of pawpaw via mound layering has great potential. Our hypotheses for this project were that: 1) IBA applied to pawpaw shoots will enhance rooting, 2) girdling of shoots will enhance rooting, 3) the combined effects of IBA application and girdling will promote rooting of shoots greater than either of these approaches used separately, and 4) that pawpaw shoots will root in a mound layering system. The objective for this study was to determine if mound layering with girdling and IBA application to shoots will promote rooting and allow clonal propagation of pawpaw.

Materials and Methods

Plant material. Nine seedling plants were selected from Kentucky State University's Pawpaw Germplasm Repository Orchard in Frankfort, Kentucky. These trees originated from seed collected from eight different geographic regions and sown in the KSU greenhouses. These seedlings were grown in the greenhouses to one-year-old and were then planted in the spring of 1995. These selections were cut back to the ground in March 2000 and 2001 in preparation for mound layering. Selected trees were 7-8 years old at the time of this study.

2002 experiment. To examine the effects of wounding and auxin on rooting in mound layering of pawpaw, a field experiment was conducted with a 2x3 factorial treatment structure, with the two main factors being auxin concentration and wounding. Trees were cut back in early May 2002 to 10-15 cm above the soil line and covered with 30 cm of fine pine shavings, contained in a wooden box to cause etiolation of the new shoots (Figure 4.1). The shoots were allowed to grow until they emerged from the shavings, a height of approximately 40 - 60 cm, and treatments were then applied to the etiolated shoots.

Treatments were applied on July 15-16, 2002 and included 0 IBA without wounding, 0 IBA with wounding; 3000 ppm IBA without wounding, 3000 ppm IBA with wounding; 6000 ppm IBA without wounding, 6000 ppm IBA with wounding; in lanolin paste applied at the wound. The experimental design for this study was a randomized block of 9 replicate blocks, with each individual tree serving as a block. Each block (tree) contained varied numbers of shoots per treatment combination per due to differences in shoot emergence. Each block (tree) had all treatment combinations, with 2-4 reps of each treatment combination for each tree. IBA was prepared from a reagent grade (H_20 insoluble) powder (Sigma-Aldrich, St. Louis, Mo.) dissolved in a small amount of 95%

ethanol to form a paste, then combined with lanolin (Fisher Scientific, Pittsburgh, Pa.) which was heated to a near-liquid state in a microwave. This paste was then placed into 50 mL syringes for ease of application. The etiolated stems were wounded by disrupting the cambial layer with sandpaper (wet/dry, 400 grade), and lanolin or lanolin with IBA was applied approximately 10 cm above the soil surface. After wounding and auxin application, the shoots were covered with 30 cm of fine pine shavings, contained in a 30 cm x 30 cm x 30 cm wooden box. The stooling box containing the pine shavings was irrigated as needed, and the mother plants were fertigated three times each summer via trickle irrigation with Peters 20-20-20 (N-P-K) water soluble fertilizer with micronutrients at about 1 oz of N/tree/year. Shoots were uncovered on November 4, 2002 to evaluate root initiation.

2003 Experiment The same nine plants used in 2002 were again cut back in early May 2003 to 10 cm and covered with 30 cm of fine pine shavings, contained in a wooden box to cause etiolation of the new shoots. The shoots were allowed to grow until they emerged from the shavings (approximately 40 cm), and treatments were then applied to the etiolated shoots on August 11, 2003. IBA treatment concentrations were increased over that applied in 2002 so that treatments included 0 IBA without wounding, 0 IBA with wounding; 5000 ppm IBA without wounding, 5000 ppm IBA with wounding; 10,000 ppm IBA without wounding, 10,000 ppm IBA with wounding, prepared in a lanolin paste and applied as described above. The shoots were uncovered on March 2, 2004 to examine for root initiation.

Results

In the 2002 experiment, only one layered shoot, which was wounded and treated with 6000 ppm IBA, produced roots. This shoot produced two roots with an average length of 12 mm (Figure 4.2). Over 22% of shoots rotted, possibly due to frequent rainfall and an unknown fungal organism in the damp pine shavings. Several shoots were broken by wind damage caused by deep girdling of the stem. Those shoots that were not broken or rotting did continue to grow after treatments were applied. Since only one out of 84 shoots produced roots and the rooted shoot that was both wounded and treated with the highest concentration of IBA tested, 6,000 ppm, higher concentrations of IBA were applied to the shoots in an experiment the following year.

In the 2003 experiment, fewer than 10% of stems rotted. Six shoots out of 66 examined had formed adventitious roots. The six shoots with roots were present in three blocks (plants). Of the two plants which had shoots that produced roots, one was a wounded shoot treated with 5000 ppm IBA (Row 5, Tree 97), and the other was a control shoot and a shoot treated with 10,000 ppm IBA but not girdled (Row 2, Tree 43). Four of the rooted shoots were from one genotype (Row 6, tree 71), and rooting was not associated with any treatments applied (Figure 4.3). Rooting had occurred far below the girdle and the area in which auxin was applied, some control shoots also rooted, and several shoots rooted that had emerged later and were not part of this mound layering study.

Discussion

With the IBA concentrations used with wounding of pawpaw shoots, mound layering does not appear to be a viable method for propagation of pawpaw. Trees used in this study were cut back to the ground for two years prior to the implementation of the experiments, so there already was an attempt to rejuvenate the plants used in this study and facilitate rooting. However, there are a number of possible problems that may have resulted in the failure of root development by shoots. Incomplete girdling of shoots may not have blocked translocation of compounds down the stem; however, when shoots were wounded with sandpaper, every effort to remove the bark was made by attempting to expose the xylem layer under the bark phloem. Timing of girdling or wounding could be important. Shoots were obviously etiolated and actively growing when treated; stems continued to grow after treatment. Various forms of auxin were used in other studies (Rashid, 1978), other forms of auxin could influence root initiation than the IBA used in this study. Finneseth used concentrations of 1,000 to 80,000 ppm IBA with cutting propagation of pawpaw and noted damage at a concentration above 40,000 ppm. Higher concentrations of IBA, greater than 10,000 ppm could be attempted in future mound layering studies, however very high concentrations (over 40,000 ppm) could also lead to damage to tissue and failure of root generation.

Rotting was also a problem in this mound layering system, particularly in 2002. Precipitation was higher in 2002 than 2003, and 22.6% of shoots rotted in 2002 compared to less than 10% rotting in 2003. These damp conditions would have been favorable for a fungal outbreak in the substrate. Utilizing a better-draining substrate, such as a sand or perlite mixture, could prevent water-logging and rotting problems. Wounding of shoots could provide an entry point for these fungal organisms; girdling of shoots using a metal ring could be a viable method of girdling shoots while not causing an open wound. Breakage of shoots was also a problem when shoots were wounded too deeply.

Although we were not successful in optimizing rooting using wounding and IBA application, the discovery of an easier-to-root pawpaw genotype (row 6, tree 71) is also important. This particular genotype could be used in future clonal propagation studies and may be examined as a potential clonal rootstock due to its ease in rooting. Since rooting occurred closer to the ground in R6T71, wounding and auxin applications closer to the ground, where plant tissue could be more juvenile in character could also enhance rooting in other genotypes. Pawpaw has proven extremely difficult to root, and has so few options in clonal propagation, therefore discovering a genotype that has greater ease in rooting may be just as important as finding new methods or treatments to enhance rooting. We do not know what fruiting characteristics this genotype possesses, but this selection could serve as a consistent rootstock. Wood (1989) reported that poorly developed root systems of pecan shoots rooted by mound layering could lead to poor field establishment rates. Solar et al. (1994) also found that while 40% of layered hazelnut shoots had strong enough root systems to be transplanted into orchards after the first year, another 35-45% of rooted stems required another year in a nursery before they were vigorous enough to be planted in orchards. Optimizing the development of a strong root system in rooted stems of pawpaw could be equally important to field establishment. The stems with roots from row 5, tree 97 and row 2, tree 43 were poorly developed (one root/stem) and would not be desirable for establishment. The rooted stems of row 6, tree 71 that developed outside the treatment areas were also few in number (1-3 roots), however, the rooted shoots were potted in 1 gallon Treepots filled with ProMix Bx and placed in the greenhouse to evaluate survivability. Unfortunately, none of the stems survived; the roots that were present at potting had apparently rotted and no new roots were observed. Although a mound layering study was not conducted in 2004, this genotype was re-covered with ProMix potting medium and rooting of this year's untreated shoots will be examined. Any rooted stems will be placed in pots, but with a better draining potting substrate such as perlite and/or sand for further evaluation.

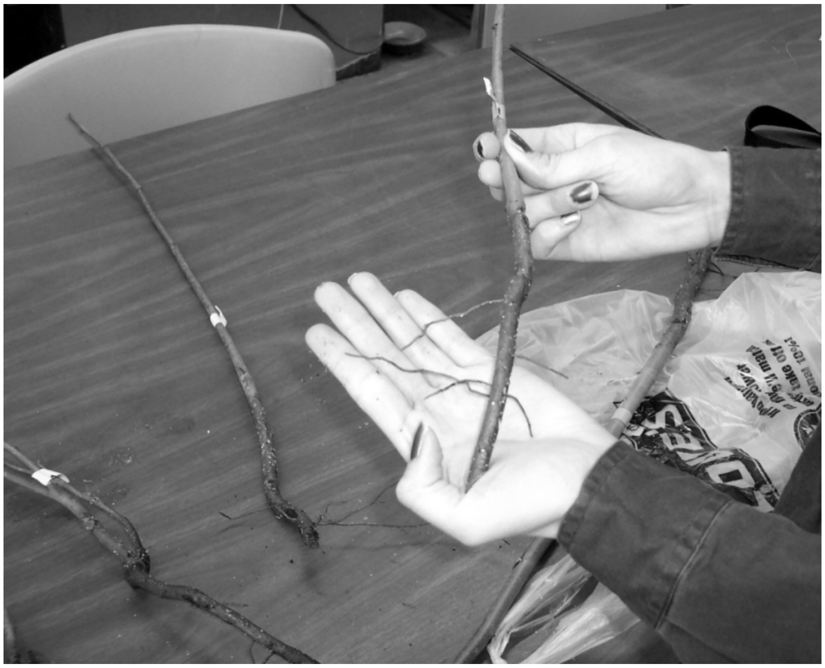
Conclusions

With the concentrations of IBA and wounding used in this experiment, mound layering was not a viable method for clonally propagating pawpaw. Further studies utilizing different methods of optimizing rooting in a mound layering system, such as different forms of auxin or wounding closer to the ground where tissue could be more juvenile in character, could make this a more feasible method of clonally propagating pawpaw. An easier-to-root genotype was discovered (R6T71), which could prove useful for future propagation studies of pawpaw.

Figure 4.1. Photograph of pawpaw in mound layering frame in Kentucky State University Orchard.

Figure 4.2. Photograph of roots on pawpaw stem from 2002 mound layering experiment.

Figure 4.3. Photograph of roots on pawpaw stems from 2003 mound layering experiment for seedling in row 6, tree 71 in Kentucky State University germplasm orchard.



Chapter 5: Diversity in Flowering and Fruiting Characteristics of North American Pawpaw

Introduction

The pawpaw [*Asimina triloba* (L.) Dunal] is a tree fruit native to many areas of the eastern and midwestern United States from USDA hardiness zones 5 through 8 (Layne, 1996; Pomper et al., 1999). The fruit has a tropical-like flavor resembling a combination of mango, banana, and papaya, and a smooth, custard-like flesh. The high fruit quality and attractive ornamental value give the pawpaw great potential as a commercial tree fruit or as a component in "edible landscapes" (Layne, 1996; Pomper and Layne, 2005). The fruit is highly nutritious and has been found to be higher in many vitamins, minerals, and amino acids than other fruits, including apples, bananas, and oranges (Peterson et al., 1982). The twig, leaf and bark tissues also produce natural defense compounds called annonaceous acetogenins, which have shown potential as a botanical pesticide and as an anti-cancer agent (McLaughlin, 1997).

In 1994, Kentucky State University (KSU) was designated as the U.S. Department of Agriculture (USDA), National Clonal Germplasm Repository for *Asimina* spp. Germplasm evaluation, preservation, and dissemination have been a high priority at KSU since that time. The repository orchards currently contain over 1700 accessions collected from the wild in 17 states and more than 40 cultivars (Pomper et al, 2003). One of the goals of the repository is to assess levels of genetic diversity in native populations. Another goal is to acquire and preserve unique germplasm in our repository collection. Vegetative and reproductive characteristics may be present in the pawpaw repository accessions that would be helpful in future breeding efforts. Molecular marker studies have been conducted to examine the level of genetic variation found in KSU repository accessions (Huang et al., 1997 and 2000; Pomper et al., 2003). These studies found that pawpaw exhibits a moderate to high level of genetic diversity compared to other woody plant species. An examination of variation in vegetative and reproductive characteristics in pawpaw repository accessions has not been conducted.

In the wild, pawpaw has been found to have very low fruit set (Peterson, 1991). There may be a number of reasons for this low fruit set. Pawpaw flowers are protogynous, so the stigmatic surface is receptive before pollen is shed from the anthers

(Willson and Schmeske, 1980). This is a mechanism which can force out-crossing. Native stands of pawpaw may be genetically identical as they spread by root suckers. Since pawpaws are believed to require cross-pollination, if genetically different patches or genotypes are not close enough for cross-pollination, fruit set in isolated patches could be low. Willson and Schemske (1980) examined flowering, pollination, and fruit production in a native Illinois pawpaw patch and found a large number of flowers (up to 500 per stem), but low fruit set, ranging from 0.01% to 1.38%. These authors suggested that the low fruit set may have been due to low light levels and thus a low rate of photosynthesis. Pawpaws are primarily pollinated by flies and beetles (Faegri and van der Piji, 1971). Limited availability of pollinators is also a contributing factor to low fruit set. Lagrange and Tramer (1985) also reported poor fruit set in wild pawpaw patches (3.4%). These authors also noted that pollinators were rarely observed visiting flowers in their study. Lagrange and Tramer (1985) also found that trunk diameter and tree height were not correlated with flower or fruit production. Low fruit set was also observed in *Asimina obovata* and *A. pygmaea* in Florida (Norman and Clayton, 1986). Fruit set did not exceed 16% without hand-pollination. With hand-pollination, *A. obovata* attained 32% fruit set, and 60% of *A. pygmaea* flowers set fruit. While hand-pollination improved fruit set, it was not high enough to lead the authors to believe that pollen transfer was the only problem leading to low fruit set in these *Asimina* species. The authors concluded that maternal resources also restricted fruit set in *A. pygmaea* and *A. obovata.* Fruit set in pawpaw has not been examined where many different genotypes are known to be nearby for cross-pollination.

There are approximately 40 commercially available pawpaw cultivars (Jones et al., 1998). Unfortunately, many pawpaw cultivars that were selected from 1900-1950 have been lost due to collection abandonment or neglect (Peterson, 2003). Improvements in fruit size, flavor, texture, storability, color break at ripening, early and/or late ripening, late flowering to avoid spring frosts, concentrated flowering time, concentrated ripening time, high fruit set, more single fruited clusters, and high fruit yields would be desirable in breeding efforts for new pawpaw cultivars. Variation in the above traits may be contained in the accessions in the repository collection at KSU.

The overall goal of this study was to evaluate the genetic diversity of reproductive characteristics of the accessions in the KSU USDA-NCGR orchards. Our hypotheses were that 1) larger trees will have greater fruit set than smaller trees; 2) trees that flower for longer periods of time will have more extended harvest times; 3) trees that flower for longer periods of time will have higher fruit set than trees with shorter flowering times; 4) greater numbers of flowers on a tree will lead to a greater number of fruit set on that tree; 5) accessions from more southern regions will flower earlier than accessions from northern regions; 6) accessions from more northern regions will require fewer degree days for fruit ripening than those from more southern regions; 7) fruit set is relatively low in pawpaw; and 8) biennial bearing does not occur in pawpaw. The objective of this study was to compare flower peaks, harvest peaks, total number of fruit produced, fruit weight, total yield by weight, trunk cross sectional area, yield efficiency, growing degree days required for ripening, total number of flowers, flower density, length of flowering in days, length of harvest in days, percentage fruit set, number of clusters, and average number of fruit per cluster in six pawpaw populations originating from six different geographic region over three consecutive years.

Materials and Methods

Plant Material Six populations of trees from different regions of the United States were selected in the KSU Germplasm Repository Orchard for evaluation of vegetative and reproductive characteristics. These populations included 10 trees from Washington Co., Ind. [(IN-1), row 2, trees 42-58]; 23 trees from Tompkins Co., N.Y. (row 5, trees 23-45), 13 trees from Tyler Co., W.V., 14 trees from Talbot Co., Md. (row 6, trees 100-120), 21 trees from Fayette Co., Ky. (row 7, trees 55-76), and 8 trees from Decatur Co., Ind. [(IN-2), row 8, trees 88-97]. Climatology data for these sites can be found in Table 5.1. Seed was collected from native stands in these six geographic regions and sown in KSU greenhouses in Spring 1994; these one-year-old seedlings were planted in the KSU orchards in Spring 1995.

2002 study Trunk diameters were measured on March 30, 2002, before spring plant growth began, with a d-tape wrapped around the trunk 30 cm from the ground, and trunk cross sectional areas (TCSA) were calculated. The number of maroon flowers on trees was counted on Mondays, Wednesdays, and Fridays from April 10, 2002 until May

20, 2002. Flowering peak was determined by taking the total flowering time in days, dividing that time span into quarters, and assigning a number accordingly: i.e. the trees with a flowering peak in the first quarter of the total flowering time were assigned a value of 1; trees with a flowering peak in the last quarter of the total flowering time were assigned a value of 4. Flower densities were determined by dividing the total number of flowers by the tree's TCSA.

A heavy frost occurred in the orchards on May 20, 2002. For an initial cluster count, the number of fruit clusters per tree was counted on May 24 since frost-damaged clusters had not yet dropped from trees. Additional cluster counts were conducted on June 3 and July 24. Fruit clusters were counted by recording the number of fruit per cluster, so as to obtain a total number of clusters, an average number of fruit per cluster, and total number of fruit per tree. To determine harvest peak, the number of ripe fruit per tree was recorded on Monday, Wednesday, and Friday from August 19 until September 23, 2002. Harvest peak was calculated in the same manner as that for determining flowering peak. Average fruit weight was determined by selecting 15 fruit at random from each tree and weighing the fruit. Yield efficiency (YE) was calculated by dividing tree yield in kg. by TCSA. Growing degree days (GDDs) were calculated using a base temperature of 10°C, which is the base temperature used in calculating GDDs in apple (Stanley et al., 2001) and beginning degree day accumulation at May 15, which was approximately the end of flowering (University of Kentucky Agricultural Weather Center calculator, http://wwwagwx.ca.uky.edu/calculators.html).

2003 study Trunk diameters were measured on March 24, 2003 to obtain trunk cross-sectional area values. The number of maroon flowers on trees was recorded every Monday, Wednesday, and Friday from April 14 to June 11. Cluster counts were performed on June 10 and July 10. A heavy frost had occurred on April 22, causing many flowers to be destroyed, however, many trees later re-bloomed, and attempts were made during the cluster counts to distinguish between fruit that had set before and after the frost, using size. The number of fruit harvested was recorded on Monday, Wednesday, and Friday from August 20 until October 10. Methods of data collection and calculations were as described above.

2004 study Trunk diameters were measured on March 3, 2004. The number of maroon flowers on trees was counted every Monday, Wednesday, and Friday from April 12 until May 10. Twice during the flowering season, on April 28 and May 4, there were frost events and moist round hay bales were burned in an effort to prevent flower and cluster loss. Cluster counts were performed on June 7 and July 29. The number of fruit harvested was recorded every Monday, Wednesday, and Friday from August 23 to October 1. Methods for data collection were as described above.

Statistical analyses Data were subjected to GLM analysis of variance and regression analysis using the statistical analysis program CoStat (CoHort Software, Monterey, Calif.). Treatment means were separated based on the least significant difference with a significance level of 0.05. Correlations were also tested between various means.

Results

2002 study Both vegetative and reproductive traits varied significantly among the six pawpaw populations evaluated (Table 5.2 and 5.3). TCSA among populations was significantly different, with the New York and Washington Co., Indiana accessions exhibiting more vigor than populations originally from Decatur Co., Indiana, and West Virginia. Total number of flowers between populations varied significantly, with trees from New York and Washington County, Indiana producing a larger number of flowers than accessions from West Virginia, Maryland, and Decatur County, Indiana. Length of flowering time also differed greatly, as populations from New York, Kentucky, and Washington County, Indiana blooming for a longer period of time than trees from West Virginia or Decatur County, Indiana. Flowering peak also differed significantly among populations, with trees from Kentucky and West Virginia having later flowering peaks than populations from Washington Co., Indiana, New York, or Maryland.

Percentage fruit set was not significantly different between populations, with an average of 15% fruit set across all populations. Total number of clusters per tree did vary significantly, with accessions from Washington Co., Indiana having the greatest number of fruit clusters per tree. Number of fruit per cluster also varied significantly, with trees from Washington Co., Indiana, New York, and Maryland averaging more fruit per cluster than West Virginia. Trees from the West Virginia had the fewest number of fruit per tree

(5) than any state except Decatur Co., Indiana (16), and a lower total yield (0.59 kg) than populations from Washington Co., Indiana, New York, or Maryland. Fruit weight was greatest in trees from Maryland (141.8 g). Yield efficiency varied greatly, and once again the West Virginia population had the poorest production, significantly lower than accessions from Washington Co., Indiana, New York, or Maryland. Accessions from Kentucky and Washington County, Indiana had a significantly longer harvest period than trees from West Virginia, Maryland, and Decatur County, Indiana. Harvest peak varied among states, with fruit from the New York accessions ripening earliest and fruit from trees from West Virginia and Kentucky ripening latest. GDDs for ripening varied among populations, with the New York population requiring the fewest GDDs, at 2492, and accessions from West Virginia requiring the highest number of GDDs, 2980.

Trees producing a higher number of flowers led to a higher number of fruit clusters produced by that tree (r=0.60, Table 5.4). An earlier flowering peak also led to an earlier harvest peak (r=0.42), and length of flowering time and length of harvest were found to be correlated (r=0.40). Trunk cross sectional area and percentage fruit set were not correlated, but a higher trunk cross sectional area was found to lead to a higher number of flowers per tree (r=0.75) higher total yield in weight per tree (r=0.44) and a higher number of fruit per tree (r=0.43). Trunk cross sectional area also did not influence the yield efficiency of the tree. Neither length of flowering time nor total number of flowers affected percentage fruit set. Length of flowering did positively affect the total number of fruit produced per tree (r=0.40) and the total yield by weight (r=0.37).

Correlations of these factors among individual states varied greatly (Table 5.5). Trunk cross sectional area was found to influence the total number of flowers on a tree for every population except IN1. Trees with a greater TCSA also produced more fruit clusters in most populations. TCSA did not generally affect yield efficiency or percentage fruit set of the accessions; however, most correlations were not consistent among states. Length of flowering generally did not affect length of harvest, percentage fruit set, or yield. There was a correlation between flowering peak and harvest peak only in the Maryland population.

2003 study Data taken in 2003 was more difficult to analyze. A frost in late April killed most pawpaw flowers and young fruit. Only 29 out of 89 trees evaluated in this study produced fruit in 2003, and two entire populations (W.V. and IN-2) did not produce any fruit. Therefore data recorded this year may be abnormal, and not representative of the true reproductive qualities of these populations. Trunk cross sectional area (TCSA) varied widely, with seedlings from New York and Washington Co., Indiana having larger TCSAs than trees from West Virginia, Maryland, or Decatur Co., Indiana (Table 5.6). Total number of flowers did differ significantly, with trees from Washington County, Indiana and Maryland producing more flowers than trees from West Virginia or Decatur County, Indiana. Length of flowering time also varied, as trees from Washington County, Indiana blooming for the longest period of time (28 days) and accessions from Kentucky and Decatur County, Indiana blooming for the shortest period of time (11-12 days). Flowering peak did not vary significantly between populations.

Percentage fruit set also did not differ significantly, with all populations having a very low fruit set, not exceeding 4%, with an average of 1.5% across all populations (Table 5.7). Accessions from Maryland produced the most fruit clusters per tree, approximately eight. Number of fruit per cluster varied significantly, with New York accessions producing more fruit per cluster than those from Maryland or Kentucky. The total number of fruit per tree and yield per tree differed significantly, with accessions from Maryland producing the largest number of fruit and the largest yields than any other populations. Average fruit weight did not differ between populations. Populations also showed great variation in yield efficiency, with accessions from Maryland having a greater yield efficiency than trees from New York, West Virginia, Kentucky, or Decatur Co., Indiana. Neither length of harvest nor harvest peak were significantly different between accessions. Growing degree days required for fruit ripening were also not found to be significantly different between populations in 2003.

Trees which had a higher total number of flowers also produced more fruit (r=0.56, Table 5.8). An earlier flowering peak did not lead to an earlier harvest peak on trees in 2003, nor did a longer total flowering time lead to a longer harvest interval. Trees with a larger trunk cross sectional area produced more flowers per tree (r=0.32), but did not have a higher percentage fruit set, nor did they produce a higher total yield by weight or higher number of fruit. Trees with a longer flowering time did have a higher total yield (r=0.32) but not a higher number of total fruit. Trees with a longer flowering time also had a higher percentage fruit set (r=0.34), and a larger total number of flowers also led to higher fruit set (r=0.25). Correlations among individual states were not analyzed due to lack of data.

2004 study. Most vegetative and reproductive characteristics varied among populations in 2004 (Tables 5.9 and 5.10). Trunk cross sectional area differed widely between populations, with trees from Washington County, Indiana and Kentucky having larger TCSA than accessions from West Virginia, Maryland, and Decatur County, Indiana. The total number of flowers produced by each tree varied greatly, with accessions from New York producing significantly more flowers than trees from West Virginia, Maryland, Kentucky, and Decatur County, Indiana. New York accessions also flowered for a longer period of time than trees from West Virginia or Kentucky. Flowering peak varied, with accessions from West Virginia and Kentucky blooming significantly later than the other populations. Trees from Washington County, Indiana, West Virginia and Kentucky had harvest peaks significantly later than those of New York and Decatur County, Indiana.

Percentage fruit set did not differ significantly between populations, with an average of approximately 12% fruit set on trees. Total number of clusters per tree did vary, with accessions from Washington Co., Indiana, New York, and Kentucky producing more clusters per tree than accessions from West Virginia or Maryland. Populations from Washington Co., Indiana and Maryland also produced more fruit per cluster, on average, than trees from New York, West Virginia, or Decatur Co., Indiana. Total number of fruit on each tree varied greatly, with accessions from Washington County, Indiana; New York, and Kentucky producing more fruit on average than trees from West Virginia and Decatur County, Indiana. Populations also showed variation in average fruit weight, with trees from West Virginia producing the largest fruit (138 g) and Kentucky accessions producing the smallest fruit (74 g), with the other four populations producing fruit of a similar, moderate size (approximately 100 g). Neither average total yield per tree nor yield efficiency was significantly different between populations. Length of harvest differed significantly, with trees from Kentucky, Washington County, Indiana, and New York producing fruit over a longer period of time than accessions from West Virginia, which had the shortest harvest interval. Harvest peak also differed between populations, with accessions from Washington Co., Indiana, West Virginia, and Kentucky exhibiting a later harvest peak than trees from New York or Decatur Co., Indiana. Growing degree days varied significantly between accessions, with trees from New York requiring the fewest GDDs to ripen, 2262, and the other 5 populations requiring a similar number of GDDs (2400-2500) for ripening.

A correlation was found in 2004 between the total number of flowers produced by a tree and the total number of fruit clusters produced by that tree (r=0.39, Table 5.11). Trees which flowered for a longer period of time did not produce more clusters per tree or a higher yield by weight. An earlier flower peak led to an earlier harvest peak (r=0.26), but there was no correlation between length of flowering and length of harvest. Neither longer flowering time nor total number of flowers led to a higher percentage of fruit set on trees. A larger trunk cross sectional area did positively affect the total number of flowers per tree (r=0.60), total yield by weight (r=0.29) and total number of fruit per tree (r=0.33), but did not affect percentage fruit set or yield efficiency. Again, correlations by individual state varied greatly (Table 5.12), with few correlations that were significant across all populations being significant by individual population.

Discussion

This study is the first of its kind to characterize vegetative and reproductive traits in pawpaw populations from various geographic regions under the same environmental conditions. Trees possessing desirable traits such as large fruit size, good flavor, texture, storability, color break at ripening, early and/or late ripening, late flowering to avoid spring frosts, concentrated flowering time, concentrated ripening time, high fruit set, more single fruited clusters, and high fruit yields could be used in breeding efforts in the development of new pawpaw cultivars by the KSU USDA-NCGR for *Asimina* species. Developing new pawpaw cultivars with these features is key to the growth and development of a commercial pawpaw industry (Peterson 1991; Peterson, 2003).

Pawpaw seedlings normally begin to flower when the trees reach about 1.8 m, but may not set fruit; cropping is achieved at five to eight years (Pomper et al., 2003). Grafted pawpaw trees often flower within 3 years after planting, but fail to set fruit (Pomper et al. 2003; Bratsch et al. 2003; Merwin et al. 2003). Failure of trees to set fruit could be due to inadequate pollination or inadequate canopy develop to support fruit development. Usually 5 to 6 years are required for grafted trees to come in to production, although some cultivars such as 'PA-Golden (#1)' may produce crops 4 to 5 years after planting (Pomper et al. 2003).

Seedling trees examined in this study had been planted for 8 years prior to the start of data collection. Vegetative vigor varied among populations in this study and a number of trees still did not produce fruit in 2002. Trees which were larger in terms of TCSA, and therefore have a higher biomass, were found to produce a greater number of clusters and have a higher total yield by weight. TCSA did not affect the percentage of flowers that formed clusters. Trees with a greater TCSA did produce more flowers than smaller trees. TCSA also increased at a greater rate between 2003 and 2004 (65% increase) than between 2002 and 2003 (39% increase), likely because very few fruit were produced on trees in 2003, leaving most of the trees resources to go into vegetative growth rather than fruit production. Although precocity data for these populations was not examined, earlier unpublished data collected by Pomper noted that trees in the New York population, that was also the largest in TCSA also flowered and produced fruit in 2001, earlier than trees in the other populations.

The tropical Annonaceae relatives of the pawpaw, cherimoya (*Annona cherimola*), sweetsop or sugar apple (*A. squamosa*), soursop (*A. muricata*), and atemoya (*A. squamosa* × *A. cherimola*), also have low yields due to low rates of natural pollination (George et al., 1992; Pena et al., 1999; Peterson, 1991). In commercial plantings, these tropical pawpaw relatives are hand pollinated to increase yields (Pena et al., 1999; Peterson, 1991). Pawpaw trees often produce large numbers of flowers but may set few fruit. This is true of many tree fruits, with typical fruit set of apple ranging from 2-8%, and an average of 15-20% fruit set in peach (Chaplin and Westwood, 1980). Pawpaws in the wild have also been reported to have very low fruit set, with less than 1.5% fruit set reported by Willson and Schmeske (1980) in native Illinois pawpaw patches, and 3.4% fruit set recorded by Lagrange and Tramer (1985). This study found that trees that produced a higher number of flowers also produced more fruit. Percentage fruit set in pawpaw ranged from 5-15% in this study, which is comparable to other tree fruits. However, fruit set was not found to be significantly different between populations except in 2003, when the Maryland population had the highest fruit set. Flies and beetles are thought to be the main pollinators of pawpaw, and many of these insects have been observed each spring in the pawpaw orchards at KSU. Low pollinator activity was observed on cool cloudy spring days during the study. This is likely because this population was located at a higher elevation in the orchard than any other group in this study, and therefore did not sustain as much frost damage, so more flowers and developing clusters survived the frost.

Pawpaw differs from many other tree fruits in that it produces flowers for an extended period of time, and fruit is also harvested over a long period of time, usually around 2 weeks per tree. Staggered bloom period in the spring also extends harvest period. Each fruit cluster develops from an individual flower, and fruit within a cluster often ripen at different times. It would be desirable for commercial pawpaw growers to identify and develop genotypes that have a more concentrated harvest time. Length of flowering time did not consistently affect the length of harvest in pawpaw. Trees with a longer flowering time were found to have a longer harvest interval in only one year. However, trees with an earlier flowering peak did generally have an earlier harvest peak.

It is possible that the long flowering period of pawpaw is a mechanism to ensure greater fruit set. By spreading flowering and fruit set out over a longer period of time, perhaps the entire crop would not be wiped out by a frost event. However, length of flowering in pawpaw was found to lead to a greater fruit set only in 2003.

Pawpaw yields are notoriously low (Peterson 1991). Pomper and Layne (2005) reported yields that averaged 6.4, 2.0, and 3.7 kg per tree for 'PA-Golden', 'Wilson', and 'Sunflower', respectively, for trees in their 5 year. In another study, yields per tree in the 7th year were: 4.4 kg for 'Sunflower', 2.3 kg for 8-20, and 2.2 kg for 'PA-Golden' (Pomper et al. 2003d). Yields among populations varied; however, the highest yielding tree in 2004 produced 28.2 kg per tree, demonstrating that some genotypes may have potential as future cultivars or in breeding efforts. The fruit weights observed (>180 g) and tendency toward few or single fruit per cluster, which is desirable to growers for easier harvest of intact fruit, of some selections were more desirable than that of some currently available commercial cultivars.

It was hypothesized that southern pawpaw accessions would flower earlier than populations from more northern areas, since more northerly pawpaws may have adapted to that colder climate and later frosts by flowering later. However, accessions from West Virginia and Kentucky consistently flowered later than any other populations in this study. West Virginia was the second most northern populations studied; however, Kentucky was the most southern population in this study. The most northern population, New York, generally flowered fairly early. Therefore it cannot be concluded that more northern accessions bloom later to avoid frost. Seedling populations from more southern regions were not available for evaluation in this current study.

Ripening of most tree fruits depends on obtaining a certain amount of growing degree days (GDDs). GDDs are calculated by subtracting a base temperature, usually 10°C, from the mean daily temperature. It is logical that trees from more northern regions of the U.S. would require fewer GDDs for fruit ripening since fall frosts would generally occur earlier in those areas; this is an adaptation to enable the fruit to ripen before it is killed by frost. GDDs required for pawpaw ripening have not been reported in any other literature. In this study, GDDs required for ripening varied between populations from different regions of the country. The New York population was the northern-most group examined and required the fewest GDDs for fruit ripening. This may represent an adaptation in northern populations to allow fruit to ripen prior to a frost or freeze. GDDs required for fruit ripening by pawpaws in this study ranged from 2200-3200 GDDs.

Many tree fruit crops experience biennial bearing (Westwood, 1993). Biennial bearing occurs when a tree has a heavy fruit crop while initiating the next year's flower buds. Most of the tree's resources must go into fruit production rather than floral bud initiation, therefore few flower buds are formed in years of heavy cropping. It is not known whether biennial bearing occurs in pawpaw. Loss of most of the fruit due to frost damage in 2003 makes it difficult to determine whether pawpaw exhibits biennial bearing, as there were not 3 consecutive years in which the trees produced a maximum fruit load. At least two more years of data would be required to determine if there is a tendency in pawpaw towards biennial bearing in the year following a year with high fruit set.

A common practice to determine maturity is to touch each fruit to determine if it is ready to harvest; ripe softening pawpaw fruit pull away from the tree when squeezed gently, as ripe peaches do, and can be picked easily with a gentle tug. Thus, fruit are harvested when they have already begun ripening and have lost some firmness. This is labor intensive and may result in slight bruising injury, perhaps leading to off-flavors (Peterson 1991). Also, a natural abscission zone forms where the fruit attaches to the peduncle when fruit ripen. “Wiggling” the fruit can determine how well this abscission zone has formed. Ripening fruit also give off a strong aroma. On current cultivars, fruit on a single tree do not ripen within close proximity in time to one another. In this study trees in the population from WV and IN2 had shorter harvest periods (9-14 days) than other selections; this was not related to a shorter bloom period. Although each fruit cluster develops from an individual flower, fruit within a cluster often ripen at different times. Currently, multiple harvests from one tree are conducted to obtain high quality fruit. A more concentrated ripening period such as that in the WV and IN2 populations would be valuable to growers.

Conclusions

The collection of pawpaws housed in the KSU USDA-NCGR for *Asimina* spp. show great diversity in reproductive characteristics. This diverse collection includes fruit with many different desirable reproductive qualities, which could be useful in future breeding efforts.

Table 5.1: Climatology data for six sites from which pawpaw seedlings included in 2002-2004 flowering and fruiting study originated

state	GPS coordinates	USDA growing zone[z]	AHS heat zone[y]	average January low temperature (°C)	average July high temperature (°C)	record low (°C)	record high (°C)	average annual precipitation (cm)	average annual growing degree day accumulation[x]
IN1	38.6°N, 86.0°W	6A	6/7	-6	31	-36	41	117	4456
IN2	39.3°N, 85.5°W	5B	5/6	-7	29	-31	41	103	3647
KY	38.0°N, 84.5°W	6A	6	-4	30	-29	39	117	3961
MD	38.8°N, 76.1°W	7A	6/7	-2	31	-21	38	117	3933
NY	42.4°N 76.5°W	5A	3/4	-9	28	-29	38	94	2861
WV	39.6°N, 81.0°W	6A	5	-7	29	-29	39	109	3625

z: Average annual minimum temperatures for United States Department of Agriculture Growing Zone 5A, 5B, 6A, and 7A are -29, -26, -23, and -18°C, respectively.

y: Average number of days per year above 30°C for American Horticultural Society Heat Zones 3, 4, 5, 6, and 7 are 7-14, 14-30, 30-45, 45-60, and 60-90 days, respectively.

x: Growing degree day data from NOAA/ National Weather Service Climate Prediction Center, http://www.cpc.ncep.noaa.gov

Table 5.2: Vegetative and flowering characteristics of six populations of pawpaw evaluated in 2002, KSU Germplasm Orchard, Frankfort, Ky.

state	trunk cross sectional area	total number of flowers	length of flowering (days)	flower peak	flower density	n
IN1	24.3±8.9 ab	276.7±62.0 ab	25.0±3.0 ab	1.7±0.3 cd	12.6±4.8 a	10
IN2	15.9±7.1 cd	109.9±122.1 cd	14.8±11.2 c	1.9±0.6 bc	5.9±5.2 b	8
KY	21.1 bc	196.4 bc	23.7 ab	2.2 ab	8.7± 5.9 b	21
MD	18.7±8.9 bcd	120.6±90.7 cd	19±8.1 bc	1.5±0.2 d	5.5±3.9 b	14
NY	25.9±8.1 a	314.8±148.1 a	26.0±2.3 a	1.9±0.2 c	11.6±3.8 a	23
WV	13.6±6.7 d	93.4±77.0 d	16.9±10.2 c	2.2±0.3 a	6.0±4.9 b	13
P-value	0.0002 ***	0.0000***	0.0019**	0.0000 ***	0.0002***	

Table 5.3: Fruiting characteristics of six populations of pawpaw evaluated in 2002, KSU Germplasm Orchard, Frankfort, Ky.

State	fruit set (%)	total number of clusters	number of fruit per cluster	number of fruit per tree	fruit weight (g)	yield (kg)	yield efficiency	length of harvest (days)	harvest peak	growing degree days for ripening
IN1	8.6 ±4.7	23.3 ±14.0 a	2.3 ±0.6 a	39.8 ±30.9 a	105.4 ± 31.8 b	4.3 ±3.5 a	0.20 ±0.19 a	16.4 ±5.9 a	2.2 ±0.9 b	2601 ±106 c
IN2	7.4 ±7.7	7.6 ±8.6 cd	1.3 ±0.6 bc	15.9 ±18.7 bc	103.4 ± 25.8 b	1.8 ±2.4 bc	0.11 ±0.16 abc	7.6 ±5.8 b	2.1 ±1.0 bc	2726 ±203 bc
KY	7.4 ±3.9	14.2 ±9.8 bc	1.7 ±0.9 abc	24.1 ±22.1 ab	88.1 ±19.5 b	2.2 ±2.1 bc	0.09 ±0.09 bc	15.5 ±8.5 a	3.0 ±0.4 a	2782 ±141 b
MD	10.3 ±7.6	11.9 ±9.9 bc	2.0 ±1.0 ab	27.9 ±29.2 ab	141.8 ± 31.5 a	3.6 ±3.6 ab	0.16 ±0.14 ab	9.7 ±6.7 b	2.1 ±0.9 b	2676 ±155 bc
NY	6.0 ±3.8	15.7 ±9.5 b	2.1 ±0.4 a	33.3 ±27.3 ab	106.1 ± 26.7 b	3.5 ±2.7 ab	0.14 ±0.10 ab	11.5 ±4.6 ab	1.4 ±0.5 c	2492 ±53.3 d
WV	4.6 ±5.6	4.2 ±5.2 d	1.2 ±1.0 c	4.8 ±7.2 c	109.9 ± 32.5 b	0.6 ±0.9 c	0.04 ±0.06 c	7.9 ±9.2 b	3.4 ±0.5 a	2980 ±244 a
P-value	0.1302	0.0002 ***	0.0081 **	0.0085 **	0.0002 ***	0.0071 **	0.0184 *	0.0133 *	0.0000 ***	0.0000 ***

Table 5.4: Correlations of vegetative and reproductive characteristics of six populations of pawpaw evaluated in 2002, KSU Germplasm Orchard, Frankfort, Ky.

x	y	correlation p-value	r
trunk cross sectional area	total number of flowers	**	0.75
trunk cross sectional area	% fruit set	NS	
trunk cross sectional area	total number of fruit	**	0.43
trunk cross sectional area	total number of clusters	**	0.44
trunk cross sectional area	yield (kg)	NS	
trunk cross sectional area	yield efficiency	**	0.45
total number of flowers	total number of fruit	**	0.60
total number of flowers	total number of clusters	NS	
total number of flowers	% fruit set	**	0.40
length of flowering	length harvest	NS	
length of flowering	% fruit set	**	0.40
length of flowering	total number of fruit	**	0.53
length of flowering	total number of clusters	**	0.37
length of flowering	yield (kg)	**	0.42

Table 5.5: Correlations of vegetative and reproductive characteristics of six populations of pawpaw evaluated in 2002, KSU Germplasm Orchard, Frankfort, Ky.

x	y	IN1	IN2	KY	MD	NY	WV
TCSA	total # flowers	N.S.	**	**	*	**	**
TCSA	% fruit set	N.S.	N.S.	N.S.	N.S.	**	N.S.
TCSA	total # fruit	N.S.	N.S.	*	*	N.S.	N.S.
TCSA	total # clusters	N.S.	*	**	**	N.S.	**
TCSA	yield (kg)	N.S.	N.S.	**	**	N.S.	N.S.
TCSA	YE	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
total # flowers	total # fruit	N.S.	N.S.	*	*	N.S.	N.S.
total # flowers	total # clusters	N.S.	**	**	**	N.S.	N.S.
total # flowers	% fruit set	N.S.	N.S.	N.S.	N.S.	**	N.S.
length flowering	length harvest	*	N.S.	N.S.	N.S.	N.S.	N.S.
length flowering	% fruit set	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
length flowering	total # fruit	N.S.	*	*	*	N.S.	N.S.
length flowering	total # clusters	N.S.	*	**	*	N.S.	N.S.
length flowering	yield (kg)	N.S.	N.S.	N.S.	*	N.S.	N.S.
flower peak	harvest peak	N.S.	N.S.	N.S.	**	N.S.	N.S.

Table 5.6: Vegetative and flowering characteristics of six populations of pawpaw evaluated in 2003, KSU Germplasm Orchard, Frankfort, Ky**.**

State	trunk cross sectional area	total number of flowers	length of flowering (days)	flower peak	flower density	n
IN1	37.8 a	278.1 a	27.8 a	1.6	8.4 ab	10
IN2	23.4 c	134.3 c	10.5 c	1.7	10.4 ab	8
KY	28.5 bc	208.9 abc	12.9 c	1.8	7.9 b	21
MD	25.3 c	278.4 a	17.6 b	1.6	13.0 a	14
NY	32.5 ab	257.7 ab	21.5 b	1.6	8.0 b	23
WV	24.1 c	175.5 bc	19.9 b	1.7	6.4 b	13
P-value	0.0115*	.0497*	.0000***	0.5811	0.1841	

Table 5.7: Fruiting characteristics of six populations of pawpaw evaluated in 2003, KSU Germplasm Orchard, Frankfort, Ky.

State	fruit set (%)	total number of clusters	number of fruit per cluster	number of fruit per tree	fruit weight (g)	Yield (kg)	Yield Efficiency	length of harvest (days)	harvest peak	growing degree days for ripening
IN1	0.96 ab	2.6 b	2.9 ab	8.2 b	103.6	.8 ab	0.03 ab	13.6	1.9	2609
IN2	0.00 b	0 b	-	0	-	0 b	0 b	-	-	-
KY	0.24 b	.6 b	2.4 b	8.3 b	102.5	.1 b	0.004 b	4	2.2	2648
MD	2.15 a	8.3 a	1.9 b	40 a	130.7	2.0 a	0.07 a	23	1.6	2524
NY	0.56 b	1.2 b	3.4 a	6.5 b	139.2	.5 b	0.02 b	5.4	2.1	2645
WV	0.0 b	0 b	-	0	-	0 b	0 b	-	-	-
P-value	.0033 **	0.0004 ***	.0051 **	.00096 **	0.2099	0.0152 *	.0184 *	.4560	0.5113	0.3346

Table 5.8: Regression and correlation of vegetative and reproductive characteristics of six populations of pawpaw evaluated in 2003, KSU Germplasm Orchard, Frankfort, Ky.

x	y	correlation P-value	r
trunk cross sectional area	total number of flowers	**	0.32
trunk cross sectional area	% fruit set	NS	
trunk cross sectional area	total number of fruit	NS	
trunk cross sectional area	total number of clusters	NS	
trunk cross sectional area	yield (kg)	NS	
trunk cross sectional area	yield efficiency	**	0.56
total number of flowers	total number of fruit	**	0.43
total number of flowers	total number of clusters	*	0.25
total number of flowers	% fruit set	NS	
length of flowering	length harvest	**	0.34
length of flowering	% fruit set	*	0.27
length of flowering	total number of fruit	**	0.30
length of flowering	total number of clusters	**	0.32
length of flowering	yield (kg)	NS	

Table 5.9: Vegetative and flowering characteristics of six populations of pawpaw evaluated in 2004, KSU Germplasm Orchard, Frankfort, Ky.

State	trunk cross sectional area	total number of flowers	length of flowering (days)	flower peak	flower density	n
IN1	58.9±16.2 a	586.7±127.6 ab	21.4±1.9 ab	2.3±0.3 b	10.4±3.1 ab	10
IN2	35.4±16.0 c	395.1±219.3 cd	22.0±4.4 ab	2.2±0.3 b	10.1±3.9 ab	8
KY	53.8±21.8 a	467.0±151.2 bc	20.0±0.6 bc	2.7±0.3 a	9.3±3.1 b	21
MD	41.0±13.2 bc	482.0±197.9 bc	21.4±2.1 ab	2.3±0.4 b	12.4±5.3 a	14
NY	51.1±16.9 ab	611.8±141.7 a	22.2±2.2 a	2.2±0.3 b	12.3±1.8 a	23
WV	39.4±16.6 bc	314.2±184.2 d	18.8±3.9 c	2.9±0.5 a	7.7±4.1 b	13
P-value	0.0098**	0.0000***	0.0024**	0.0000***	0.0018**	

Table 5.10: Fruiting characteristics of six populations of pawpaw evaluated in 2004, KSU Germplasm Orchard, Frankfort, Ky.

State	fruit set (%)	total number of clusters	number of fruit per cluster	number of fruit per tree	fruit weight (g)	Yield (kg)	Yield Efficiency	length of harvest (days)	harvest peak	growing degree days for ripening
IN1	14.3 ± 8.6	83.1 ±43.9 a	2.7 ±0.7 ab	127.8 ±50.3 a	98.9 ±23.1 b	12.8 ± 6.2	0.23 ±0.13	20.4 ±2.3 ab	2.1 ±0.6 a	2527 ±150 ab
IN2	11.8 ± 7.5	55.9 ±45.1 ab	1.8 ±0.5 d	72.6 ±54.1 bc	100.1 ±19.1 b	8.1 ± 4.8	0.20 ±0.12	17.0 ±8.7 bc	1.5 ±0.4 bc	2396 ±163 b
KY	15.2 ± 10.0	69.4 ±46.7 a	2.3 ±0.8 bc	117.4 ±60.6 a	73.9 ±21.5 c	9.0 ± 5.8	0.18 ±0.09	23.3 ±5.0 a	2.1 ±0.5 a	2561 ±112 a
MD	8.8 ±4.9	37.8 ±20.5 bc	3.0 ±1.9 a	98.7 ±5.6 ab	112.4 ±31.8 b	10.9 ± 5.6	0.26 ±0.10	18.4 ±5.9 b	1.8 ±0.8 ab	2457 ±204 b
NY	11.4 ± 5.5	65.1 ±24.1 a	2.3 ±0.5 c	130.6 ±50.0 a	95.9 ±24.3 b	12.1 ± 4.8	0.25 ±0.10	21.0 ±5.9 ab	1.2 ±0.4 c	2262 ±93 c
WV	8.0 ±6.8	24.6 ±21.5 c	1.8 ±0.8 d	39.1 ±7.5 c	137.6 ±44.4 a	7.2 ± 7.5	0.16 ±0.16	13.1 ±9.4 c	2.2 ±0.7 a	2577 ±129 a
P-value	.0552	0.0004 ***	0.0000 ***	.0000 ***	.0000 ***	0.1047	0.0904	.0026 **	.0000 *	.0000 *

Table 5.11: Regression and correlation of vegetative and reproductive characteristics of six populations of pawpaw evaluated in 2004, KSU Germplasm Orchard, Frankfort, Ky.

x	y	correlation P-value	r
trunk cross sectional area	total number of flowers	**	0.60
trunk cross sectional area	% fruit set	NS	
trunk cross sectional area	total number of fruit	**	0.33
trunk cross sectional area	total number of clusters	**	0.29
trunk cross sectional area	yield (kg)	NS	
trunk cross sectional area	yield efficiency	**	0.46
total number of flowers	total number of fruit	NS	
total number of flowers	total number of clusters	**	0.39
total number of flowers	% fruit set	NS	
length of flowering	length harvest	NS	
length of flowering	% fruit set	NS	
length of flowering	total number of fruit	*	0.23
length of flowering	total number of clusters	NS	
length of flowering	yield (kg)	*	0.26

Table 5.12 Correlation of vegetative and reproductive characteristics of six populations of pawpaw evaluated in 2004, KSU Germplasm Orchard, Frankfort, Ky.

x	y	IN1	IN2	KY	MD	NY	WV
TCSA	total # flowers	N.S.	**	N.S.	N.S.	**	**
TCSA	% fruit set	N.S.	N.S.	N.S.	N.S.	*	N.S.
TCSA	tota l# fruit	N.S.	N.S.	N.S.	N.S.	N.S.	*
TCSA	total # clusters	N.S.	N.S.	N.S.	N.S.	N.S.	*
TCSA	yield (kg)	N.S.	N.S.	N.S.	*	N.S.	N.S.
TCSA	YE	N.S.	N.S.	N.S.	N.S.	*	N.S.
total # flowers	total # fruit	N.S.	*	N.S.	N.S.	N.S.	N.S.
total # flowers	total # clusters	N.S.	*	N.S.	N.S.	N.S.	N.S.
total # flowers	% fruit set	N.S.	N.S.	N.S.	N.S.	**	N.S.
length of flowering	length of harvest	N.S.	*	N.S.	N.S.	N.S.	N.S.
length of flowering	% fruit set	**	N.S.	N.S.	N.S.	*	N.S.
length of flowering	total # fruit	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
length of flowering	total # clusters	*	N.S.	N.S.	N.S.	*	N.S.
length of flowering	yield (kg)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
flower peak	harvest peak	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

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